PARP inhibitors in ovarian cancer: where are we now?

C. Marchetti[1], P. Benedetti Panici[1], S. Kaye[2]

Abstract
Ovarian cancer is the most important cause of gynaecological cancer-related mortality. Although the combination of radical surgery and platinum-taxane-based chemotherapy is initially effective, most women will relapse, requiring further treatments. The poor outcome and the lack of effective chemotherapeutic regimens at recurrence have stimulated the exploration of new strategies. Research has been recently focused on targeted therapies and the family of polyadenosine diphosphate-ribose polymerase (PARP) inhibitors seems to be one of the most promising alternatives. The aim of this review is to summarize recent research and clinical progress with PARP inhibitors as novel targeting agents in ovarian cancer.

Key words: BRCA1/2, ovarian cancer, polyadenosine diphosphate-ribose polymerase (PARP) inhibitors

Introduction

Ovarian cancer represents the most important cause of gynaecological cancer-related mortality with a 5-year survival rate of approximately 50% for advanced disease, when most patients are diagnosed [1]. The traditional treatment of advanced ovarian cancer is based on cytoreductive surgery, followed by platinum-based chemotherapy. Unfortunately, despite the high response rate to the initial chemotherapy, there remains a significant risk for recurrence of the disease and resistance to the chemotherapy. These discouraging data have stimulated the exploration of new strategies such as targeted therapies, in order to achieve greater selectivity and reduced toxicities [2-4]. In this regard, the strategy of targeting DNA repair pathways has been explored and the family of poly-ADP ribose polymerase (PARP) inhibitors has proved particularly promising. They have been investigated in various cancers, with interesting results especially in women with hereditary breast and ovarian cancers associated with BRCA1 and BRCA2 (BRCA1/2) mutations [5]. In this review we will summarize the recent research and clinical progress with PARP inhibitors as novel targeting agents in ovarian cancer.

BRCA1 and BRCA2 and ovarian cancer

The inheritance of mutations in either BRCA1 or BRCA2 is associated with an estimated lifetime risk of developing ovarian cancer of up to 40-60% for BRCA 1 mutation carriers [6]. Hereditary germline mutations in either of these BRCA mutations account for about 10% of invasive ovarian carcinomas in unselected cases [7-9]. A key focus for BRCA1 and BRCA2 carriers has been prevention through early cancer detection with screening and prophylactic surgical measures. Although prophylactic oophorectomy reduces the risk of ovarian cancer by approximately 90%, BRCA mutation carriers are still at increased risk of developing ovarian and other malignancies and may already have developed cancer prior to screening. Recent data indicate that the fallopian tube rather than the ovary is the organ most at risk for malignancy and in the future prophylactic surgery should certainly take this into account [10].

Although the oncological management of BRCA mutation ovarian cancer patients has not differed from that of non-hereditary carriers, recent retrospective studies evaluating the clinical impact of germline BRCA1/2 mutations in ovarian cancer patients, found that BRCA carriers may have better clinical outcomes and higher response rates than non-hereditary ovarian cancer patients to first and subsequent lines of platinum-based chemotherapy [11, 12]. Common characteristics included serous ovarian cancer histology, longer treatment-free intervals between disease relapses, and improved overall survival (OS).

BRCAness, PARP1 inhibitors and synthetic lethality

It is now well established that 10–15% of women with

[1]Department of Gynaecological and Obstetrical Sciences and Urological Sciences, Università di Roma "Sapienza", Roma, Italy.
[2]The Royal Marsden National Health Service Foundation Trust and The Institute of Cancer Research, Sycamore House, Downs Rd, Sutton, Surrey SM2 5PT, UK.

Correspondence to: Dr. Claudia Marchetti, Dipartimento di Scienze Ginecologiche-Ostetriche e Urologiche, Università di Roma "Sapienza", Viale del Policlinico 155, 00161 Roma, Italy. Phone: +39 338 4652744; Fax: +39 06 49972564; E-mail: clamarchetti@libero.it

CANCER BREAKING NEWS 2013;1(1):4-8

ovarian cancer have germline BRCA1 or BRCA2 mutations [7-9]. In addition, data from studies such as the Cancer Genome Atlas [13] indicate that up to 50% of women with high-grade epithelial ovarian cancer (EOC) could have functional loss of proteins involved in the homologous recombination repair (HRR) pathways of DNA repair and behave like BRCA1/2 mutant cancers, even in the absence of germline BRCA mutations. This is the phenomenon called 'BRCAness' and the identification of the BRCA-like or BRCAness ovarian cancer population could define a larger population of patients who might potentially benefit from PARP inhibition.

PARP is a complex enzyme initially identified in 1963 involved in DNA repair. PARP-1 and PARP-2 represent the best-characterized subtypes of the 17 members of the PARP family [14]. Mechanisms of DNA repair can be grouped into those following single-stranded DNA-binding protein (SSB), which include base excision repair (BER), nucleotide excision repair and mismatch excision repair; or those following double-strand breaks (DSB), which comprise non-homologous end-joining (NHEJ) and HRR. PARPs are involved in DNA repair that utilizes the BER pathway. DNA damage stimulates the catalytic [15, 16] 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. Inhibition of PARP blocks the BER pathway leading to the generation of DSB. Normal cells can readily repair this DNA damage through the HRR pathway. However, cells with deficient HRR, such as BRCA-mutated ovarian cancer cells, without both copies of BRCA1 or BRCA2, have to use alternative pathways such as NHEJ; this is error-prone and eventually leads to cell death. In normal cells, patients are heterozygous for the defect, with one functional allele, and therefore retain BRCA1/2 protein expression and maintain a functional HRR pathway. This difference between tumour and normal cells means that PARP inhibitors kill tumour cells selectively compared with the effects in normal cells and this has led to the concept of 'synthetic lethality', which describes the situation whereby one pathway is mutated in the cancer cell and another pathway is inhibited by the drugs [17, 18].

PARP inhibitors in hereditary epithelial ovarian cancers

A recent Cochrane review summarized the evidence for using PARP inhibitors in clinical trials in BRCA1/2 mutated women [19]. From this and other reports the following conclusions can be drawn.

Olaparib is the most investigated PARP inhibitor; it was initially assessed in a phase I trial by Fong et al. with a dose escalation from 10 mg daily to 600 mg twice daily (bid) in a cohort which included recurrent/refractory ovarian cancer patients [5]. Of the 60 recruited patients, 22 (37%) were carriers of BRCA1 or BRCA2 mutation and one had a strong family history of BRCA-associated cancer. In this trial, dose-limiting toxicities were observed at olaparib 400 and 600 mg bid; the maximum tolerated dose (MTD) was 400 mg bid. Most frequently reported adverse events were gastrointestinal effects and fatigue. Clinical benefit was reached in 63% (12/19) of patients with BRCA-related cancer, including eight patients with ovarian cancer. It was noteworthy that anti-tumour activity was seen in platinum-resistant patients even at a dosage below the recommended/maximum tolerated doses [5, 20]. An expanded cohort of 50 patients affected by advanced BRCA1/2 mutation-associated ovarian, primary peritoneal and fallopian tube cancers received olaparib 200 mg bid; the results in the initial cohort were largely confirmed with an indication that efficacy was greater in platinum-sensitive than platinum-resistant patients [21].

A subsequent international phase II trial in patients with recurrent EOC and BRCA1/2 mutation suggested a dose-response relationship; although not randomized, greater activity for olaparib was seen at the dose of 400 mg bid, compared with the 100 mg bid dose (RECIST objective tumour response rate: 33% vs. 12.5%, $p < 0.05$, progression-free survival [PFS] 5.8 months), with an acceptable toxicity profile (grade 3 nausea in 7% and leucopoenia in 5% of patients) [22].

The first randomized trial investigated two different dosages of olaparib in 97 patients with BRCA-mutated progressive or recurrent disease (<12 months after last platinum administration) [23]. Patients were randomized to receive olaparib 200 mg bid or 400 mg continuously or intravenously pegylated liposomal doxorubicin (PLD) 50 mg/m^2. Although objective response rates favoured olaparib (25%, 31% and 18% for olaparib 200 mg, olaparib 400 mg and PLD, respectively), there was no statistically significant difference in progression-free survival (PFS), the primary endpoint of the trial (olaparib 200 mg: 6.5 months; olaparib 400 mg: 8.8 months; PLD: 7.1 months; hazard ratio [HR] 0.88, $p = 0.6$) [23]. The explanation was the unexpectedly high level of activity of PLD in patients with BRCA mutations and this has been confirmed in other studies.

Finally, positive results for mutated BRCA1/2 patients have recently been reported in a large multicentre non-comparative study conducted by Kaufman et al. and presented at ASCO 2013 [24]. Two hundred and ninety-eight BRCA1/2 mutated patients with various advanced cancers refractory to standard therapy received olaparib 400 mg bid until disease progression. The median duration

of treatment was 5.5 months (range 1–28.5 months) and most common toxicities were (generally mild/moderate) fatigue (59%), nausea (59%) and vomiting (37%). The most common grade ≥3 adverse event (AE) was anaemia (17%). Among 193 heavily pre-treated patients with ovarian cancer, the overall response rate was 31%; 124 patients (64,4%) were alive after 1 year of treatment, with a progression-free interval of 6 months in 105 patients (PFS 54.6%) [24].

PARP inhibitors in sporadic epithelial ovarian cancers

The question as to whether or not activity of PARP inhibitors is limited to tumours with BRCA mutations, or is also seen in those tumours having the property of 'BRCAness' has been addressed in a phase II single-arm study of olaparib [25]. Gelmon et al. enrolled 90 patients with high-grade serous/undifferentiated ovarian cancer and breast cancer including those with unknown BRCA status and those known to be BRCA mutation-negative as well as a small number of BRCA mutation-positive patients. All 90 enrolled patients (64 with ovarian cancer and 26 breast cancer) were treated with olaparib 400 mg bid. Seventeen patients had BRCA mutations. Objective responses were seen in 7/17 patients with BRCA1 or BRCA2 mutations (41%; 95% confidence interval [CI] 22–64) and 11/46 without mutations (24%; 95% CI 14–38) with a median response duration of 31 weeks. For those patients without mutations, platinum-sensitive patients obtained a higher response rate (radiological 50%, biochemical 40%) compared with resistant/refractory disease (radiological 3.8%, biochemical 17.4%). No severe toxicities were registered [25].

Finally, Ledermann et al. [26] have recently reported the results of a key trial in patients with relapsed ovarian cancer. In a randomized, double blind, placebo-controlled trial, patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer were randomized to receive olaparib or placebo as maintenance treatment, irrespective of BRCA1/2 mutations. Patients were eligible if they had received two or more platinum-based regimens and had a partial or complete response to their most recent platinum-based schedule: 136 received olaparib (400 mg bid) and 129 received placebo. There was an advantage of approximately 4 months for olaparib versus placebo group in median PFS (HR for progression or death 0.35; 95% CI, 0.25–0.49; p <0.001), although no significant differences in OS between the two groups of treatment were reported at the first interim analysis (p = 0.75). More common AEs reported in the olaparib group, usually grade 1 or 2, included: nausea (68% vs. 35%), fatigue (49% vs. 38%), vomiting (32% vs. 14%), and anaemia (17% vs. 5%). Initially the BRCA mutation status was unknown for 64% of the patients, but blood samples had been stored for retrospective mutation testing in most cases. A subsequent analysis was recently presented, which reassessed the PFS and OS results according to BRCA mutation status. A total of 136 (51%) proved to have either germline or somatic BRCA mutations and 118 (41%) were BRCA wild-type [27]. The data indicated that olaparib led to a greater PFS and OS benefit in those women with BRCA1/2 mutations. Specifically, the mutated patients showed the greatest PFS benefit with olaparib maintenance vs. placebo (median: 11.2 vs. 4.1 months; p < 0.001) and a significant quality of life (QoL) improvement (p = 0.03). In addition, the subgroup OS analysis limited to patients with BRCA1/2 mutations resulted in an OS HR of 0.74 (median: 34.9 vs. 31.9 months), i.e. a trend towards benefit. Analysis after longer follow-up is awaited in order to clarify the extent of OS benefit, but the investigators have pointed out that crossover from placebo to PARP inhibitors took place in over 20% of cases and this is likely to affect the OS results. Interestingly, at the time of the presentation, 21% of the patients were still receiving olaparib.

Future challenges – resistance to PARP inhibitors and the development of a predictive biomarker

Resistance

Deficiencies in BRCA function and the HRR pathway confer profound genome instability in cancers. Therefore, as the disease progresses, these cancer cells tend to evolve into subpopulations, each of which may possess distinct phenotypes with various 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. In cell lines it has been shown that an acquired secondary mutation can allow a BRCA1/2-deficient tumour to regain BRCA function and homologous recombination competency, so that PARP inhibition can no longer be synthetically lethal.

Interestingly, some patients who have responded to olaparib and then developed resistance have been reported to retain sensitivity to further platinum-based treatment [28], indicating that PARP inhibitors and platinum are not completely cross-resistant. Nevertheless, secondary mutations which have been associated with platinum resistance have also been noted in tumours from some patients with PARP inhibitor resistance, suggesting that this might be part of the explanation [29, 30]. Another possible mechanism for PARP inhibitor resistance is up-

regulation of the p-glycoprotein efflux pump, thus reducing intracellular PARP inhibitor concentrations [31]. Careful analysis of tumour tissue acquired from patients with PARP inhibitor resistance would be necessary in order to fully understand the underlying mechanisms, which are likely to be multiple.

Predictive biomarkers

To identify patients who are likely to benefit from treatment, a major aim of current research is the recognition of biomarkers to detect HRR-deficient cancers, and this includes developing functional assays of homologous recombination. Gene expression or immuno-histochemical signatures of deficiency of BRCA 1 and BRCA 2 expression or HRR defects need also to be explored [32] as well as functional assays performed on tumour cells derived from ascites and circulating cells.

Conclusion

PARP inhibition shows great potential as a valid treatment opportunity for ovarian cancer patients. Olaparib demonstrated a significantly improved PFS used as maintenance therapy in the randomized trial from Ledermann et al. [26], with an attractive toxicity profile. Data are particularly encouraging in BRCA1/2 mutated patients. While final OS results are awaited, in our view, the substantial improvement in PFS in itself is sufficient to encourage those involved to move towards a registration strategy in BRCA-mutated ovarian cancer patients. It is this subgroup of patients who will probably benefit most from synthetic-lethality based therapy in the era of targeted therapies. The identification of a broader susceptible patient population through robust predictive biomarker screening for faulty HRR pathways, and hence efficiency for PARP inhibition, should be a primary objective for future clinical investigation.

References

1. Heintz AP, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. 2006 Nov;95(Suppl 1):S161-92.
2. Bellati F, Napoletano C, Gasparri ML, et al. Current knowledge and open issues regarding bevacizumab in gynaecological neoplasms. Crit Rev Oncol Hematol. 2012 Jul;83(1):35-46.
3. Hall M, Gourley C, McNeish I, et al. Targeted anti-vascular therapies for ovarian cancer: current evidence. Br J Cancer. 2013 Feb 5;108(2):250-8.
4. Marchetti C, Imperiale L, Gasparri ML, et al. Olaparib, PARP1 inhibitor in ovarian cancer. Expert Opin Investig Drugs. 2012 Oct;21(10):1575-84.
5. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009 Jul 9;361(2):123-34.
6. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003 Oct 24;302(5645):643-6.
7. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005 Dec 15;104(12):2807-16.
8. Soegaard M, Kjaer SK, Cox M, et al. BRCA1 and BRCA2 mutation prevalence and clinical characteristics of a population-based series of ovarian cancer cases from Denmark. Clin Cancer Res. 2008 Jun 15;14(12):3761-7.
9. Zhang S, Royer R, Li S, et al. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecol Oncol. 2011 May 1;121(2):353-7.
10. Vang R, Shih Ie M, Kurman RJ. Fallopian tube precursors of ovarian low- and high-grade serous neoplasms. Histopathology. 2013 Jan;62(1):44-58.
11. Bolton KL, Chenevix-Trench G, Goh C, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012 Jan 25;307(4):382-90.
12. Yang D, Khan S, Sun Y, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA. 2011 Oct 12;306(14):1557-65.
13. Integrated genomic analyses of ovarian carcinoma. Nature. 2011 Jun 30;474(7353):609-15.
14. Chambon P, Weill JD, Mandel P. Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem Biophys Res Commun. 1963 Apr 2;11:39-43.
15. de Murcia JM, Niedergang C, Trucco C, et al. Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc Natl Acad Sci U S A. 1997 Jul 8;94(14):7303-7.
16. Szabo C, Zingarelli B, O'Connor M, et al. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci U S A. 1996 Mar 5;93(5):1753-8.
17. Ashworth A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol. 2008 Aug 1;26(22):3785-90.
18. Mendes-Pereira AM, Martin SA, Brough R, et al. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med. 2009 Sep;1(6-7):315-22.
19. Martinek I, Haldar K, Gaitskell K, et al. DNA-repair pathway inhibitors for the treatment of ovarian cancer. Cochrane Database Syst Rev. 2010(6):CD007929.
20. Sessa C. Update on PARP1 inhibitors in ovarian cancer. Ann Oncol. 2011 Dec;22(Suppl 8):viii72-viii6.
21. Fong PC, Yap TA, Boss DS, et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol. 2010 May 20;28(15):2512-9.
22. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in pa-

tients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet. 2010 Jul 24;376(9737):245-51.
23. Kaye SB, Lubinski J, Matulonis U, et al. Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. J Clin Oncol. 2012 Feb 1;30(4):372-9.
24. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germ-line BRCA1/2 mutation: An open-label phase II study. J Clin Oncol. 2013;31(suppl): abstr 11024.
25. Gelmon KA, Tischkowitz M, Mackay H, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. Lancet Oncol. 2011 Sep;12(9):852-61.
26. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012 Apr 12;366(15):1382-92.
27. Ledermann JA, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer (SOC) and a BRCA mutation (BRCAm). J Clin Oncol. 2013;31(suppl):abstr 5505.
28. Ang JE, Gourley C, Powell B, et al. Efficacy of chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of poly(ADP-ribose) polymerase inhibitor resistance: a multi-institutional study. Clin Cancer Res. 2013 Aug 6 [E-pub ahead of print].
29. Barber LJ, Sandhu S, Chen L, et al. Secondary mutations in BRCA2 associated with clinical resistance to a PARP inhibitor. J Pathol. 2013 Feb;229(3):422-9.
30. Norquist B, Wurz KA, Pennil CC, et al. Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. J Clin Oncol. 2011 Aug 1;29(22):3008-15.
31. Lee SA, Roques C, Magwood AC, et al. Recovery of deficient homologous recombination in Brca2-depleted mouse cells by wild-type Rad51 expression. DNA Repair (Amst). 2009 Feb 1;8(2):170-81.
32. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990 Jul 4;82(13):1107-12.