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PARP inhibition: A promising therapeutic target in ovarian cancer

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Abstract

Ovarian cancer is burdened by the highest mortality rate among gynecological cancers. Gold standard is represented by the association of platinum- taxane -based chemotherapy and radical surgery. Despite several adjustments occurred in cytotoxic drug in last decades, most patients continue to relapse, and no significant enhancement has been reached in the overall survival. The development of drug resistance and the recurrence of disease have prompted the investigations of other targets that can be used in the treatment of ovarian cancers. Among such targets, polyadenosine diphosphate-ribose polymerase (PARP) represents a novel way to target specific pathways involved in tumor growth. PARP accelerates the reaction of the polyADP-ribosylation of proteins implicated in DNA repair. PARP inhibitors have shown activity in cancers with BRCA mutations, with other deficient DNA repair genes or signaling pathways that modulate DNA repair, or in association with DNA damaging agents not involved in DNA repair dysfunction. A number of inhibitors for PARP has been developed, and such drugs are under investigation in clinical trials to identify their impact in the treatment of ovarian cancers. This review aims to summarize the recent researches and clinical progress on PARP inhibitors as novel target agents in ovarian cancer.

Key words: Ovarian Cancer, PARP inhibitors, Target Therapies, Synthetic Lethality 1.

Introduction

Despite several improvements in gynaecological malignancy scenario, ovarian cancer still represents the most important cause of women cancer-related mortality, with a 5-year overall survival rate of approximately 49.7%, ranging from 47% to 19% for advanced stages when most patients are diagnosed (1). The combination of radical surgery and platinum-taxane-based chemotherapy is originally effective, however most patients relapse and develop drug-resistant disease. The poor outcome of advanced ovarian cancer under conventional therapy and the lack of effective chemotherapeutic regimens at recurrence have led to the exploration of new strategies that are mainly oriented into planning chemotherapy upfront when disease presented uncompletely resectable (2), changing of dose and schedule of various chemotherapeutic agents (3), and the research on molecular targeting drugs with or without chemotherapy (4), as well as immunotherapy (5). Lately, target therapies have gained great attention, because such therapies interfere solely with specific molecular targets, holding the promise of greater selectivity and lower toxicities (6-8).

Translational research in cancer has recently reached many goals in understanding DNA repair pathways in order to developing target therapy. A novel group of chemotherapeutic agents consists of polyadenosine diphosphate-ribose polymerase (PARP) inhibitors. These therapies have been investigated in several cancers. In particular, phase II clinical trials have demonstrated promising results in women affected by hereditary breast and ovarian cancers linked with BRCA1 and BRCA2 (referred as BRCA1/2) mutations (9). This

review summarizes the recent researches and clinical progress on PARP inhibitors as novel targeting agents in ovarian cancer.

PARP inhibitors and synthetic lethality

PARP is an enzymatic complex that was firstly discovered in 1963. However, the potential of PARP inhibition to increase DNA damage caused by cytotoxic chemotherapy was first speculated in 1980.

PARP-1 and PARP-2 represent the best-characterized subtypes of the 17 members of the PARP super family (10).

PARP-1 is expressed in all nucleated human cells except neutrophils and has been demonstrated to be over-expressed in some tumor types (11). Its expression has also been related with overall prognosis in cancer, particularly for breast cancer (12).

Each cell has a series of pathways to protect its genome from DNA damage. DNA injury appears as result of cell cycling, endogenous events, or cytotoxic agents. Once DNA damage alterations occur in these DNA repair pathways, there is an augmented risk of malignant transformation and resistance to chemotherapy.

Schematically, DNA repair can be categorized into single- stranded DNA-binding proteins (SSB), which include base excision repair (BER), nucleotide excision repair, and mismatch excision repair, or double-strand breaks (DSB), which comprise non-homologous end-joining and homologous recombination repair (HRR).

PARPs are implicated in DNA repair that utilizes the BER pathway (13) and share enzymatic and scaffolding properties.

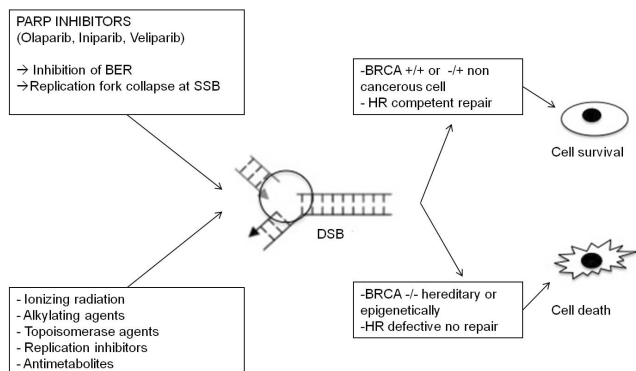


Figure 1. DNA repair and PARP inhibitors mechanism. DNA damage by ionizing radiation, alkylating agents, topoisomerase inhibitors, replication inhibitors and antimetabolites may induce double-strand breaks. PARP inhibition impairs the base excision repair (BER) pathway. In cells with deficient homologous recombination (HR) pathway, like BRCA-mutated cancer cells, which have lost both copies of BRCA1/2, DNA damage accumulates, leading to programmed cellular death. Non-cancer cells, less subjected to DNA damage, are heterozygous for the defect, with one functional allele, and therefore retain BRCA1/2 protein expression and maintain homologous recombination pathway. BER: Base excision repair; DSB: Double-strand break; HR: Homologous recombination; SSB: Single-strand break.

In fact, DNA damage stimulates the catalytic (14-15) activity of PARP-1, which by two zinc finger motifs in the DNA-binding domain binds to DNA SSB, thus activating the BER machinery to repair the SSB. Therefore, inhibition of PARP blocks the BER pathway.

Normal cells can restore the DNA injury using different pathways, habitually the homologous recombination (HR) pathway, a route in which an intricate machinery is built to permit the sister chromatid to act as a specimen for the DNA repair process.

In contrast, in BRCA-mutated ovarian cancer cells that missed both copies of BRCA1 or BRCA2, DNA damage advances and leads to cell death. Non-cancer cells, less subjected to DNA damage, are heterozygous for the defect, with one functional allele, and consequently retain BRCA1/2 protein expression and maintain the HR pathway (see Figure 1).

This dissimilarity between tumor and normal cells means that PARP inhibitors eliminate tumor cells selectively compared to the effects in normal cells. This has led to the idea of ‘synthetic lethality,’ which means that one pathway is mutated in the cancer cell and the other pathway is blocked by the drugs. Individually, not even the pathway is fundamental, but when both are annulled the cell cannot survive (16).

PARP inhibitors in hereditary and sporadic ovarian cancers

BRCA1 and BRCA2 proteins have a central role via homologous recombination in the complex of DNA repair machinery, whereas PARP is the key component in BER of DNA.

PARP inhibitors have shown encouraging efficacy results in BRCA-mutated ovarian cancer, which represents approximately 10% to 15% of epithelial ovarian cancer (EOC).

BRCA1 has a multidimensional role in DNA repair, cell cycle checkpoints, transcription, chromatin remod-

eling, and ubiquitination (17). In spite of its wide roles in DNA damage response, BRCA1 is indispensable for HRR by contribution in initial DSB processing while BRCA2 has a more precise role in the regulation of Rad51 recombinase, a key mediator of HRR.

Absence in either BRCA1 or BRCA2 function brings to an amplified use of error-prone and mutagenic non-homologous end-joining repair (18). Consequently, loss-of-function mutations in the BRCA1 or BRCA2 gene augment genome instability that causes hereditary ovarian carcinogenesis.

Furthermore, the phenomenon of ‘BRCAness,’ characterized by HR deficiency, should be underlined: up to 50% of women with high-grade EOC could have functional loss of proteins implicated in the HRR pathways of DNA repair, and consequently these tumors will phenotypically work like BRCA1/2 mutant cancers, even if they do not have specific mutations. In vitro experiments have demonstrated that inhibition of the PARP enzyme, which is implicated in the base excision DNA repair pathway, results in the lack of DNA repair and death in cells exhibiting defective HR (19).

Current data propose that sporadic high-grade EOCs can allow developed genetic and epigenetic defects in BRCA and in other DNA repair genes that may contribute to the ‘BRCAness profile’ associated with an improved prognosis after platinum-based chemotherapy, even in absence of gBRCA1/2 mutations. Several major processes seem to be involved in the development of the BRCAness pattern. The BRCA1 promoter hypermethylation has been shown to lead to BRCA1 silencing in 5% to 30% of cases (9). Other defective genetic factors who affect or are affected by normal BRCA gene have been investigated, including the amplification of EMSY that brings to BRCA silencing in 20% of cases (10). Furthermore, BRCA collaborates with the proteins of the Fanconi anemia (FA) complex in the pathway of DNA repair, and consequently defects in members of the FA complex have been shown to reproduce the BRCA-deficient phenotype (11). Other proteins implicated in DNA repair in addition to BRCA could also lead to the BRCAness profile in over 10% of cases: hypermethylation of Rad51C, a protein that traces DNA repair machinery to the damaged strand; mutation/deletion in ATM, ATR, and PTEN, which is involved in transcription regulation of Rad51(9). Nonetheless, the evidence of involved mechanisms is still fragmented, and the idea of BRCAness for patients with the sporadic form of the disease has not found a concrete base, yet. The precise characterization of the BRCAness phenotype in high-grade EOC represents today a pivotal challenge to enable patients to access new therapeutic strategies, thus impacting the survival perspective. Based on the above preclinical data on observed synthetic lethality in BRCA1/2-deficient cancers, a number of PARP inhibitors have been developed for clinical use by various pharmaceutical companies. At present, the most investigated PARP inhibitor is olaparib, which has shown promising results. An increasing number of clinical trials on iniparib, veliparib, rucaparib, niraparib, and BMN673 are also underway in gynecological malignancies, and many phase I and II studies are currently ongoing (Table 1).

Table 1. Ongoing phase I-II clinical trials of polyadenosine diphosphate- ribose polymerase inhibitors (Olaparib, Iniparib, Veliparib, Rucaparib, Niraparib, BMN 673) as single agent or in combination with other agents.

PARP Inhibitor	Phase of Development	Combination	Type of tumor	Status of study
OLAPARIB (AZD2281)	1	Cisplatin, Gemcitabine	S	C
	1	Carboplatin, Paclitaxel	S	A
	1	Carboplatin	CC, OC	Rec
	1	Topotecan	S	C
	1	None	S	C
	1	Carboplatin	OC	Rec
	1	None	OC	Rec
	1	None	OC	A
	1	Cisplatin	S	A
	1-2	Cediranib	OC	A
	2	None	OC, TC	W
	2	None	OC	C
	2	Doxorubicin	OC	A
	2	Carboplatin, Paclitaxel	OC	A
	2	None	OC	A
	2	Carboplatin, Paclitaxel	OC	A
	2	None	OC	A
	2	None	OC, PC, PrC, BC	C
	2	Carboplatin, Paclitaxel	OC	C
	INIPARIB (BSI-201)	1	None	S
1		Gemcitabine, Carboplatin, Paclitaxel, Doxorubicin	S	Rec
1		Irinotecan	S	C
2		None	OC	C
2		Carboplatin, Gemcitabine	OC	C
2		Carboplatin, Paclitaxel	US	C
2		Carboplatin, Gemcitabine	OC	A
VELIPARIB (ABT-888)	1	None	S	C
	1	Carboplatin, paclitaxel, bevacizumab, ABT-888	OC,TC,P	Rec
	1	Doxorubicin	OC	U
	1	Mitomycin C	S	Rec
	1	Dinaciclib with or without Carboplatin	S	Rec
	1	Carboplatin, Paclitaxel, Bevacizumab	OC, TC	Rec
	1	Radiation therapy	S with peritoneal carcinomatosis	C
	1	Carboplatin, Gemcitabine	S	Rec
	1	Paclitaxel, Carboplatin	S	Rec
	1	Gemcitabine	S	Rec
	1	Topotecan	S	C
	1	Temozolomide	OC	C
	1	Bevacizumab, Carboplatin, Cisplatin, Paclitaxel	OC	Rec
	1	Carboplatin, Paclitaxel	OC	Rec
	1	Carboplatin, Gemcitabine	S	Rec
	1	Floxuridine	OC, TC	Rec
	1	Doxorubicin, Carboplatin	OC, TC	Rec
	1	Oxaliplatin, Capecitabine	S	Rec
	1-2	Topotecan	OC, S	A
	1-2	Topotecan	OC	Rec
2	Temozolomide or Doxorubicin	OC	A	
2	None	OC	Rec	
2	Ciclofosfamide	OC	A	
RUCAPARIB	2	Platinum based chemotherapy	Relapsed OC, TC, P	Rec
NIRAPARIB	3	None	OC	Rec
BMN 673	1	None	S, OC	Rec

S: solid tumors; OC: ovarian cancer; CC: cervical cancer; PrC: prostate cancer; PC: pancreatic cancer; BC: breast cancer; TC: tubal cancer; US: uterine carcinosarcoma; Rec: recruiting; A: active not recruiting; C: completed; T: terminated; W: withdrawn; U: unknown

Olaparib and ovarian cancer

Pharmacodynamics, pharmacokinetics, and metabolism

Olaparib (AZD2281, KU-0059436) is a strong inhibitor of PARP that provokes synthetic lethality in BRCA1/2-deficient tumor cells.

It is being developed as oral monotherapy and for combination schedules with other anticancer agents.

The median effective concentration necessary to induce a 50% effect (EC50) of olaparib is approximately 6 nM. In cancer patients, following a single capsule oral dose, olaparib was rapidly absorbed. Exposure increased proportionally with doses up to 100 mg twice daily but augmented in a less proportional fashion at higher doses. Following dosing to cancer patients at doses of 400 mg bid (twice daily), the population estimated maximum plasma concentration at steady state (C_{max} ss) ranged from 1.45 to 11.0 µg/mL (17). Drug-related material was eliminated in the urine (35%-50%) and in the feces (12%-60%), with 10% to 20% and 0.6% to 14% of the dosed material recovered as unchanged drug in the urine and feces, respectively (21).

Clinical development

Phase I-II studies

'Synthetic lethality' concept was firstly tested in a phase I trial by Fong *et al.* with a dose escalation of olaparib from 10 mg daily to 600 mg twice daily in a population of patients affected by recurrent/refractory tumors including EOC (22). Sixty patients were recruited: 22 (37%) were carriers of a BRCA1 or BRCA2 mutation, and 1 (2%) had a robust family history of BRCA-associated cancer but refused to undergo mutational testing.

At a dosage of 600 mg twice daily or more of olaparib, there was an inhibition of PARP activity by more than 90%, measured in peripheral mononuclear cells.

The maximum tolerated dose (MTD) was 400 mg twice daily. Minimal adverse events were reported, mostly gastrointestinal and fatigue. Radiological or biochemical (CA 125) response or stable disease for a period of 4 months or more was achieved in 63% (12/19) of patients. An antitumor activity was reported in platinum-resistant patients at a dosage underneath the recommended/maximum tolerated doses (21). These hopeful results were lately confirmed by an expanded cohort of 50 patients affected by BRCA1/2 mutation-associated advanced ovarian, primary peritoneal, and fallopian tube cancers. With the administration of 200 mg twice daily a clinical benefit rate of 46% was achieved, with median response duration of 28 weeks (22). The overall clinical benefit rate increased notably with platinum sensitivity, and a significantly positive connection between the overall platinum-free interval and response to olaparib was found ($p = 0.002$), suggesting that PARP inhibition correlates with platinum sensitivity in addition to showing a benefit in resistant and refractory patients. Finally, in a dose-finding phase I trial in Japanese population that included ovarian cancer patients, olaparib was well tolerated up to the 400 mg dosage, and preliminary evidence of antitumor activity was reg-

istered (23). Olaparib administration with topotecan, dacarbazine, paclitaxel, cisplatin, and gemcitabine has been described (24), with reports of an overlapping, dose-limiting toxicity, particularly myelosuppression, more pronounced compared with chemotherapeutic agents alone. Olaparib has also been associated with bevacizumab in a phase I trial in patients affected by advanced solid tumors, the hypothesis being that the vessel regression produced by the anti-angiogenic agent induces hypoxia and results in an intensification of DNA damage and genetic instability (25). Dean *et al.* reported the administration of growing doses of continuous oral olaparib (100, 200, and 400 mg) in combination with intravenous bevacizumab (10 mg/kg every 2 weeks).

The most common adverse events (AEs) related to olaparib were gastrointestinal (grade 1-2 nausea) and fatigue. Three serious AEs and/or dose-limiting toxicities were reported (26). The latest phase I trial on olaparib in association with chemotherapy was conducted by Van der Noll *et al.* and presented at the American Society of Clinical Oncology (ASCO) meeting 2013 (27). In this study olaparib was associated with carboplatin, paclitaxel, or both (CPa) in 87 patients with advanced solid tumors, mostly breast (26%), melanoma (10%), and ovarian (7%), refractory to standard therapies. Escalating doses of olaparib capsule and tablet formulations were studied. Twelve patients (14%) had known germline BRCA1/2 mutation (gBRCA1/2m). The most common AEs included myelosuppression (71%), notably neutropenia (54%), thrombocytopenia (26%), and fatigue (77%). Two doses were identified as tolerable: continuous olaparib 100 mg bid with weekly paclitaxel 80 mg/m² and intermittent olaparib 200 mg bid (d1-10) with CPa AUC4/175 mg/m² every 3 weeks. Fourteen patients (16%) revealed an objective response (complete response 5%; partial response 11%); 28% had stable disease for ≥4 months with a greater drug activity in BRCA1/2 mutated patients (complete response 17%; partial response 33%). Tolerability was improved with intermittent olaparib. Given the promising results of phase I studies (see Table 2), phase II clinical trials involving patients affected by advanced/recurrent ovarian cancer have been conducted (28-29) (Table 3).

A phase II international multicenter single-arm, open-label, sequential-dosing cohort study was initiated, recruiting BRCA1/2 mutation carriers with recurrent EOC. This trial suggested a dose-response relationship; in fact greater olaparib activity was seen at a dose of 400 mg twice daily rather than at 100 mg twice daily (RECIST objective tumor response rate: 33% vs. 12.5%, $p < 0.05$, progression free survival (PFS) 5.8 vs. 1.9 months), with an acceptable toxicity profile (grade 3 nausea in 7% and leucopenia in 5% of patients). Remarkably, two patients in the 400 mg cohort reached complete responses, whereas there was none in the lower dose group.

Favorable survival data in BRCA1/2 mutated patients treated with olaparib, have been recently reported in a multicenter non-comparative study conducted by Kaufman and presented at ASCO 2013 (30). Two hundred ninety-eight heavily pretreated patients with advanced cancer refractory to standard therapy and with a gBRCA1/2m, including 193 patients with advanced ovarian cancer, received olaparib 400 mg bid (capsule)

Table 2. Phase I trials of Olaparib in EOC.

Authors, year	Patients	Schedule	Response rate	Toxicity (grade I-II)	Toxicity (grade III-IV)
Fong et al, 2013	15 BRCA1 or BRCA2 OC	<u>Olaparib:</u> 100mg bid	PR/CR: 6%	<u>400mg bid group:</u> Diarrhea: 12% Dyspepsia: 12%	<u>400mg bid group:</u> Lymphopenia: 12% Nausea:12%
		200mg bid	PR/CR:40%;	Fatigue: 62% Digeusia: 12%	Vomiting: 12% Dizziness: 12%
	50 advanced B R C A m OC, primary peritoneal and fallopian tube cancer	600mg bid	PR/CR: 6%	Anemia: 6%	Lymphopenia: 8%
		Dose-escalation cohort: from 40mg daily for 2 of 3 weeks, to 600mg bid. Dose-expansion cohort: 200 mg twice daily in 28-days cycles	PR/CR: 40% SD:6%	Nausea: 42% Vomiting:18% Diarrhea: 6% Dyspepsia: 16% Anorexia :16% Fatigue:42%	Anemia: 8% Nausea: 6% Vomiting:2% Diarrhea: 2% Fatigue:4%
Martinek et al, 2010	12 solid tumor with BRCAm (including OC)	<u>Olaparib:</u> 100mg bid	CR: 0%, PR:8,3%, SD:0%,PD: 16,6%	<u>400mg bid group:</u> Nausea: 66% Leukopenia: 50% Anemia: 50%	
		200mg bid	CR: 0%, PR:0%, SD:16,6%, PD: 8,3%	Anorexia: 33% Vomiting: 16% Fatigue:16%	Not observed
	400mg bid	CR:0%, PR:0%, SD:16,6%, PD: 33,3%			
Kaufman et al, 2013	12 solid tumor with BRCAm (including OC)	Increasing doses of continuous oral Olaparib (100, 200 and 400 mg) in combination with bevacizumab (10mg/kg every 2 weeks)	NA	No serious adverse effects were reported	

OC: ovarian cancer; bid: twice daily; CR: complete response; PR: partial response; SD: stable disease; PD: progression disease; NA: not available.

until disease progression. The median duration of treatment was 5.5 months (range 1- 28.5 months). The most common AEs reported (generally of grade 1/2) were fatigue (59%), nausea (59%), and vomiting (37%), whereas the most common grade ≥ 3 AE was anemia (17%).

Among patients with ovarian cancer, the overall response rate was 31%; 124 patients (64.4%) were alive 1 year after treatment, with a progression-free interval of 6 months in 105 patients (PFS 54.6%). These results seem to indicate the antitumor activity of olaparib in monotherapy in gBRCAm patients affected by advanced cancer refractory to standard therapy, support-

ing the hypothesis that therapy focused against a genetically defined target is effective regardless of the anatomic origin of the tumor. Gelmon et al. investigated the role of olaparib (400 mg) in a phase II single-arm study on patients affected by high-grade serous/undifferentiated ovarian cancer and with unknown BRCA status or BRCA-negative. The study also included a reference group known to have germline BRCA mutations. Ninety patients were enrolled (64 with ovarian cancer and 26 with breast cancer) and treated with olaparib 400 mg bid. Biopsies were taken before starting treatment, after two cycles, and at the time of progression.

Table 3. Phase II trials of Olaparib in EOC.

AUTHORS, year	PTS	SCHEDULE	RR	PFS	OS	TOXICITY (grade III-IV)
Ledermann et al, 2012	57 BRCA1/2 m recurrent OC	<u>Olaparib:</u> - 400mg bid (33 pts) - 100mg bid (24 pts)	CR: 6%; PR: 27%; SD: 36%; PD: 30%; CR:0%; PR: 13%; SD: 29%; PD:58%;	5.8 mths (median) 1.9 mths (median)	NA	<u>400mg bid group:</u> Nausea: 6% Fatigue: 3% Anaemia: 3% Vomiting: 3% Neutropenia: 9%
Ledermann et al, 2014	265 pts recurrent OC BRCA 1/2 m	<u>Olaparib:</u> -400 mg bid (136 pts) <u>Placebo</u> (129 pts)	NA	11.2 mths (median) 4.3 mths (median)	29.8 mths (median) 27.8 mths (median)	severe adverse events: 18% 9% (most common: bowel obstruction)
Liu et al, 2013	90 pts recurrent OC, TC, PeC	<u>Olaparib:</u> -400 bid (46 pts) <u>Olaparib 200 mg bid plus Cediranib 30 mg</u> (44 pts)	47.8% 79.6%	9.0 mths (median) 17.7 mths (median)	65% at 24 mths 81% at 24 mths	<u>Combination group:</u> Diarrhoea: 23% Fatigue: 27% Hypertension:41%
Kaufman et al, 2014	298 BRCA1/2 m OC, PrC, PC, BC	<u>Olaparib:</u> -400 mg bid (193 OC pts)	OC: 31.1%	OC: 7.0 mths (median)	OC: 16.6 mths	<u>OC group:</u> Nausea: 0.5% Fatigue: 6.2% Anaemia:18.7% Vomiting: 2.6% Abdominal pain: 7.3%
Oza et al, 2014	162 recurrent BRCA1/2 m OC	<u>Olaparib 200 mg bid plus TXL175 mg/mq and CBDCA AUC 4 followed by Olaparib 400 mg bid</u> (81 pts) <u>TXL175 mg/mq and CBDCA AUC 6</u> (81 pts)	CR:10% PR: 54% OR: 64% CR: 7% PR: 51% OR: 58%	12.2 mths (median) 9.6 mths (median)	33.8 mths (median) 37.6 mths (median)	<u>Olaparib plus CHT group:</u> Headache: 1% Fatigue: 7% Neutropenia: 43%

PTS: patients; RR: response rate; PFS: progression free survival; OS: overall survival; m: mutated; nm: not-mutated; OC: ovarian cancer; OR: objective response; OOR: overall objective response; CR: complete response; PR: partial response; SD: steady disease; PD: progression of disease; mths: months; PLD: pegylated liposomal doxorubicin; I.A.: interim analysis; NA: not available OC: ovarian cancer; PrC: prostate cancer; PC: pancreatic cancer; BC: breast cancer; PeC: peritoneal cancer; OR: overall response.

After BRCA testing, 17 patients showed BRCA mutations. Consistent with previous studies, objective responses were reported in 7 of 17 patients with BRCA1 or BRCA2 mutations (41%; 95% confidence interval (CI), 22–64) and 11 of 46 without mutations (24%; 95% CI, 14–38), with a median response duration of 31 weeks. In the ovarian-cancer cohort without BRCA1- or BRCA2-associated mutations, a CA-125 response rate of 26% (95% CI, 15–42; 10 of 38) and a combined RECIST and CA-125 response rate of 30% (95% CI, 19–44; 14 of 47) were recorded.

Of the totality of patients with ovarian cancer, the disease-control rate (partial response plus stable disease at 8 weeks) was 66% (42 of 64): in BRCA1- or BRCA2-negative mutation cohorts the disease control rate was 76% (13 of 17), and in BRCA1- or BRCA2-positive cohorts it was 62% (29 of 47). Responses were positively correlated with platinum sensitivity (radiological 50%, biochemical 40%) compared with resistant/refractory disease (radiological 3.8%, biochemical 17.4%), consistent with other studies on BRCA1 or BRCA2 mutation carriers. Adverse events were only of grade 1–2, including fatigue (70% of patients with ovarian cancer), nausea (66%), vomiting (39%), and decreased appetite (36%) (28). This study clearly showed that patients with platinum-sensitive high-grade serous ovarian cancer without a BRCA1/2 germline mutation responded to olaparib. This also suggests that a greater susceptibility to platinum and other DNA damaging agents can be considered as BRCA-like behavior. A possible explanation for between platinum sensitivity and olaparib response is that intra- and inter-strand platinum-DNA network can generate torsion on the double helix and lead to DSBs (31), needing HR for proper and efficacious correction. Without repair, additional genomic damage is continued, leading to cell death.

Randomized phase II trials

To address the role of olaparib as a second-line treatment in BRCA1/2-mutated EOC patients, a three-arm study evaluating two different dosages of olaparib with the reference dose was planned. Ninety-seven patients with BRCA-mutated progressive or recurrent disease < 12 months after their last platinum administration were randomized in a 1:1:1 ratio to receive olaparib 200 mg bid or 400 mg continuously or pegylated liposomal doxorubicin (PLD) 50 mg/m² intravenously (32). RECIST-assessed objective response rates were statistically comparable across the three arms (25%, 31%, and 18% for olaparib 200 mg, olaparib 400 mg, and PLD, respectively). No statistically important differences in terms of PFS (olaparib 200 mg: 6.5 months; olaparib 400 mg: 8.8 months; PLD: 7.1 months; hazard ratio 0.88, $p=0.6$) were found, thus failing the primary end point of the study. Nevertheless, it should be underlined that the median PFS of 7.1 months of PLD observed in this trial is considerably greater than the 4 months expected, taking as a reference the randomized trial by Gordon *et al.* (33), in a comparable mix of platinum-resistant and platinum-sensitive cancers.

Accordingly, Adams and colleagues published retrospective data (34) that showed an increased activity of PLD in BRCA-mutated ovarian cancer patients, suggesting that HR-deficient ovarian cancer may have an

improved clinical outcome from anthracyclines such as PLD compared with not selected cases.

Notably, data from study of Kaye *et al.* confirmed that the higher dose of olaparib (400 mg twice daily) is suitable and more effective (35). Similar data for relapsed ovarian cancer, irrespective of BRCA1/2 mutations, were presented in a randomized, double blind, placebo-controlled phase II study by Ledermann *et al.* (36). Patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer were included in order to test the role of olaparib as maintenance treatment in this setting. Patients submitted to two or more platinum-based regimens and with a partial or complete response to their most recent platinum-based schedule were recruited to receive olaparib in monotherapy with the aim of evaluating its efficacy as maintenance treatment. The results from 256 randomized patients (136 received olaparib and 129 received placebo) showed an advantage of approximately 4 months for olaparib versus placebo in median PFS (hazard ratio for progression or death 0.35; 95% CI, 0.25–0.49; $p < 0.001$), even if no significant differences in overall survival (OS) between the two treatment groups were reported ($p = 0.75$). Side effects more frequently reported in the olaparib group than in the placebo one included nausea (68% vs. 35%), fatigue (49% vs. 38%), vomiting (32% vs. 14%), and anemia (17% vs. 5%); however, the majority of these were globally of grade 1 or 2.

Nevertheless, no significant difference in terms of OS between groups was found at interim analysis (38% mortality, meaning that 38% of the patients had deceased; hazard ratio with olaparib, 0.94; 95% CI, 0.63–1.39; $p = 0.75$). A preplanned subgroup analysis of 218 out of 256 (82%) patients with a known gBRCAm from the trial by Ledermann *et al.* was recently performed; the results, presented at ASCO 2013 (37), suggested that olaparib (400 mg bid) may lead to a greater PFS and an OS benefit in those women with gBRCAm. In particular, gBRCA1/2m patients showed greater PFS benefit with olaparib maintenance versus placebo (median: 11.2 vs. 4.1 months; $p < 0.001$) and a significant quality of life (QoL) improvement, as measured with the Trial Outcome Index ($p = 0.03$). Furthermore, even if at the interim analysis of OS the comparison of olaparib versus placebo in the overall population led to a hazard ratio of 0.88 with medians of 29.8 versus 27.8 months, respectively, the subgroup analysis limited to gBRCAm patients resulted in an OS hazard ratio of 0.74 (median: 34.9 for olaparib group vs. 31.9 months for placebo group). Moreover, olaparib was recently combined with paclitaxel plus carboplatin (P/C) followed by olaparib as maintenance treatment in patients with platinum-sensitive recurrent serous ovarian cancer enrolled in a multicenter randomized open-label, phase II study (38). Arm A, which included patients submitted to olaparib capsules plus P/C for 6 cycles followed by maintenance olaparib monotherapy, was compared with Arm B, which included patients receiving P/C alone for 6 cycles and no further therapy.

Of 162 patients randomized ($n=81$ per arm), 156 received treatment (Arm A, $n=81$; Arm B, $n=75$) and 121 started the maintenance/no further therapy phase (Arm A, $n=66$; Arm B, $n=55$). Olaparib + P/C (AUC4) followed by maintenance olaparib showed a significant

improvement in PFS versus P/C (AUC6) alone (hazard ratio 0.51, 95% CI, 0.34–0.77; $p = 0.0012$; median = 12.2 vs. 9.6 months). Data on OS are still immature (total events: 14%). ORR was similar for Arm A and Arm B (64% vs. 58%). The most common AEs during the combination phase included alopecia (74% vs. 59% for Arm A versus Arm B, respectively), nausea (69% vs. 57%), and fatigue (64% vs. 57%). There were no fatal AEs.

Phase III trials are warranted to confirm these data. A second interim analysis of OS and a retrospective, preplanned analysis of data by BRCA mutation status from the a randomised, double-blind, multicenter phase 2 study assessing the maintenance treatment with olaparib 400 mg twice daily versus placebo in platinum-sensitive recurrent ovarian cancer patients (36) has been recently conducted by Ledermann *et al* (36). BRCA status was known for 131 (96%) patients in the olaparib group versus 123 (95%) in the placebo group, of whom 74 (56%) versus 62 (50%) had a detrimental or suspected detrimental germline or tumour BRCA mutation. A significantly longer PFS has been documented in the olaparib group than in placebo one both for BRCA mutated patients (11,2 mo vs 4,3 mo for olaparib and placebo group respectively, $p < 0.0001$) and for wild-type BRCA (7,4 mo vs 5,5 mo for olaparib and placebo group respectively, $p < 0.0075$), resulting in a greater benefit than that previously reported in the overall population (36). However, overall survival did not differ significantly between groups both for BRCA mutated patients ($p = 0.44$) and wild-type BRCA ($p = 0.96$). Moreover, in the overall population, median time to first subsequent therapy or death was significantly longer in the olaparib population than in the placebo, regardless of BRCA mutation ($p < 0.0001$). The most common adverse events reported were grade 1-2 fatigue and anemia, with grade 3 or worse events in 10 (7%) patients of olaparib group versus four (3%) in the placebo one (39). These promising results have led to the approval of the drug in the maintenance treatment of platinum sensitive disease.

A recent randomized open-label phase II study performed by Liu *et al* investigated the effects of the combination of olaparib plus cediranib ($n = 44$) versus olaparib alone ($n = 46$) in women with measurable platinum-sensitive, relapsed, high-grade serous or endometrioid ovarian, fallopian tube, or primary peritoneal cancer or with detrimental germline BRCA1/2 mutations (40).

Cediranib is an antiangiogenic agent active against VEGF receptor type 1, 2, 3.

Cediranib plus olaparib significantly improved progression-free survival (9.0 vs 17.7 months or olaparib and combination respectively; $p = 0.005$) and the proportion of patients who achieved an objective response compared with olaparib alone.

These results suggest that the combination of a PARP inhibitor and an anti-angiogenic drug could be synergistic and have increased activity in patients with platinum sensitive high-grade serous ovarian cancers, as compared with either agent alone, consistent with hypotheses generated by preclinical data. In exploratory analyses, the activity of cediranib plus olaparib seemed to be robust in both BRCA mutated and BRCA wild-type or unknown populations. Nota-

ble side-effects occurred with cediranib plus olaparib, resulting in dose reductions in more than 75% of patients. Drug-related adverse events were more frequent with cediranib plus olaparib than with monotherapy, with 70% of patients having a grade 3 or higher event (diarrhoea, fatigue, hypertension). Another more recent multicenter phase II study enrolling 298 individuals with a germline BRCA1/2 mutation and recurrent cancer (ovarian, breast, pancreatic or prostate), investigated tumor response rate to olaparib in these patients (41). One hundred and seventy-eight out of 193 patients of ovarian cancer cohort, presented ovarian cancer, four had fallopian tube cancer, and 11 had primary peritoneal cancer.

BRCA1 germline mutation affected 148 (77%) of those in the ovarian cancer cohort, while 44 patients (23%) had a BRCA2 mutation, and one had a germline mutation in both BRCA1 and BCRA2. These patients were heavily pretreated with meanly 4,3 prior regimens and presented platinum-resistant disease. Patients received olaparib 400 mg twice per day until disease progression. The tumor response rate was 26.2% and 31.1% for all and ovarian cancer patients respectively. Stable disease that continued ≥ 8 weeks was observed in 40.4% of OC patients.

Overall median duration of response was 208 days (225 days for ovarian cancer). In these patients median PFS and OS were 7 and 16.6 months respectively. At 6 months of follow up, 54,6% of OC patients were progression free, while the proportion of patients alive at 12 months was

64.4%. The most common adverse events reported included fatigue, nausea, and vomiting with grade ≥ 3 AEs considered causally related to olaparib in 30,9% of patients (41). In addition, in a latter randomized, open-label, phase 2 study 162 selected patients with platinum-sensitive, recurrent, high-grade serous ovarian cancer submitted to up to three previous courses of platinum-based chemotherapy and who were progression free for at least 6 months before randomization were randomized to receive chemotherapy plus olaparib or chemotherapy alone (42). Patients received either olaparib (200 mg capsules twice daily, administered orally on days 1–10 of each 21-day cycle) plus paclitaxel (175 mg/m², administered intravenously on day 1) and carboplatin (area under the curve (AUC) 4 mg/mL per min, administered intravenously on day 1), then olaparib monotherapy (400 mg capsules twice daily, given continuously) until progression (the olaparib plus chemotherapy group), or paclitaxel (175 mg/m² on day 1) and carboplatin (AUC 6 mg/mL per min on day 1) followed by no further treatment (the chemotherapy alone group). One hundred and fifty-six patients were treated in the combination phase and 121 continued to the maintenance or no further treatment phase. BRCA mutation status was known for 107 patients of which 41 (38%) had a BRCA mutation (20 in the olaparib plus chemotherapy group and 21 in the chemotherapy alone group). The study results showed a significantly longer PFS in the combination group (median 12.2 months) than in the chemotherapy alone group (median 9.6 months; HR=0.51, $p = 0.0012$), especially in patients with BRCA mutations (HR=0.21, $p = 0.0015$). Most common adverse events reported at least 10% more

frequently with olaparib plus chemotherapy than with chemotherapy alone were typically of grade 1-2 with exception of neutropenia and included alopecia, nausea, neutropenia, diarrhea, headache, peripheral neuropathy and dyspepsia. The most common grade 3 or higher adverse events during the combination phase were neutropenia (43% vs 35% in the olaparib plus chemotherapy group and chemotherapy alone one respectively) and anaemia (9% vs 7%, respectively). Serious adverse events were reported in 12 patients (15%) in the olaparib plus chemotherapy group and 16 patients (21%) in the chemotherapy alone group; thus showing an acceptable and manageable tolerability profile (42).

Iniparib and ovarian cancer

Pharmacodynamics, pharmacokinetics, and metabolism

Iniparib (BSI 201) belongs to the benzamide family of compounds; it is enzymatically reduced in malignant cells to the cytotoxic metabolite 4-iodo-3-nitrosoamide. Initial mechanistic studies showed that 4-iodo-3-nitrosobenzamide inactivated PARP-1 by removing a single zinc finger. However, recent studies have shown that the mechanism of action of iniparib is not specific for the PARP-1 pathway. Iniparib also failed to potentiate the effects of topoisomerase, a hallmark of PARP inhibitors. It has been proposed that iniparib induces cell death by forming protein adducts with cysteine-containing proteins, an interaction that is not limited to PARP. These protein adducts can cause sufficient alterations in intracellular protein function to trigger cell death via a variety of pathways (32). Iniparib is administered intravenously and excreted in the urine (43). The half-life of the drug in human studies is 4 minutes, with evidence of longer-lasting active metabolites.

Clinical development

Iniparib has been mostly studied in breast cancer, showing improvements in PFS and OS in a phase II trial for the treatment of metastatic triple-negative breast cancer

but failing to demonstrate improvement in survival in a phase III study (44-45). It was studied in combination with carboplatin and paclitaxel in a phase II trial involving women affected by uterine carcinosarcoma, but the response rate was not adequate to warrant further studies (46). Clinical data on iniparib in ovarian cancer are still limited, with preliminary but interesting data on tolerability and efficacy obtained from phase I-II studies. Phase III study results are not available. Phase I studies involving patients affected by solid tumors, conducted by Mahany *et al.* and Kopetz *et al.*, have shown a maximum tolerated dose of 8 mg/kg for iniparib (47- 48).

The most common side effects reported were gastrointestinal and respiratory, with no report of grade 3 or 4 toxicities. Of the 23 patients enrolled by Kopetz *et al.*, 6 patients had stable disease for about 2 months with iniparib administration (48). Mahany *et al.* enrolled 55 patients with solid tumors and assigned to 1 of 4 combinations of iniparib plus topotecan or gemcitabine or temozolomide (TMZ) or carboplatin, according to physician preference. All regimens were well tolerated.

One patient with ovarian cancer showed complete

response at 6 months. Partial response was seen in 9 (16%) patients, and 19 (34%) patients had stable disease for at least 2 months (47).

Additional phase I dose escalation and pharmacokinetic studies in advanced stage solid

cancers are currently ongoing (Table 1). Preliminary data from phase II studies of iniparib for the treatment of platinum-sensitive or resistant recurrent ovarian cancer show improvements in survival compared with historic controls.

Notably, data from a phase II trial of iniparib (5.6 mg/kg) in combination with gemcitabine (1000 mg/m² on days 1 and 8) and Carboplatin (AUC 4 on day 1) in women with recurrent platinum-sensitive ovarian cancer (ClinicalTrials.gov number NCT01033123) showed a 70.6% overall response rate in the first 17 patients enrolled (49). The same dosage has been used in a phase II study including platinum-resistant ovarian cancer patients (ClinicalTrials.gov number NCT01033292). Preliminary data in 19 treated patients showed an objective response rate of 31.6% and PFS of 5.6 months, significantly improved if compared with the response rates of prior studies in platinum-resistant patients (50). The results of a third, completed phase II study of patients with BRCA1- and BRCA2- associated advanced ovarian, fallopian tube, or primary peritoneal cancers are eagerly awaited (ClinicalTrials.gov number NCT00677079). At present, iniparib has not yet been explored in phase III trials for the treatment of EOC.

Veliparib and ovarian cancer

Pharmacodynamics, pharmacokinetics, and metabolism

Veliparib (ABT-888) is a PARP inhibitor with excellent potency (KI, 5.2 and 2.9 nmol/L, PARP-1/ PARP-2) and oral bioavailability (51). In preclinical studies, veliparib was shown to be a potent inhibitor of PARP and was found to potentiate the effect of platinum agents, cyclophosphamide, and radiation in syngeneic and xenograft tumor models (52). It was also reported to have good bioavailability and the capability to cross the blood-brain barrier (53).

The first phase 0 study performed under the guidance on exploratory investigational new drugs, issued by the U.S. Food and Drug Administration (FDA), was conducted by the National Cancer Institute with veliparib. It was chosen because of its wide therapeutic index. Veliparib enhanced the activity of multiple DNA-damaging agents, including, irinotecan, carboplatin, cisplatin, cyclophosphamide, radiation, and temozolomide, in various syngeneic and xenograft preclinical models (54). A validated pharmacodynamics assay was developed for assessing PARP inhibition by measuring PAR, a product of PARP. The pharmacokinetics and pharmacodynamics were evaluated over a short time period after a single dose of nontoxic veliparib (10, 25, and 50 mg, each tested in three patients). A significant reduction in PAR was seen at the 25 and 50 mg dose 3-6 h after dosing for both tumor and PBMC, respectively. Even at 24 h after dosing, a 49% reduction below baseline PAR level was reported (54).

Plasma veliparib concentrations were calculated with a liquid chromatography-mass spectrometry assay

validated by FDA guidelines (55). Majority of drug-related material was excreted in urine as unchanged drug.

Clinical development

Several studies have suggested that veliparib was effective in combination with chemotherapy for gynecologic cancers (56-58). An open-label, multicenter, single-arm phase I combination study of ABT-888 and metronomic oral cyclophosphamide in patients with advanced malignancies was recently published by Kummar *et al.* The study included 11 patients affected by advanced ovarian cancer, treated with a dose escalation design of the combined drugs. The study treatment was well tolerated, with an acceptable profile of toxicity rates and the maximum tolerated dose (MTD) was established as veliparib 60 mg with cyclophosphamide 50 mg given once daily. Seven patients had a partial response (5 patients with ovarian cancer) and an additional six patients had disease stabilization for at least six cycles (1 patient with ovarian cancer). These results suggest that also at lower doses, veliparib created sufficient inhibition of PARP activity to provide benefit to BRCA-positive patients receiving DNA-damaging chemotherapy (56). These positive results encouraged the beginning of a multicenter, randomized phase II study comparing metronomic cyclophosphamide alone versus metronomic cyclophosphamide in combination with veliparib in patients affected by advanced ovarian cancer and BRCA mutations, high-grade serous ovarian cancers, triple-negative breast cancers, and low-grade lymphomas (ClinicalTrials.gov identifier: NCT01306032). This study is ongoing, but is not recruiting participants. Veliparib has also been associated with topotecan in an open-label, single-arm phase I study testing the combination of ABT-888 administered orally with topotecan hydrochloride administered intravenously in patients with advanced malignancies. Twenty-four patients with refractory solid tumors and lymphomas, including five patients with ovarian cancer, were enrolled. The study was carried out to determine the MTD, safety, pharmacokinetics, and pharmacodynamics of the combination in these patients.

Various schedules and doses of intravenous topotecan in combination with ABT-888 (10 mg) administered orally bid were evaluated.

All patients had previously received standard therapy and had evidence of disease progression.

Significant myelosuppression limited the ability to co-administer ABT-888 with standard doses of topotecan, requiring dose reductions.

The MTD was established as topotecan 0.6 mg/m²/d and ABT-888 10 mg bid on days 1 to 5 of 21-day cycles. Myelosuppression was the principal toxicity on this trial. This is the first clinical study showing significant reduction in PARP levels (more than 75%) in all three paired tumor biopsies compared with baseline after administration of ABT-888 at 10 mg. Whether this degree of PARP inhibition is sufficient (or a higher degree of inhibition is needed) to derive clinical benefit is currently unknown, making it difficult to define the 'optimal biologic dose' of ABT-888 in combination with chemotherapy. Escalating the PARP inhibitor dose to the MTD may not be necessary to derive clinical benefit, especially given the narrow therapeutic index of

certain combination regimens (57). In addition to chemotherapeutic agents, veliparib has also been combined with anti-angiogenic agents. The rationale behind this combination is based on the observation that vascular endothelial growth factor receptor (VEGFR) inhibition may lead to increased DNA damage through down-regulation of DNA repair proteins (58) and may stop the growth of tumor cells by blocking blood flow to the tumor.

The multicenter, dose-escalation phase I study of carboplatin, paclitaxel, bevacizumab, and ABT-888 in treating patients with newly diagnosed stage II to IV ovarian epithelial cancer, fallopian tube cancer, or primary peritoneal cancer is actively enrolling patients (ClinicalTrials.gov identifier: NCT00989651). In the experimental regimen (Arm I) patients receive paclitaxel over 3 hours, carboplatin over 30 minutes, and bevacizumab over 30 to 90 minutes (beginning in course 2) on day 1. Patients also receive oral ABT-888 bid on days 1-21.

Patients belonging to Arm II receive paclitaxel over 1 hour on days 1, 8, and 15. Patients also take carboplatin, bevacizumab, and ABT-888 as in regimen I. For both arms treatment repeats every 21 days for 6 courses, and patients then receive bevacizumab alone on day 1. The treatment with bevacizumab repeats every 21 days for 16 courses in the absence of disease progression or unacceptable toxicity.

The primary objectives of the study are to determine the MTD and dose-limiting toxicities of ABT-888 when associated to carboplatin, paclitaxel, and bevacizumab using two different treatment regimens, and the feasibility and the toxicity related to these regimens over four courses once the MTD is established. In the last multi-institutional phase I study, veliparib was combined with low-dose fractionated whole abdominal radiation (LDFWAR) to assess the safety profile of the combination in patients with advanced solid malignancies and peritoneal carcinomatosis (59). Twenty-two patients were recruited, 8 patients with ovarian or fallopian cancer. Patients were treated with veliparib for a total of 3 cycles. At the time of study enrollment, 16 of 22 patients had exclusively abdominal disease and 6 of 22 patients had both intra-abdominal and extra-abdominal disease. In the subset of 8 ovarian and fallopian cancers, mPFS was 6.77 months and mOS was 17.54 months compared with mPFS 2.71 months and mOS 13.01 months in others. Patients with ovarian and fallopian cancers had better QoL over time than those with other cancers. Treatment-related grade 3-4 toxicities included lymphopenia (68%), anemia (9%), thrombocytopenia (14%), neutropenia (4%), leukopenia 9%), ascites (4%), vomiting (4%), and dyspnea (4%). No objective responses were observed. Thus, for some patients with advanced solid tumors and carcinomatosis, particularly in the ovarian and fallopian cancer subpopulation combined veliparib and LDFWAR could be a well-tolerated regimen that resulted in prolonged disease stability (59).

Recently a multicenter phase II trial presented at the SGO meeting reported the clinical activity of the single-agent veliparib in BRCA mutation carriers affected by ovarian, fallopian tube or peritoneal cancer (60). The study enrolled 52 patients (50 of them evaluable for efficacy and toxicity) with recurrent or persistent measur-

able disease and germline mutations in BRCA1 (78%) or BRCA2 (22%). The majority of cancers (82%) were high-grade serous cell carcinomas. Patients underwent up to three prior therapies (with the exception of a prior PARP inhibitor). Thirty patients were platinum-resistant and 20% were platinum-sensitive.

Veliparib was started at 400 mg twice daily for 28 days (one cycle), and dose reductions were allowed for toxicity. Overall response rate was 26%, including 2 complete responses and 11 partial responses. The response rate was 20% in platinum-resistant patients, 35% in platinum-sensitive ones, 26% and 27% in BRCA1 and BRCA2 carriers respectively. At the time of the first analysis, median progression-free survival was 8.11 months. At 6 months, 60% of patients were disease-free. Overall survival was estimated at 19.7 months. Toxicity profile was satisfactory in these heavily pretreated patients. Only one case of grade 4 toxicity (thrombocytopenia) was reported. Grade 3 adverse events included fatigue ($n = 2$), nausea ($n = 2$), leukopenia ($n = 1$), neutropenia ($n = 1$), dehydration ($n = 1$), and elevated alanine transaminase level ($n = 1$). Grade 2 toxicities, reported in more than 10% of patients, included nausea (46%), fatigue (26%), vomiting (16%), and anemia (14%). Dose reductions for toxicity were required in 24 patients (48%). The finding of somewhat activity of veliparib in various platinum-resistant patients with recurrent or persistent disease suggests the importance of further investigations.

Rucaparib and ovarian cancer

Pharmacodynamics, pharmacokinetics, and metabolism

Rucaparib (CO338, AGO14699, and PF01367338) is another PARP-1 and PARP-2 oral inhibitor (with an inhibition constant of <5 nM) that has entered into clinical trial testing for recurrent ovarian cancer showing anti-ovarian cancer activity both in vitro and in vivo studies (60). The mean plasma half-life of oral rucaparib is approximately 17 h (61–62). In studies on female nude mice with heterozygous BRCA2 mutation inoculated with cancer cells with mutated BRCA1/2 or XRCC3 or with epigenetically silenced BRCA1, a 30-minute exposure to 10 μ M AGO14699 caused more than 94% inhibition of PARP activity, compared with untreated controls. This result demonstrated that AGO14699 freely permeates the cells, binds to and inactivates PARP, and that the inhibition persists during cell permeabilization and subsequent PARP enzyme stimulation.

Moreover, combination treatment with AGO14699 plus carboplatin reduced tumor growth better than treatment with each drug alone (15).

Clinical development

The phase I study of oral rucaparib tested doses of 40 mg up to 500 mg once per day continuously as well as 240 mg to 840 mg bid; the recommended phase II dose of single-agent rucaparib was determined to be 600 mg bid.

Rucaparib demonstrated anticancer responses in an ovarian and peritoneal cancer subgroup, both in platinum-resistant and platinum-sensitive recurrence (61). In addition to being explored as a single-agent, it has

also been combined with temozolomide, carboplatin, carboplatin and paclitaxel, and cisplatin and pemetrexed (62–65). Rucaparib at 600 mg bid has been selected as the recommended Phase II dose in an ongoing Phase I/II study in patients affected by various solid tumors (63–68). Subsequent analysis described the patient population with ovarian or primary peritoneal cancer enrolled in the Phase I portion. The treatment was well tolerated, drug-related adverse events resulting mild or moderate (Grade 1 (37%), Grade 2 (22%)) with low incidence of Grade 3 (6%) and no Grade 4. The safety profile in ovarian and primary peritoneal patients was reliable with the overall profile in all patients and nausea, vomiting, diarrhea and fatigue events were controllable, with no discontinuations of the treatment. It has also demonstrated an enduring clinical benefit both in platinum-sensitive and resistant ovarian and primary peritoneal cancer patients. In fact, three RECIST PR and two GCIG CA-125 responses has been accounted at the time of last analysis with 4 out of them being platinum resistant. Disease control rate (CR, PR, SD at 12 weeks/24 weeks) were 93% and 70% in germline BRCA ovarian cancer patients, respectively (69).

Rucaparib is currently undergoing further testing at the 600 mg bid dose in recurrent ovarian cancer as part of two clinical trials: ARIEL2 and ARIEL3. ARIEL2 is a phase II biomarker study of 180 patients with platinum-sensitive, relapsed high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer who have received one or more prior platinum-based chemotherapy regimens and whose last treatment regimen was platinum-based; fresh tumor biopsy and archival tumor are both required to participate. ARIEL3 is a phase III randomized trial of oral rucaparib versus placebo (2:1 randomization) following platinum-based therapy in patients with platinum-sensitive high-grade serous or endometrioid epithelial ovarian, primary peritoneal, or fallopian tube cancer. Both trials are still recruiting patients.

Niraparib and ovarian cancer

Pharmacodynamics, pharmacokinetics, and metabolism

Niraparib, 2-{4-[(3S)-piperidin-3-yl]phenyl}-2H-indazole-7-carboxamide (MK4827), is a selective PARP-1 and PARP-2 inhibitor, orally bioavailable, tested in a phase I trial in both patients with recurrent germline BRCA -ovarian cancer and patients with sporadic BRCA-proficient cancers (44–45). It presents a half maximum inhibitory concentration (IC₅₀) of 3.8 nmol/L for PARP-1 and 2.1 nmol/L for PARP-2 (66).

In a phase I dose-escalation study, mean plasma concentrations of niraparib peaked 3 to 4 h after a dose with a subsequent biphasic decrease in concentration. The mean terminal elimination half-life was 36.4 h (range 32.8–46.0 h). Pharmacodynamic analyses confirmed PARP inhibition exceeded 50% at doses greater than 80 mg/day, and antitumor activity was documented beyond doses of 60 mg/day (65–67). Niraparib inhibits tumor growth in models with loss of BRCA and PTEN function (70).

Clinical development

In a phase 1 dose-escalation multicentre study, 100 patients with advanced solid tumours were enrolled (67). In part A, cohorts of three to six patients, enriched for BRCA1 and BRCA2 mutation carriers, received niraparib daily at ten escalating doses from 30 mg to 400 mg in a 21-day cycle to establish the maximum tolerated dose. In part B, the maximum tolerated dose was further investigated in patients with sporadic platinum-resistant high-grade serous ovarian cancer (HGSC) and sporadic prostate cancer. Sixty patients were enrolled in the part A of the study, including 49 patients with ovarian or peritoneal cancer. Twenty-two patients had known germline BRCA mutations and 27 were sporadic HGSC. A dose of 300 mg/day was found to be the MTD. Two patients developed grade 4 thrombocytopenia at the 400 mg dose level. DLTs during the first cycle of all doses were grade 3 fatigue (one patient dosed at 30 mg/day), grade 3 pneumonitis (one patient dosed at 60 mg/day), and grade 4 thrombocytopenia (2 patients dosed at 400 mg/day). Twenty of the 22 patients with germline BRCA mutations had RECIST measurable cancer, and 8 of these 20 (40%; 95% CI, 19–64) had a confirmed RECIST and CA125 partial response; these patients received doses of niraparib between 80 mg and 400 mg PO/day and the median response duration was 387 days (range 159–518 days). Ten patients with known BRCA mutations had platinum-sensitive cancer, and the ORR by RECIST and CA125 was 50% (95% CI, 19–81); median duration of the response was 431 days (range 159–518 days). In the sporadic HGSC group that included 27 patients, 22 patients had RECIST measurable cancer; two of three patients with platinum-sensitive sporadic HGSC had responses by RECIST or CA125, and the doses received by these responders were 30 mg and 60 mg. Of 19 patients with platinum-resistant sporadic HGSC, 3 responded according to RECIST or CA125 (16%; 95% CI, 3–40). Toxicities at the recommended phase II dose of 300 mg/day (50 patients tested) were mostly myelosuppression and gastrointestinal.

Randomized trials

Currently, a phase III study of niraparib versus placebo as maintenance therapy, called NOVA, is open and still accruing patients with platinum-sensitive recurrent HGSC who have achieved a partial or complete response to both their current and their penultimate platinum course. This is a double-blind study with a 2:1 randomization of niraparib versus placebo for either germline BRCA or sporadic BRCA recurrent platinum-sensitive HGSC.

BMN 673 and ovarian cancer

Pharmacodynamics, pharmacokinetics, and metabolism

BMN 673 is an oral PARP-1 and PARP-2 inhibitor (70) (PARP1 IC₅₀ = 0.57 nmol/L). It shows selective antitumor cytotoxicity and elicits DNA repair biomarkers at very lower concentrations than earlier generation PARP1/2 inhibitors (olaparib, rucaparib, veliparib). In vitro, BMN 673 selectively targets tumor cells with BRCA1-2 or PTEN gene defects with 20- to more than 200-fold superior potency than existing PARP inhibi-

tors. It is quickly orally bioavailable, with more than 40% absolute oral bioavailability in rats when dosed in carboxymethyl cellulose. Xenografted tumors that transport defects in DNA repair due to BRCA mutations or PTEN deficiency were greatly sensitive to oral BMN 673 treatment at well tolerated doses (0.33 mg/kg or 0.1 mg/kg once a day for 28 days) in mice. Synergistic or additive antitumor effects were also found when BMN 673 was associated to temozolomide, SN38, or platinum drugs.

To assess the in vivo pharmacodynamics of BMN 673, it has been administered orally in a single dose of 1 mg/kg: intratumoral PAR levels drastically decreased at 2 and 8 hours following oral administration, with partial recovery of basal PAR levels at 24 hours after dosing, an effect probably due to the clearance of BMN 673 (71–72). 9.2 Clinical development An open-label phase I study tested once-daily orally administered BMN 673 in patients with advanced or recurrent solid tumors, the primary objective of the study being to establish the MTD of daily oral BMN 673 (71). Thirty-nine patients were enrolled in 9 cohorts testing from 25 to 1100 µg PO daily that defined a maximally tolerated dose of 1000 µg/day. The patient population included 23 patients with either ovarian or primary peritoneal cancer, of which 17 presented a germline BRCA mutation. Dose-limiting thrombocytopenia occurred in 1 of 6 patients and in 2 of 5 patients at the 900 and 1100 µg/day, respectively. Potentially related AEs in >10% of patients, mostly of grade 1–2, included fatigue, nausea, flatulence, anemia, neutropenia, thrombocytopenia, and grade 1 alopecia. RECIST and/or CA-125 responses occurred at doses ≥100 µg/day in 11 out of 17 ovarian or peritoneal cancer patients who had a germline BRCA mutation. The recommended phase II dose was established at 1000 µg/day PO (61). Currently, phase I and phase I–II studies, both on patients affected by advanced or recurrent solid tumors, are still accruing patients. Moreover, a phase III study testing BMN 673 in patients with advanced or metastatic breast cancer who are carriers of BRCA mutation and a phase II cohort study in germline BRCA mutation subjects with locally advanced and/or metastatic breast cancer are ongoing. Other PARP inhibitors Other PARP inhibitors that are or have been in clinical testing but do not have any associated ovarian cancer patient data or any ongoing or completed ovarian cancer studies include AZD2461 (NCT01247168), CEP9722 (NCT00920595), E7449 alone or in several combinations (NCT01618136), E7016 in combination with temozolomide (NCT01127178), and INO-1001 plus temozolomide (NCT00272415).

Resistance to PARP inhibitors

The development of resistance to PARP inhibitors has been reported, and several mechanisms of potential resistance in BRCA-related tumors have been suggested.

Defects in BRCA function and HRR pathway give great genome instability in cancers. Therefore, as the disease progresses, these cancer cells tend to evolve into subpopulations, each of which may own distinct phenotypes with several degrees of response to PARP inhibitors.

BRCA deficiency may be reverted by changes in the mutational reading frame, resulting in production of wild-type BRCA protein. Mounting evidence has proved that secondary somatic mutations of mutated BRCA1 or BRCA2 genes reestablish proficiency in HRR and give resistance to platinum chemotherapy and PARP inhibitors (73). These changes in the mutational reading frame of BRCA may potentially occur through second mutations, compensatory mutations, or crossover (71-72). This may explain why not all BRCA-mutation tumors respond to PARP inhibitors. Another mechanism hypothesized includes up-regulation of the p-glycoprotein efflux pump reducing intracellular PARP inhibitor concentrations (74).

Ongoing studies are evaluating the chemo-sensitivity in women non-responders to PARP inhibitors and their mechanism of action.

Studies conducted on cell lines have demonstrated that an acquired secondary mutation can allow a BRCA1/2-deficient tumor to recover BRCA function and homologous recombination competency, so that PARP inhibition can no longer be synthetically lethal (75-76).

Remarkably, some patients who responded to olaparib and then developed resistance have been described to retain sensitivity to further platinum-based treatment (77).

To describe a gene expression profile of BRCAness related to chemotherapy response and outcome in epithelial ovarian cancer, Kostantinopolos *et al.* (78) made a publicly available microarray data set that includes 61 patients affected by EOC with either sporadic disease or BRCA1/2 germline mutations and a second cohort of 70 EOC patients submitted to exploratory laparotomy for staging, diagnosis and debulking, followed by first-line platinum-based chemotherapy.

Combination with radiation-induced RAD51 foci formation and with PARP inhibitor responsiveness was assessed in cisplatin-resistant clones of the BRCA2-mutated pancreatic cancer cell line Capan-1.

The BRCAness profile was confirmed in 70 patients enriched for sporadic disease to assess its association with outcome.

Association with platinum responsiveness was assessed in platinum-sensitive and resistant tumor biopsy specimens from six patients with BRCA germline mutations.

The BRCAness profile accurately predicted platinum responsiveness in 8 of 10 patient-derived tumor specimens and the correlation between PARP-inhibitor sensitivity and resistance in four out of four Capan-1 clones. Moreover, in terms of the 70 patients with sporadic disease, patients with the BRCA-like (BL) profile showed improvements compared with patients with a non-BRCA-like (NBL) profile in disease-free survival (34 months vs. 15 months, respectively; $p = 0.013$) and overall survival (72 months vs. 41 months, respectively; $p = 0.006$), and this result was independent of standard prognostic factors such as age, grade, histology, stage, and debulking status (78). However, these data are not so strong as to assess whether the association between the BRCA-like profile and improved survival is indicative of enhanced platinum responsiveness or, instead, might identify patients with a more weak natural his-

tory (78). Interestingly, the proportion of patients with a complete clinical remission at the end of first-line chemotherapy was higher in the BL population (90%) than in patients with the NBL signature (74%), although this was not statistically significant ($p = 0.2$). Additional studies are needed for the recognition of biomarkers to detect HR-deficient cancers. However, the finding of a gene expression profile that seems to relate with BRCAness may make it possible to eventually offer PARP inhibitors to a much bigger number of patients with epithelial ovarian cancer, regardless of their BRCA1 or BRCA2 mutation status.

Gene expression or immune-histochemical signatures of deficiency of BRCA 1 and BRCA 2 expression or HR defects need also to be explored (26), and functional assays should be performed on tumor cells derived from ascites and circulating cells. In order to overcome resistance to olaparib, PARP inhibitors have also been combined with chemotherapeutic agents in several studies, and several trials investigating the combined regimens are still ongoing. In particular, because of the potential synergy of PARP inhibitors with inhibitors of other signaling pathways, combinations of PARP inhibitors with other targeted biologic agents have been explored in clinical trials, including trials combining PARP inhibitors with anti-angiogenic agents and with pi3-kinase (pi3k) inhibitors. A preclinical rationale exists for combining anti-angiogenic agents: PARP inhibitors including HR can be suppressed by hypoxia through down-regulation of HR proteins, and PARP inhibitor sensitivity is enhanced in hypoxic states (58-60). Olaparib was combined with bevacizumab to examine doses of continuous oral olaparib (100, 200, and 400 mg bid) in combination with 10 mg/kg of bevacizumab IV every 2 weeks (72). Twelve patients were enrolled, and the most common toxicities observed were grade 1/2 nausea and fatigue. The recommended phase II dose established was olaparib 400 mg bid with bevacizumab 10 mg/kg IV every 2 weeks.

Moreover, a phase I trial of oral cediranib, an oral VEGFR2 inhibitor, and olaparib enrolled 28 patients (20 ovarian cancer and 8 breast cancer patients) to four dose levels (73, 75). Cediranib may help keep cancer cells from growing by affecting their blood supply, whereas olaparib may stop cancer cells from growing abnormally. Thus, the combination of cediranib and olaparib may help to keep cancer from growing. Two DLTs (one grade 4 neutropenia and one grade 4 thrombocytopenia) followed at the highest dose level (cediranib 30 mg daily and olaparib capsules 400 mg bid). The recommended phase II dose was cediranib 30 mg daily and olaparib 200 mg bid. In the 18 ovarian cancer patients with RECIST-evaluable disease, the ORR was 44%. Furthermore, we are still waiting for definitive results of a randomized phase II study testing the combination of cediranib plus olaparib versus olaparib alone in women with platinum-sensitive recurrent ovarian cancer not previously submitted to anti-angiogenic in the recurrent setting (NCT01116648). A rationale also exists for combining PARP inhibitors and PI3kinase inhibitors: a phase I study of olaparib plus oral BKM120 (an oral PI3kinase inhibitor) is currently ongoing (NCT01623349). The foundation for this approach is based on the observation that activa-

tion of the PI3K pathway occurs in as much as 70% of all ovarian cancers: 30% to 40% of type I ovarian cancers have activating mutations of PIK3CA (encoding the p110 α subunit of PI3K), whereas 17% to 25% of type II ovarian cancers show genomic amplification of the PIK3CA gene (79, 80). The PI3K pathway has been shown to be activated in a mouse model of BRCA1-related breast cancer, and the combination of olaparib and BKM120 was synergistic, leading to delayed tumor doubling compared with each agent alone (81). In addition, PI3K p110 α inhibition was found to render BRCA1-proficient tumors sensitive to the anti-cancer effects of olaparib using a murine breast cancer model (82). The combination of PARP inhibitors with chemotherapeutic or biologic agents could represent a favorable challenge by the increasing population of patients who may benefit from PARP inhibitors beyond BRCA-associated and/or HR-deficient cancers. However, both the choice on the timing of the introduction of a PARP inhibitor and the decision about combining drugs and sequencing of administration should be accurately weighted on patient characteristics and tumor features, in order to increase patient benefits by minimizing treatment-related toxicities (19).

Future directions

The best way to counteract tumour growth is a synergistic action between different strategies. Surgery is the milestone of ovarian cancer treatment, however it needs to be followed by chemotherapy to maximize the efficacy. Similarly, immunologic effects have been studied both after chemotherapy and surgery. No data are completed now on the effect of molecular therapy alone or in association of standard treatment.

PARP inhibitors have been precursors in introducing the concept of personalized medicine in cancer therapy, showing great clinical promise, especially in olaparib-based studies. Nevertheless, some issues remain controversial. PARP inhibitors are poised to change how BRCA-related ovarian cancer is treated, but one of the major challenges remains the identification of patients who are most likely to benefit from treatment. At present, relatively little biomarker information is available for the stratification of cancer patients eligible for PARP inhibitor therapy. Moreover, the tests that have been developed, such as PARP, γ -H2AX foci, and RAD51 (79-84), are too unwieldy for routine clinical use, although they appear to be able to exactly define PARP inhibitor-sensitive tumors. The systematic use of these biomarkers in tumor biopsies or patient blood prior to, during, and after treatment would allow to discriminate patient populations responding or resistant to PARP inhibitors. Unfortunately, there is still no prospectively validated biomarker of HR-deficient ovarian cancers that accurately predict defective HR and responsiveness to PARP inhibitors, and this is an area of high priority for ovarian cancer research. The discovery of biomarkers that can identify patients most likely to benefit from PARP inhibition has the potential to maximize benefit while minimizing health care costs.

PARP inhibition in an unselected population improved PFS and prolonged disease control but an overall survival advantage has not been reported yet (83, 84).

The lack of survival improvement could be overcome by identifying a biomarker to direct therapy. Compared with the universal use of PARP inhibition, or the use of other biologic maintenance therapies, the BRCA1/2 test to direct personalized PARP inhibition treatment may symbolize a cost-reducing strategy, but further clinical trials are needed to confirm that BRCA1/2 mutations are predictive biomarkers to direct anti-PARP therapies. Understanding the real mechanism of resistance to PARP would allow the definition of optimal sequencing of PARP inhibitors and platinum compounds. It is important that caution would be exercised for long-term use of these drugs, especially due to the lack of knowledge on the effects of long-term inhibition of base excision repair in normal cells. The most investigated PARP inhibitor, olaparib, showed a significantly improved progression-free survival as maintenance therapy in the phase II randomized trial from Ledermann *et al.* (36-37), with an extremely attractive toxicity profile, especially in those women with known germline BRCA mutations. These preliminary results encouraged further studies to characterize the subgroup of the ovarian cancer population that will probably benefit from synthetic lethality-based therapy. Results of SOLO1 and SOLO2 randomized trials, still in recruitment phase, will provide definitive results on the role of olaparib as maintenance therapy in women with newly diagnosed and recurrent BRCA-positive ovarian cancer. Moreover, with great nosiness we are waiting the results of the ongoing randomized, double-blind, multicenter, phase III trial of olaparib vs placebo in association with bevacizumab in patients not progressed after first-line chemotherapy plus bevacizumab for advanced high grade epithelial ovarian, fallopian tube, or primary peritoneal cancer (PAOLA1), to assess the role of this combination in this setting of patients.

Furthermore, an immunological point of view on the effects of these new drugs could be a new scenario, similarly to what concern for chemotherapy (85), in which the sole different of timing (neoadjuvant vs adjuvant chemotherapy) could differently affect immune system against tumor.

Another challenge will be the rational development of combinations of chemotherapy and PARP inhibitors with non-overlapping or minimally overlapping toxicities and defining clinically optimal schedules using sequential therapy versus continuous or both. In conclusion, a new individualized chemotherapeutic approach to patient based on the genomic characteristics of the cancer is urgently needed. Synthetic lethality is defined as a promising idea in ovarian cancer targeting. The best example of synthetic lethality is the interaction between PARP inhibition and BRCA deleterious mutations or BRCAness profile of sporadic ovarian cancer.

Given the predictable increase in the global problem of cancer and limited health care resources, it is imperative that researches will be conducted to define patient populations that will really benefit from novel therapies. Appropriate identification of susceptible patient gene characteristics as a predictive and prognostic degree of the efficacy of the PARP inhibitor targeting is paramount.

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