Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Poly(ADP-ribose) polymerase inhibitors in breast cancer and other tumors: advances and challenges

Clin. Invest. (2011) 1(11), 1545–1554

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Poly(ADP-ribose) polymerase (PARP) inhibitors are currently in development for the treatment of cancer. PARP-1 and PARP-2 are important in the repair of DNA damage. PARP inhibitors used either as single agents or in combination with other cytotoxics, aim to increase efficacy of DNA damage. When PARP is inactivated in cells lacking functional *BRCA1* or *BRCA2*, cells reach a high degree of genomic instability and die. Populations studied in ongoing and reported clinical trials include BRCA mutation-associated cancers, as well as sporadic breast cancers, ovarian cancers and other malignancies. In this review, PARP inhibitors undergoing clinical trial evaluation in humans (olaparib, iniparib, veliparib, rucaparib, MK-4827 and INO-1001) are discussed, with a focus on breast cancer, but also other tumors and the recently reported study results.

Keywords: BRCA • DNA repair • poly(ADP-ribose) polymerase inhibitors • triple-negative breast cancer

Poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi) are a class of novel anticancer agents undergoing clinical evaluation. PARPi have attracted considerable attention and created enthusiasm in the oncology community. This is due to their unique mechanism of action and also because they have proven remarkably tolerable.

There is ongoing research using PARPi in different tumor types, including BRCA mutation-associated cancers, as well as other cancers and sporadic breast cancers, either as single agents or in combination with other cytotoxic medications to increase the efficacy of DNA damage. In this review we discuss the biological background of the agents and discuss the published early phase studies and also explore ongoing research in the field, as well as future directions.

What are PARPi & how do they work?

PARPs are a class of proteins that play major roles in a wide range of biologic processes, including the maintenance of genomic stability, transcriptional regulation, energy metabolism and cell death. PARP-1 and PARP-2 are important in the repair of DNA damage. Most cellular PARP activity is attributable to PARP-1, which is a nuclear protein that localizes to sites of ssDNA damage that in turn recruits DNA repair proteins that execute base excision repair. PARP-1 and PARP-2 possess overlapping and nonredundant functions that are important in maintaining the stability of the genome [1].

DNA repair mechanisms & the biology of PARP inhibition

Although PARP-1 is involved in ssDNA break repair, the mechanism of action of PARPi relates to the inability to repair double strand breaks in cells lacking functional *BRCA1* or *BRCA2* genes. Mutations that affect BRCA genes lead to increased cancer risk in humans and, when homozygous, cause embryonic lethality in mice [2]. PARP-deficient cells, although impaired in their ability to repair

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single-strand breaks, are nonetheless able to carry out error-free DNA repair through homologous recombination (HR), a process mediated by large protein complexes, whose components include proteins encoded by *BRCA1* or *BRCA2*.

Nicotinamide adenine dinucleotide (NAD^+) is a cofactor for oxidation–reduction reactions. NAD^+ is also a substrate for several important biochemical reactions, such as histone deacetylation and ADP-ribosylation of proteins, such as those catalyzed by the PARPs [1]. Both PARP-1 and -2 use NAD^+ as a substrate and catalyze poly-(ADP-ribosyl)-ation of proteins in response to ssDNA damage, the first step in base excision repair [3]. If PARP activity is lost, single-strand breaks are converted to double-strand breaks during DNA replication. dsDNA breaks (DSBs) can be repaired by two different pathways; HR including double-strand break repair or via nonhomologous end-joining (NHEJ). PARP inhibition induces phosphorylation of DNA-dependent protein kinase substrates and stimulates error-prone NHEJ selectively in HR-deficient cells [4]. Disabling NHEJ by using either genetic or pharmacologic methods rescues the lethality of PARP inhibition or downregulation in cell lines lacking *BRCA2* and *BRCA1* [4]. Abnormal NHEJ is essential in creating genomic instability and cytotoxicity in HR-deficient cells treated with PARPi [4].

BRCA1 may function partly as a scaffold protein, coordinating the assembly of protein complexes involved in mediating HR-mediated double-strand break repair and checkpoint function, *BRCA2* appears to function further down the repair pathway, serving as part of the effector arm of DSB repair.

When PARP is inactivated in cells lacking functional *BRCA1* or *BRCA2*, the cells will eventually become overloaded with DSBs and reach a high degree of genomic instability, eventually leading to cell death. This is an example of a phenomenon termed 'synthetic lethality', a state where two mutations, each having a viable phenotype, generate a lethal phenotype in combination. The geneticist Theodosius Dobzhansky first described the term synthetic lethality in 1946 in highly genetically malleable organisms including *Drosophila* and *Candida* [5,6]. This concept is now important in PARP inhibition.

■ Why is PARP inhibition relevant in breast cancer?

Prognosis and survival rates of breast cancer vary greatly depending on the extent of the disease, performance status of the patients and the type of the tumor, including the status of estrogen receptor (ER), progesterone receptor (PgR) and HER2. Expression of ER and PgR confers a better prognosis than the expression of HER2 and the lack of expression of ER, PgR or HER2 (triple-negative breast cancer [TNBC]) tend to be indicative of a more aggressive cancer [7]. Basal-like breast cancers (BLBC) are a group of breast tumors characterized by gene-expression profiling, rather than immunohistochemistry, with significant overlap with TNBC and a similarly poor prognosis in many cases. Ongoing studies have shed light on important genetic abnormalities in TNBC, BLBC and BRCA mutation-associated tumors. TNBC and BLBC often have shared morphological and genetic features, but they are not identical entities. TNBC and BLBC occur most frequently in young women and they respond to conventional chemotherapy, but relapse earlier and more frequently than hormone receptor-positive breast cancer. Breast cancers found in patients with *BRCA1* mutations are often triple negative and basal-like. While 80% of *BRCA1*-associated breast cancers are triple negative, the vast majority of triple-negative tumors arise sporadically in noncarriers, yet these two subsets share many distinct features [7]. Sporadic triple-negative tumors may express low levels of *BRCA1* [8], have DNA damage response and repair defects ('BRCAness'). However, it has been challenging to demonstrate a profound defect in HR in sporadic triple-negative tumors and the use of PARPi in non-*BRCA1/2*-associated tumors is an uncertain endeavor. Unlike BRCA mutation carriers, patients with sporadic tumors have not demonstrated responses in small clinical studies using single agent olaparib [9]. However, early-phase clinical studies of certain combinations of PARPi and conventional cytotoxics in unselected metastatic breast cancer were encouraging [10]. These experiences have prompted the testing of PARP inhibition in combination with DNA-damaging chemotherapeutics that, in theory, could kill tumor cells by exhausting DNA repair mechanisms, even in the absence of an intrinsic HR defect.

Clinical trials of PARPi

■ PARPi investigated as single agents

Cancers in patients with germline *BRCA1* or *-2* mutations have lost both copies of *BRCA1* or *-2* and are deficient in HR-mediated DNA repair. However, nontumor cells in these individuals are heterozygous, retaining one functional copy of the gene, and are fully capable of HR and hence efficient DNA repair. In this setting, the use of single-agent PARPi would be expected to be lethal for the cancer cells (synthetic lethality), but completely nontoxic to normal tissue elsewhere in the body. If proven correct, this construct is the ideal situation for the future of anticancer drug development, where the tumor is targeted and responding to treatment, while the patient remains free of side effects.

■ Studies with single agent PARPi olaparib (previously known as KU-0059436/AZD2281)

A study to assess the safety & pharmacokinetics of an inhibitor of PARP

The initial report of activity of olaparib was presented at the 2006 American Society of Clinical Oncology annual meeting, with preliminary findings in a study involving 12 patients with advanced tumors unselected for a BRCA mutation [11]. This initial study was later enriched with BRCA mutation carriers and updated annually with more patients and also with an expansion phase, including women with ovarian cancer harboring BRCA mutations [9,11,12].

The Phase I study ultimately included 60 patients with 21 ovarian (15 *BRCA1* and one *BRCA2*), nine breast (three *BRCA2*), eight colorectal and 22 patients with other solid tumors [9]. Two patients with mutations had tumors not typically associated with BRCA-carrier status: one with small-cell lung cancer and one with vaginal adenocarcinoma [9]. The maximum administered olaparib dose in the study was 600 mg twice-daily and the maximum tolerated dose (MTD) was 400 mg twice-daily [9]. Durable objective antitumor activity was observed only in confirmed carriers of a *BRCA1* or *BRCA2* mutation. All responses were measured by response evaluation criteria in solid tumors (RECIST) [13]. Eight patients with advanced ovarian cancer had a partial response (PR). Six patients with a BRCA mutation had a decline of more than 50% in their CA125 tumor marker.

A complete remission was seen in one of the three patients, with *BRCA2* breast cancer lasting more than 60 weeks. Another *BRCA2* patient had stable disease for 7 months. Both had an associated decline in tumor markers. A 50% reduction in the PSA level and resolution of bone metastases was seen in castration-resistant prostate cancer with *BRCA2* mutation [9].

■ Expansion cohort of the Phase I olaparib study

To explore response in greater detail, an expansion cohort was increased in size with a protocol amendment to enrich that cohort with *BRCA1*- and *BRCA2*-mutation carriers with ovarian, primary peritoneal and fallopian tube cancer. A *post hoc* analysis was conducted to assess any association between response to olaparib and platinum sensitivity [12]. A total of 50 patients were treated with olaparib, 48 had germline *BRCA1* and/or *BRCA2* mutations; one had a *BRCA2* germline sequence change of unknown significance and another had a strong family history of *BRCA1/2*-associated cancers, but declined mutation testing. The maximum administered dose was defined as 600 mg of olaparib twice-daily and the MTD as 400 mg twice-daily. Of the 50 patients, 20 (40%) achieved RECIST complete response (CR) or PR and/or tumor marker (CA125) responses and three (6%) maintained RECIST disease stabilization for more than 4 months, giving an overall clinical benefit rate of 46% (95% CI: 32–61%). Median response duration was 28 weeks [12].

Platinum-sensitive ovarian cancer is defined by a relapse-free period of 6 months following a response to the final dose of platinum treatment [14]. Of the 50 patients, 13 had platinum-sensitive disease, 24 had platinum-resistant disease and 13 had platinum-refractory disease (according to platinum-free interval). The antitumor activity of olaparib was noted to be associated with platinum sensitivity ($p = 0.001$) [12].

Two parallel open-label, multicenter Phase II studies were conducted to assess the efficacy and safety of olaparib for the treatment of cancers in germline *BRCA1/2* mutation carriers with advanced breast (ICEBERG1) [9] or ovarian (ICEBERG2) [15] cancers. A total of 54 patients with breast cancer in the ICEBERG1 study [9] and 56 patients with ovarian cancer in the ICEBERG2 study [15] received olaparib at either a pharmacodynamically active dose of 100 mg twice-daily or the previously established MTD of 400 mg twice-daily.

ICEBERG1: study to assess the efficacy & safety of a PARPi for the treatment of BRCA-positive advanced breast cancer

ICEBERG1 was initially carried out in a group of heavily pretreated patients with recurrent, measurable chemotherapy refractory BRCA mutation-associated breast cancers [16]. A total of 27 patients were given continuous oral olaparib at 400 mg twice-daily (MTD), and another 27 given the pharmacodynamically active lower dose of 100 mg twice-daily. The objective response rate, which was the primary efficacy end point, was 41% (11/27) in the cohort assigned to 400 mg twice-daily, and 22% (6/27) in the cohort assigned to 100 mg twice-daily. The lower response rate (RR) in the 100 mg twice-daily cohort may suggest that the dose of the PARPi is important for response, but this was not a randomized Phase II study [16]. Toxicities were mainly low grade, and included fatigue, GI disturbances and anemia. A functional assay of PARP inhibition carried out in surrogate samples of peripheral blood mononuclear cell and removed hair follicles revealed >90% inhibition of PARP functional activity, as compared with the value at baseline [9]. This was seen in peripheral blood monocytes at doses >60 mg twice-daily. Of note, an accumulation of DSBs in plucked eyebrow hair follicles was demonstrated in pharmacodynamic assays 6 h after olaparib treatment and levels remained elevated on treatment [16]. Toxicity in *BRCA1/2* carriers was similar to that reported previously in noncarriers [17].

ICEBERG2: study to assess the efficacy & safety of a PARPi for the treatment of BRCA-positive advanced ovarian cancer.

The promising activity of olaparib in patients with BRCA-associated ovarian cancer, seen in the Phase I study [9], was confirmed in a Phase II report [15]. An analysis of 57 assessable patients with *BRCA1* and *-2* mutations, treated at two dose levels (400 and 100 mg twice-daily), was reported.

RR were 33 and 12.5%, respectively. Toxicity was mild; the only reported grade 3 toxic effects were nausea (7%) and leukopenia (5%). Overall RR was 33% (11/33) patients in the cohort assigned to olaparib 400 mg twice-daily, and 13% (3/24) in the cohort assigned to 100 mg twice-daily. Findings from this Phase II study reconfirmed proof-of-concept of the efficacy and tolerability of genetically targeted treatment with olaparib in BRCA-mutated advanced ovarian cancer [15].

Overall, both Phase II ICEBERG studies confirmed the tolerability of olaparib in BRCA mutation carriers, with mainly mild-to-moderate nausea, fatigue and hematological events observed, which is in line with safety data from the Phase I clinical trial and randomized studies that are currently ongoing, at least in ovarian cancer. Compared with previous studies using conventional chemotherapy, olaparib was well tolerated.

Other Phase II studies in BRCA associated breast and/or ovarian cancers with single-agent PARPi are ongoing (Table 1), including the following agents AG014699 (also known as PF-01367338), ABT-888 (veliparib) and BSI-201 (iniparib). In addition, a Phase I study with MK4827, both in solid tumors that have failed standard chemotherapy and in BRCA mutation-associated cancers, is ongoing.

■ Studies using PARPi in combination with chemotherapy

It has been postulated that PARPi can act as chemosensitizers in combination with other cytotoxic agents or radiotherapy. There is preclinical evidence of synergy when PARPi is administered concurrently with radiotherapy [18], and that they can increase tumor responses to ionizing radiation in xenograft models [19,20]. To the best of our knowledge, there are no published data in humans on the combination of PARPi and radiation therapy, although clinical trials are ongoing.

Concurrent cytotoxic chemotherapy and PARPi has been investigated in multiple clinical studies (Table 2). Mostly, they have been investigated without considering BRCA function and with different cytotoxic agents (methylating agents, platinum drugs, alkylating agents, and topoisomerase I and II inhibitors).

Olaparib combinations with cytotoxic chemotherapy

Studies confirmed that combinations of olaparib with cisplatin and gemcitabine are associated with significant myelosuppression [21]. The combination of olaparib and weekly paclitaxel was well tolerated, but acceptable dose intensity (i.e., missed/delayed doses) was not achieved due to neutropenia, despite secondary prophylaxis with granulocyte colony stimulating factor [22]. Alternative schedules and dosing of olaparib are being considered [22]. However, robust response data are not currently available.

Iniparib (BSI-201)

Iniparib has been developed as a noncompetitive inhibitor of PARP1 that disrupts the interaction between PARP1 and DNA. It is now clear that the clinical effects of iniparib are not mediated by PARP inhibition, as a pharmacodynamic study presented at the last annual meeting of the American Association of Cancer Research (AACR) suggested that the compound does not inhibit PARP at the doses given in human subjects [23]. Nevertheless, it will be discussed here, as the original presentations suggested that the compound was acting as a PARPi.

The first reported Phase I study with iniparib involved 23 heavily pretreated patients who received iniparib at seven dose levels ranging from 0.5 to 8.0 mg/kg. All

Table 1. Reported studies with single agent poly(ADP-ribose) polymerase inhibitors.

Pharmaceutical agent	Route	Tumor type	Phase	Ref.
Olaparib KU-0059436/AZD2281 (Kudos/Astra-Zeneca)	p.o.	Solid tumors	I	[9]
		Solid tumors enriched with 22 mutation carriers	I	[12]
		Advanced breast cancers in BRCA mutation carriers	II	[16]
		Advanced ovarian cancers in BRCA mutation carriers	II	[15]
Iniparib BSI-201 (BiPar/Sanofi-Aventis)	iv.	Solid tumors	I	[24]
MK-4827 (Merck, Sharp & Dohme)	p.o.	*BRCA*-deficient and sporadic ovarian cancers	I	[39]
ABT-888 (Abbott)	iv.	Solid tumors	0	[50]

iv.: Intravenous; p.o.: Per orem.

Table 2. Reported studies with cytotoxic chemotherapy combined with poly(ADP-ribose) polymerase inhibitors.

Pharmaceutical agent	Route	Accompanying cytotoxic	Tumor type	Phase	Ref.
AG014699/Rucaparib/CO-338 (Pfizer/Clovis)	iv./p.o.†	TMZ	Solid tumors, melanoma	I	[51]
		TMZ	Melanoma	II	[52]
INO-1001 (Inotek/Genentech)	iv.	TMZ	Melanoma, glioblastoma multiform	I	[40]
Olaparib KU-0059436/AZD2281 (Kudos/Astra-Zeneca)	p.o.	Gemcitabine plus cisplatin	Solid tumors	I	[53,54]
		Paclitaxel	TNBC	I/II	[22]
ABT-888 (Abbott)	iv.	Topotecan	Solid tumors and lymphoid malignancies	I	[55]
		Irinotecan		I	
Iniparib-BSI-201 (BiPar/Sanofi-Aventis)	iv.	Topotecan, gemcitabine, TMZ or carboplatin plus paclitaxel	Solid tumors	I	[25]
		Gemcitabine plus carboplatin	TNBC	II	[10,27]
		Gemcitabine plus carboplatin	TNBC	III	[29]

†Studies reported to date have used the iv. formulation (AG014699) and there are no published results yet using the p.o. formulation (rucaparib/CO-338).
iv.: Intravenous; p.o.: Per orem; TMZ: Temozolomide; TNBC: Triple-negative breast cancer.

doses were well tolerated without an identified MTD. The best response of stable disease for 2 months or more was seen for 6/23 subjects [24].

A 55-patient Phase I study was conducted in patients with advanced solid tumors to assess safety and establish the MTD of the combination of iniparib with different chemotherapy agents, including topotecan, gemcitabine, temozolomide (TMZ) and carboplatin/paclitaxel (Taxol) [25]. Patients were treated with iniparib doses from 1.1 to 8.0 mg/kg twice weekly, in combination with the cytotoxic chemotherapy at standard doses. All dose combinations were well tolerated with a total of 21 serious adverse events reported for 10 trial participants, none of which were attributed to the study drug. A CR at 6 months was reported for one subject with ovarian cancer, and five subjects with renal, breast (2), uterine and sarcoma experienced a PR. Significant PARP inhibition was reported at 2.8 mg/kg or higher [25].

In a 30 patient Phase I study enrolling newly diagnosed malignant gliomas, the combination of iniparib given with TMZ was reported to be safe. Iniparib was well tolerated with conventionally prescribed doses of TMZ given during and following radiotherapy. No MTD of iniparib with TMZ has been reached. Peak plasma levels at the end-of-infusion were higher than those associated with efficacy in preclinical models. This may be related to the higher doses required to cross the blood–brain barrier. The combination has progressed to Phase II development [26].

O'Shaughnessy *et al.* [27] reported an open-label, randomized Phase II study of 123 patients with advanced TNBC, who were not selected for *BRCA1/2* mutation status and who had received only one or two lines of previous chemotherapy. Efficacy and safety of gemcitabine and carboplatin with or without iniparib were compared. Doses were gemcitabine (1000 mg/m^2 of body surface area) and carboplatin (at a dose equivalent to an area under the concentration–time curve of 2) on days 1 and 8 with or without iniparib (at a dose of 5.6 mg/kg of body weight) on days 1, 4, 8 and 11 every 21 days. There was improved clinical benefit from 34 to 56% ($p = 0.01$) and the rate of overall response from 32 to 52% ($p = 0.02$). The addition of iniparib also prolonged the median progression-free survival from 3.6 to 5.9 months ($p = 0.01$) and the median overall survival from 7.7 to 12.3 months ($p = 0.01$). No significant difference was seen between the two groups in terms of toxicity [27]. Traditionally, it is uncommon to observe improvement in overall survival in these studies. This is mainly because once their tumor begins to progress, patients are allowed to 'crossover' from the control arm to the experimental arm that includes the study drug. Remarkably, however, the addition of iniparib in the O'Shaughnessy study increased the overall survival of these patients from 7.7 to 12.3 months. The number of *BRCA1/2* mutation carriers enrolled in this trial was not reported, but based on population frequencies it can be assumed that the majority of patients were not BRCA mutation carriers and, therefore, some of the clinical benefit occurred in noncarriers. There was no significant combined toxicity with the addition of iniparib to chemotherapy including no additive myelotoxicity, even though marked bone marrow toxicity was reported with other PARPi chemotherapy combinations [28].

Therefore, these results generated significant enthusiasm and expectation regarding this agent, and PARPi in general. Unfortunately, the ensuing randomized Phase III trial assessing essentially the same regimen (gemcitabine–carboplatin–iniparib) in 519 patients with metastatic TNBC did not meet the prespecified criteria for significance for the end points of overall survival (11.1 vs 11.8 months; HR = 0.88; p = 0.28) and progression-free survival (4.1 vs 5.1 months; HR = 0.79; p = 0.027) [29]. The results of a prespecified analysis in patients treated in the second- and third-line setting (43% of subjects) demonstrated an improvement in overall survival and progression-free survival. The overall safety analysis indicates that the addition of iniparib did not significantly add to the toxicity profile of gemcitabine and carboplatin [29]. Multivariate analyses that adjusted for several prespecified baselines factors and replaced 'time since diagnosis of metastatic disease' with 'disease-free interval from primary breast cancer surgery to onset of metastatic disease' showed a significant improvement in overall survival (HR = 0.78; p = 0.05) in the overall population and in second- and third-line patients (HR = 0.71; p = 0.05). It is unknown if the Phase III trial was enriched with patients who had *BRCA1/2* genetic mutations. Testing for the genetic mutation was voluntary and was completed by only a minority of patients.

There are a number of other ongoing studies with iniparib, including a trial evaluating the efficacy and safety of iniparib plus irinotecan in patients with triple negative breast cancer brain metastases [30].

Veliparib (ABT888)

Veliparib is a competitive inhibitor that also mimics nicotinamide and has shown promise in early phase development [31]. Veliparib and TMZ are synergistic in breast cancer xenograft models. TMZ has minimal activity in breast cancer, probably due to robust repair of methylated DNA adducts by the base excision repair pathway and *O*-6-methylguanine-DNA methyltransferase. Inhibition of base excision repair by PARPi could, therefore, be expected to improve the efficacy of TMZ. Isakoff *et al.* reported a single arm Phase II trial of veliparib and TMZ in 41 pretreated MBC patients who received veliparib (40 mg p.o. b.i.d. days 1–7) and TMZ (150 mg/m^2 p.o. days 1–5) on a 28 day cycle. After higher than expected grade 4 thrombocytopenias, the dose of veliparib was reduced to 30 mg b.i.d. RECIST response was evaluated every two cycles. The best response for the 24 patients evaluable included one CR, two PR and seven stable disease [28]. Clinical benefit was largely seen in mutation carriers, leading to enrollment of an expansion cohort. Results of the expanded study have not yet been reported.

A Phase I study of veliparib in combination with irinotecan (CPT-11) in 32 patients (two lung, 14 breast, four esophageal, seven ovarian, four colon and one anal) reported an MTD and recommended a Phase II dose of 100 mg/m^2 of irinotecan given on days 1 and 8, combined with veliparib 40 mg b.i.d. 15 days on/6 days off (21 day cycle). This irinotecan and ABT 888 study showed responses outside of the BRCA population [28]. Another Phase I trial of veliparib in combination with doxorubicin and cyclophosphamide (AC) in breast cancer and other solid tumors reported a MTD of 100 mg every 12 h for veliparib, in combination with AC every 21 days. Objective antitumor activity was seen in BRCA mutation carriers. Veliparib inhibited PARP in peripheral monocytes at all dose levels, and the study continues in a breast cancer dose expansion cohort [32].

A number of ongoing veliparib studies in different tumor types, including breast cancer, were reported at the trial in the progress section of the recent 2011 American Society of Clinical Oncology annual meeting. One Phase II study randomizes patients with chemotherapy-resistant ER and/or PR-positive, HER2/neu-negative metastatic breast cancer to low-dose metronomic cyclophosphamide alone or in combination with veliparib [33]. Another Phase I dose-escalation study investigates veliparib in combination with carboplatin in HER2-negative metastatic breast cancer [34].

Rucaparib (AG014699/ PF-01367338/ CO338):

AG014699 or PF-01367338 was probably the first PARPi tested in humans. Clovis Oncology has recently in-licensed the rights to global development of this compound from Pfizer and it is now called rucaparib (CO-338) (courtesy of Clovis Oncology).

Human cancer cells or xenograft tumors with mutated or epigenetically silenced *BRCA1/2* have been shown to be sensitive to AG014699 monotherapy [35]. This supports a potential role of this PARPi in sporadic cancers with HR defects.

A 33 patient Phase I dose escalation study has reported safety and tolerability of AG014699 combined with TMZ five-times every 28 days. The study achieved dose escalation to MTD of 12 mg/m^2 for AG014699 and 200 mg/m^2 for TMZ. PARP inhibition was demonstrated at all doses. No toxicity attributable to AG014699 alone was observed [36]. Responses were seen in patients with melanoma, desmoid tumor, pancreas cancer, prostate cancer and leiomyosarcoma.

A subsequent Phase II study of 40 chemotherapy-naive patients with good performance status was conducted. Of the 20 patients assessable at reporting, four PR were seen and an additional four patients had prolonged disease stabilization. 40 patients who fulfilled the eligibility criteria were recruited and treated. More

enhancement of TMZ associated myelosuppression by the addition of AG014699 has been observed compared with the Phase I study. There was one toxic death in cycle 1 from febrile neutropenia [37].

Rucaparib will be developed further as an oral formulation, not as it was initially given in an iv. formulation. Current efforts focus on development of rucaparib as monotherapy and in combination with platinum-based chemotherapy (courtesy of Clovis Oncology).

MK-4827

MK-4827 is a PARPi with antiproliferative activities against *BRCA1* and *BRCA2* deficient cancer cells, with high selectivity over BRCA proficient cells [38]. It displays good pharmacokinetic properties and is currently in Phase I clinical trials. A 59 patient study (13 males, 46 females; 23 BRCA mutation carriers) reported that the drug was well tolerated. Dose-limiting toxicities (DLTs) observed were fatigue, reversible pneumonitis and two cases of reversible thrombocytopenia, a total of four DLTs. The MTD was established at 300 mg and linear pharmacokinetics was observed. The mean half-life was 40 h (37–42 h), which is longer than any of the other available PARPi. PARP inhibition in peripheral blood mononuclear cells was confirmed. Antitumor activity was observed in both sporadic and BRCA cancers. There were 11 patients with PR (nine ovarian, two breast, 9/11 BRCA cancers) and four patients with stable disease (two ovarian, two NSCLC, 2/4 BRCA) [39]. Cohort expansions are ongoing, and updated safety and response data are awaited.

INO-1001

A 12 patient Phase I study of the combination of INO-1001 plus TMZ was recently published [40]. Administration of this PARPi is more challenging than some of the orally bioavailable agents and was given iv. for a period of 1 h, every 12 h, for 5 days. The DLTs were myelosuppression and elevated hepatic transaminases.

Hurdles in development of PARPi in breast cancer

Recently, the development of PARPi in breast cancer has been hampered by a number of setbacks. The previously planned Phase III development of olaparib in hereditary *BRCA1*- and *-2*-associated breast cancer is delayed, although there have been some successes in the development of this agent in ovarian cancer [41]. Also, the negative results of the Phase III iniparib study have led to a degree of disappointment [42,43]. A major issue with respect to the iniparib Phase III trial is that the number of carriers of BRCA mutations in the Phase III study population is unknown [29]. There is preclinical work suggesting that iniparib is not a PARPi after all [23]. However, there is evidence of anticancer activity, both preclinically and clinically [23]. Treatment of BRCA-deficient cells and healthy donor peripheral blood monocytes confirmed lack of PARP inhibition by BSI-201 and the authors concluded that iniparib is not a PARPi in contrast to veliparib, olaparib and MK-4827 that demonstrated inhibition of PARP-1/2 [23]. Histone H2A phosphorylation is an important process in DNA double-strand break repair. A dose- and time-dependent increase in γH2AX occurred in cells treated with iniparib, independent of PARP inhibition. DNA damage response, and increases in γH2AX after iniparib treatment, indicate that the agent is causing DNA damage through an uncertain mechanism.

Resistance can arise to a synthetic lethal therapy via different mechanisms, such as deletion of a mutation in *BRCA2* [44]. Secondary somatic mutations that restore *BRCA1/2* in carcinomas in carriers of germline *BRCA1/2* mutations may predict resistance to platinum chemotherapy and, subsequently, to PARPi. In a retrospective series, these mutations were only found in ovarian carcinomas previously treated with chemotherapy for either ovarian or breast cancer [45]. However, this series did not represent all women with *BRCA1/2*-associated ovarian carcinoma, and only cases with known mutations were included. This has enriched the population for patients with two primary cancers and those who survived the initial cancer and underwent genetic testing [45].

Future perspective

Despite the recent hurdles in the development of PARPi, it is still hoped that these agents will play a significant role in treatment of cancers, including those arising in *BRCA1/2* carriers. The work that has been done so far raises the possibility that future studies will uncover additional synthetic lethal relationships between PARP-dependent pathways and tumor-specific defects present in sporadic cancers [31].

The optimal PARPi–chemotherapy drug combination remains to be established, with a wide range of trials ongoing. Additive or synergistic cytotoxic effects generated through such combinations of treatment may potentially permit lower doses of chemotherapy to be utilized in conjunction with standard doses of PARPi to achieve similar benefits. More rational combinations with targeted agents, with particular emphasis on blocking the HR pathway, may be needed, possibly with biologically likely partners, such as histone deacetylase inhibitors or metronomic or dose dense chemotherapy schedules. The concern with the development of these combinations is the potential abrogation of regulatory pathways required for normal cellular functions and the potential subsequent narrowing of the therapeutic window.

PARPi have demonstrated the ability to selectively kill *BRCA2*-deficient cells *in vivo* while sparing normal tissue with heterozygous *BRCA2* loss. These data support the study of PARPi in chemoprophylaxis studies to prevent the development of cancer [46]. However, the possible role in prevention needs to be weighed against potential toxicities, given the role of PARP in DNA repair and cell function [3]. There is particular concern that long-term inhibition of a DNA repair pathway could potentially result in mutations and subsequent secondary cancers, such as myelodysplasia [47,48].

A major challenge is to accurately identify the right target population and/or to identify non-BRCA patients with a 'BRCAness' phenotype, for instance by measuring HR or NHEJ activity [49]. Prognostic markers, such as 'BRCAness', mutational status and functional tests for DNA repair deficiencies, will need to be emphasized in the design of future studies.

Ongoing clinical and translational studies will shed more light on the clinical impact, as well as the biology of PARP inhibition. Future research will hopefully provide more insight into the unanswered questions. It will take a few more years until a place for PARPi is established in the anticancer armamentarium.

Financial & competing interests disclosure

M Robson has served on advisory boards for Sanofi-Aventis, AstraZeneca, Pfizer and Abbott Pharmaceuticals, all of which have PARP inhibitors under development. M Robson's institution also received funding from Astra-Zeneca to conduct a clinical trial of a PARP inhibitor. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Poly(ADP-ribose) polymerase (PARP)-1 and PARP-2 are important in the repair of DNA damage.
- PARP-deficient cells carry out DNA repair through homologous recombination.
- Homologous recombination is mediated by proteins encoded by *BRCA1* or *BRCA2*.
- Repair can also be done by nonhomologous end-joining.
- Many *BRCA1*-associated breast cancers are triple negative (TNBC), do not express estrogen, progesterone or HER2 receptors.
- Many TNBC are sporadic and may express low levels of *BRCA1*.
- 'BRCAness' describes TNBC without BRCA mutation that may have DNA repair defects and may respond to treatment with PARP inhibitors.
- Most studies including either BRCA mutation carriers or patients with tumors exhibiting 'BRCAness', have demonstrated a benefit by using PARP inhibitors. This is true for breast and ovarian cancer.

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