Update on PARP1 inhibitors in ovarian cancer

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The clinical development of PARP inhibitors for the treatment of tumors deficient in BRCA1 or BRCA2 is based on the concept of synthetic lethality. From the initial proof of concept study with the PARP1 inhibitor olaparib (AZD2281) in BRCA mutation carriers, in which 28% of ovarian cancer patients achieved an objective response, the target population of ovarian patients potentially sensitive to treatment with PARP inhibitors has greatly increased. Objective responses have been observed in both platinum-sensitive and platinum-resistant BRCA mutation carriers but, more recently, also in BRCA negative ‘BRCAness’ patients, those with no BRCA mutations but with a dysfunction of the homologous recombination (HR) system, which makes them more sensitive to the antitumor agents which cause double strand breaks of DNA. The recent results achieved with olaparib, given as maintenance in platinum sensitive recurrent high grade serous ovarian cancer, in response after reinduction with platinum, confirm the antitumor effect of single agent olaparib in BRCAness patients. Main topics of investigations in this field are the identification of BRCAness phenotype and the definition of tests to identify BRCAness patients. More in general, additional preclinical studies are needed to further improve clinical results in order to define the optimal regimen of combination with PARP1 inhibitor and cytotoxics or molecular targeted agents (sequence of administration, interval between dosing of the agents, duration of treatment).

Key words: PARP, PARP inhibition, DNA repair, ovarian cancer

PARP1 and DNA repair

Poly(ADP-ribose) polymerase (PARP) is a family of nuclear proteins with enzymatic, scaffolding properties and recruiting ability for DNA repair proteins [1].

The most important and best understood member of the PARP family is PARP1, which is involved in the base excision repair system that repairs DNA damage induced by radiation and alkylating agents. Once activated by DNA strand breaks (SSB), PARP1 binds to the strand break and starts the synthesis of PAR on acceptor proteins, located on PARP1 or other proteins involved in DNA repair. PARP1 then loses affinity for DNA and repair proteins are recruited by PAR to the damaged DNA (Figure 1).

PARP1 is also involved in the repair of double-strand breaks (DSB). Here, PARP1 attaches to the DNA protein kinase catalytic subunit, and recruits ATM (ataxia telangiectasia mutated), MRE11 and topoisomerase I, all involved in DSB repair [2].

The homologous recombination (HR) system works to correct DSB. When damage occurs, ATM mobilizes proteins, such as the products of the tumor suppressor genes BRCA1 and BRCA2 (which carries RAD51, the recombination enzyme), to the DSB site for error-free DNA repair (Figure 2). If HR is active, RAD51 foci appear on DNA and could act as biomarkers of the HR function.

If there is defect in BRCA1 or BRCA2, DSB repair is carried out by an error-prone mechanism, the nonhomologous end-joining repair system, with an increased risk of chromosomal alterations.

Within 30 min of DSB formation, a large amount of the phosphorylated form of the histone protein H2AX (γH2AX) appears in the chromatin around the break, with the accumulation of proteins involved in DNA repair. This focus of accumulation can be detected and visualized by a specific antibody, which is a potential means of quantifying DSB in circulating mononuclear and tumor cells [3].

PARP1 inhibitors and synthetic lethality

The concept of synthetic lethality was the rationale for developing PARP inhibitors for the treatment of tumors deficient in BRCA1 or BRCA2. Synthetic lethality is a phenomenon in which the individual deletion of two independent genes does not cause cell death, but the combined deletion is cytotoxic [4].

Initial observations showed that PARP1 inhibitors had cytotoxic effects on BRCA1- or BRCA2- deficient cells and human tumors. This was caused by the lack of repair of SSB due to PARP1 inhibition and the lack of DSB repair because of HR dysfunction due to BRCA mutations.

The advantages of synthetic lethality are the possible avoidance of the toxic effects of chemotherapy and its selectivity, because of the heterozygous status of normal tissues and the homozygous status of the genotype of tumors.
Among the most interesting recent preclinical data are those achieved in a series of human tumor cells with PTEN deficiency, in which sensitivity to PARP1 inhibitors and cisplatin, but not to PARP1 inhibitors and paclitaxel, was higher than in the wild-type because of an HR defect [5]. These results were confirmed in PTEN-deficient HCT116 tumor models xenografted into mice. PTEN mutant tumors had a reduced capacity to form nuclear RAD51 foci, indicative of HR dysfunction, possibly due to the PTEN deletion.

These data support the clinical evaluation of PARP inhibitors in PTEN-deficient tumors, possibly in combination with PI3K/AKT inhibitors.

**BRCAness**

BRCAness is the phenotype that some sporadic tumors share with familial BRCA cancers (such as improved response and survival with exposure to platinum agents in ovarian cancer) [6]. These features are due to specific DNA repair defects consistent with HR dysfunction caused by mutations and inactivation of the BRCA/FA pathways as well as mutations in BRCA1/BRCA2 genes (such as reduced expression of BRCA1, hypermethylation of the BRCA1 promoter and amplification of the EMS1 gene with inactivation of BRCA2), decreased expression of proteins involved in HR (like RAD51, ATM) and PTEN deficiency [2].

The overall frequency of the BRCAness phenotype and HR dysfunction in ovarian cancer is unknown, but it is estimated to be present in up to 50% of high-grade serous ovarian cancers due to genetic (germline or somatic) or epigenetic inactivation of BRCA1/BRCA2 or independent defects of proteins involved in the HR pathway [6].

**Clinical development of PARP inhibitors in advanced ovarian cancer**

single agent in platinum pretreated patients. In the initial proof-of-concept study with the PARP1 inhibitor olaparib (AZD2281) in BRCA mutation carriers, 28% of patients with ovarian cancer achieved an objective response (RECIST...
criteria) of a median duration of 7.0 months [7]. The important finding of this study was the observation of objective antitumor activity in platinum-resistant patients at dosages well below the recommended/maximum tolerated doses.

These promising results were confirmed in the expansion cohort study, in which a total of 50 BRCA1/BRCA2-mutated patients (13 platinum sensitive, 24 platinum resistant and 13 platinum refractory) were treated with olaparib 200 mg bid continuously [8]. Objective response rates (RECIST criteria) were 46%, 33% and 0%, respectively, confirming the activity of olaparib in patients with platinum-resistant disease.

Comparable results were achieved in the international multicentric study in which two subsequent cohorts of patients received either 400 mg bid (33 patients) or 100 mg bid (24 patients) of olaparib [9]. A significant antitumor activity of 38% was again observed with the higher dose in patients with platinum-resistant disease (N = 26), while no responses were observed with 100 mg bid.

The subsequent steps in the clinical development were the evaluation of the role of PARP1 inhibitors as second-line treatment in BRCA1/BRCA2-mutated patients with tumor recurrence within 12 months [10] and the role of PARP1 inhibitors as salvage therapy, irrespective of BRCA1/BRCA2 mutation [11].

To answer the first question, a three-arm study comparing two different dosages (400 and 200 mg) of olaparib with the reference treatment doxorubicin (Caelyx) was performed in patients with progressive or recurrent disease <12 months after their last platinum [12]. Confirmed response rates were 31%, 25% and 18% with a similar progression-free survival (PFS) of 8.8, 6.5 and 7.1 months, respectively. Prognostic factors for PFS could not be identified while the toxicity of olaparib 400 mg was limited.

The presence of a mutation in BRCA was not predictive of response, as also shown in a Canadian multicentric study in which 55 patients with known or unknown BRCA status but high-grade serous histotype received olaparib 400 mg bid [11]. The antitumor activity in BRCA-mutated patients was 41% in the wild-type BRCA patients while it was 24%.

**combinations in platinum pretreated patients**

The rationale for developing PARP inhibitors in combination with chemotherapy is the potentiation of the DNA-damaging effects of the cytotoxic compounds (usually platinum agents and topotecan).

Notwithstanding differences in the myelosuppressive effects of the PARP inhibitor tested [with olaparib being more myelotoxic than iniparib (BSI 201)], the addition of PARP inhibitors resulted in greater than expected potentiation of the myelotoxicity of chemotherapy, requiring dose reductions and treatment delays [12].

So far, the most promising combination is that of iniparib with gemcitabine/carboplatin, which has already been evaluated in triple-negative breast cancer [13].

The rationales for developing iniparib in ovarian cancer were the preclinical data showing the appearance of $\gamma$H2AX at

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**Figure 2.** Proteins involved in homologous recombination. (From N Engl J Med 2003; 348: 1917-1919.)
concentrations lower than expected by PARP inhibition and the potentiation of the cell-cycle arrest induced by DNA-damaging agents, such as platinum and gemcitabine.

In 41 platinum-sensitive high-grade serous ovarian cancer patients, irrespective of their mutational status, the antitumor activity of iniparib (3–6 mg/kg, days 1, 4, 8 and 11 q3wks) with gemcitabine (1000 mg/m², days 1 and 8) and carboplatin (AUC 4, day 1) was 65% (60% in mutated and 71% in wild-type BRCA), with a PFS of 9.5 months [14]. As expected, toxicity was remarkable, with 42% grade 3–4 thrombocytopenia and 59% grade 3–4 neutropenia requiring dose reductions in 41% of patients.

In 34 platinum-resistant recurrent patients, treatment with iniparib with the same combination of gemcitabine and carboplatin produced an overall response rate of 25% (50% in mutated and 17% in wild-type BRCA), thus, confirming the activity of combinations of PARP inhibitors with DNA-damaging agents in ovarian cancer, mainly in cases with HR dysfunction [15].

Toxicity was remarkable, with 71% fatigue (6% grade 3–4), 26% grade 3–4 thrombocytopenia and 46% neutropenia, with dose reductions and dose delays in 85% and 27% of patients, respectively.

Overall, combinations with PARP inhibitors are worthy of further clinical investigation. However, the toxicity profile should be improved; this could be done in preclinical/clinical studies to define the optimal schedule of treatment through pharmacokinetic/pharmacodynamic evaluations [16].

**maintenance in advanced disease**

The efficacy of olaparib as maintenance in patients with platinum-sensitive high-grade serous ovarian cancer has been assessed in a randomized double-blind placebo-controlled phase II study, the results of which have been reported recently [17].

The study enrolled patients who had received at least two prior platinum regimens, who responded to the last platinum-based chemotherapy and who didn’t have any evidence of tumor recurrence at trial entry.

The rationales for the study were the notion that up to 50% of patients with high-grade serous ovarian cancer have an HR dysfunction and the clinical experience in some patients (often those with a family history of ovarian/breast cancer) that repeated platinum therapies are associated with repeated responses, albeit of progressively shorter duration, possibly due to an intrinsic weakness in DNA repair.

After stratification by objective response to last platinum and time to disease progression (TTP) after penultimate platinum therapy, 250 patients were randomized to receive olaparib (n = 136) or placebo (n = 129) until progression. The two groups were balanced in terms of presence of BRCA1/BRCA2 mutation (>20%), >12 months TTP after penultimate platinum regimen (60%) and objective complete response to last platinum (40%).

The primary analysis, performed after the occurrence of 153 events, showed that treatment with olaparib was associated with a significantly longer PFS, as evaluated by CA125 or RECIST criteria (8.3 versus 3.7 months), irrespective of BRCA
status, TTP after penultimate platinum and response to last platinum (Figure 3). Objective responses were seen in 12% and 4% of patients receiving olaparib and placebo, respectively. Toxicity, consisting mainly of nausea, fatigue and vomiting, was moderate; nevertheless, ~50% of patients on olaparib required some treatment modifications.

The conclusions were that maintenance with a PARP inhibitor in selected patients (mainly those who are likely to have an HR dysfunction because of their histotype and clinical history) could prolong PFS.

These results support the clinical development of PARP inhibitors in a broad population of patients with a high-grade serous histotype, irrespective of their BRCA status; they confirm the results of the Canadian study and reinforce the importance of BRCAness and HR dysfunction in the selection of patients; they indicate the prolonged follow-up of patients and need of the acquisition of detailed information on the long-term side-effects of the treatment, because of the postulated PARP1 tumor-suppressive effect [1].

issues in clinical development

The clinical development of PARP inhibitors in ovarian cancer, from the first proof-of-concept phase I study to the results achieved in platinum-resistant patients and as maintenance in high-grade serous histotype, has shown that a molecular-based approach to treatment is feasible. To improve further, we need tests to identify BRCAness phenotypes; gene expression profiles or functional assays have been proposed, but they are of limited practical application [18, 19]. We should try to obtain repeated biopsies of the tumor at the time of recurrence to allow screening for defects in the HR repair. We should increase our knowledge on the biology of PARPs other than PARP1, on the effects of PARP inhibitors on enzymes other than PARP1 and on the different biological properties of PARP1 inhibitors.

Finally, in view of the future broader development and of the effect on transcription of PARP1, we should assess the long-term inhibition of PARP1 in combination with DNA-damaging agents in animal models, to exclude deleterious effects.

At this point of development it is important to start evaluating, case by case, the risk of secondary malignancies and the benefits derived from the improved treatment of an incurable disease.

disclosures

Member of Advisory Board of OSI, Sigma Tau.

references

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