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Current Perspective

Poly(ADP-ribose) polymerase inhibitors in cancer treatment: A clinical perspective

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ABSTRACT

Inbuilt mechanisms of DNA surveillance and repair are integral to the maintenance of genomic stability. Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme that plays a critical role in DNA damage response processes. PARP inhibition has been successfully employed as a novel therapeutic strategy to enhance the cytotoxic effects of DNA-damaging agents. We have shown that PARP inhibition has substantial single agent antitumour activity with a wide therapeutic index in homologous DNA repair-defective tumours such as those arising in *BRCA1* and *BRCA2* mutation carriers. This is the first successful clinical application of a synthetic lethal approach to targeting cancer. Exploitation of defects in DNA repair pathways through targeted inhibition of salvage repair pathways is an exciting anticancer approach, with potentially broad clinical applicability. Several PARP inhibitors are now in clinical development. This review outlines the biological function and rationale of targeting PARP, details pre-clinical and clinical data and discusses the promises and challenges involved in developing these antitumour agents.

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1. Maintenance of genomic integrity

DNA is continually exposed to genotoxic stresses, which are either exogenous (e.g. ultraviolet or ionising radiation and genotoxic chemicals) or endogenous (e.g. cellular metabolism and free radical generation) in origin, with an estimated DNA damage rate of 10^4 events per cell/day.[1,2] If left unchecked, these DNA lesions may lead to mutagenesis and predispose to tumourigenesis or trigger cell death. There is an integrated network of DNA damage response mechanisms in place to cope with damage signalling, repair and maintenance of genomic integrity.[3] Specific DNA repair cascades are recruited according to the types of lesions and repair required. There is, however, sufficient interplay and redundancy between these DNA repair pathways to provide backup in the event that a particular pathway is deficient. Nucleotide-excision repair (NER), base-excision repair (BER) or mismatch repair (MMR) pathways are utilised for single strand breaks (SSBs) where the complementary DNA strand is intact and serves as a template. In contrast, homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways are recruited for the repair of DNA double-strand breaks (DSBs), which are devoid of a viable chromatid template.[3–5]

The preferred form of DSB repair is the highly conserved system of HR, which is reliant on sequence homology. Ataxia-telangeiectasia-mutated (ATM) and ataxia-telangeiectasia

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RAD3-related (ATR) recruitment mediates the DSB DNA damage response, by phosphorylating a range of key proteins such as CHK1, CHK2, histone H2AX, SMC1, FANCD2, BRCA1 and BRCA2, to initiate cell cycle arrest and DNA repair.[4,6] In contrast, NHEJ is a non-conserved pathway, which dissects the DNA damage and anneals DNA strands either through the addition or removal of nucleotides, or by connecting ends of previously unrelated DNA sequences. NHEJ is error-prone, thereby potentially resulting in genomic instability with altered nucleotide sequences, chromosomal rearrangements and mutations.[7]

Oxidative stress from background cellular metabolism predominantly results in SSBs and nucleotide base damage, which is mainly repaired by BER.[2] Poly(ADP-ribose) polymerase (PARP) is a critical component of this repair pathway. PARP-1 was identified 40 years ago and is the best defined of a family of 17 PARP enzymes.[8,9] PARP-1 and PARP-2 are the only members of the family known to be involved in DNA repair. PARP-2 has been shown to partly compensate for PARP-1 function, suggesting a degree of functional redundancy between these enzymes.[10,11] PARP-1 has three conserved structural and functional domains: the NH2-terminal domain which acts as the DNA nick sensor and binding domain, the central automodification domain with multiple glutamate residues for auto-poly(ADP-ribosyl)ation and the C-terminal catalytic domain of the enzyme.[9,12,13]

PARP-1 is a nuclear zinc-finger, DNA-repair enzyme that binds to the DNA strand break, signals the presence of DNA damage and initiates repair. PARP-1 is catalytically activated in the setting of DNA breaks. The polymerase utilises nicotinamide adenine dinucleotide (NAD)+ as a substrate, catalysing the synthesis and successive attachment of branched ADP-ribose polymers onto DNA, histones, PARP-1 and other DNA repair proteins.[13,14] These negatively charged ribosylated products in the vicinity of the break affect the unwinding of the DNA helix for repair access, form a scaffold for recruitment of various BER components and modulate a range of important cellular processes such as transcription, replication and differentiation. The presence of poly(ADP-ribose) chains at the site of DNA damage also acts as an anti-recombinogenic factor, preventing the inappropriate recombination of homologous DNA.[12,13,15] PARP has also recently been implicated in DSB repair responses.[15–17]

2. Pre-clinical development of PARP inhibitors

PARP cleaves NAD^+ to generate ADP and ADP-ribose which is then added to acceptor proteins through a process of ADP-ribosylation. PARP inhibitors resemble the NAD^+ moiety and are designed to competitively block the catalytic domain of the PARP enzyme. The earliest non-selective PARP inhibitor to enhance the cytotoxicity of a DNA-methylating agent in a murine model was the nicotinamide analogue 3-aminobenzamide (3-AB).[18] Early PARP inhibitors lacked potency and have since been superseded with more potent and specific PARP inhibitors through design optimisation of the NAD^+-binding site.[12,19,20] Many PARP inhibitors have been developed from structural modification of 3-AB with variable biochemical, pharmacokinetic and PARP selectivity properties. The most promising of these compounds include the benzimidazoles (ABT-888),[21,22] pthalazinones (AZD2281 or Olaparib),[23–25] ideno[1,2-c]isoquinolinones (INO-1001)[26,27] tricyclic indoles (AG-014699)[19,28–30]; BSI-201[31–33]; MK-4826[34] and most recently pyrrolocarbazole (CEP-9722).[34]

3. Therapeutic rationale for targeting PARP

Targeting DNA repair with PARP inhibitors has great promise as an anticancer therapeutic strategy either as a single agent, mediating selective killing in tumours with pre-existing HR DNA repair pathway defects, or as a resistance modifier in conjunction with other DNA-damaging agents including radiation therapy.

3.1. *Targeting tumours with HR DNA deficiency*

BRCA1 and *BRCA2* are tumour suppressor genes implicated in a variety of critical cellular processes, including cell cycle control, transcription regulation and DSB DNA repair.[7,35,36] The *BRCA1* gene is involved in DNA damage signalling, cell cycle checkpoint regulation and recruitment of repair enzymes, while *BRCA2* binds and translocates RAD51 to the site of DNA damage to initiate repair.[36–38] RAD51 forms a nucleoprotein filament which invades a homologous sequence of the sister chromatid to facilitate DNA sequencing and restoration of DNA to its original form.[37,39]

Loss of both wild-type alleles in tumours of *BRCA1* and *BRCA2* germline mutation carriers leads to dysfunctional HR DNA repair. Reliance on upregulated, lower fidelity and potentially mutagenic DSB DNA repair pathways confers cancer susceptibility through genomic instability.[40] *BRCA1* and *BRCA2* mutation carriers have a cumulative lifetime tumour risk of 84% and 60–80%, respectively, for breast cancer, and 40–50% and 10–20%, respectively, for ovarian cancer. They also have an enhanced predisposition for the development of other cancers.[41–44] A defining feature of tumours arising in *BRCA1* and *BRCA2* mutant carriers is the tumour-specific loss of DSB repair via HR and therefore selective sensitivity to DNA-damaging agents that induce DSBs.[45]

Synthetic lethality was first described more than 60 years ago as two non-lethal genetic defects, which are innocuous when occurring singularly, but which result in lethality in combination.[46] *BRCA1* and *BRCA2* deficient tumour cells lacking HR capacity are unable to repair the replication-associated DNA DSB damage that accumulates in the setting of suppressed PARP activity, leading to chromatin instability and promotion of cell death. PARP inhibition, exploiting the framework of synthetic lethality is therefore able to transform background endogenous DNA SSBs into persistent DSBs which are cytotoxic in the absence of HR repair capability (Fig. 1).[47,48]

Two groups have elegantly proven this concept pre-clinically by demonstrating that *BRCA1* and *BRCA2* deficient cells are exquisitely sensitive to PARP inhibition, in contrast to heterozygous mutant or wild-type cells. This confirmed that PARP-mediated BER is a synthetic lethal partner of complete *BRCA1* or *BRCA2* functional loss.[7,49,50] Inhibition of PARP-1-dependent SSB repair by BER results in the accumulation of SSBs, spontaneous stalled replication forks and the conver-

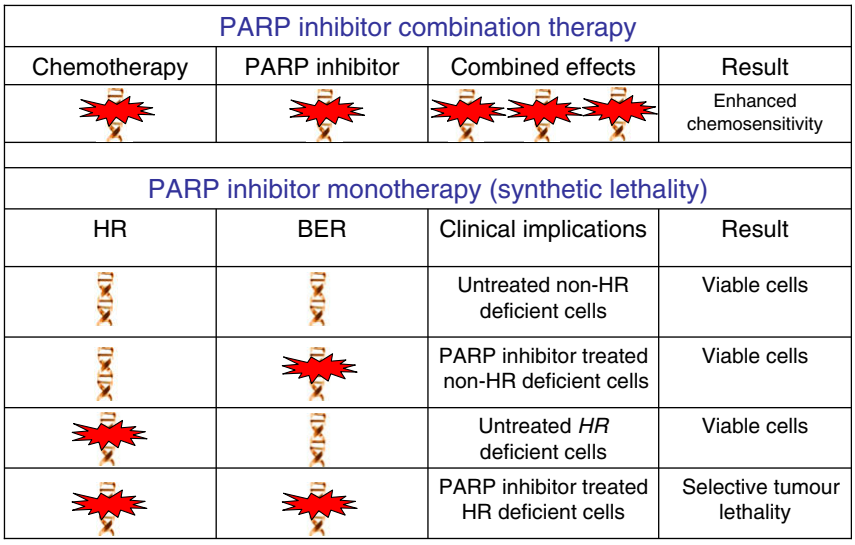

Fig. 1 – This schematic diagram highlights the ways in which PARP inhibitors are currently being utilised in the clinic. The PARP enzyme plays an important role in repairing DNA SSBs through the BER pathway to maintain genomic stability. SSBs produced by genotoxic stress from DNA-damaging chemotherapies, radiotherapy or endogenous insults are repaired through the BER pathway following activation of PARP. In the event that PARP activity is silenced, the SSBs persist and are converted to DSBs during DNA synthesis. These DSBs under normal circumstances are preferentially repaired by HR repair. Concurrent use of PARP inhibitors and DNA-damaging agents enhance the extent of DSBs which overwhelms the DNA damage repair capacity, resulting in increased cell apoptosis and potentiation of cytotoxic effects. In patients with deficiency in HR owing to loss of *BRCA1* and *BRCA2* function, the DSBs are repaired by alternative error-prone repair mechanisms such as NHEJ and SSA, resulting in genomic instability which leads to cell death.

sion of SSBs into potentially lethal DSBs.[49–51] These replication-associated DSBs are preferentially repaired by *BRCA*-dependent HR function in normal tissue, thus maintaining chromosomal stability and cell viability.[7,52] However, tumour cells devoid of *BRCA*-mediated HR function resort to compensatory error-prone NHEJ and single strand annealing (SSA) repair mechanisms, leading to chromosomal rearrangements, cell cycle arrest and apoptosis.[39,44] This differential ability in repairing DSBs in HR-proficient normal tissue which retains at least one wild-type *BRCA* allele versus HR-deficient tumours with biallelic *BRCA* loss provides a wide therapeutic window for improved efficacy and tolerability, which may be rationally exploited with potent and specific PARP inhibitors.[53,54]

Cells deficient in *BRCA2* (V-C8) were hypersensitive to the PARP inhibitors AG14361 and NU1025, with tumour regression noted in 3/5 and complete regression noted in 1/5 *BRCA2* defective xenografts.[49] Similarly, Farmer and colleagues reported selective killing of *BRCA1*- and *BRCA2*-deficient cell lines to the PARP inhibitors KU0058684 and KU0058948.[50] These pivotal results of profound cytotoxic activity in *BRCA*-mutated cell lines with sparing of wild-type *BRCA1* or *BRCA2* cells provided us with the impetus to rapidly apply this synthetic lethal strategy in patients with *BRCA*-deficient cancers.[24] It should be noted that the selective sensitivity of *BRCA1* and *BRCA2*-deficient cells is contingent on the potency and the specificity of the PARP inhibitor. CAPAN1 pancreatic cancer cell lines with *BRCA2* mutations were sensitive to KU0058948 but not to less potent PARP inhibitors such as 3-aminobenzamide and NU1025.[53,54]

Emerging evidence indicates that in addition to the small number of germline *BRCA1* and *BRCA2* mutation-related tumours there is a wider group of sporadic cancers which manifest clinical features of *BRCA*-like functional loss either through epigenetic inactivation of the *BRCA* genes or disruption of other non-redundant genes in the *BRCA*-associated HR repair pathway. Proficient, high fidelity DSB repair is tightly coordinated and involves a multitude of different interacting proteins, the disruption of which imposes the same functional HR repair defect and clinical outcome akin to cancers with *BRCA* mutations.[55,56]

These sporadic tumours display *BRCA*-like clinical properties reminiscent of hereditary cancers without *BRCA* gene loss, a phenomenon that has been described as 'BRCAness'.[55] Such sporadic tumours may therefore similarly benefit from the therapeutic approach of synthetic lethality with PARP inhibition. A striking example of this is the clinical and pathological likeness between triple negative (ER, PR and HER2 negative), basal-like breast cancers and hereditary *BRCA1* breast cancers suggesting a common pathogenic pathway.[55,57–60]

Some of the mechanisms accounting for the *BRCA*-like phenotype in sporadic tumours include epigenetic silencing of *BRCA* and *FANCF* genes through promoter methylation.[55,61,62] *BRCA1* gene inactivation via promoter methylation is implicated in up to 10–15% of sporadic breast cancers and 5–30% of sporadic ovarian cancers.[63–66] In contrast, the role of epigenetic silencing in disrupting BRCA2 protein function is less clear.[67] Overexpression of the *EMSY* gene which inversely suppresses *BRCA2* transcription has however been reported in 13% of sporadic breast cancers and 17% of

sporadic ovarian cancers.[68] In addition, disruption to other critical components of the HR repair pathway, such as RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA or FANCC has been shown to confer selective hypersensitivity to PARP inhibition.[55,56] Moreover, a tumour cell line harbouring epigenetic silencing of *BRCA1* function was selectively hypersensitive to PARP inhibition.[69] Taken collectively, this data supports the potential therapeutic role of PARP inhibition in a wider subgroup of sporadic tumours with defective HR function.

Overall, it is increasingly evident that genomic instability due to defects in DNA repair pathways is a common characteristic of many tumours.[70] This suggests that similar synthetic lethal strategies targeting DNA repair pathways may be broadly applicable to multiple tumour types. Greater insights into the specific DNA repair pathways that are dysfunctional in sporadic tumours, and their corresponding residual complementary pathways critical to tumour survival, will facilitate the development of targeted antitumour agents that exploit this paradigm of synthetic lethality.[7]

3.2. *Potentiating the cytotoxic effects of DNA-damaging agents*

DNA-damaging agents confer antitumour activity by inducing DNA damage; if unrepaired this damage leads to the intended outcome of tumour cell death. However, the intrinsic ability of tumour cells to repair the DNA damage incurred during genotoxic stress confers treatment resistance. PARP is a key player in DNA repair[14,71]; and is therefore an ideal therapeutic target for mitigating resistance and enhancing the cytotoxic effects of DNA-damaging agents (Fig. 1).[28,72] There is abundant preclinical data demonstrating that concurrent PARP inhibition sensitises tumour cells to cytotoxic therapy, including alkylating agents (cyclophosphamide and temozolomide), platinums (cisplatin and carboplatin), topoisomerase inhibitors (irinotecan and topotecan), anthracyclins (doxorubicin) and radiotherapy.[19,21,72–78]

The PARP inhibitors NU1025, NU1085,[75] CEP-6800 (a 3-aminomethyl carbazole imide),[73] INO-1001,[79] ABT-888[21,78] GPI 15427[80] and AG1436[72,19,48] have shown chemosensitisation with enhanced tumour regression, when combined with a range of cytotoxic chemotherapies in different tumour cell lines and xenograft models. A number of combination chemotherapy and PARP inhibitor clinical trials are currently underway. It however remains to be seen if a wide therapeutic window of selective tumour cell killing will hold true for other PARP inhibitor combination studies in unselected sporadic tumours.

Ionising radiation is also an efficacious and commonly utilised anticancer modality that induces SSBs and DSBs through the generation of oxidative free radicals. PARP plays a central role in repairing radiotherapy-induced DNA strand breaks, minimising potentially lethal radiation-induced damage and conferring resistance. PARP inhibition is therefore a rational therapeutic approach for radiosensitisation.[78,81,82] Several PARP inhibitors including AG14361, ABT-888 and CEP-9722 have shown radiosensitising properties in pre-clinical studies.[77,78,19,72]

4. Clinical development of PARP inhibitors

The clinical development of PARP inhibitors in cancer treatment initially focused on the robust pre-clinical evidence for chemo- or radiopotentiating effects (Table 1); this was followed by single agent studies based on the pre-clinical activity in *BRCA1*- and *BRCA2*-deficient cell lines (Table 2). Recent emerging data suggest that this therapeutic strategy may be more broadly relevant in HR defective sporadic tumours as well. There are currently several PARP inhibitors in various stages of clinical development. The safety, pharmacokinetic profile and preliminary efficacy of some of the agents already in clinical trials are summarised below. Early biomarker development in this field is also discussed.

4.1. *AG014699 (Pfizer Inc.)*

AG014699, a potent tricyclic indole PARP-1 inhibitor (IC_{50} < 5 nM) was combined with the DNA-methylating agent temozolomide in a phase I study,[29] based on pre-clinical data showing significant potentiation of temozolomide cytotoxic activity when co-administered with AG014699.[72] This study was the first ever PARP inhibitor study to establish a biologically effective dose based on target enzyme inhibition in surrogate tissue and to confirm corresponding PARP inhibition in tumour tissue. AG014699 was initially escalated with a fixed reduced dose of temozolomide (100 mg/m^2) until the PARP-inhibitory dose (PID), defined as PARP inhibition of >50% in peripheral blood lymphocytes (PBLs) 24 h post dosing was attained. The PID dose of AG014699 was established at 12 mg/m^2 based on PARP inhibition of between 74% and 97% in PBLs. AG014699 was subsequently fixed at 12 mg/m^2 whilst temozolomide was escalated to 200 mg/m^2 daily for 5 days, every 4 weeks. A further dose increase of AG014699 to 18 mg/m^2 was evaluated with no additional evidence of PARP inhibition in PBLs, but a trend toward a dose-dependent increase in PARP inhibition in tumour tissue. This dose however was intolerable with significant myelosuppression, necessitating dose delays and reductions. The mean terminal half-life of AG014699 was 9.5 h when given as a daily infusion. At the recommended phase II dose of 12 mg/m^2, AG014699 in combination with 200 mg/m^2 of temozolomide, mean tumour PARP inhibition of 92% (range 46–97%) was detected with no dose-limiting toxicities (DLT) observed.[29] One complete response (CR) and 2 partial responses (PRs) in 15 evaluable metastatic melanoma patients were seen at this dose level.

The phase II study of this combination reported a PR rate of 18% and prolonged disease stabilisation (>6 months) of 40% which is marginally better than the reported 13.5% objective response rate (ORR) and 18% stable disease (SD) rate seen with single agent temozolomide, suggesting a possible chemopotentiating effect.[30,83] There was however an increased frequency of myelosuppression with grade 4 neutropaenia and thrombocytopaenia being reported in 12–15% of cycles, necessitating a 25% dose reduction of temozolomide in 12/40 patients. A phase II evaluation of single agent AG014699 in *BRCA*-deficient advanced breast and ovarian cancers is ongoing.[34]

Table 1 – Key combination parp inhibitor clinical trials (*source*: *www.clinicaltrials.gov*).

PARP inhibitor	Company	Combination agent	Patient population	Phase of development	Clinical status	Route of PARP inhibitor
AG014699	Pfizer	Temozolomide	Advanced solid tumours	I	Completed	IV
AG014699	Pfizer	Temozolomide	Metastatic melanoma	II	Completed	IV
Olaparib	KuDOS/ AstraZeneca	Carboplatin and/or paclitaxel	Advanced solid tumours	I	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Dacarbazine	Advanced melanoma patients who have not received systemic cytotoxic chemotherapy	I	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Liposomal doxorubicin	Advanced solid tumours	I	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Paclitaxel	1st or 2nd line treatment of patients with metastatic triple-negative breast cancer	I/II	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Topotecan	Advanced solid tumours	I	Completed	PO
Olaparib	KuDOS/ AstraZeneca	Gemcitabine	Advanced solid tumours (escalation); pancreatic tumours (expansion)	I	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Cisplatin and gemcitabine	Patients with unresectable or metastatic solid tumours	I	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Bevacizumab	Advanced solid tumours	I	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Cisplatin	Advanced solid tumours (I); adjuvant triple-negative breast cancer (II)	I/II	Ongoing	PO
Olaparib	KuDOS/ AstraZeneca	Irinotecan	Advanced colorectal cancer	I	Ongoing	PO
ABT-888	Abbott Laboratories	Whole Brain Radiation	Patients with brain metastases	I	Ongoing	PO
ABT-888	Abbott Laboratories	Temozolomide	Advanced solid tumours	I	Ongoing	PO
ABT-888	Abbott Laboratories	Cyclophosphamide	Advanced solid tumours	I	Ongoing	PO
ABT-888	Abbott Laboratories	Topotecan	Advanced solid tumours	I	Ongoing	PO
BSI-201	BiPar Sciences	Gemcitabine + carboplatin	Triple-negative breast cancer patients	II	Ongoing	IV
BSI-201	BiPar Sciences	Temozolomide	Newly Diagnosed Malignant Glioma	I/II	Ongoing	IV
CEP-9722	Cephalon	Temozolomide	Advanced solid tumours	I	Ongoing	PO
INO-1001	Genentech/ Inotek Pharmaceuticals Corporation	Temozolomide	Newly diagnosed or recurrent unresectable stage III or stage IV melanoma	I	Terminated	IV

4.2. *Olaparib (KuDOS/AstraZeneca Pharmaceuticals)*

Olaparib (previously known as KU-0059436 and AZD2281) is a selective and potent type I and II PARP inhibitor with an IC_{50} of $\leqslant$2 nM. The first human phase I study of this drug was enriched with *BRCA1* and *BRCA2* mutation carriers with advanced tumours during the escalation phase, with *BRCA* carrier status mandated in the expansion phase. The maximum tolerated dose (MTD) of olaparib was established at 400 mg bd, continuously.[24,25] One case of DLT was observed at the dose of 400 mg bd (grade 3 fatigue) and two cases of DLT were seen at 600 mg bd, with grade 3 somnolence and grade 4 myelosuppression. At doses of 400 mg bd and below, olaparib was well tolerated with mild toxicities, predominantly gastrointestinal symptoms and fatigue. Pharmacokinetics were dose proportional with a terminal-elimination half-life of 5–7 h, supporting twice daily dosing. Pharmacodynamic assessment demonstrated significant PARP inhibition by more than 90% in normal tissue from doses $\geqslant$60 mg. In this study, 41% of 46 evaluable patients with *BRCA*-deficient ovarian cancers achieved objective responses (CR and PR) either by Gynaecologic Cancer Intergroup (GCIG) CA125 or

Table 2 – Key single agent parp inhibitor clinical trials (*source*: *www.clinicaltrials.gov*).

PARP inhibitor	Company	Patient population	Phase of development	Clinical status	Route
Olaparib	KuDOS/AstraZeneca	Advanced solid tumours enriched with BRCA1/2 mutation carriers	I	Completed	PO
AG014699	Pfizer	BRCA1/2 mutation carriers with advanced breast or ovarian cancer	II	Ongoing	IV
Olaparib	KuDOS/AstraZeneca	BRCA1- or BRCA2-positive advanced breast cancer (ICEBERG 1)	II	Ongoing	PO
Olaparib	KuDOS/AstraZeneca	Advanced BRCA1- or BRCA2-associated ovarian cancer (ICEBERG 2)	II	Ongoing	PO
Olaparib	KuDOS/AstraZeneca	2 doses of drug AZD2281 versus liposomal doxorubicin in BRCA1/2-advanced ovarian cancer patients who have failed platinum therapy (ICEBERG 3)	II	Ongoing	PO
Olaparib	KuDOS/AstraZeneca	Randomised, double blind, multicentre study in platinum sensitive serous ovarian cancer following treatment with $\geqslant$2 platinum-containing regimens	II	Ongoing	PO
Olaparib	KuDOS/AstraZeneca	Known BRCA or recurrent high grade serous/ undifferentiated tubo-ovarian carcinoma and in known BRCA or triple-negative breast cancer	II	Ongoing	PO
ABT-888	Abbott Laboratories	Refractory solid tumours and lymphoid malignancies	0/I	Completed	PO
MK-4827	Merck	Advanced solid tumour and BRCA1/2 mutation ovarian cancer	I	Ongoing	PO
BSI-201	BiPar Sciences	BRCA1- or BRCA2-associated advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer	II	Ongoing	IV
CEP-9722	Cephalon	Advanced solid tumour	I	Ongoing	PO
INO-1001	Inotek Pharmaceuticals Corporation	Subjects with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention	II	Completed	IV
INO-1001	Inotek Pharmaceuticals Corporation	High risk subjects undergoing cardiopulmonary bypass for coronary revascularisation and/or valve surgery	II	Completed	IV

by RECIST criteria and a further 11% of patients' attained RECIST disease stabilisation. The encouraging clinical benefit rate of >50% in the subpopulation of patients with *BRCA* mutanted disease provides clinical validation for single agent PARP inhibitor activity in *BRCA*-deficient cancer patients.[25] Responses were seen in patients with platinum-refractory (18%), - resistant (44%) and - sensitive (80%) ovarian cancers, being most frequent in the latter group. Apart from ovarian cancer, objective antitumour activity was also seen in *BRCA*-mutated breast and prostate cancer patients.[24,25]

Two parallel open-labelled multicentre phase II studies of olaparib in *BRCA1* and *BRCA2* mutation carriers with advanced breast and ovarian cancer recently confirmed significant therapeutic efficacy and established proof-of-concept for targeting cancers in *BRCA* mutation carriers with PARP inhibitors.[84,85] The design of the two studies was similar;

54 breast cancer patients and 56 ovarian cancer patients with advanced heavily pre-treated disease were recruited to two non-randomised sequential dose cohorts of 100 mg bd (a pharmacodynamically active dose) and 400 mg bd (previously established MTD) of olaparib. Following efficacy assessment of the two dose levels, patients on the 100 mg bd cohort in both studies were allowed to cross over to the higher dose cohort of 400 mg bd, given improved antitumour activity seen at this dose level. The breast cancer study reported an ORR of 41% (11/27) with 1 CR and 10 PRs and a median progression-free survival (PFS) of 5.7 months (range 4.6–7.4 months) at 400 mg bd in contrast to an ORR of 22% (6/27) with 6 PRs and a median PFS of 3.8 months (range 1.9–5.5 months) at the lower dose level.[84] In the ovarian study, patients on the 400 mg bd and 100 mg bd showed an ORR of 33% (11/33) versus 13% (3/24) by RECIST criteria, a response by either RECIST or GCIG criteria of 61% (20/33) versus 17% (4/24) and a median duration of response of 290 d (range: 126–513 d) versus 269 d (range: 169–288), respectively.[85] Of note, 20–30% of the patients on the breast study had prior platinum exposure whilst more than three quarters of patients on the ovarian study were defined as platinum resistant.[84,85] The clinical implication of responses to prior platinum therapy in relation to subsequent PARP inhibitor therapy requires further study.

Overall, olapranib was well tolerated with a similar toxicity profile to that observed in the phase I study. Both dose levels demonstrated clinical activity, however, the 400 mg bd appeared to be more efficacious with improved outcomes. Both studies have confirmed the impressive activity of olaparib at a dose of 400 mg bd in heavily pre-treated, *BRCA* mutation carriers with advanced breast and ovarian cancer.[84,85] A number of combination studies with carboplatin and paclitaxel, topoisomerase inhibitors, gemcitabine and bevacizumab in advanced solid tumours are ongoing.[34] A neoadjuvant study of cisplatin and olaparib is planned for patients with triple-negative breast cancer.[34]

4.3. *ABT-888 (Abbott Laboratories)*

ABT-888 was the first anticancer compound to be evaluated in a phase 0 clinical trial. This biomarker-driven study was designed to elucidate the dose range at which effective target modulation is observed in peripheral blood mononuclear cells (PBMCs) and tumour tissue, as measured by an enzyme-linked immunosorbent assay (ELISA), and to determine the pharmacokinetic profile of ABT-888.[22] ABT-888 demonstrated good bioavailability with a half-life of several hours and clearance after 24 h of dosing. PARP inhibition of >85% lasting up to 24 h post-ABT-888 administration was observed in tumour tissue and PBMCs at the dose levels of 25 and 50 mg. Tumour biopsies performed at baseline and 3–6 h post treatment demonstrated PARP inhibition of 92–100%, thus validating the ELISA assay.[22] The pharmacokinetic and pharmacodynamic data obtained from this study served as a platform for several phase Ib combination studies of ABT-888 with temozolomide, irinotecan, cyclophosphamide and carboplatin. A phase I study of ABT-888 and whole brain radiotherapy for cerebral metastasis has started accrual.[34]

4.4. *BSI-201 (BiPar Sciences Inc.)*

BSI-201 is a small molecule intravenous PARP-1 inhibitor. The phase I study of BSI-201 evaluated escalating doses (0.5–8 mg/kg) delivered twice weekly in 23 patients with advanced solid tumours. BSI-201 was well tolerated without a defined MTD. The biologically active dose of BSI-201 was established at $\geqslant$2.8 mg/kg based on pharmacodynamic data of effective target modulation (PARP inhibition of >50% and 80% after single and multiple dosing in PBMCs), as well as the knowledge that, trough concentration at this dose exceeds the pre-clinically defined biologically effective dose. The $t_{1/2}$ is several minutes with pharmacokinetic evidence of longer acting active intermediate metabolites accounting for prolonged PARP inhibition. A subsequent phase Ib study evaluated escalating doses of BSI-201 (2.0–11.2 mg/kg) twice weekly combined with multiple cytotoxic chemotherapy including topotecan (1.5 mg/m^2 or 1.1 mg/m^2 twice a day for 5 out of 21 d, gemcitabine (1000 mg/m^2, weekly, 7 out of 8 weeks), temozolomide (75 mg/m^2 twice a day for 21 out of 28 d) or carboplatin (AUC6) and paclitaxel (200 mg/m^2) in a 4-arm non-randomised study.[31,32] The cytotoxic chemotherapy and BSI-201 combinations were well tolerated at all dose levels studied with no DLT reported. Of the 66 evaluable patients recruited, there was 1 CR in an ovarian cancer patient and a further 6 PRs observed in patients with renal cell ($n = 1$), breast ($n = 2$), uterine ($n = 1$), and ovarian cancers ($n = 1$) and sarcoma ($n = 1$). However, as with any combination study, it is difficult to tease out the potential contributions of PARP inhibitor versus cytotoxic agent in terms of accounting for the efficacy observed in these different tumour types.

A multicentre, open-labelled randomised phase II study of gemcitabine plus carboplatin, with and without BSI-201 recently reported promising chemopotentiating effects without additional chemotherapy-related toxicities in patients with advanced triple-negative (ER/PR/HER2 negative) breast cancer.[33] A total of 116 patients received gemcitabine 1000 mg/m^2 plus carboplatin AUC2 on days 1 and 8, with or without intravenous BSI-201 at 5.6 mg/kg on days 1, 4, 8 and 11, of a 21 day cycle. Patients on the chemotherapy alone arm were allowed to cross over to the triple combination on disease progression. The preliminary clinical benefit (PR and SD) rate was 62% versus 21%, ($p = 0.0002$) in favour of the experimental arm. Likewise, the ORR of 48% versus 16% ($p = 0.002$), median PFS of 6.9 months versus 3.3 months, $p < 0.0001$ [HR 0.342 (95% CI, 0.200–0.584)] and median overall survival (OS) of 9.2 months versus 5.7 months, $p = 0.0005$ [HR = 0.348 (95% CI, 0.189–0.649)] significantly favoured the carboplatin, gemcitabine and BSI-201 arm. This study has provided clinical evidence for the chemopotentiating effects of PARP inhibition although concerns remain that patients in the control arm of this study received a sub-therapeutic dose of carboplatin, resulting in a lower response rate and PFS compared with an ORR of 38–41% and PFS of 5.8–6 months observed with platinum-based combinations in this setting.[86,87] Nonetheless, this study supports further evaluation of PARP inhibitors in sporadic tumours which potentially harbour defective HR DNA repair pathways, including triple-negative breast cancers. BSI-201 is also currently being evaluated as a single

agent in *BRCA1* and *BRCA2*-associated ovarian, primary peritoneal and fallopian tube cancers.[34]

4.5. *INO-1001 (Inotek Pharmaceuticals)*

INO-1001 was granted orphan drug status for cardiovascular indications based on the clinical evidence of its ability to blunt reperfusion injuries and postoperative complications. A phase Ib study of INO-1001 evaluated escalating doses (100–400 mg) of intravenous INO-1001 administered twice daily for 5 days in combination with temozolomide at standard doses (200 mg/m^2 days 1–5), every 4 weeks in patients with stage III/IV melanoma.[27] Of the 12 evaluable patients, 1 patient had a PR and a further 4 patients had SD, with a median PFS of 2.2 months and median survival of 17 months. Notably, grade 3 or 4 myelosuppression was observed in 7/12 (58%), requiring the use of granulocyte colony-stimulating factor and/or dose reduction of temozolomide. Dose-limiting myelosuppression and hepatic toxicity were observed at a dose of 400 mg. INO-1001 at 200 mg BD for 5 days in combination with temozolomide delivered at a standard dosing schedule was well tolerated. This combination is being evaluated in patients with malignant glioma.[14]

4.6. *MK-4827 (Merck Inc.) and CEP-9722 (Cephalon Inc.)*

A phase I clinical trial of MK-4827 is currently ongoing in patients with advanced solid tumours recruited to the dose escalation phase of the study. Once the biologically relevant dose is achieved, a phase Ib expansion in *BRCA*-mutant ovarian cancer patients is planned.[34] CEP-9722, either as single agent or in combination with temozolomide is currently being tested in patients with advanced solid tumours in the phase I setting.[34]

5. Resistance to PARP inhibitors

Mechanism-based, acquired resistance is a common feature of molecular targeted agents and arises as a consequence of selection pressures following drug exposure.[88,7] Increased reliance on upregulated, less conserved, DNA repair pathways such as SSA[40] in the context of *BRCA* deficiency is felt to promote intragenic deletions, which on occasion can rectify the pre-existing frameshift truncating mutation, and consequently reinstate the *BRCA* reading frame and *BRCA* function.[88,89,7] These resistant cancer clones with restored BRCA-mediated HR function have a survival advantage and are therefore naturally selected for with the ongoing treatment. This model of acquired resistance was reported using the CAPAN1 cell line, which carries a protein-truncating c.6174delT *BRCA2* frameshift mutation and is devoid of the wild-type *BRCA2* allele. The truncated *BRCA2* protein lacks prerequisite functional elements for *BRCA2*-mediated HR repair[88–93,38] and consequently CAPAN1 cells are highly sensitive to PARP inhibition.[53,88] Selection pressure from protracted high dose exposure to KU0058948 results in multiple highly resistant clones with evidence of secondary intragenic deletions of the c.6174delT mutation that restored *BRCA2* open reading frame and protein function.[89] It is not surprising that these PARP inhibitor-resistant clones were also found to be cross-resistant to cisplatin since platinum sensitivity may also be in part related to the BRCA*ness* phenotype by inducing increased DNA crosslinks and DSBs which go unrepaired in the absence of HR DNA repair.[89,50,45,7]

This proposed mechanism of *in vivo* resistance acquisition is further supported by the identification of similar secondary open reading frame restoring mutations in ovarian cancer patients with previously known *BRCA2* c.6174delT mutations who have subsequently developed platinum resistance.[89,94] Secondary genetic mutations that reinstate the wild-type *BRCA1* reading frame have also been observed in *BRCA1*-mutated platinum-resistant ovarian tumours and may account for acquired resistance in these patients.[95]

Cisplatin-resistant CAPAN1 cells with a secondary *BRCA2*-restoring mutation acquired through cisplatin selection were also cross-resistant to the PARP inhibitor AG14361. Of interest however was the fact that not all the CAPAN1 platinum-resistant cells acquired in this way had a secondary *BRCA2*-restoring mutation suggesting that other potential mechanisms of platinum resistance are at play. Cisplatin-resistant CAPAN1 cells without the secondary *BRCA2* mutation reversion remained sensitive to AG14361.[94] These findings mirror what has been observed clinically. Platinum-sensitive, - resistant or - refractory ovarian and breast cancer patients with inherited *BRCA* mutations had responses to PARP inhibition in phase I and II studies of olaparib,[24,25,84,85] making any clinical interpretations about possible shared resistance mechanisms and cross resistance between PARP inhibitors and platinum compounds difficult at this time. It is plausible that other *BRCA1* or *BRCA2* mutations may result in genetic reversion in a similar way, accounting for *de novo* or acquired resistance.[95] It is likely, however, that other resistance mechanisms independent of restoration of BRCA function may also be relevant. These remain to be defined.[94]

6. Challenges and future directions

PARP inhibitors were initially developed as a therapeutic sensitiser to enhance the cytotoxic effects of DNA-damaging agents by preventing the repair of lethal DNA lesions incurred in the context of genomic stress. Fuelled by robust pre-clinical data supporting synergism, combination therapies with multiple cytotoxic agents are currently being extensively evaluated. The optimal PARP inhibitor–chemotherapy drug combination however remains to be established. Another challenge of concurrent treatment is selecting the appropriate dose and schedule of both PARP inhibitor and cytotoxic agent to optimise efficacy while mitigating side-effects. Concurrent therapies can potentially amplify toxicities and will require cautious drug escalation if additional toxicies are to be avoided. While the mechanism of action of PARP inhibitors suggests that their administration should precede DNA-damaging agents, this remains unresolved.[14] Pharmacodynamic markers of PARP inhibition in PBMC correlate with target modulation in tumour and are useful to ensure that biologically active doses of PARP inhibitors are used in combination studies. Despite the importance of such pharmacodynamic studies to reject the 'No–Go' decision, it is unclear whether

these agents are best dosed at the maximum tolerated dose to maximise the induction of double-strand DNA breaks or at lower PARP inhibitory doses.

The best developed of these pharmacodynamic assays for PARP inhibition is the ELISA for ADP-ribose polymer (PAR) activity. This has been incorporated into the trial design of multiple PARP inhibitor studies.[22,24,96] Such pharmacodynamic markers are not, however, direct surrogates for antitumour activity and will thus require correlation with clinical outcome in larger clinical studies. There is a clear unmet need for the development of intermediate surrogate markers of clinical benefit which are likely to be of particular value in the context of developing these agents as maintenance or prophylactic therapy.

Despite *in vivo* evidence of radiosensitisation and multiple potential clinical settings where radiotherapy constitutes the dominant treatment modality, the clinical evaluation of combined PARP inhibition with radiotherapy has lagged behind other strategies. Registration studies for radiotherapy combinations remain difficult to pursue. Such combinations require careful consideration of the complex interaction of dose and schedule for both radiotherapy and PARP inhibitors, as well as the potential for delayed and cumulative toxicity.

PARP inhibitors have shown exciting single agent activity in patients with *BRCA*-deficient tumours. The concept of 'BRCAness' is likely to extend the benefits of single agent PARP inhibition to a much wider group of patients with sporadic tumours than previously anticipated.[55] The challenge remains identifying this subgroup of patients with non-*BRCA* mutation-related HR repair-deficient tumours. There are a multitude of genes known to be involved in the process of HR DNA repair and it is increasingly understood that disturbance at multiple points in the repair process can lead to the same functional loss and clinical outcome.[55] Validated, readily applicable predictive determinants of response are essential for optimal patient identification to improve clinical outcomes. High throughput PARP-inhibitor synthetic lethal short interfering RNA (siRNA) screens may provide a useful approach to identify new predictors of PARP inhibitor sensitivity for therapeutic exploitation in the clinic.[97]

Cancer prevention strategies such as prophylactic risk-reducing surgery, hormonal treatments and surveillance are currently accepted ways of managing *BRCA1* and *BRCA2* mutation carriers who are at increased risk of developing malignancies.[98] Despite these efforts, however, many of these patients still develop *BRCA* mutation-related cancers. PARP inhibitors are generally well tolerated as single agents and have been suggested as a possible chemoprophylactic measure to avert cancer development in *BRCA* mutation carriers by selectively eliminating clones with biallelic *BRCA* loss prior to tumourigenesis.[99,100] The potential therapeutic utility of such an approach is attractive but it is premature to suggest that this is a feasible strategy without longer term safety data to exclude detrimental effects.[99] There are valid concerns that the chronic inhibition of an essential DNA repair pathway could potentially lead to enhanced mutagenesis and development of secondary malignancies.[99,101]

Tumours commonly possess underlying DNA repair defects that foster genomic instability, carcinogenesis but also provide a therapeutic target for rationally designed treatments.[39] Identifying key gene–gene interactions within tumours that can be targeted may provide novel therapeutic approaches for selective cytotoxicity to tumour tissue, while sparing normal tissue.[7] There is a great interest in the therapeutic application of agents that impair other DNA-repair enzymes apart from PARP, such as inhibitors of O-methylguanine–DNA methyltransferase (MGMT) or excision repair cross-complementation 1 (ERCC1).[102] Reduced expression of these DNA repair proteins is linked with enhanced sensitivity to certain cytotoxic agents.[103,104] Likewise, a number of emerging key targets of DSB repair including ATM, ATR and DNA-PK, as well as CHK1 and CHK2 kinases are currently under evaluation. Small-molecule inhibitors of these proteins sensitise cells to genotoxic damage, suggesting a possible cytotoxic potentiating role in combination with DNA-damaging agents.[39] Inhibition of both DNA-dependent protein kinases and PARP enzymes prevent 90% of DSB repair and have shown additive radiosensitisation.[81,105] Such agents currently in development include KU55933, a potent small-molecule inhibitor of ATM, several inhibitors of DNA-PK, including Vanillin, Salvicine, OK-1035, NU7026, NU7441 and IC87102, as well as selective CHK1 inhibitors (CEP-3891), selective CHK2 inhibitor (CEP-6367) and dual CHK1 and 2 inhibitors (XL844).[39,106,107] A key challenge to the study of these agents is the identification of broad therapeutic windows for selective tumour cell cytotoxicity.

Although PARP inhibition is an exciting novel therapy in cancer with potentially broad applications, there remain several unresolved issues. These include the establishment of the optimal selectivity and potency of PARP inhibitors necessary for the specific targeting of HR-deficient tumours over normal tissue. Identifying predictive biomarkers to broaden the clinical application of these agents and characterising mechanisms of drug resistance are also key. Finally, other strategies for utilising these agents in the clinic such as the role of PARP inhibitors in chemoprevention, maintenance, adjuvant and as single agent therapy in sporadic tumours will need to be clearly defined in the future.

7. Conclusion

PARP inhibitors offer the promise of effective chemo- and radiopotentiation effects and have demonstrated compelling single agent antitumour activity in *BRCA*-deficient tumours, providing the first example of successful exploitation of tumour synthetic lethality in the clinic. The prospects of abrogating a complementary tumour-reliant pathway in the context of disrupted DNA damage responses provide a framework that could potentially find broad application in many tumour types. The concept of BRCA*ness* suggests that the therapeutic utility of PARP inhibitors may be extended to non-*BRCA* mutation-related HR-deficient tumours. The challenge however remains the identification of such tumours. The use of rationally designed and clinically validated pharmacodynamic, prognostic and predictive biomarker studies will be imperative to facilitate the optimal development of these agents and selection of appropriate patients to improve clinical outcomes.

Conflict of interest statement

None declared.

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