A review of PARP inhibitors: from bench to bedside

C. Underhill1,2, M. Toulmonde1 & H. Bonnefoi1*

1Department of Medical Oncology, Institut Bergonie Cancer Center and University of Bordeaux, Bordeaux, France; 2University of New South Wales, Rural Clinical School, Albury, Australia

Received 2 February 2010; revised 28 April 2010; accepted 28 April 2010

Background: Poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors, with novel and selective mechanisms of action, have moved from the laboratory to the clinic in just the last few years.

Design: We conducted an extensive review of PARP inhibitors using a Medline search. We also searched abstracts in databases of major international oncology meetings from the last 4 years.

Results: To understand the mechanisms of action of PARP inhibitors requires a basic understanding of DNA repair mechanisms and the critical role of the PARP enzyme. We briefly review these DNA repair mechanisms, the concept of ‘synthetic lethality’, and how PARP inhibitors play a role to selectively disrupt DNA repair in cells with absent or dysfunctional BRCA genes. We review the preclinical data highlighting this unique and selective mechanism of action and we discuss early but highly promising clinical data and ongoing studies.

Conclusion: PARP inhibitors show promise as a powerful therapeutic tool, especially in the management of BRCA-associated breast and ovarian cancers but also in tumours where BRCA genes may be dysfunctional. Clinical studies are ongoing and many translational questions remain unanswered that will help clarify how to determine the best way to use PARP inhibitors.

Key words: BRCA, DNA repair, PARP inhibitors, triple-negative breast cancer

introduction

The discovery of new targeted therapies has offered dramatic therapeutic improvements in oncology over the last decade. Many research groups are currently trying to identify new targets or pathways whose overexpression or activation accelerate cancer growth. On the other hand, two groups, instead of targeting a strength, have concentrated on a weakness of specific tumours, namely their inability to repair double-strand breaks (DSBs) that occur as a consequence of a deficiency of BRCA1 or BRCA2 genes, two well known tumour-suppressor genes [1, 2]. In brief, they have demonstrated in preclinical models that flooding BRCA-deficient cells (a weakness) with DSBs will lead to cell death, a result which may have obvious implications when attempting to treat cancers. To flood these cells with DSBs, they used a novel strategy by increasing the number of single-strand breaks (SSBs) blocking a specific enzyme, the poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP), whose physiological function is to repair these SSBs. These unrepaird SSBs are converted into DSBs at fork replication. Consequently, the cells are flooded with these DSBs and die. To block this enzyme they used various methods including drugs known to inhibit this enzyme known as PARP inhibitors.

What is the clinical impact of these findings? To begin with, these results have revolutionised the strategic clinical development of this class of compounds in two ways. Firstly, they have pushed researchers to redefine the patient population eligible for trials with PARP inhibitors. Until 2005, these agents were used in clinical trials as chemosensitisers independently of the DNA repair function. In 2005, the concept of ‘treating cancer by targeting a weakness’ was born [3] and trials began to focus on cancers in BRCA1 and BRCA2 mutation carriers. Less than 2 years later, the results of a phase 1 trial enriched with BRCA-mutated patients were presented at the 2007 American Society of Clinical Oncology (ASCO) meeting [4] and the final results were published in 2009 [5]. Secondly, these results show once again a case in the history of medicine where the study of a rare disease provides treatment solutions for a broader range of patients. In this particular case, the effect of PARP inhibitors was first identified in patients with hereditary cancers related to a mutation of BRCA1 and BRCA2 genes and was then extended to tumours with abnormal function of these two genes or other genes implicated in similar DNA repair pathways to BRCA1 and BRCA2. This abnormal function is called BRCAness and its clinical relevance was recently highlighted in triple-negative breast cancers [6].

We believe that such rapid development from bench to bedside deserves to be reviewed. We first provide a comprehensive review of DNA repair mechanisms in normal cells and BRCA-deficient cells and the impact of PARP inhibition in both cases. Then we summarise the preclinical
development of PARP inhibitors as single agents or in combination with cytotoxic agents. Finally, we present available clinical data and ongoing studies.

**understand DNA repair mechanisms and role of PARP inhibition**

DNA is unstable, and alterations occur from three main causes: firstly, after exposure to environmental agents; secondly, as a result of by-products of normal cellular metabolism; and finally, by spontaneous disruption of chemical bonds in DNA [7]. It causes a variety of lesions including base modifications, SSBs, DSBs, and intrastrand or interstrand cross-links. Four principal and partially overlapping DNA repair mechanisms are involved in repairing these lesions and maintaining genomic integrity in mammals. These are base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), and recombinational repair [with homologous recombination (HR) and non-homologous end-joining (NHEJ)]. Repair of SSBs involves BER, NER, and MMR while repair of DSBs involves HR and NHEJ. In addition, some damage to DNA can be repaired directly; for example, methylation of guanine bases is directly reversed by the protein O6-methylguanine-DNA methyltransferase (MGMT).

In this paper, we will briefly summarise these repair mechanisms. More detailed information on DNA repair mechanisms can be found in a review article by Hoeijmakers [7].

**cells with normal DNA repair mechanisms**

*single DNA strand repair including SSBs and role of PARP.* Alterations of a single DNA strand, including SSBs, are mainly endogenous and are the most common DNA aberration. They are repaired using the intact complementary strand as a template by BER, NER, and MMR [7]. About 10 000 spontaneous SSBs occur in each cell every day [8]. The BER pathway is the main repair mechanism of these lesions and involves a family of enzymes called PARP [7]. PARP was first described in 1963 [9]. There are 17 members of the PARP superfamily described [10, 11]. PARP1 is the most important member of this family and its primary function is to bind to PARP1 enzyme (PARP1) is crucial for the repair of SSBs, especially by the process of base-excision repair (BER). PARP1 catalyses the formation of large branched chains of poly(ADP)-ribose from its substrate nicotinamide adenine dinucleotide (NAD+). These chains recruit other DNA repair proteins [10, 11] (Figure 1A). In addition to BER, PARP1 might also be involved in NER [15]. Thus, PARP1 appears to play a pivotal role in repair of damaged DNA and in stabilisation of the genome.

*DSBs repair.* DSBs can be induced by X-rays or chemicals during repair of interstrand cross-link and during replication of SSBs converted into DSBs at replication forks and repaired by HR (Figure 1B) [7]. DSBs are potentially more of a problem to a mammalian cell than SSBs because both strands are affected and the complementary DNA strand is not available as a template. DSBs are primarily repaired by HR, which tends to be error-free, and NHEJ, which is more prone to error and can lead to changes in the DNA sequence at the break site [7].

**cells with abnormal DNA repair mechanisms and effect of PARP deficiency or inhibition**

*abnormal SSBs repair and PARP deficiency.* There are two nuclear PARP activated by DNA damage: PARP1 and PARP2 (less abundant). Interestingly, despite the important role of the PARP1 enzyme in SSBs repair described in ‘Single DNA strand repair including SSBs and role of PARP’ section, PARP1 deficiency does not seem to be such a problem for non-malignant cells. As a matter of fact, mice engineered to lack PARP1 enzyme (PARP1−/−) are both viable and fertile [13, 16, 17]. These PARP1−/− mice do not seem to develop early-onset tumours [18]. However, conflicting results have been reported in another paper suggesting that PARP genetic ablation may predispose to mammary cancer [19]. The explanation for this apparent lack of ‘severe consequences’ as a result of PARP deficiency can be explained by an understanding of DNA repair mechanisms. The loss of PARP1 activity affects repair of SSBs via BER, resulting in cells using different DNA repair pathways. At the time of DNA replication, unrepaired SSBs are converted into DSBs at replication forks and repaired by HR (Figure 2A). PARP1 inhibition blocks repair of SSBs but repair of DSBs is able to proceed [20–22]. In this circumstance, HR activity is increased and acts as a very efficient error-free rescue mechanism [12, 13, 17, 23].

PARP2 also plays a role. In a cell line model, the clonogenic survival of normal human cells is decreased by 20% when both PARP1 and PARP2 proteins are co-depleted with short interfering RNA [1]. These results are consistent with the
PARP deficiency or inhibition becomes essential only in BRCA-deficient cells.

Summary of two seminal papers: When exposed to PARP inhibitors, these cells are flooded with SSBs that are converted into DSBs at replication forks but these BRCA-deficient cells are unable to utilise the HR pathway that would normally be the ‘rescue’ mechanism. Instead, DSBs are repaired via NHEJ or the single-strand annealing sub-pathway of HR with large numbers of chromatid aberrations leading to cell lethality (Figure 2C). We summarise in Appendix 1, the two Letters to Nature, simultaneously published in 2005, which demonstrated the importance of PARP inhibition in BRCA-deficient or HR-deficient cells.

Concept of synthetic lethality: The use of a PARP inhibitor in a BRCA-deficient cancer is probably the first example of the clinical application of the concept of synthetic lethality [1, 2]. Dobzhansky [31] first described ‘Synthetic lethality’ in 1946 as the situation when a mutation in one of two genes individually has no effect but combining the mutations leads to cell death. In 1997, Hartwell et al. [32] made the first suggestion that the concept of synthetic lethality could be applied to cancer therapeutics.

Concept of ‘BRCAness’: Deficiency in other genes involved in the complex HR pathway also confers sensitivity to PARP inhibitors [33]. These findings raise the possibility that PARP inhibitors may play a role not only in BRCA-mutated tumours but also in a broad range of tumours with dysfunction of HR or identified as ‘BRCAness’. BRCAness defines characteristics that some sporadic cancers share with BRCA1 or BRCA2 cancers, which suggests a common underlying DNA repair defect with a loss of HR. BRCAness is important as it may have important therapeutic consequences. The first way to approach or identify BRCAness is to consider the similar phenotype of some sporadic cancers and BRCA1 or BRCA2 cancers. The second, more ambitious, way is to consider BRCAness at a functional level. With this approach, Turner et al. [34] have estimated that up to 25% of sporadic breast cancers could show BRCAness phenotypes. We will briefly consider these two approaches.

The phenotypic presentation of BRCA1-mutated and basal-like and/or triple-negative breast cancers is very similar [35–37]. In addition, they have very similar gene expression profiles [38]. In contrast, there is no histopathological characteristic specific for BRCA2-mutated breast cancers. However, a BRCA2 gene expression profile has been described which differentiates these tumours from BRCA1 and sporadic tumours [39–41]. BRCA1 gene expression may be lost in both hereditary and high-grade sporadic epithelial ovarian cancer and play a role in tumour development [42–45]. Acquired loss of BRCA expression conveys similar clinicopathological characteristics (similar microarray-based genetic profile, morphological high grade, aggressive clinical behaviour, and poor prognosis) [45].

Mutations of BRCA1 and BRCA2 genes are very rare in sporadic cancers. Silencing of BRCA1 or BRCA2 or of other genes acting in similar pathways might be important in the pathogenesis of sporadic cancers. The first example of silencing is through aberrant methylation of the BRCA1 promoter found
in 11%–14% of sporadic breast cancers and in 5%–31% of sporadic ovarian cancers (the second allele being lost) [34]. The second example relates to the silencing by aberrant methylation of the gene F of Fanconi anaemia (FANCF) which has been identified in several tumour types: breast cancer, ovarian cancer, head and neck squamous-cell carcinoma, non-small-cell lung cancer, and cervical carcinoma [46].

How this preclinical knowledge might be applied in the clinic for BRCA-mutated and BRCAness tumours will be referred to in ‘Clinical trials of PARP inhibitors selecting the tumours for BRCA mutation or BRCAness’ section.

preclinical development of PARP inhibitors

development of PARP inhibitors
PARP enzymes catalyse the transfer of ADP-ribose units from intracellular NAD+ to nuclear acceptor proteins, leading to the formation of ADP-ribose polymers. Nicotinamide, which is realised in the process, was the first PARP inhibitor identified [47]. A second generation of PARP inhibitors was then developed through screening of chemical libraries leading to an improved potency and a better understanding of the structure–activity relationship [48]. Several third generation molecules have been or are being evaluated in clinical trials and are summarised in ‘Clinical development’ section of this review. PARP inhibitors under clinical investigation do not discriminate between PARP1 and 2. Interestingly, preclinical data indicate that PARP1 inhibitors should be more selective and cause less toxicity to normal cells than those that inhibit both PARP1 and 2 [1]. In this study, the clonogenic survival of normal cells was worse when both PARP1 and 2 were silenced than when only PARP1 or PARP2 was silenced. Ongoing clinical trials may give us answers regarding the clinical impact of this lack of selectivity seen in preclinical models.

preclinical data of PARP inhibitors without considering BRCA function
Interestingly, before a role for PARP inhibitors was suspected in BRCA-deficient tumours, these molecules were developed as chemosensitisers. Experimental data have shown a synergistic effect between PARP inhibitors and specific cytotoxic agents [49–55]. These data are summarised in Appendix 2 (available in Annals of Oncology online).

preclinical data of PARP inhibitors in BRCA-deficient tumours
The ‘proof of principle’ regarding efficacy of PARP inhibitors as single agents in BRCA-deficient cell lines (embryonic stem cells and Chinese hamster cells) and xenografts derived from these cell lines has been demonstrated in 2005 in two seminal papers that we have summarised in the Introduction [1, 2] and in two additional publications [33, 56]. These experiments demonstrated that only cell lines or xenografts dysfunctional in either BRCA genes were exceptionally sensitive to PARP inhibition. Farmer et al. [2] showed that some cell lines were 1000 times more sensitive than wild-type cells. In addition, the effect of AZD2281 was demonstrated recently in BRCA2−/− mammary tumours arising spontaneously in a mouse model, which is a relevant preclinical model with important advantages over the xenograft models [56].

In addition, the synergy of AZD2281 in combination with platinum drugs has been demonstrated in vivo with both BRCA1−/− p53−/− mammary tumours transplanted into wild-type mice [57]. In vitro, there is a synergy between AZD2281 and cisplatin in BRCA2-deficient cell lines [58]. However, in vivo data with BRCA2 are more difficult to interpret [56]. With another PARP inhibitor, BSI-201, a synergistic effect with gemcitabine and carboplatin has been demonstrated in triple-negative MDA-MB-468 cell line (D. Roychowdhury, personal communication).

PARP inhibitors in combination with radiotherapy
Since radiotherapy damages cells by causing DNA breaks, and PARP inhibitors disrupt DNA repair mechanisms, there was an expectation that PARP inhibitors would be synergistic with radiotherapy. Several studies have shown that this is indeed the case both in vitro [59, 60] and in vivo models [59, 61–63].

clinical development

The first clinical development strategy of potent PARP inhibitors began in 2003 using them without tumour selection for their DNA repair function (or lack of DNA repair) either in combination with different cytotoxic agents as chemosensitisers or as single agents after failure of standard chemotherapy agents. The second strategy, after the demonstration of the role of BRCA selectivity in 2005 [1, 2], has been to use them after selecting for BRCA mutations or BRCAness either as single agents or in combination with chemotherapy. We present available data obtained from these two strategies (much of which has been presented in abstract form only) (Table 1) and summarise ongoing studies. This summary has been done based on available data on the www.clinicaltrials.gov website with a cut-off date of 15 January 2010. Trials are ongoing with at least eight PARP inhibitors.

clinical trials of PARP inhibitors without tumour selection for BRCA mutation or BRCAness

PARP inhibitors as chemosensitisers in combination with cytotoxic agents or radiotherapy. Based on data presented in ‘Preclinical data of PARP inhibitors without considering BRCA function’ section, specific cytotoxic agents (methylating agents, platinum drugs, alkylating agents, and topoisomerase I and II inhibitors) in combination with PARP inhibitors have been and are still evaluated in clinical trials. Of note, interest in the use of agents that inhibit DNA repair such as PARP inhibitors for the treatment of glioblastoma was sparked by the knowledge that DNA repair mechanisms may play an important role in the response of glioblastoma to temozolomide. Translational studies suggest that epigenetic silencing of the MGMT DNA repair enzyme by methylation of its promoter inhibits DNA repair and may lead to enhanced response to alkylating agents such as temozolomide and to longer survival in patients with glioblastoma [64–66].
Conversely, glioblastomas with MGMT intact are resistant to alkylating agents. Thus, a number of clinical studies have been initiated in glioma using PARP inhibitors in combination with temozolomide.

**AG014699:** The first in human phase I trial of a PARP inhibitor was a combination of AG014699 (Pfizer) and temozolomide, given to 27 patients with solid cancers, was initially reported at the ASCO annual scientific meeting in 2005 [67] and subsequently updated and published in 2008 with a total of 33 patients [68]. The goal of the first part of this trial was to define the PARP inhibitory dose. The PARP inhibitory dose (based on 74%–97% inhibition of peripheral blood lymphocyte PARP activity) was 12 mg/m² of AG014699 [68]. In the second part, the maximum dose of AG014699 was defined as 18 mg/m²/day, given with temozolomide 200 mg/m² for 5 days every 28 days with one grade 3 neutropenia. The combination was well tolerated. PARP inhibition was seen at all dose levels. Evidence of increased SSBs was seen in all patients treated at the PARP inhibitory dose using a previously validated method [69]. Responses were seen in patients with melanoma, desmoid tumour, pancreas cancer, prostate cancer, and leiomyosarcoma.

Based on these results, a phase II study was conducted in patients with metastatic malignant melanoma. The results were reported at ASCO 2006 [70]. Using the above-recommended doses, 40 chemotherapy-naïve patients with good performance scores were included. Of the 20 patients assessable at the time of the report, 4 partial responses were seen and an additional 4 patients had ‘prolonged disease stabilisation’.

**INO-1001:** A phase I study of the combination of INO-1001 plus temozolomide was recently published [71]. This PARP inhibitor is less convenient to administer than some of the orally bioavailable agents and was given i.v. for a period of 1 h, every 12 h, for 5 days. The dose-limiting toxicities (DLTs) were myelosuppression and elevated hepatic transaminases.

**KU-0059436/AZD2281 (olaparib) and ABT-888:** Myelosuppression is a problem when these two drugs are combined with chemotherapy.

A phase I of AZD2281 with cisplatin plus gemcitabine reported DLT of myelosuppression at the first dose level explored. The dose was de-escalated [72, 73]. Similarly, a phase I of ABT-888 in combination with topotecan reported DLT of myelosuppression at the first dose explored [74].

**BSI-201:** A phase Ib study was conducted in patients with advanced solid tumours to assess safety and establish the maximum tolerated dose (MTD) of the combination of BSI-201 with topotecan, gemcitabine, temozolomide, and carboplatin/paclitaxel (Taxol) [75]. Fifty-five patients were treated with BSI-201 doses from 1.1 to 8.0 mg/kg twice weekly,
in combination with the cytotoxic chemotherapy at standard doses. The investigators reported that all dose combinations were well tolerated. A complete response at 6 months was reported for one patient (ovarian cancer), and five patients experienced a partial response (one renal carcinoma, two breast cancers, one uterine carcinoma, and one sarcoma).

This lack of myelosuppression is in contradiction with the toxicity results seen with AZD2281 and ABT-888. A possible explanation for this lack of normal tissue toxicity might be related with the intermittent dosing regimen [76].

other PARP inhibitors: Several phase I studies are ongoing and are listed in Table 2. PARP inhibitors as single agents.

KU-0059436/AZD2281 (olaparib): A phase I study was first presented at ASCO 2006 [77] with preliminary results on 12 patients showing PARP inhibition of >50% at 40 mg/day. This trial was enriched in BRCA mutation carriers and is described below in ‘KU-0059436/AZD2281 (olaparib)’ section. Of note, the final results of this phase I have been published recently and no response was seen in non-BRCA mutation carriers [5].

Other phase I trials are ongoing with olaparib in solid tumours where chemotherapy has failed (Table 3). In addition, a phase 2 trial is ongoing in metastatic colorectal cancer.

BSI-201: A phase I study of BSI-201 has been conducted in subjects with advanced solid tumours [78]. A pharmacodynamic approach was taken by the investigators to determine a 'biologically relevant dose' by measuring PARP inhibition in peripheral mononuclear cells. Eligible patients had refractory solid tumours and they were heavily pretreated. Dose was escalated from 0.5 to 8.0 mg/kg given i.v. twice a week. All doses were well tolerated with no MTD identified. At a dose of 2.8 mg/kg, PARP was inhibited in peripheral blood mononuclear cells (PBMCs) by >50% after a single dose and 280% with multiple doses. No responses were seen by RECIST criteria but stable disease was observed for >2 months in 6/23 subjects. A phase II trial is ongoing in BRCA-positive ovarian cancer but none in an unselected solid tumours population.

other PARP inhibitors: A phase 0 study with ABT-888 in patients with advanced tumours used pharmacokinetic and pharmacodynamic analyses to determine a dose range at which ABT-888 inhibits PARP in tumour tissue and PBMCs [79]. This was the first phase 0 trial of a therapeutic agent in oncology with target modulation as the primary end point conducted under the Exploratory Investigational New Drug Guidance of the USA Food and Drug Administration.

Several phase 1 studies in patients with solid tumours that have failed standard chemotherapy are ongoing with ABT-888, MK4827, and 14C-radiolabelled AZD2281 (Table 3).

clinical trials of PARP inhibitors selecting the tumours for BRCA mutation or BRCAness

In ongoing studies, the definition of BRCA-ness is very pragmatic and based on a simple (albeit imperfect) phenotypic description: triple-negative breast cancers and serous ovarian cancers (this histological subtype being used as stratification criteria in trials allowing for inclusion of other subtypes) [80].

PARP inhibitors as single agents.

KU-0059436/AZD2281 (olaparib):

- phase I trials: As mentioned above, preliminary results were first presented at the ASCO meeting in 2006 with 12 patients unselected for a BRCA mutation [77]. Based on preclinical data published in 2005 [1, 20], this phase I was then enriched with BRCA mutation carriers. The study was updated in 2007, with a population enriched with patients with known germline mutations in BRCA1 or BRCA2 genes (11 of 44 recruited) [4], and a focus on the expansion phase in BRCA-deficient ovarian cancer (BDOC) was presented in 2008 (44 BDOC of 92 treated) with 14 patients with BDOC of 32 assessable achieving partial response [81]. The final results of the phase I including several advanced solid tumours and subsequently enriched in BRCA mutation carriers was published recently in The New England Journal of Medicine [5]. Sixty patients were treated, 22 of which had BRCA1 or BRCA2 mutations and 1 had a strong family history of BRCA-associated cancer. A DLT was seen in one of eight patients at a dose of 400 mg bd continuously, with grade 3 neurocognitive toxicity (mood alteration and fatigue), and two of five patients at a dose of 600 mg bd (grade 4 thrombocytopenia in a patient previously treated with chemotherapy that had resulted in prolonged myelosuppression and grade 3 somnolence), all which resolved rapidly with discontinuation of therapy. No other grade 3 or 4 toxic effects were reported. The maximum administered dose was defined as 600 mg of olaparib twice daily and the maximum tolerated dose as 400 mg twice daily. Objective antitumour activity was noted in patients with BRCA1/2 mutations and in one patient with a strong family history of BRCA mutation: 9 of 19 (47%) assessable patients with BRCA mutations (plus the patient with a strong family history of BRCA mutation) had a response by RECIST criteria. Twelve of 19 (64%) assessable patients had a clinical benefit (reported as radiological or CA-125 response or meaningful disease stabilisation for ≥4 months). A functional assay of PARP inhibition was carried out in surrogate samples of PBMC in order to investigate the pharmacodynamic of olaparib administration. More than 90% inhibition of PARP functional activity, as compared with the value at baseline, was seen in PBMC at doses >60 mg twice daily. Of note, an accumulation of DSBs in normal tissue (plucked eyebrow hair follicles) was demonstrated in pharmacodynamic assays carried out in this trial 6 h after olaparib treatment and levels remained elevated on treatment. The formation of foci of gammaH2AX, the phosphorylated form of histone H2A at serine 129, was measured as a marker of DNA DSBs. Does this mean patients could be at risk of developing second tumours or other problems? It certainly casts some doubt on the widely held view that PARP inhibition results in little toxicity in normal tissue (as discussed in a recent review by Drew and Plummer [76]). PARP1 is a DNA repair protein and its genetic ablation in mice has been shown to predispose to mammary neoplasia [19].

- phase II trials in patients with BRCA mutation-associated breast cancers: A phase II of olaparib, ICEBERG 1, was
Table 2. Ongoing clinical trials with PARP inhibitors as chemosensitisers in combination with chemotherapy or radiotherapy without selecting tumours for a BRCA mutation or a possible BRCAness

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial identifier</th>
<th>Phase</th>
<th>Tumour type/line</th>
<th>Combined with</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG014699</td>
<td>NCT01009190</td>
<td>I</td>
<td>Advanced solid tumours</td>
<td>Carboplatin, paclitaxel, cisplatin or pemetrexed</td>
<td>N</td>
</tr>
<tr>
<td>BSI-201</td>
<td>NCT00687765</td>
<td>I/II</td>
<td>Glioma/second line</td>
<td>Temozolomide</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00422682</td>
<td>I</td>
<td>Advanced solid tumours</td>
<td>Topotecan, temozolomide, gemcitabine, carboplatin/ paclitaxel</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT010687687</td>
<td>II</td>
<td>Advanced/persistent/ recurrent uterine carcinomas</td>
<td>Carboplatin/paclitaxel</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00298675</td>
<td>Ib/II</td>
<td>Metastatic breast cancer</td>
<td>Irinotecan</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT01033123</td>
<td>II</td>
<td>Platinum-sensitive recurrent ovarian cancer</td>
<td>Carboplatin/gemcitabine</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT01033292</td>
<td>II</td>
<td>Platinum-resistant recurrent ovarian cancer</td>
<td>Carboplatin/gemcitabine</td>
<td>R</td>
</tr>
<tr>
<td>AZD2281</td>
<td>NCT00516724</td>
<td>I</td>
<td>Metastatic solid tumours/ third line</td>
<td>Carboplatin or paclitaxel or carboplatin/paclitaxel</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00515866</td>
<td>I</td>
<td>Pancreas cancer/second line</td>
<td>Gemcitabine</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00678132</td>
<td>I</td>
<td>Solid tumours/second or third line</td>
<td>Cisplatin/gemcitabine</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00516438</td>
<td>I</td>
<td>Solid tumours/third line</td>
<td>Topotecan</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>NCT00535353</td>
<td>I</td>
<td>Colorectal cancer/second or third line</td>
<td>Irinotecan</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00516802</td>
<td>I</td>
<td>Melanoma/first line</td>
<td>Dacarbazine</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>NCT00710268</td>
<td>I</td>
<td>Solid tumours/failed standard therapy</td>
<td>Bevacizumab</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>NCT008189221</td>
<td>I</td>
<td>Solid tumours/&lt;fifth line</td>
<td>Liposomal doxorubicin</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00553189</td>
<td>I</td>
<td>Solid tumours, non-Hodgkin lymphoma, and chronic lymphocytic leukaemia/failed standard chemotherapy</td>
<td>Topotecan</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00810966</td>
<td>I</td>
<td>Solid tumours and lymphoma/refractory or failed standard chemotherapy</td>
<td>Cyclophosphamide (oral, metronomic doses)</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00649207</td>
<td>I</td>
<td>Solid tumours with brain metastases</td>
<td>Whole-brain radiation</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00526617</td>
<td>I</td>
<td>Solid tumours/failed standard therapy</td>
<td>Temozolomide</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00804908</td>
<td>II (randomised)</td>
<td>Melanoma/no prior dacarbazine or temozolomide</td>
<td>Dacarbazine</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00576654</td>
<td>I</td>
<td>Lymphoma, unspecified tumours</td>
<td>Irinotecan</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>NCT00946335</td>
<td>I</td>
<td>Recurrent/refractory brain and CNS tumours, age up to 21 years</td>
<td>Temozolomide</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT01009788</td>
<td>II</td>
<td>Metastatic breast cancer/ second line and more</td>
<td>Temozolomide</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00588991</td>
<td>I</td>
<td>Relapsed/refractory acute leukaemia, high-risk myelodysplasia or myeloproliferative disorders</td>
<td>Carboplatin, topotecan</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT01017640</td>
<td>I</td>
<td>Unspecified tumours/ &lt;fourth line</td>
<td>Mitomycin</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>NCT00770471</td>
<td>I/II</td>
<td>Brain and CNS tumours/ first line</td>
<td>Temozolomide + radiation</td>
<td>R</td>
</tr>
</tbody>
</table>
carried out in a group of heavily pretreated patients with recurrent, measurable, chemotherapy refractory BRCA mutation-associated breast cancers (median 3 prior lines of chemotherapy). This trial showed high levels of response [82]. A total of 54 patients received olaparib at two different dose levels: 400 mg twice daily and subsequently 100 mg twice daily (a PARP inhibitory dose). Efficacy data were presented for the patients treated at the higher dose level and showed an overall response rate (ORR) of 41% (11/27) in this single-agent trial in heavily pretreated patients. Toxicity was mild. The ORR falls to 22% (6/27) in the 100-mg twice daily cohort suggesting that the degree of PARP inhibition is important for response.

Table 3. Ongoing clinical trials with single-agent PARP inhibitors without selecting tumours for a BRCA mutation or a possible BRCA1ess

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial identifier</th>
<th>Phase</th>
<th>Tumour type/line</th>
<th>Combined with</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD2281</td>
<td>NCT00777582</td>
<td>I</td>
<td>Solid tumours/refractory</td>
<td>Solid tumours (in Japanese patients)/failed standard chemotherapy</td>
<td>A</td>
</tr>
<tr>
<td>BSI-201</td>
<td>NCT00298675</td>
<td>I</td>
<td>Advanced solid tumours/refractory</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>14C-radiolabelled AZD2281</td>
<td>NCT00633269</td>
<td>I</td>
<td>Solid tumours/failed standard chemotherapy</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>MK4827</td>
<td>NCT00129502</td>
<td>I</td>
<td>Solid tumours (phase 1a)/failed standard therapy</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>ABT-888</td>
<td>NCT00387608</td>
<td>I</td>
<td>Solid tumours or haematologic malignancies/failed standard therapy</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

A = active, not recruiting: study is ongoing (i.e. patients are being treated or examined), but enrolment has completed. C = completed: the study has concluded normally; participants are no longer being examined or treated (i.e. last patient’s last visit has occurred). R = recruiting: participants are currently being recruited and enrolled.

Table 2. (Continued)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial identifier</th>
<th>Phase</th>
<th>Tumour type/line</th>
<th>Combined with</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00740805</td>
<td>I</td>
<td>Metastatic or unresectable solid tumours or non-Hodgkin lymphoma</td>
<td>+ Cyclophosphamide ± Doxorubicin</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>NCT01026493</td>
<td>I/II</td>
<td>Recurrent brain and CNS tumours</td>
<td>Temozolomide</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>NCT01012817</td>
<td>I/II</td>
<td>Solid tumours or relapsed/refractory ovarian cancer after platinum-based first line</td>
<td>Topotecan</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>CEP-9722</td>
<td>NCT00920595</td>
<td>I</td>
<td>Advanced solid tumours</td>
<td>Temozolomide</td>
<td>R</td>
</tr>
</tbody>
</table>

PARP = poly(adenosine diphosphate [ADP]-ribose) polymerase. N = not yet recruiting: the study is not open yet. R = recruiting: participants are currently being recruited and enrolled. C = completed: the study has concluded normally; participants are no longer being examined or treated (i.e. last patient’s last visit has occurred).

phase II trials in patients with BRCA mutation-associated ovarian cancers: The promising activity of olaparib in patients with BRCA1- and BRCA2-mutated ovarian cancer, seen in the phase I study [81], was recently confirmed in a phase II report [83]. An interim analysis of 57 assessable patients with BRCA1 and BRCA2 mutations, treated at two dose levels (400 and 100 mg twice daily), was reported. Response rates were 33% and 12.5%, respectively. Toxicity was mild; the only reported grade 3 toxic effects were nausea (7%) and leukopenia (5%). A randomised phase II in ovarian cancer is ongoing (Table 4).

other PARP inhibitors: Phase II studies in BRCA-mutated breast and/or ovarian cancers with AG014699, ABT-888, and BSI-201 are ongoing (Table 4). A phase I study with MK4827 both in solid tumours that have failed standard chemotherapy and in BRCA2-positive cancers is ongoing (Tables 3 and 4).

trials with PARP inhibitors in combination with chemotherapy. The first randomised phase II study in triple-negative breast cancer patients of a PARP inhibitor (BSI-201 at 5.6 mg/kg i.v. biweekly) in combination with chemotherapy (gemcitabine 1000 mg/m^2 and carboplatin AUC2, both given on days 1 and 8 every 21 days) showed remarkable efficacy [84].
Patients with measurable disease could have had up to two prior cytotoxic regimens. The clinical benefit rate (complete response rate, partial response, and stable disease) was 12% for chemotherapy alone and 52% for the combination. Median progression-free survival was 87 versus 211 days [HR 0.3; 95% confidence interval (CI) 0.15–0.59] and median overall survival 25 versus 452 days [HR 0.3; 95% CI 0.2–0.6].

Table 4. Ongoing clinical trials with single-agent PARP inhibitors selecting tumours for a BRCA mutation or a possible BRCAness

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial identifier</th>
<th>Phase</th>
<th>Tumour type/line</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD2281</td>
<td>NCT00679783</td>
<td>II</td>
<td>BRCA-mutated ovarian cancer or recurrent high-grade ovarian cancer or triple-negative breast cancer/failed standard chemotherapy</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>NCT00735345</td>
<td>II (randomised versus placebo)</td>
<td>Platinum-sensitive ovarian cancer, third line</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00494234</td>
<td>II</td>
<td>BRCA-mutated breast cancer, &gt;first line</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>NCT00494442</td>
<td>II</td>
<td>Ovarian cancer, &gt;first line</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>NCT00628235</td>
<td>II (randomised)</td>
<td>BRCA-positive ovarian cancer/failed platinum-based treatment/AZD2281 v liposomal doxorubicin</td>
<td>A</td>
</tr>
<tr>
<td>AG014699</td>
<td>NCT00664781</td>
<td>II</td>
<td>BRCA-mutated breast or ovarian cancer/≤four prior lines</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00677079</td>
<td>II</td>
<td>BRCA-mutated ovarian cancer/second line</td>
<td>A</td>
</tr>
<tr>
<td>MK4827</td>
<td>NCT00749502</td>
<td>I</td>
<td>BRCA-mutated cancers (phase 1b)/failed standard therapy</td>
<td>R</td>
</tr>
</tbody>
</table>

A = active, not recruiting: study is ongoing (i.e. patients are being treated or examined), but enrolment has completed. R = recruiting: participants are currently being recruited and enrolled. C = completed: the study has concluded normally; participants are no longer being examined or treated (i.e. last patient’s last visit has occurred).

Table 5. Ongoing clinical trials with PARP inhibitors in combination with chemotherapy or radiotherapy selecting tumours for a BRCA mutation or a possible BRCAness

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial identifier</th>
<th>Phase</th>
<th>Tumour type/line</th>
<th>Combined with</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI-201</td>
<td>NCT00813956</td>
<td>II</td>
<td>Triple-negative breast cancer/neoadjuvante</td>
<td>Gemcitabine/carboplatin + BSI-201</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00938652</td>
<td>III</td>
<td>Triple-negative breast cancer/≤3 lines in metastatic setting</td>
<td>Gemcitabine/carboplatin ± BSI-201</td>
<td>R</td>
</tr>
<tr>
<td>AZD2281</td>
<td>NCT00647062</td>
<td>I</td>
<td>BRCA1/BRCA2-associated, hereditary, triple-negative metastatic or unresectable breast cancer or ovarian cancer/failed standard chemotherapy</td>
<td>Carboplatin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT00782574</td>
<td>I</td>
<td>Triple-negative breast cancer/neoadjuvante</td>
<td>Cisplatin</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>NCT00707707</td>
<td>I</td>
<td>Metastatic triple-negative breast cancer</td>
<td>Paclitaxel</td>
<td>A</td>
</tr>
<tr>
<td>ABT-888</td>
<td>NCT00535119</td>
<td>I</td>
<td>BRCA-mutated, hereditary breast, and ovarian cancer, unspecified</td>
<td>Carboplatin/paclitaxel</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT01012817</td>
<td>I/II</td>
<td>Ovarian cancer</td>
<td>Topotecan</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>NCT00989651</td>
<td>I</td>
<td>Ovarian cancer</td>
<td>Carboplatin, paclitaxel, bevacizumab</td>
<td>R</td>
</tr>
</tbody>
</table>

R = recruiting: participants are currently being recruited and enrolled. A = active, not recruiting: study is ongoing (i.e. patients are being treated or examined), but enrolment has completed.

Patients with measurable disease could have had up to two prior cytotoxic regimens. The clinical benefit rate (complete response rate, partial response, and stable disease) was 12% for chemotherapy alone and 52% for the combination. Median progression-free survival was 87 versus 211 days [HR 0.3; 95% confidence interval (CI) 0.15–0.59] and median overall survival 25 versus 452 days [HR 0.3; 95% CI 0.2–0.6].
survival (OS) 169 versus >254 days, respectively (HR 0.24; 95% CI 0.09–0.61). Results updated at the San Antonio Breast Cancer Symposium in December 2009, with a longer follow-up, showed a significantly improved median OS (12.2 months) compared with gemcitabine/carboplatin alone (7.7 months; \( P = 0.005; \) HR = 0.5; 95% CI 0.30–0.82). There was no statistically significant difference in the frequency and nature of adverse events between the two arms [6]. The results of this study provided proof of concept that patients with triple-negative breast cancer are likely to share a common functional BRCA dysfunction to patients with BRCA-mutated tumours. A phase III is now ongoing (Table 5).

**Conclusion**

Recent clinical studies with PARP inhibitors have confirmed what was initially indicated by the preclinical data. Further research is ongoing and will hopefully confirm these results.

At the same time, many questions have been raised by the clinical studies to date. We will mention two of these. The first question is how to identify tumours that will benefit from these new drugs? BRCA-needs are not restricted to triple-negative breast cancers and can occur in other subtypes (in total, BRCA-needs may affect 25% of breast cancers) [34]. In addition, BRCA-needs can present in other tumour types such as head and neck squamous-cell carcinomas, non-small-cell lung cancers, and uterine cervical carcinomas. A major challenge in the coming years will be to identify these tumours with BRCA-needs. The second question is to understand to what extent the PARP enzyme must be inhibited. Results from phase II studies with olaparib suggest that the degree of PARP inhibition is important for response [82, 83].

Lastly, in the rush to bring PARP inhibitors to the clinic, some cautionary data are beginning to emerge. This includes evidence of an accumulation of DNA damage reported in pharmacodynamic sub-study from the phase I study of olaparib in patients with known BRCA mutations [see ‘KU-0099436/AZD2281 (olaparib)’ section] [5]. These data warrant careful consideration especially for the development of clinical trials in the adjuvant setting or for long-term usage in prevention studies in known BRCA mutation carriers.

**Funding**

Royal Australasian College of Physicians (to C.U.).

**Acknowledgement**

We thank Ms Pippa McKeve-Sebilleau for editorial assistance.

**Disclosure**

The authors declared no conflicts of interest.

**References**


63. Noel G, Godin C, Fernet M et al. Radiosensitization by the poly(ADP-ribose) polymerase inhibitor 4-amino-1,8-naphthalimide is specific of the S phase of the cell cycle and involves arrest of DNA synthesis. Mol Cancer Ther 2006; 5: 564–574.


