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Molecular Mechanisms of PARP Inhibitors in BRCA-related Ovarian Cancer

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Abstract

Ovarian cancer continues to be the main cause of death among all gynecological tumors. After standard treatments, most of patients are destined to recur within a short period, thus new therapies are urgently needed. The increasing knowledge of molecular mechanisms in ovarian cancer pathogenesis allowed identifying several targeted agents that are now entering in clinical practice. The family of poly(ADP-ribose) polymerase inhibitors represents a widely investigated and promising alternative for the targeted therapy of ovarian malignancies. PARP inhibitors exploit the synthetic lethality concept to prevent the repair of DNA damage, causing cancer cell death. This review describes the molecular mechanisms at the basis of PARP inhibition, particularly in BRCA-related ovarian malignancies and analyzes the main agents under investigations in preclinical and clinical studies.

Introduction

Ovarian cancer still represents the main cause of death in women with gynaecological cancers, counting in the United States about 22,280 estimated new cancer cases in 2012 and about 15,500 estimated deaths. The prevalence of ovarian cancer among gynaecologic malignances is rising; unfortunately, most of patients are diagnosed at advanced stages with consequently worse prognosis. Thus, overall survival is the poorest of all gynaecologic malignances, with a five-year relative survival rate of 44% for all stages [1].

Currently, the standard treatment in advanced disease remains optimal surgical debulking followed by a chemotherapy regimen based on taxane and platinum [2,3]. Despite surgical cytoreduction and chemotherapy, a large proportion of patients are at high risk for recurrent disease and are candidates for a second-line treatment. Recurrent ovarian cancer is currently classified according to sensitivity to platinum-based chemotherapy. Patients with a complete response after a platinum-based treatment who achieve a platinum-free interval more than 6 months before recurrence are classified as having platinum-sensitive disease (partially platinum-sensitive if platinum-free interval is between 6 and 12 months) and should be treated again with platinum-derived combinations. Women who progress during chemotherapy or experience a response of less than 6 months duration should be classified as having chemorefractary or chemoresistant (platinum-resistant) disease, respectively, and should be treated with a non-platinum single agent. However, cancer recurrences show low chemo-sensitivity and poor prognosis, thus new treatment strategies are urgently needed to improve outcomes [4,5].

The various histological subtypes of ovarian cancer are determined by different molecular alterations. Understanding the tumour molecular biology and identifying predictive indicators of outcome and response to therapy are essential steps in selecting the novel treatment strategies. The wide knowledge of molecular mechanisms in ovarian cancer pathogenesis allowed to identify several molecular targets, thus several agents targeted at these molecules are now entering in clinical practice [6]. The family of poly(ADP-ribose) polymerase (PARP) inhibitors represents a widely investigated and promising alternative for the targeted therapy of ovarian malignancies.

Ovarian Cancer Pathogenesis and Hereditary Cancer Syndromes

For a long period of time, ovarian cancer has been defined as one single disorder. Nowadays, we know that ovarian cancer is a heterogeneous disease that includes various biological behaviour from a clinical and molecular point of view. Epithelial ovarian cancer is characterized by four main histotypes that show differentiation resembling normal tissues of genital apparatus. Serous ovarian cancer seems to derive from the cells that line the fallopian tube, endometrioid tumors from endometrium, mucinous tumors from endocervix and clear cell tumors from the vagina epithelium. Even from a molecular point of view, the genetic profile of each histotype is similar to that of the histological counterparts in normal cells [7]. On this basis, Kurman et al. has recently reconsidered the function of the ovarian surface epithelium in tumorigenesis of epithelial ovarian cancer. The author emphasises the role of the fimbriae of fallopian tube in the pathogenesis of serous ovarian carcinomas and foci of endometriosis in endometrioid and clear cell ovarian cancers [8].

Regarding genetic pathogenesis, sporadic ovarian cancer is

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characterized by marked genetic instability caused by the modulation of several gene expressions. At the present time, a total of 16 tumor suppressor genes, a total of 15 oncogenes and three imprinted tumor suppressor genes have been described (Table 1) [9,10]. Depending on the gene expression profile, two diverse types of ovarian cancer have been described. Type I ovarian cancer includes low-grade and borderline serous cancers, endometrioid, mucinous and clear-cell tumors. The most frequent mutations in type I tumors involve *PTEN*, *PI3K* catalytic subunit-α (*PIK3CA*), *KRAS*, *BRAF* and b-catenin (*CTNNB1*) genes. On the other hand, high-grade serous carcinomas, mixed malignant mesodermal tumors, carcinosarcomas and undifferentiated cancers are included in type II ovarian cancers. Type II tumors express high genomic instability and in up to 80% of patients *TP53* is affected by the mutation. Moreover, this type of tumor is characteristic of *BRCA1* and *BRCA2* mutated patients and mostly arises from the fallopian tubes and the peritoneum [11].

BRCA1 and *BRCA2* mutation carriers have an increased lifetime risk of developing breast and ovarian cancer (up to an 85% for breast cancer and up to a 54% for ovarian cancer), and other cancer types as pancreatic and prostate [12-15]. About 10-15% of all ovarian cancers have been associated to hereditary DNA repair defects, and in about 90% of hereditary cancers the repair defect is caused by a germline mutation in *BRCA* genes. However, several other DNA repair genes have been linked to hereditary breast and gynaecological cancers, such as *TP53, PTEN, BARD1, CHEK2, RAD51* and *PALB2* [16-18]. At least 16 genes, mostly involved in the DNA repair pathways, have been showed to play a role in hereditary ovarian tumorigenesis [10]. Nevertheless, several hereditary ovarian malignancies are currently associated to unknown mutations and thus they cannot be detected by specific tests.

The identification and management of women at high risk for hereditary ovarian cancer should be carried out in a specialized family cancer center. Family-based care programs provide genetic counseling in order to inform women and their families about primary and secondary cancer prevention. To date, in healthy women carrying a *BRCA* mutation, surveillance programs for ovarian cancer have not been proven to be effective. Empirical ovarian cancer screenings are based on annual or semi-annual gynecological exams, transvaginal ultrasound, and evaluation of serum CA 125 concentrations. Prophylactic salpingo-oophorectomy (removal of both ovaries and fallopian tubes) is strongly recommended by the age of 35 or 40 years, even before the natural menopause, as primary prevention for ovarian and fallopian tube cancer. Alternatively, women at increased risk should be informed about the opportunity to join prevention clinical trials, such as chemoprevention trials. In particular, oral contraceptives could play an important role as chemopreventive agents for young women carrying a *BRCA1* mutation who refused risk-reducing salpingo-oophorectomy (RRSO). Furthermore, cyclo-oxygenase inhibitors, retinoids, analgesic drugs, peroxisome proliferator-activated receptor gamma ligands and vitamin D are currently under investigation and represent the most promising future chemopreventive agents for cancer prevention [19].
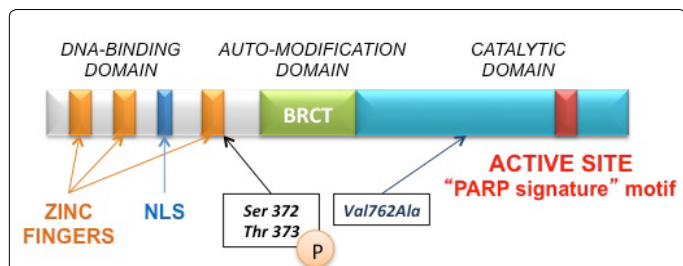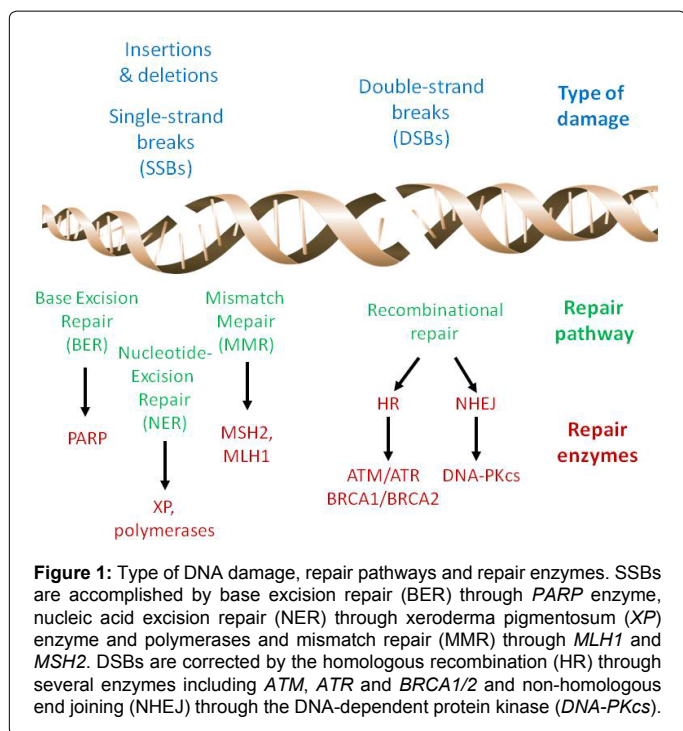
DNA Repair Mechanisms

Due to the high frequency of replication and their genetic profile, tumor cells have high genomic instability with increased probability of DNA mutations. Several DNA repair mechanisms are employed to remove single-strand breaks (SSBs) and double-strand breaks (DSBs) (Figure 1). The single strand break repair is accomplished by base excision repair (BER), nucleic acid excision repair (NER) and mismatch repair (MMR). BER is important for removing damaged bases by a DNA glycosylase and it is involved in the damage induced by radiation and alkylating agents. MMR recognizes and corrects mismatched bases that can result from DNA replication and recombination. NER removes short single-stranded DNA segment around the lesion and repairs mutations resulting from UV light and hydrocarbons.

Poly(ADP-ribose) polymerase (*PARP*) is a crucial enzyme involved in BER pathway (Figure 2). *PARP* has been described for the first time in 1963 and in 1980 his modulation has been proposed to increase the efficacy of alkylator chemotherapy [20,21]. Seventeen structurally similar proteins compose the PARP family. PARP proteins play several roles in different biological pathways, from DNA damage repair to differentiation and cell death. Particularly, research on *PARP* enzyme as target for cancer treatment has focused on *PARP1*, the best characterized protein of the family. Consequently to SSBs, *PARP1* detect DNA strand interruptions and promote the synthesis of poly(ADP-ribose) (PAR) using NAD^+ as a substrate. Poly (ADP-ribosylation) of histones and their release from DNA permit chromatin relaxation to facilitate the access of more repair components (Figure 3). *PARP1* account for more than 90% of ADP-ribosylation in cells, while *PARP2* is only responsible for 15% of the cell's PAR production and its precise functions remains to be explained [22,23]. Furthermore, some *PARP1* polymorphisms have been associated with increased risk of developing solid tumours, such as germ cell tumour, breast cancer, bladder cancer, lung cancer, gastric cancer and prostate cancer. Particularly, previous in vitro and in vivo clinical trials highlighted that Val762Ala in the catalytic domain might influence clinical outcome in ovarian cancer [24].

Several exogenous agents, such as alkylating drugs or ionizing radiations, and endogenous processes, for example resulting from an error in SSB repair, may produce double-strand breaks. DSBs are corrected by the homologous recombination (HR) and non-homologous end joining (NHEJ). Homologous recombination provides accurate recombination using a sister chromatid as a template, maintaining genomic stability. However, due to the need for a sister chromatid, HR is limited to the S-phase and G2-phase of cell cycle. Several proteins are largely involved in the HR pathway, such as *BRCA 1/2*, *ATM*, *CHEK2, RAD51* and Fanconi's anemia proteins (Figure 4) [25]. The *BRCA 1* and *BRCA 2* proteins play crucial roles in promoting the repair by HR. Particularly, DSBs activates the kinases *ATM*, *ATR* and *CHK2*, which in turn phosphorylate *BRCA1* on several different residues modulating its function. The role of *BRCA1* consist in DNA repair and in cell cycle regulation, causing G1-S, S or G2-M phase arrest depending on the residues phosphorylated. *BRCA1* forms a complex with *BARD1*, a protein with structural similarity, important for *BRCA1* stability. Lately, the BRCA1-BARD1 complex

Tumor suppressor genes	Oncogenes	Imprinted tumor suppressor genes
ARHI, RASSF1A, DLEC1, SPARC, DAB2, PLAG1, RPS6KA2, PTEN, OPCML, BRCA2, ARL11, WWOX, TP53, DPH1, BRCA1, PEG3	*RAB25, EVI1, EIF5A2, PRKCI, PIK3CA, MYC, EGFR, NOTCH3, KRAS, ERBB2, PIK3R1, CCNE1, AKT2, AURKA*	*ARHI, PLAGL1, PEG3*

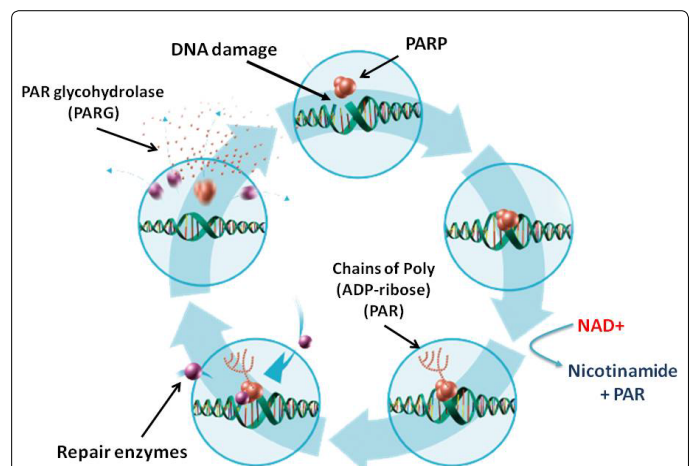**Table 1:** Wide genetic panel involved in ovarian cancer pathogenesis.

Figure 1: Type of DNA damage, repair pathways and repair enzymes. SSBs are accomplished by base excision repair (BER) through *PARP* enzyme, nucleic acid excision repair (NER) through xeroderma pigmentosum (*XP*) enzyme and polymerases and mismatch repair (MMR) through *MLH1* and *MSH2*. DSBs are corrected by the homologous recombination (HR) through several enzymes including *ATM*, *ATR* and *BRCA1/2* and non-homologous end joining (NHEJ) through the DNA-dependent protein kinase (*DNA-PKcs*).

Figure 2: PARP-1 proteins domains. *PARP-1* has a carboxyl-terminal domain (catalytic domain) with an enzymatic activity in the "PARP signature" motif that catalyzes the cleavage of the coenzyme nicotinamide adenine dinucleotide (NAD+) into nicotinamide and ADP-ribose. *PARP-1* also has an amino-terminal DNA binding domain containing three zinc finger motifs, a nuclear localization signal (NLS), and an auto-modification domain that functions as the target of covalent auto-poly(ADP-ribosyl)ation. The phosphorylation of *PARP-1* at Ser 372 and Thr 373 residues is required for the maximal activation of the enzyme in response to DNA damage. PARP-inhibitors bind to the donor site of the "PARP signature" motif in the catalytic domain causing reversible inhibition of PARP enzyme. Val762Ala polymorphism in the catalytic domain represents the most frequent variant of *PARP1*, associated with an increased risk of many tumors.
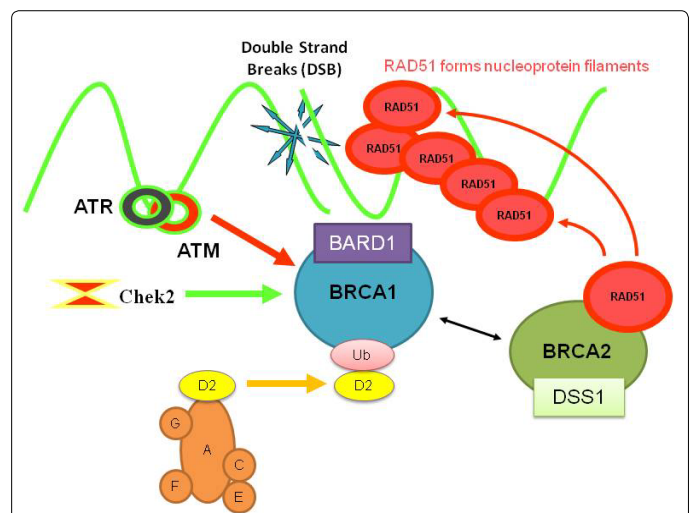
has been shown to play a role in ubiquitination and degradation of RNA polymerase II, inhibiting transcription and RNA processing, in order to eliminate prematurely terminated transcripts and clear the damaged DNA region for the intervention of DNA repair enzymes. Parallel, *BRCA2* participates in the repair of DSBs modulating the recombinase function of *RAD51*. *BRCA2* is necessary for the transport of *RAD51* into the nucleus and to the site of DNA damage, where *RAD51* is released to form the nucleoprotein filament required for recombination. About 20% of *RAD51* is bound to *BRCA2* in a relatively immobile fraction; the remaining 80% is composed by immobile oligomerized fractions or relatively mobile fractions. On the other hand, the BRCA2-binding protein *DSS1* is essential in controlling BRCA2-dependent recombination. *DSS1* showed to be necessary

for the interaction between *BRCA2* and *RAD51* and is implicated in maintaining the correct conformation of *BRCA2*. On this basis, the DNA repair pathway disruptions have represented the best approach for the development of targeted therapy in *BRCA1/2* carriers [26-28].

Although less accurate, NHEJ plays a crucial role in minimizing DNA damage in both G0 and G1 phases of cell cycle, when HR cannot be supplied. Moreover, when a defect occurs in one of the enzymes involved in HR, the DSBs are repaired from error prone mechanisms,

Figure 3: PARP-1 mediates the repair of SSBs via the activation and recruitment of repair enzymes. Clockwise: *PARP-1* binds to the DNA adjacent to the damage detecting and signaling the presence of an SSB. Once bound, *PARP-1* catalyzes the cleavage of the coenzyme nicotinamide adenine dinucleotide (NAD+) into nicotinamide and ADP-ribose to produce highly charged branched chains of poly(ADP-ribose) (PAR). Then DNA ligase III (*LigIII*), DNA polymerase beta (polβ), and scaffolding proteins such as x-ray repair complementing gene 1 (*XRCC1*) are recruited to the site of damage, to repairing the damaged DNA. After repair, the PAR chains are degraded via PAR glycohydrolase (*PARG*).

Figure 4: Proteins involved in the HR pathway. When DSBs occur, *ATM, ATR* and *CHEK2* kinases phosphorylates *BRCA1* that is stabilized by *BARD1*. At the same time a complex of Fanconi anemia proteins (A, C, D2, E, F and G) permit the ubiquitinization of D2 protein and the consequent interaction between D2 and *BRCA1. BRCA2* carries *RAD51*, the recombination enzyme, to the site of DNA damage. DSS1 is a BRCA2-binding protein essential in controlling BRCA2-dependent recombination and involved in maintaining the correct conformation of BRCA2.
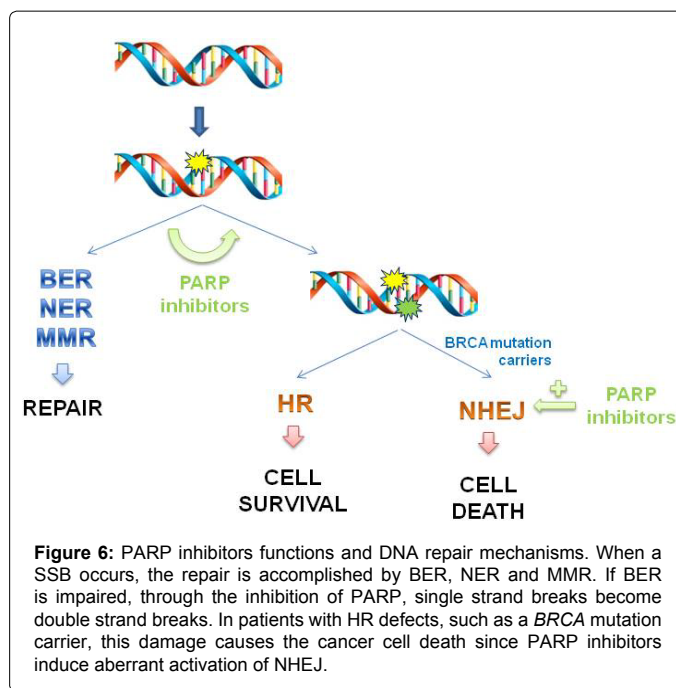
mostly NHEJ, resulting in increased risk of new chromosomal defects and thus the development of cancer [23]. In the first step of NHEJ the heterodimer Ku70/Ku80 breaks the DNA ends and improves the stability of the NHEJ enzymes at the DNA termini. Two Ku70/Ku80 heterodimers recruit DNA-dependent protein kinases (DNA-PKcs) to the DNA ends. The resulting complex of DNA-PKcs and his substrate Artemis has shown an endonuclease activity, thus it processes the DNA termini in order to prepare them for the intervention of XRCC4-Ligase IV. The nuclease functions of Artemis seem to be accomplished by the complex of RAD50, MRE11 and NBS1, which in vitro models interacts also with Ligase IV and Ku homologues (Figure 5) [29].

Finally, also *PARP1* is involved in the two principal mechanisms of DSB repair: HR and NHEJ. Particularly, *PARP* prevents NHEJ components from binding to site of DNA damage [30].

Synthetic Lethality and PARP Inhibitors Trials

Synthetic lethality occurs when a combination of different events, which singularly are not lethal, causes cell death. Particularly, if BER is impaired, through the inhibition of *PARP*, single strand breaks, e.g. caused by alkylant agents, can not be correct and become double strand breaks. In patients with HR defects, such as a *BRCA* mutation carrier, this damage causes the cancer cell death since PARP inhibitors induce aberrant activation of NHEJ (Figure 6). Thus, tumor cells with defective HR are highly sensitive to blockade of the BER pathway by PARP inhibitors when associated with alkylant agents [31]. In fact, in 2005 two seminal preclinical studies pointed out that BRCA-mutated cell are more sensitive to PARP inhibitors than heterozygous mutant

Figure 5: NHEJ pathway. When DSBs occur, the heterodimer Ku70/Ku80 recognizes the DNA ends and recruits the DNA-dependent protein kinase (DNA-PKcs). The complex of DNA-PKcs and his substrate Artemis processes the DNA ends preparing them for ligation by XRCC4-Ligase IV. The RAD50-MRE11-NBS1 complex seems to cooperate with the other enzymes, mostly Artemis and Ligase IV, to relegate the broken ends.

Figure 6: PARP inhibitors functions and DNA repair mechanisms. When a SSB occurs, the repair is accomplished by BER, NER and MMR. If BER is impaired, through the inhibition of PARP, single strand breaks become double strand breaks. In patients with HR defects, such as a *BRCA* mutation carrier, this damage causes the cancer cell death since PARP inhibitors induce aberrant activation of NHEJ.

and wild-type cells, highlighting the promising role of PARP inhibition in treatment of BRCA-mutated patients [32,33].

To date, several PARP inhibitors have been investigated and mentioned in literature. These molecules act binding to the donor site of the catalytic domain and causing reversible inhibition of PARP enzyme. In clinical trials, the most widely studied reversible PARP inhibitors are AZD2281 (Olaparib) and ABT-888 (Veliparib). BSI-201 (Iniparib), initially considered as a PARP inhibitor, has still unclear mechanism of activity and it does not seem to inhibit PARP enzymes at the clinically used dose [34].

In 2009 a phase 1 trial of Olaparib in *BRCA* mutation carriers has been published. The authors enrolled and treated 60 patients with different doses of Olaparib and analyzed the pharmacokinetic and pharmacodynamic characteristics of the agent. The maximum tolerated dose was established at 400 mg twice daily. The dose of 200 mg twice per day showed a favourable tolerability with an objective antitumor activity in *BRCA1* or *BRCA2* patients [35]. The successive expansion cohort study confirmed these notable results, highlighting that the clinical benefit rate is significantly associated with platinum-free interval and increases through platinum-refractory, resistant and sensitive subgroups (23%, 45%, and 69% respectively). These data suggested that PARP inhibitors anti-tumor activity is effective even in platinum-resistant disease but sensitivity to these agents' decreases with the raising resistance to platinum [36].

In 2010 an international, multicentre, phase 2 study with a cohort sequential design compared the continuous administration of Olaparib at the dose of 400 mg twice a day to Olaparib at 100 mg twice a day, in *BRCA1* or *BRCA2* mutated patients with recurrent ovarian cancer. Both cohorts of patients showed a significant antitumor efficacy with an objective response rate (ORR) of 33% and median response duration of 9.5 months at the dose of 400 mg twice a day, and an ORR of 13% with median response duration of 8.8 months at the dose of 100 mg twice a day. The tolerability profile and related adverse events were quite similar between the two cohorts of patients with nausea, fatigue
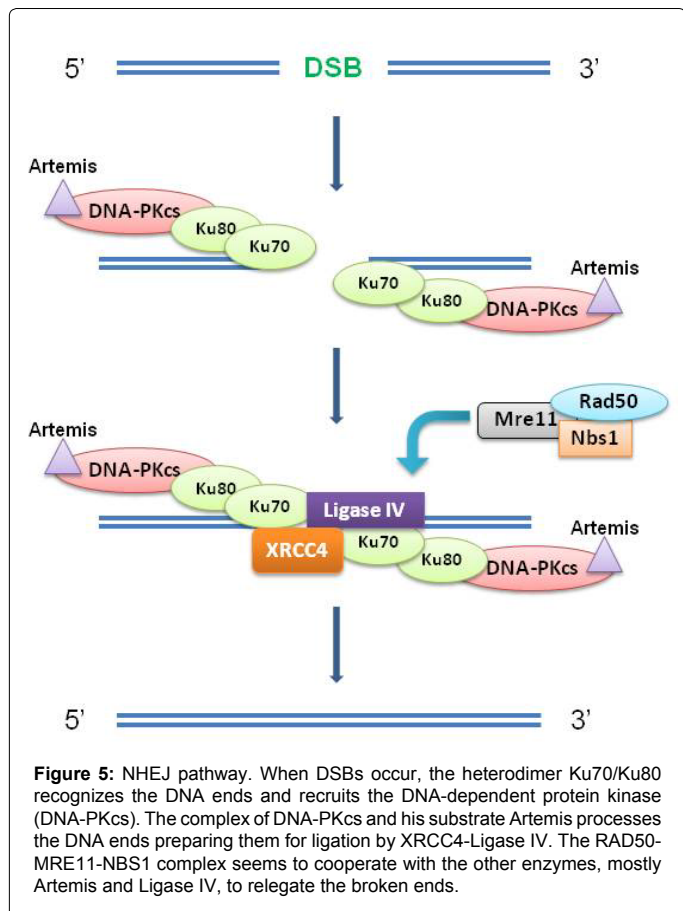
and anaemia (all events mostly grade 1 or 2) in patients who assumed Olaparib at the dose of 400 mg and nausea and fatigue (mostly grade 1 or 2) in the other cohort [37].

The role of *BRCA* mutation status in patients treated with PARP inhibitors has long been discussed. A randomized double-blind placebo-controlled phase 2 study enrolled patients with platinum-sensitive high-grade serous ovarian cancer to investigate the role of Olaparib maintenance therapy. Two hundred and fifty patients with objective complete response to the last platinum-based treatment were randomized to receive Olaparib or placebo until progression. The results highlighted that progression-free survival (PFS) in the Olaparib arm improved significantly compared to placebo [38]. In 2012, the last interim analysis of the study was published and confirmed that Olaparib as maintenance treatment significantly increased PFS (from 4.8 to 8.4 months) among patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer, regardless of the *BRCA* gene mutation. When the interim analysis was published, there was no evidence of overall survival benefit [39]. These data confirmed the results of a Canadian multicentric study, in which 55 high-grade serous ovarian cancer patients received Olaparib 400 mg twice daily. The study included BRCA carriers and women with unknown BRCA status and concluded that the efficacy of Olaparib is not related to the *BRCA* genes mutation status [40]. These results suggested that there is a specific phenotype of *BRCA* negative tumor (BRCAness) with a defect in the HR system, thus with features and behaviour similar to BRCA-related cancers even if *BRCA* mutation negative.

The successive step in the clinical research has been the study of PARP inhibitors as second-line treatment in *BRCA*-related ovarian cancers. In 2012 a phase 2 multicenter three-arm study compared two diverse dosage of Olaparib (200 and 400 mg twice per day continuously) to Pegylated liposomal doxorubicin (PLD) 50 mg/m^2 by IV infusion every 4 weeks, in 97 *BRCA1* or *BRCA2* mutation carriers affected by partially platinum-sensitive or platinum-resistant ovarian cancer. Median PFS was 6.5 months for the Olaparib 200 mg, 8.8 months for the Olaparib 400 mg and 7.1 months for PLD group. The difference in PFS between the Olaparib and PLD group was not statistically significant. To conclude, the activity of Olaparib in this study showed to be consistent with previous research whereas PLD has proven to be more effective than previously described [41]. Three possible explanations for these negative results have been listed by Konstantinopoulos et al. [42]. First, in the PLD group there was a relatively higher frequency of platinum-sensitive ovarian cancers (57.6%) than in Olaparib groups (46.9% in 400 mg dose and 43.8% in 200 mg dose). Considering the higher efficacy of Olaparib in platinum-sensitive disease [36], this unbalanced distribution could have led to an underestimation of Olaparib activity. Furthermore, in 2011 an observational study of multidimensional genomics and clinical data on 316 high-grade serous ovarian cancer patients investigated the relationships between *BRCA1/2* mutations and overall survival (OS), progression-free survival (PFS) and chemotherapy response. Interestingly, *BRCA2* mutation status in ovarian cancer patients has proven to be an independent predictive factor for OS, while *BRCA1* mutation status was not significantly associated with increased survival. No differences in PFS between *BRCA1* mutation carriers and wild-type *BRCA* patients were found, while *BRCA2* mutation carriers showed significantly longer PFS than the other two groups. Finally, analyses of chemotherapy response revealed that *BRCA2*-mutated ovarian cancer were more chemo-sensitive and showed longer platinum-free intervals than *BRCA1*-mutated and wild-type *BRCA* diseases [43]. In the study

comparing different dosage of Olaparib to PLD, the higher proportion of *BRCA1*-mutated cases over *BRCA2*-mutated in each group might be another plausible explanation for the negative results. Finally, the predominance of more heavily treated patients in the Olaparib 400 mg group than PLD one (78.2% vs. 51.5%) could have contributed to the development of subsequent somatic mutations that, restoring *BRCA1/2* functions, could have conferred resistance to Olaparib [44]. In the same year, a phase 1 trial evaluated the role of Veliparib in association to metronomic Cyclophosphamide in patients with refractory solid tumors and lymphoid malignancies. Of the 35 patients enrolled, 11 had ovarian cancer and 12 had breast cancer. The maximum dose tolerated was defined as Veliparib 60 mg plus Cyclophosphamide 50 mg once a day. Seven cases, 5 of which were *BRCA 2*-related ovarian cancers, achieved partial responses; additional 6 patients, one of which was *BRCA 2*-related ovarian cancer, achieved stable disease for at least six cycles. The study showed promising activity of the combination in particular in the subgroup of *BRCA* mutation carriers [45]. Currently, the combination compared to Cyclophosphamide monotherapy is under investigation in a phase 2 trial enrolling *BRCA*-related ovarian cancers, triple-negative breast cancers, and low-grade lymphomas.

Recently, the association between PARP inhibition and anti-angiogenic strategies has been analyzed in a phase 1 trial. This study investigated the combination of Cediranib with Olaparib in patients with recurrent ovarian cancer and breast cancer. ORR was achieved in 44% of ovarian cancer cases, and the clinical benefit rate (defined as ORR plus stable disease >24 weeks) was 61%. Conversely, no clinical response was observed in the seven evaluable breast cancer cases. In conclusion, this study showed promising evidence of activity of the combination Cediranib and Olaparib in ovarian cancer patients [46].

In 2011, two single-arms phase 2 trials investigating the combination of Iniparib (BSI-201) with Gemcitabine/Carboplatin in patients with platinum-sensitive and platinum-resistant ovarian cancer has been presented at the ASCO Annual Meeting. In the first trial in platinum-sensitive disease, analysis from the first 17 patients demonstrated an increase in ORR (70.6%) compared with previous data. In the preliminary analysis, no significant association between *BRCA* mutation status and objective response rate has been observed, and no unexpected toxicities have been reported [47]. On the other hand, in platinum-resistant ovarian cancer, the combination showed promising evidence of response (ORR 31.6%) and median PFS substantially improved (5.9 months) [48]. In 2013, a Phase 1/1b study analyzing the combination of Olaparib and Carboplatin in *BRCA1/2*-related breast and ovarian cancer has been presented in the poster discussion session of ASCO Annual Meeting. The analysis of results concluded that Olaparib 400 mg twice daily with Carboplatin AUC5 every three weeks is active and tolerable in *BRCA* mutated patients despite interactive marrow suppression. Moreover, exploratory translational studies indicated *FOXO3* and *NFkB1* as possible predictive factors for response to therapy, requiring a prospective validation [49].

As previously mentioned, defects in the BER system have particular impact on the repair of the damage induced by alkylating agents and ionizing radiation. On this basis, PARP-inhibitors have been studied in association to alkylating agents as a potential approach to increase cytotoxicity of radiotherapy. Recently, Veliparib has been investigated combined with radiotherapy and temozolomide in glioblastoma, showing clinically significant benefit particularly in MGMT-unmethylated tumors [50]. The role of PARP inhibitors in association with chemotherapy as radiosensitizers has been analysed in

several other settings where radiotherapy represents a crucial tool for the control of the disease. For instance, Rucaparib has been studied in BRCA-2-deficient and wild type pancreatic cancer cells [51], Olaparib has been evaluated in nasopharyngeal carcinoma cells [52], in non-small cell lung carcinoma [53], and in Ewing Sarcoma [54] while Veliparib has been investigated in colorectal cancer cells [55].

STUDY	PARP inhibitor	PHASE	STATUS
Olaparib for patients with recurrent BRCA deficient ovarian cancer	olaparib	Phase 2	Withdrawn
AZD2281 Plus Carboplatin to treat breast and ovarian cancer	AZD2281	Phase 1	Recruiting
A study to assess the safety and pharmacokinetics of an inhibitor of Poly-ADP-Ribose Polymerase-1 (PARP)	AZD2281	Phase 1	Active, not recruiting
A single-arm study evaluating carboplatin/gemcitabine in combination with BSI-201 in patients with platinum-Sensitive recurrent ovarian cancer	Iniparib	Phase 2	Completed
A single-arm study evaluating carboplatin/gemcitabine in combination with BSI-201 in patients with platinum-resistant recurrent ovarian cancer	Iniparib	Phase 2	Completed
An open-label, multicenter, phase 1/2 study of Poly(ADP-ribose) Polymerase (PARP) Inhibitor E7449 as single agent in subjects with advanced solid tumors or with B-cell malignancies and in combination with Temozolomide (TMZ) or with Carboplatin and Paclitaxel in subjects with advanced solid tumors	E7449	Phase 1, Phase 2	Recruiting
Study to assess the efficacy and safety of a PARP Inhibitor for the treatment of BRCA-positive advanced ovarian cancer	AZD2281	Phase 2	Completed
Rucaparib(CO-338;Formally Called AG-014699 or PF-0136738) in treating patients with locally advanced or metastatic breast cancer or advanced ovarian cancer	Rucaparib	Phase 2	Recruiting
Olaparib in combination with carboplatin for refractory or recurrent women's cancers	Olaparib	Phase 1	Recruiting
Study to assess the safety and tolerability of a parp inhibitor in combination with carboplatin and/or paclitaxel	AZD2281	Phase 1	Active, not recruiting
Dose-finding study comparing efficacy and safety of a PARP inhibitor against Doxil in BRCA+ve advanced ovarian cancer	AZD2281	Phase 2	Active, not recruiting
A study of MK4827 in participants with advanced solid tumors or hematologic malignancies (MK-4827-001 AM8)	MK-4827	Phase 1	Completed
Study to compare the efficacy and safety of Olaparib when given in combination with Carboplatin and Paclitaxel, compared with Carboplatin and Paclitaxel in patients with advanced ovarian cancer	Olaparib	Phase 2	Active, not recruiting
Phase I of BKM120/Olaparib for triple negative breast cancer or high grade serous ovarian cancer	BKM120 and Olaparib	Phase 1	Recruiting
Veliparib and Topotecan Hydrochloride in treating patients with solid tumors, relapsed or refractory ovarian cancer, or primary peritoneal cancer	Veliparib	Phase 1, Phase 2	Recruiting
Phase II study of AZD2281 in patients with known BRCA mutation status or recurrent high grade ovarian cancer or Patients with known BRCA mutation status/triple neg breast cancer	AZD2281	Phase 2	Active, not recruiting
Veliparib and pegylated liposomal doxorubicin hydrochloride in treating patients with recurrent ovarian Cancer, Fallopian Tube Cancer, or Primary Peritoneal Cancer or Metastatic Breast Cancer	Veliparib	Phase 1	Recruiting
A study of Oral Rucaparib in Patients with a Solid Tumor (Phase I) or with gBRCA Mutation Ovarian Cancer (Phase II)	Rucaparib	Phase 1, Phase 2	Recruiting
Veliparib Monotherapy for Relapsed Ovarian Cancer with BRCA Mutation	Veliparib	Phase 1, Phase 2	Recruiting
Olaparib treatment in BRCA mutated ovarian cancer Patients After Complete or Partial Response to Platinum Chemotherapy	Olaparib 300 mg tablets	Phase 3	Not yet recruiting
Olaparib Monotherapy in Patients with BRCA mutated ovarian cancer following first line platinum based chemotherapy	Olaparib 300 mg tablets	Phase 3	Not yet recruiting
Assessment of efficacy of AZD2281 in platinum sensitive relapsed serous ovarian cancer	AZD2281	Phase 2	Completed
A study of Rucaparib in patients with platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer (ARIEL2)	Oral Rucaparib	Phase 2	Not yet recruiting
Study of BMN 673, a PARP Inhibitor, in Patients with Advanced or Recurrent Solid Tumors	BMN 673	Phase 1	Recruiting
ABT-888 with Cyclophosphamide in Refractory BRCA-Positive ovarian, primary peritoneal or ovarian high-grade serous carcinoma, fallopian tube cancer, triple-negative breast cancer, and low-grade non-hodgkin's lymphoma	ABT-888	Phase 2	Active, not recruiting
A Phase I study of ABT-888 in combination with Temozolomide in Cancer Patients	ABT-888	Phase 1	Completed
Veliparib, Cisplatin, and Vinorelbine Ditartrate in treating patients with Recurrent and/or Metastatic Breast Cancer	Veliparib	Phase 1	Recruiting
Single arm study of BSI-201 in Patients with BRCA-1 or BRCA-2 associated advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer	Iniparib	Phase 2	Completed
Veliparib in treating patients with malignant solid tumors that did not respond to previous therapy	Veliparib	Phase 1	Recruiting
Veliparib and Floxuridine in treating patients with metastatic epithelial ovarian, primary peritoneal cavity, or fallopian tube cancer	Veliparib	Phase 1	Recruiting
Veliparib in treating patients with persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer	Veliparib	Phase 2	Active, not recruiting
Carboplatin, Paclitaxel, Bevacizumab, and ABT-888 in treating patients with newly diagnosed Stage II, Stage III, or Stage IV ovarian epithelial cancer, fallopian tube cancer, or primary peritoneal cancer	Veliparib	Phase 1	Recruiting
Cediranib and Olaparib in combination for recurrent ovarian or Triple-Negative Breast Cancer	Olaparib, Cediranib	Phase 1, Phase 2	Active, not recruiting
Open label study to assess efficacy and safety of Olaparib in confirmed genetic BRCA1 or BRCA2 mutation pats	Olaparib	Phase 2	Active, not recruiting

Table 2: Ongoing studies of PARP inhibitor in ovarian cancer [50].

To date, other novel PARP inhibitors have been proposed and are being studied in preclinical and clinical setting. For instance, in preclinical tumor models with defects in *BRCA* and *PTEN* function, Niraparib (MK4827) has been shown to inhibit selectively PARP-1 and PARP-2 inducing synthetic lethality. In a phase 1 study that enrolled patients affected by advanced stage solid tumors, Niraparib (maximum tolerated dose of 300 mg/day) showed antitumor activity in eight of 20 patients with *BRCA*-related ovarian cancer and in two of four patients with *BRCA*-related breast cancer. Anti-tumor efficacy was also observed in sporadic high-grade serous ovarian cancer, non-small-cell lung cancer, and prostate cancer [56]. Moreover, a recent preclinical study investigated growth inhibitory effects of the PARP inhibitor Rucaparib in a set of 39 ovarian cancer cell lines [57].

In conclusion, Table 2 lists and describes current studies of PARP inhibitor as mono-therapy or combined with different agents in ovarian cancer patients (Table 2) [58].

Conclusion

In the recent years, the increasing knowledge of molecular mechanisms in ovarian cancer pathogenesis allowed to identify several targeted agents that are now entering in clinical practice. Nowadays, the family of poly(ADP-ribose) polymerase (PARP) inhibitors represents a widely investigated and promising alternative for the targeted therapy of ovarian malignancies. PARP inhibitors exploit the synthetic lethality concept to prevent the DNA damage repair, causing cancer cell death. Several agents have already been identified and studied in phase 1 and 2 trials and others are still under investigations in preclinical and clinical studies. The first published phase 1 and 2 studies analyzed the role of PARP inhibitors as single agent in recurrent ovarian cancer. However, to date available data in literature and ongoing trials [58] are mostly related to the association of PARP inhibitors and chemotherapy. This trend suggests a future prevalent role for PARP inhibitors as combination rather than monotherapy, probably confining the use of PARP inhibitors as single agent for the maintenance therapy.

Despite the enrolment of an adequate number of participants in order to obtain significant statistical power could be a challenge, randomized phase 3 trials are urgently needed to compare PARP inhibitors to standard therapies. The evidence of BRCAness represents a resource to extend the amount of patients who might benefit from PARP inhibitors activity. However, while genetic testing helps to find *BRCA* mutation carriers, to date we still need tests to allow identifying BRCAness patients, carrying dysfunctions in HR pathway. Future research should be directed to define the cases that may truly benefit from the synthetic lethality approach and thus from PARP inhibition strategy.

Moreover, the resistance mechanisms to PARP inhibitors still represent a crucial issue for the proper development of these promising agents. To date, several mechanism have been described including restoration of *BRCA* function, up regulation of NHEJ system, induction of P-glycoprotein efflux pump expression and the loss of the protein 53BP1 which avoids HR proceeding in DNA repair [59].

Another important issue must be discussed in order to ensure an effective use of these agents. The long-term effects of inhibition of PARP enzymes in combination with DNA-damaging agents should be evaluated in animal models, in order to define the risk of secondary malignancies.

Finally, further research should focus on the structure, mechanism and function of PARP enzymes other than *PARP1*. Improved understanding of the function of the other PARP enzymes may lead to better interpret the wider consequences of PARP inhibition and may help to suggest novel treatment approaches, identifying further molecular targets.

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