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The resistance to PARP inhibitors in epithelial ovarian cancer: State of art and perspectives of biological and clinical research

Angiolo Gadducci* and Stefania Cosio

#Contributed equally

Department of Clinical and Experimental Medicine, Division of Gynecology and Obstetrics, University of Pisa, Pisa, Italy

Abstract

PARP inhibitors are commonly used as maintenance therapy after response to platinum- based chemotherapy both in patients with newly diagnosed advanced epithelial ovarian cancer [EOC] and in those with platinum- sensitive recurrent EOC. The selective pressure of PARPi can drive the emergence of neoplastic resistant clones which represent a serious obstacle to the prolonged use of these drugs. Secondary BRCA somatic reversion mutations, BRCA1 promoter demethylation, altered expression of 53BP1, miR-622, RIF and Shieldin complex, altered expression of c-MET, PI3K/AKT/mTOR and ATM/ATR, and upregulation of P-glycoprotein, NF-κB and ALDH have found to correlate with PARPi resistance in preclinical studies. However, only secondary BRCA reversion mutations or demethylation of the BRCA promoter have been detected as events able to induce PARPi resistance in the clinical setting. The present review has assessed the possible strategies to increase the sensitivity and to overcome the resistance to PARPi in EOC.

Introduction

Poly(ADP-ribose) [PAR] polymerase [PARP] inhibitors [PARP]i have been widely investigated and are commonly used as maintenance therapy after complete or partial response to platinum- based chemotherapy both in patients with newly diagnosed advanced epithelial ovarian cancer [EOC] [1-4] and in those with platinum-sensitive recurrent EOC [5-8]. Three PARPi have been approved in these clinical settings by regulatory agencies: olaparib, niraparib and rucaparib.

Also single-agent PARPi have shown activity in EOC. Matulonis, *et al.* [9] assessed pooled data from six phase I or II trials that enrolled 300 patients with recurrent BRCA- mutated EOC treated ith olaparib monotherapy at the time of relapse. The response rate was 36% in the entire population, and ranged from 50% for women who had received one prior regimen to 31% for those who had received > 3 lines and to 24% for those who had received > 6 prior regimens. The Food and Drug Administration [FDA] approved olaparib monotherapy for patients with germline BRCA-mutated EOC who had received > 3 prior lines of chemotherapy.

SOLO 3 randomized phase III trial compared olaparib monotherapy *versus* non-platinum-based chemotherapy (pegylated liposomal doxorubicin [PLD], paclitaxel, gemcitabine, or topotecan) in patients with platinum-sensitive recurrent BRCA-mutated EOC pretreated with at least two platinum-based lines [10]. Both response rates and median PFS were significantly better in olaparib arm (84.6% *versus* 61.5%, Odds Ratio [OR]= 3.44, 95% confidence interval [CI]=1.42–8.54, and, respectively, 13.4 *versus.* 9.2 months, Hazard ratio= 0.62, 95% CI=0.43–0.91).

In the Quadra study single- agent niraparib achieved an objective response in 28% of 47 patients with recurrent platinum-sensitive, homologous recombination [HR] deficient [HRD] EOC (with and without BRCA mutations) responsive to their last platinum-based therapy who had received three or four previous regimens [11]. The FDA approved niraparib monotherapy for patients with HRD EOC treated with ≥3 prior chemotherapy regimens

The ARIEL2 trial, that administered single- agent rucaparib to patients with platinum- sensitive recurrent EOC, reported that PFS was significantly longer in the BRCA- mutant subgroup (HR= 0·27, 95% CI =0·16–0·44) and high loss of heterozygosity [LOH] subgroup (HR= 0·62, 95% CI=0·42–0·90) than in the low LOH subgroup [12]. Rucaparib has been approved by European Medicines Agency [EMA] also as monotherapy for patients with platinum-sensitive, recurrent, BRCA-mutated high grade EOC who have received > two prior platinum-based regimens and could not tolerate further platinum-based therapy.

The selective pressure of PARPi can drive the emergence of resistant neoplastic clones which represent a serious obstacle to the prolonged use of these drugs [13].

The present paper assesses the mechanisms of resistance to PARPi and the strategies able to delay or revert the development of PARPi resistance in patients with EOC.

Mechanisms of DNA damage repair: Several noxae, such as reactive oxygen species, ionizing radiation and chemical exposures,

Correspondence to: Angiolo Gadducci, MD, Department of Clinical and Experimental Medicine, Division of Gynecology and Obstetrics, University of Pisa, Via Roma 56, Pisa, 56127, Italy, E-mail: a.gadducci@med.unipi.it

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damage DNA and generate single strand break [SSB]s and double strand break [DSB]s which can be repaired by different mechanisms, including base excision repair [BER], HR and non-homologous end joining [NHEJ] [14-19].

The nuclear proteins belonging to the PARP superfamily are critical sensors that bind SSBs and catalyze the PARylation, i.e, the polymerization of ADP-ribose units from donor nicotinamide adenine dinucleotide [NAD+] molecules on target proteins, resulting in the attachment of linear or branched polymers [20-23]. The consequent activation of BER pathway recruits both DNA-repairing enzymes, such as DNA ligase III and DNA polymerase-β, and scaffolding proteins, such as X-ray cross-complementing gene 1 [XRCC1], that restore DNA integrity [24]. PARP1, PARP2, and PARP3 have all been involved in DNA repair, with PARP1 being the major member of the superfamily [25]. PARP1 also inhibits NHEJ by preventing the binding of the Ku70–Ku80 complex to free DNA ends [26].

DSBs can be repaired by HR or NHEJ [14,16-19]. HR, which uses a sister chromatidis as a template, is a high fidelity, error-free repair system. Conversely, NHEJ which directly ligates the ends of DSBs together without a homologous template is an error-prone repair mechanism leading to genetic aberrations, chromosomal instability, cell cycle arrest and apoptosis. Ataxia telangiectasia mutated [ATM] and ataxia telangiectasia and Rad-3-related protein [ATR] are two phosphatidyl- inositol 3-kinase- [PI3-K]-related kinases that recognize genotoxic stress, inhibit cell cycle progression and enhance DNA repair or, if this fails, activate the apoptotic cascade [27,28]. The three proteins meiotic recombination 11 [MRE11], RAD50 and inibrin [MRN complex] are needed to recruit ATM to DSBs [29,30]. Checkpoint kinase [CHK]1 and CHK2 activated by ATR and ATM phosphorylate and inhibit phosphatases CDC25B and CDC25C, leading to G2 arrest and giving sufficient time for DNA repair [31-33].

BRCA1 and BRCA 2 play a major in HR [34-36]. BRCA1 promotes the phosphorylation of TP53-binding protein [53BP1], a chromatin-binding protein that suppresses the nucleolytic resection of DNA ends [37,38]. Different proteins are involved in BRCA1 regulation. BRCA1-associated ring domain protein1 [BARD1] heterodimerizes with BRCA1 and this interaction is essential for BRCA1 stability and its rapid location to DNA damage sites [39]. Moreover, the BRCA1/BARD1 heterodimer ubiquinates RNA polymerase II, preventing the transcription of the damaged DNA and restoring genetic stability [40]. The BRCA1 interacting protein c-terminal helicase 1[BRIP1] gene encodes a helicase that interacts with BRCA1 and contributes to BRCA1-associated DNA repair function [41]. CDK12 is involved in the transcription of BRCA1 and other DNA repair genes [42]).

BRCA2 interacts with RAD51 recombinase at sites of DNA breakage, stimulates RAD51 nucleoprotein filament formation and catalyzes strand exchange with homologous duplex DNA [43,44]. Partner and localizer of BRCA2 [PALB2] enhances the recruitment of BRCA2 and RAD51 recombinase to sites of DNA damage [45,46]. Conversely, EMSY is a BRCA2-binding protein which when over-expressed blocks BRCA2 function [47].

In NHEJ repair system the heterodimer Ku70-Ku80 complex binds to DSBs and acts as a loading protein through which other NHEJ proteins, including polymerases, nucleases and ligases, can be recruited to join DNA ends [48,49]. NHEJ is active throughout the cell cycle but especially in G0/G1 phase, whereas HR is nearly absent in G1 and is most effective in the S phase, due to the high DNA replication and available sister template [19,50-52].

A critical factor for choosing between these two repair pathways is the competition between DNA end protection essential for NHEJ and DNA end resection necessary for HR [53-56]. 53BP1 antagonizes the resection of DSBs and favors repair by NHEJ, whereas 53BP1 loss allows DSB resection and RAD51 recruitment thus restoring a competent HR [36,53-56]. The Rap1 interacting factor 1 [RIF1] and the Shieldin complex, consisting of four subunits termed REV7, SHLD1, SHLD2 and SHLD3, act as the key downstream effectors of 53BP1 [57-61]. Whereas Ku70–Ku80 complex promotes NHEJ, miR-622 suppresses this repair system through targeting Ku70–Ku80 complex and activates HR [48,49,56]. Ku70–Ku80 complex and MRE11 complex antagonize each other to influence the choice between NHEJ and HR [62].

Mechanisms of actions of PARPi: The Cancer Genome Atlas [TCGA] project detected germline or somatic BRCA mutations in 20% and epigenetic silencing of BRCA due to promoter hypermethylation in 11% of 489 high-grade serous EOCs, respectively [63]. The study revealed mutations or methylations of other HR-related genes, and overall 50% of these tumors showed a signature of HRD and were potentially sensitive to blockade of the repair of SSBs through PARP inhibition [16,64,65].

PARPi suppress BER pathway and promote the synthetic lethality, which occurs when two genetic lesions each having no effect on the cellular outcome become lethal when combined together [16,20]. If not repaired by BER due to PARP inhibition, SSBs are converted in the more cytotoxic DSBs which are normally repaired by HR in HR proficient cells whereas these cause cytotoxicity in HRD cells [66,67].

Besides inhibiting parylation, PARPi block PARP-1 ability to dissociate from damaged DNA, thus establishing a stable complex of PARP-1 with DNA in a process called PARP trapping [13,68]. These trapped complexes are highly cytotoxic and lead to replication forks obstruction [13]. All PARPi contain a benzamide moiety essential for binding to PARP-1, but each inhibitor differs in size and flexibility accounting for different trapping abilities [13]. Talazoparib is □100-fold more potent than olaparib and rucaparib at trapping PARP1- and PARP-2, whereas it is only slightly more potent than olaparib and rucaparib at inhibiting PARP catalytic activity [68].

Mechanisms of resistance to PARPi

Different mechanisms of resistance to PARPI have been detected in preclinical studies (Table 1).

Secondary BRCA somatic reversion mutations: Secondary BRCA1 somatic reversion mutations leading to HR restoration have been detected in patients with BRCA-mutated EOC after platinum-based chemotherapy [13,69-72]. These mutations have been also found in circulating free DNA [cfDNA] in patients with different malignancies [73,74].

Nordquist, *et al.* [70], who assessed a series of primary and recurrent BRCA -mutated EOCs, found a secondary BRCA mutation in 2 of 64 (3.1%) primary carcinomas compared with 13 of 46 (28.3%) recurrent

Table 1. Mechanisms of resistance to PARP inhibitors (PARPi)s

Secondary BRCA somatic reversion mutations
BRCA1 promoter demethylation
Altered expression of 53BP1, miR-622 , RIF and Shieldin complex
Altered expression of c-MET, PI3K/AKT/mTOR, and ATM/ATR
P-glycoprotein upregulation
NF-κB upregulation
ALDH upregulations

carcinomas (p= 0.0003). It is noteworthy that a secondary mutation occurred in 46.2% and 5.3% of platinum-resistant and platinum-sensitive relapses, respectively (p= 0.003). The next-generation sequencing [NGS] of cfDNA drawn before rucaparib treatment in 112 patients with recurrent germline or somatic BRCA-mutant EOC enrolled in ARIEL2 trial detected BRCA reversion mutations in 18% of patients with platinum-refractory disease and 13% of those with platinum-resistant disease compared with 2% of those with platinum-sensitive disease (p= 0.049) [75]. Median PFS was longer in patients without pretreatment BRCA reversion mutations than in those with reversion mutations (9.0 *versus* 1.8 months; HR= 0.12; 95% CI= 0.05–0.26). Sequencing post-progression cfDNA identified additional eight patients with BRCA reversion mutations not found in pretreatment samples.

Kondrashova, *et al.* [76] sequenced HR genes in 12 matched pretreatment and post-progression tumor biopsies from patients enrolled in ARIEL2 trial. Deleterious mutations of BRCA1, RAD51C and RAD51D were detected in 4, one, and one, respectively, of 6 pretreatment biopsies. In 5 of these 6 cases, post-progression biopsies had at least one secondary mutation restoring the open reading frame of the genes. The secondary mutations detected in the BRCA1-mutated cases were deletions that restored the open reading frame by either deleting the primary frameshift mutation or shifting the reading frame back into the correct state. In a patient with RAD51C mutation post-progression biopsy contained 4 distinct secondary mutations that all restored the open reading frame of the gene. In another patient with RAD51D mutation post-progression tumor samples were collected from a liver metastasis still responding to rucuparib and from a spleen metastasis progressing on rucaparib. The RAD51D secondary mutation was found only in this latter lesion.

BRCA1 promoter demethylation: HR repair can also be restored by BRCA1 promoter demethylation [**72,77**].

Silencing of all BRCA1 copies predicted rucaparib response in BRCA1-methylated, EOC patient-derived xenografts in mice, whereas heterozygous methylation was associated with PARPi resistance [78]. The analysis of pre-treatment tumor samples from patients enrolled in the ARIEL2 trial showed a median PFS of 14.5 months and 12.8 months for the 6 patients with homozygous methylated BRCA1 and the 40 patients with mutant BRCA1/2, respectively, and of 5.5 months for the 143 patients with non-methylated BRCA1. Multiple chemotherapy lines could induce loss of BRCA1 promoter methylation, with consequent rescued BRCA1 expression [19].

Altered expression of 53BP1, miR 622, RIF and Shieldin complex : Loss of 53BP1 promotes DNA end resection, HR restoration and PARPi resistance [53,79]. Low 53BP1 expression in tumor samples correlated with a poorer response to PARPi in HRD EOC [79]. Similarly, HR can be recruited by over-expression of miR-622 through targeting the Ku70–Ku80 complex [56]. Choi, *et al.* [56] assessed 89 EOC patients with BRCA1-inactivation due to mutation or promoter hypermethylation who underwent surgery followed by platinum-based chemotherapy. Patients high miR-622 expression experienced shorter PFS (median, 14.7 *versus* 19.8 months, p= 0.03) and shorter OS (median, 39 *versus* 49.3 months, p= 0.03) compared to those with low miR-622 expression. Patients, whose miR-622 expression levels were in the highest quintile, had the worse PFS (median, 13.7 *versus* 18.1 months p= 0.005) and OS (median, 35.3 *versus* 48.3 months, p= 0.001) also the loss of downstream effectors of 53BP1, such as RIF1 and Shielding complex, can restore HR [57-60].

Altered expression of c-MET, PI3K/AKT/mTOR, and ATM/ATR: Aberrant expression of mesenchymal-epithelial transition factor [c-MET], PI3K/AKT/mammalian target of rapamycin [mTOR] and ATM/ATR could be associated with PARPi resistance.

c-MET is a proto-oncogene encoding a receptor tyrosine kinase which plays critical roles in cell proliferation, motility and survival [80]. High c-Met expression is a poor prognostic factor in EOC [81,82]. PARPi resistance is often detected in cells overexpressing c-MET, which phosphorylates PARP-1 and reduces its binding affinity to PARPi [82,83]. c-Met inhibitors may enhance the activity of PARPi and overcome PARPi resistance in different malignancies [84,85].

PARPi can induce upregulation of PI3K/AKT/mTOR signaling pathway, which is involved in the progression and in the development of chemoresistance of EOC [86,87], whereas PI3K AKT/mTOR pathway inhibition in BRCA1- deficient cancer cells increase their sensitivity to PARPi [88,89].

P-glycoprotein upregulation: The overexpression of the P-glycoprotein is involved in the resistance to different anticancer agents as well as to PARPi [77,90,91]. In a mouse model of BRCA1-mutated breast carcinoma, PARPi resistance was mediated through upregulation of Abcb genes encoding for this protein and the same mechanism of resistance could develop in human malignancies [77]. Abcb upregulation has been detected in 8% of tumor samples from recurrent high-grade serous EOC [91,92].

NF-κB upregulation: Nuclear factor kappa-light-chain-enhancer of activated B cells [NF-κB] signaling pathway is a complex of transcription factors which regulates both cell proliferation, inflammation and apoptosis as well as lymphogenesis and B cell maturation [93].

An *in vitro* study showed that this pathway is upregulated in established BRCA1-mutated breast and EOC cell lines rendered resistant to PARPi after repeated exposure to different doses of olaparib [94].

Aldehyde dehydrogenase upregulation: High expression of aldehyde dehydrogenase [ALDH], a marker of cancer stem cells, has been correlated with chemoresistance in several tumors including EOC [95,96].

Olaparib-resistant EOC cells showed an elevated ALDH activity, mainly due to increased expression of the isoform ALDH1A1 [97]. A selective inhibitor of ALDH1A1 was found to synergize with olaparib in killing BRCA2-mutated EOC cells both *in vitro* and the *in vivo* xenograft animal model. Therefore, ALDH1A1 inhibition should be further investigated as a therapeutic option for preventing and overcoming PARPi resistance.

Anticancer treatment in patients with progression after PARPi

Different options could be tested to increase the sensitivity and to overcome the resistance to PARPi (Table 2 and Table 3).

Chemotherapy: The development of pharmacological mechanisms of resistance could impact on the ability of time interval after the last administration of platinum (PFI) to predict the responsiveness to further chemotherapy in patients with progressive disease after PARPi maintenance [91].

A multicenter retrospective study assessed 66 patients with recurrent, platinum -sensitive BRCA- mutated EOC who had received olaparib maintenance after one or more lines of platinum rechallenge

Table 2. Promising strategies to increase the sensitivity and to overcome the resistance to PARP inhibitors (PARPi)

Trabectedin-based chemotherapy
PARPi rechallenge
c-Met inhibitors
NF-kB inhibitors
Combinations of PARPi and immune checkpoint inhibitors
Combinations of PARPi and anti-angiogenic agents
Combination of PARPi and PI3K/AKT/mTOR inhibitors

Table 3. Ongoing trials of PARP inhibitors (PARPi) and immune-checkpoint inhibitors in epithelial ovarian cancer

NCT04034927. A Phase II Randomized Trial of Olaparib Versus Olaparib Plus Tremelimumab in Platinum-Sensitive Recurrent Ovarian Cancer
NCT02657889. Phase 1/2 Clinical Study of Niraparib in Combination with Pembrolizumab (MK-3475) in Patients with Advanced or Metastatic Triple-Negative Breast Cancer and in Patients With Recurrent Ovarian Cancer
NCT03522246 ATHENA - (A Multicenter, Randomized, Double-Blind, Placebo-Controlled Phase 3 Study in Ovarian Cancer Patients Evaluating Rucaparib and Nivolumab as Maintenance Treatment Following Response to Front-Line Platinum-Based Chemotherapy
NCT03602859. ENGOT-0V44 The FIRST (First-line Ovarian Cancer Treatment with Niraparib Plus TSR-042) Study: A Randomized, Double-blind, Phase 3 Comparison of Platinum-based Therapy With TSR-042(Dostarlimab) and Niraparib Versus Standard of Care Platinum-based Therapy as First-line Treatment of Stage III or IV Nonmucinous Epithelial Ovarian Cancer
NCT02734004. A Phase I/II Study of MEDI4736 (Anti-PD-L1 Antibody) in Combination with Olaparib (PARP Inhibitor) in Patients with Advanced Solid Tumors (Ovarian Cancer, Breast Cancer, Squamous Cell Lung Cancer, Gastric Cancer)
NCT03737643. A Phase III Randomised, Double-Blind, Placebo-Controlled, Multicentre Study of Durvalumab in Combination with Chemotherapy and Bevacizumab, Followed by Maintenance Durvalumab, Bevacizumab and Olaparib in Newly Diagnosed Advanced Ovarian Cancer Patients (DUO-O).
NCT02953457. A Phase I/II Evaluation of Olaparib in Combination with Durvalumab and Tremelimumab in the Treatment of Recurrent Platinum Sensitive or Resistant or Refractory Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer in Patients Who Carry a BRCA1 or BRCA2 Mutation
NCT03574779. Phase 2 Multicohort Study to Evaluate the Safety and Efficacy of Novel Treatment Combinations (Niraparib+ Dostarlimab + Bevacizumab) in Patients with Recurrent Ovarian Cancer
NCT03806049. ENGOT-OV42-NSGO/AVANOVA-Triplet: A Randomized Study to Evaluate the Efficacy of Niraparib-bevacizumab-TSR042 (Dostarlimab) Triplet Against Niraparib-bevacizumab Doublet and Against Standard of Care in Women with Platinum-sensitive Ovarian Cancer
NCT03598270. A Phase III Randomized, Double-blinded Trial of Platinum-based Chemotherapy with or Without Atezolizumab Followed by Niraparib Maintenance with or Without Atezolizumab in Patients With Recurrent Ovarian, Tubal or Peritoneal Cancer and Platinum Treatment-free Interval >6 Months.

and who afterwards had undergone further chemotherapy for progression [98]. Eighteen patients had a PFI >12 months and 14 of these underwent platinum- based chemotherapy. Twenty-seven patients had a PFI of 6-12 months, and of these 14 were treated with platinum -based chemotherapy and the others with trabectedin ± PLD. Twenty-one patients had a PFI < 6 months and usually received single- agent therapy, being weekly paclitaxel and trabectedin the most frequently used drugs. The response rate was 22.2%, 11.1% and 9.5% in patients with a PFI > 12 months, 6-12 months, and < 6 months, respectively. Therefore, the response rates to salvage chemotherapy after olaparib progression were lower than expected according to PFI.

Frenel, *et al.* [99] analyzed the patients enrolled in SOLO2 trial who progressed after olaparib (n. 195) or placebo (n.99). First subsequent treatment consisted of non platinum-based chemotherapy and platinum- based chemotherapy in 44% and 56%, respectively, of patients of placebo arm, and in 37% and 63%, respectively, of those of olaparib arm. Time to further progression was longer in the placebo arm compared to olaparib arm in the entire population (12.6 *versus* 6.9 months, HR= 2.17; 95% CI= 1.47-3.19) and even more in the patients treated with platinum-based chemotherapy (14.3 *versus* 7 months; HR= 2.89; 95% CI= 1.73-4.82). Therefore, some degree of resistance to subsequent chemotherapy was observed in olaparib arm.

One strategy to expand the number of patients who could benefit from PARPi could be the combination of these agents with DNA damaging drugs [100,101]. Trabectedin could be an ideal drug for both its favorable hematopoietic toxicity profile and its unique mechanisms of action [102]. Trabectedin forms adducts in the minor groove of DNA bending it towards the major one. The transcription-coupled nucleotide excision repair system in the attempt to remove trabectedin adducts generates SSBs and DSBs and triggers a cascade of events that interfere with transcription factors, DNA binding proteins and DNA repair pathways, resulting in G2-M phase cell cycle arrest and apoptosis. Moreover, trabectedin modifies tumor microenvironment, especially by reducing the number of tumor associated macrophages and the production of inflammatory cytokines and chemokines. In sarcoma preclinical models, trabectedin activates PARP1 and the combination with olaparib shows a higher antitumor activity than either single agent [103]. Trabectedin appears to be more effective in BRCA-mutated and/or BRCAness phenotype EOC [104,105]. The phase III randomized OVA-301 trial showed that trabectedin + PLD achieved a significantly better PFS and OS over PLD monotherapy in patients with recurrent partially platinum-sensitive EOC with PFI of 6-12 months [106]. A subset analysis showed the superiority of the combination in patients with BRCA- mutated but not in those with BRCA wild-type [107]. Conversely, a prospective European phase IV trial of trabectedin + PLD failed to detect significant differences in terms of either response rates or PFS according to BRCA status in patients with platinum- sensitive recurrent EOC [108].

In vitro and *in vivo* studies on trabectedin-resistant EOC and myxoid liposarcoma cell lines after repeated exposures to the drug have shown that persistent neoplastic cells after trabectedin were nucleotide excision repair [NER] deficient and sensitive to platinum compounds [109]. Therefore, trabectedin might resensitize neoplastic cells to platinum rechallenge. Clinical trials should be planned to investigate the activity of trabectedin-based treatment in patients with progressive EOC after PARPi, which probably have developed secondary BRCA reversion mutations recovering HR.

PARPi rechallenge: Rechallenge with PARPi is currently under clinical investigation, either as a maintenance option after subsequent platinum retreatment (OreO study, NCT03106987) or in combination with another drug after progression on olaparib (NCT02340611) [91].

NCT03106987 is a phase 3b, randomised, double-blind, placebo-controlled, multicentre study of olaparib maintenance retreatment in patients with non mucinous EOC previously treated with a PARPi and responding to repeat platinum-based chemotherapy.

NCT02340611 is a phase 2 study testing the combination cediranib + olaparib after disease progression on olaparib alone in patients with EOC.

The efficacy of intermittent (on/off) strategies or the sequential use of different PARPi is still unproven [91].

c-Met inhibitors and NF-kB inhibitors: c-Met inhibition downregulates RAD51 and sensitizes tumor cells to DNA damaging agents [80]. Whereas the anti-c-Met monoclonal antibody rilotumumab achieved a complete response rate of 3.2% and a 6-month PFS rate of

6.5% only in 31 women with persistent or recurrent EOC [110], the c-Met inhibitor cabozantinib obtained an objective response and a 12-week disease control in 21% and 50%, respectively, of 70 - heavily pretreated EOC patients, of whom 50% were platinum-refractory/ resistant [111].

The proteasome inhibitor bortezomib, approved by FDA for the treatment of multiple myeloma, affects the NF-κB pathway through protection of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha [IkBa] from the proteolysis by ubiquitin proteasome system [112,113]. Nagahawa, *et al.* [94] demonstrated that NF-κB signaling was up-regulated in established PARPi resistant breast carcinoma and EOC cell lines and that knockdown of core components of this signaling could restore PARPi sensitivity. Exposure to bortezomib resulted in cell death of the PARPi -resistant cells but not of parental cells. Therefore, thistable agent could represent a novel therapeutic option for reversing resistance to PARPi.

Combinations of PARPi with other molecularly targeted agents

Combinations of PARPi and immune checkpoint inhibitors: Anti CTLA-4 monoclonal antibodies synergize with veliparib to prolong survival of mice bearing BRCA1-mutant EOC, and this effect is- mediated by increased interferon- γ release by immune cells [114]. The analysis of 245 high-grade serous EOCs included in the TCGA dataset revealed a higher tumor neoantigen load, increased CD3+ and CD8+ tumor infiltrating lymphocytes and increased programmed death protein [PD-1] and PD-ligand [L]-1 expression in BRCA-mutated tumors compared to HR proficient ones, which suggested that the former could might be more sensitive to immune checkpoint inhibitors [115].

The combination of niraparib + the anti-PD1 monoclonal antibody pembrolizumab obtained an objective response and a disease control in 18% and 65%, respectively, of 60 heavily pretreated EOC patients [116]. More than 80% of patients were BRCA wild-type or unknown, 64% were HR proficient or unknown, and 35% were PD-L1 negative.

Table 3 reports the ongoing trials with combinations of PARPi and immune-checkpoint inhibitors in EOC.

Combinations of PARPi and antiangiogenic agents: Antiangiogenic agent-induced hypoxia downregulates the expression of BRCA and RAD51, thus inducing HRD [117]. The combination of olaparib + cediranib achieved a significantly longer PFS compared with olaparib monotherapy in 90 patients with recurrent, platinum -sensitive EOC with either high-grade serous or endometrioid histology or germline BRCA mutation (median, 16.5 *versus* 8.2 months, HR= 0.50; 95% CI=0.30–0.83) [118]. A subset analyses demonstrated a significant improvement in PFS (median, 23.7 *versus* 5.7 months, p= 0.0013) and OS (median, 37.8 *versus* 23.0 months, p= 0.047) in BRCA wild-type/ unknown patients, whereas the clinical outcome was similar between the two arms in BRCA. mutated patients. In a phase 3 trial olaparib + cediranib obtained a PFS similar to that of standard chemotherapy in platinum-sensitive recurrent EOC patients with either BRCA-wild type or BRCA- mutated [119].

The PAOLA1 trial reported a longer PFS for the combination of bevacizumab + olaparib compared with bevacizumab + placebo as maintenance treatment in patients with advanced, high-grade serous and endometrioid EOC in complete or partial response after carboplatin/paclitaxel-based first-line chemotherpy + bevacizumab (22.1 months *versus* 16.6 months, HR= 0.59; 95% CI= 0.49-0.72) [2]. Preplanned analysis revealed that the benefit was evident in either HRD patients with BRCA-mutated (HR= 0.33 ,95% CI= 0.25- 0.45) or HRD patients with BRCA wild- type (HR= 0.43, 95% CI=0.28 -0.66), but not in HR proficient patients (HR= 1.00; 95% CI=0.75-1.35).

A randomized phase 2 trial, including 97 patients with platinum-sensitive, recurrent high-grade serous or endometrioid EOC, showed that niraparib + bevacizumab significantly improved PFS compared with niraparib alone (median, 11·9 *versus* 5·5 months, HR= 0.35,95% CI=0.21-0.57) [120]. Based on these results, the NCT03806049 phase 3 trial has been planned to compare doublet platinum-based chemotherapy +bevacizumab *versus* niraparib + bevacizumab *versus* niraparib + bevacizumab + the anti-PD-1 monoclonal antibody dostarlimab in platinum-sensitive recurrent EOC.

Combination of PARPi and PI3K/AKT/mTOR inhibitors: The inhibition of PI3K/AKT/mTOR signaling pathway downregulates BRCA expression in breast carcinoma and EOC, thus enhancing cancer cells sensitivity to PARPi [121,122].

The combination of the PI3K inhibitor BKM120 + olaparib delayed tumor doubling to more than 70 days in a mouse model of BRCA1-related breast carcinoma and more than 50 days in xenotransplants from human BRCA1-related carcinomas, whereas olaparib alone reduced tumor growth modestly [123]. Analogously BKM120 synergized with olaparib both to induce apoptosis and to inhibit migration and invasion of PI3K- mutated EOC cells *in vitro* [122]. The combination of these two agents yielded a strong therapeutic effect in an intraperitoneal dissemination xenograft mouse model of EOC.

In a phase 1b study, the administration of the PI3K inhibitor alpesilib + olaparib achieved a partial response and a stable either disease in 36% and 50%, respectively, of 28 recurrent EOC patients with high-grade serous histology or any histology but with germline BRCA mutations [124]. In another phase I study, the combined use of BKM120 + olaparib obtained a partial response and a stable disease in 29% and 49% of 41 EOC patients, respectively, and in 28% and 44% of 18 breast carcinoma patients, respectively. Anticancer activity was detected in both BRCA- mutated and BRCA wild-type patients [125].

Carboplatin, olaparib, niraparib and the PI3K inhibitor LY294002 at 5μM concentrations and the c-Met inhibitor crizotinib at 2.5μM provided a 20-30% growth inhibition of high- grade serous EOC cell lines *in vitro* [82]. The sequential combinations of crizotinib + olaparib or crizotinib + niraparib were more synergistic compared to the combination of either carboplatin or LY294002 + PARPi, and induced cell cycle arrest and apoptosis through activation of ATM/CHK2 and inhibition of c-Met pathways. The synergistic effect of co-targeting c-Met and PARP may suggest a novel approach to overcome PARPi resistance in high-grade serous EOC, and the combination of crizotinib with a PARPi should be further explored in this malignancy.

Conclusions

PARPi have significantly improved the clinical outcome of EOC patients when administered as maintenance treatment after response to platinum-based chemotherapy. Although PARPi exert the greatest anticancer activity in patients with BRCA-mutated, these agents can be effective regardless of BRCA or HR status, and therefore a broad range of patients may benefit from their use. However, patients with and without BRCA-mutant will eventually become resistant to PARPi [72]. In the clinical setting only secondary BRCA reversion mutations or demethylation of the BRCA promoter have been detected as events able to induce PARPi resistance. The frequency of mutations can be

assessed by analyzing cfDNA which seems to offer a reliable non-invasive tool to identify patients who are more likely to benefit from PARPi treatment. Trabectedin, c-Met inhibitors and NF-kB inhibitors should be investigated to reverse PARPi resistance. Moreover, novel combination therapies may increase PARPi efficacy and overcome resistance to these agents. Additional biological studies are strongly warranted to investigate the pharmacological mechanisms of resistance to PARPi and to detect novel predictive biomarkers to better plan clinical trials of combination therapies with the highest chances of synergistic effects [13,126].

Conflicts of interest

The Authors declare no conflict of interest in relation to this study.

Authors' contributions

Conceptualization, Writing - original draft: AG; Data curation, Formal analysis, Methodology, Writing-review & editing: AG, SC.

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