

The potential for poly (ADP-ribose) polymerase inhibitors in cancer therapy

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Abstract: The modulation of DNA repair pathways for therapeutic benefit in cancer has now become a reality with the development of poly (ADP-ribose) polymerase inhibitors (PARPi). PARP is involved in single-strand DNA breaks, which in the presence of defective homologous recombination repair lead to double-strand DNA breaks, the most lethal form of DNA damage. These agents therefore may be the drugs of choice for BRCA mutant breast and ovarian cancers. PARPi result in synergistic antitumor effects when combined with cisplatin, temozolomide, topoisomerase inhibitors and ionizing radiation. The indications for PARPi lie beyond BRCA mutations and may include genomic and functional defects in DNA repair and damage response pathways. Several PARPi are in the clinical development phase at this time and, given the recent failure of a phase III clinical trial of iniparib in triple-negative breast cancer, the identification of structural and functional differences between these inhibitors becomes critical. Acquired resistance to PARPi is being noted and represents an important limitation in this field. A concise review of the literature in this field is presented.

Keywords: BRCA2 gene, DNA repair-deficiency disorders, poly (ADP-ribose) polymerases

Genomic instability: an 'enabling' characterisation of cancer

The human genome is continually exposed to potentially deleterious genotoxic events. These events could be endogenous, including oxidative stress from normal metabolism and DNA replication and recombination aberrations or exogenous, resulting from exposure to genotoxic agents such as chemical mutagens and ultraviolet light. Several detection and signaling pathways are activated by DNA damage, resulting in the recruitment and activation of groups of proteins to repair the incurred damage by the appropriate DNA repair pathway. This results either in cell cycle arrest, thereby allowing sufficient time for repair to take place, or in the case of irreparable damage, programmed cell death.

It follows therefore that the genes responsible for DNA damage detection and repair essentially behave as tumor suppressors and their defects would enable tumorigenesis in the presence of ongoing genotoxic stress. The consequent genetic or epigenetic alterations such as mutations, methylation or histone modifications result in genomic instability, which can lead to the evolution of a subclone of cells with a selective growth

advantage. These variant subclones may outgrow normal, unaffected tissues and successive clonal expansions of mutated cells may be responsible for the multistep process of tumorigenesis. While hematologic malignancies have only a limited number of genetic alterations, in the case of solid tumors, myriad genetic alterations lead to genetic heterogeneity. Genomic instability has been described as an enabling characteristic of cancer and is broadly classified into microsatellite instability associated with the mutator phenotype, and chromosome instability recognized by gross chromosomal abnormalities [Hanahan and Weinberg, 2011].

Several DNA repair pathways are responsible for the maintenance of genomic integrity, each of which repairs a specific type of DNA damage. These include nonhomologous end joining (NHEJ), homologous recombination (HR), base excision repair (BER, also called single-strand break repair [SSBR]), nucleotide excision repair (NER), mismatch repair (MMR) and translesion synthesis (TLS). Each of these pathways repairs a specific DNA defect. For instance, MMR is involved in the detection and repair of base mismatches, insertions and deletions, BER/SSBR

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removes incorrect bases and repairs resultant nicks while NHEJ and HR are involved in the repair of DNA double-strand breaks (DSBs) and repair of DNA crosslinks is complex involving NER, HR and possibly other pathways. Interestingly, although DNA repair defects lead to increased tumorigenesis, a paradoxical situation arises from the fact that in cancer cells, sustained replication in the presence of genotoxic stress also requires some intact DNA repair pathways. Thus, cancers with aberrant DNA repair pathways become ‘addicted’ to one or more retained intact repair pathways to preserve their growth. This can represent a resistance mechanism to certain types of DNA damaging chemotherapy and radiotherapy. Targeting the upregulated DNA repair pathway can enhance the DNA damage and antitumor activity induced by radiotherapy and chemotherapy. These upregulated DNA damage signaling and repair pathways that cancer cells are addicted to may also represent the cancer’s ‘Achilles’ heel’. A specific inhibitor to the pathway could potentially lead to a selective antitumor effect by preventing the repair of intrinsic DNA damage by exploiting the principle of synthetic lethality.

Synthetic lethality

Originally described by the geneticist Dobzhansky in the 1940s, synthetic lethality refers to an interaction in which the individual deletion of either of two genes has no effect but the combined deletion of both genes is cytotoxic (Figure 1). Synthetic lethality can also be exploited in the treatment of cancer as in the case with cancer susceptibility syndromes, such as *BRCA1* or *BRCA2* mutations. The latter genes play a key role in maintaining genomic integrity due to their involvement in HR, an important repair pathway for DNA DSBs. Cancer cells with aberrant HR secondary to *BRCA* mutations are critically dependent on BER/SSBR for viability. The enzyme, poly (ADP-ribose) polymerase 1 (PARP-1) is critical for BER/SSBR (described below). Inhibition of PARP-1 leads to an accumulation of unrepaired SSBs and therefore is synthetically lethal in the case of *BRCA1* or *BRCA2* mutations due to the accumulation of fatal replication fork collapse and DSBs as was demonstrated by two independent groups [Bryant *et al.* 2005; Farmer *et al.* 2005]. Recent evidence suggests that activation of NHEJ is necessary for synthetic lethality, suggesting that the error-prone repair of replication-associated DSBs is associated with the

cytotoxicity of PARP inhibitors (PARPi) in HR-defective cells [Patel *et al.* 2011]. While PARPi are effective in the case of *BRCA1* or *BRCA2* mutations, the paradigm of synthetic lethality can also be extended to other cancers, including sporadic cases. HR is a complex process involving many components including ATM, ATR, CHK1, RAD51 and its homologues, the FANC proteins, MRE11/RAD50/NBS1 (MRN) and loss of function in any of these components may confer sensitivity to PARPi [McCabe *et al.* 2006]. PARPi may also be synthetically lethal in cases where epigenetic silencing of *BRCA* occurs [Drew *et al.* 2010]. This effect in sporadic breast and ovarian cancers was referred to as ‘BRCA-ness’ [Ashworth, 2008] but it is now apparent that this BRCA-centric view is misleading as defects in other HR components are associated with a variety of cancers, e.g. ATM defects in mantle-cell lymphoma, which may also benefit from PARPi therapy [Williamson *et al.* 2010]. EMSY and PTEN have also been implicated as they regulate the activity of other components of HR [Cousineau and Belmaaza, 2011; McCellin *et al.* 2010].

PARP structure–function relationship

At the current time, a total of 16 PARP family members have been identified of which PARP1, PARP2, PARP4 (Vault-PARP), Tankyrase-1 and 2 have confirmed poly (ADP)-ribosylating activity and only PARP-1 and PARP-2 are involved in DNA repair [Schreiber *et al.* 2006]. Recently, PARP-3 was identified as cooperating with PARP-1 in DNA DSB repair, but deletion of PARP-3 alone does not compromise survival after DNA damage and the mechanisms remain to be fully elucidated [Boehler *et al.* 2011]. PARP-1 was the first member of this family to be discovered and its function in the maintenance of genomic integrity has been well documented. In response to DNA single-strand breaks resulting from genotoxic stimuli, the PARP reaction uses nicotinamide adenine dinucleotide (NAD) as a substrate to generate poly (ADP-ribose) (PAR) [De Murcia, G. and Menissier De Murcia, J. 1994; De Murcia, G *et al.* 1994].

PARP-1 and PARP-2 form homodimers and heterodimers at DNA breaks catalyzing the formation of long PAR chains covalently attached to PARP-1 itself (automodification) or other nuclear proteins, e.g. histone H1 heteromodification adjacent to the DNA breaks. These negatively charged polymers form a scaffold and

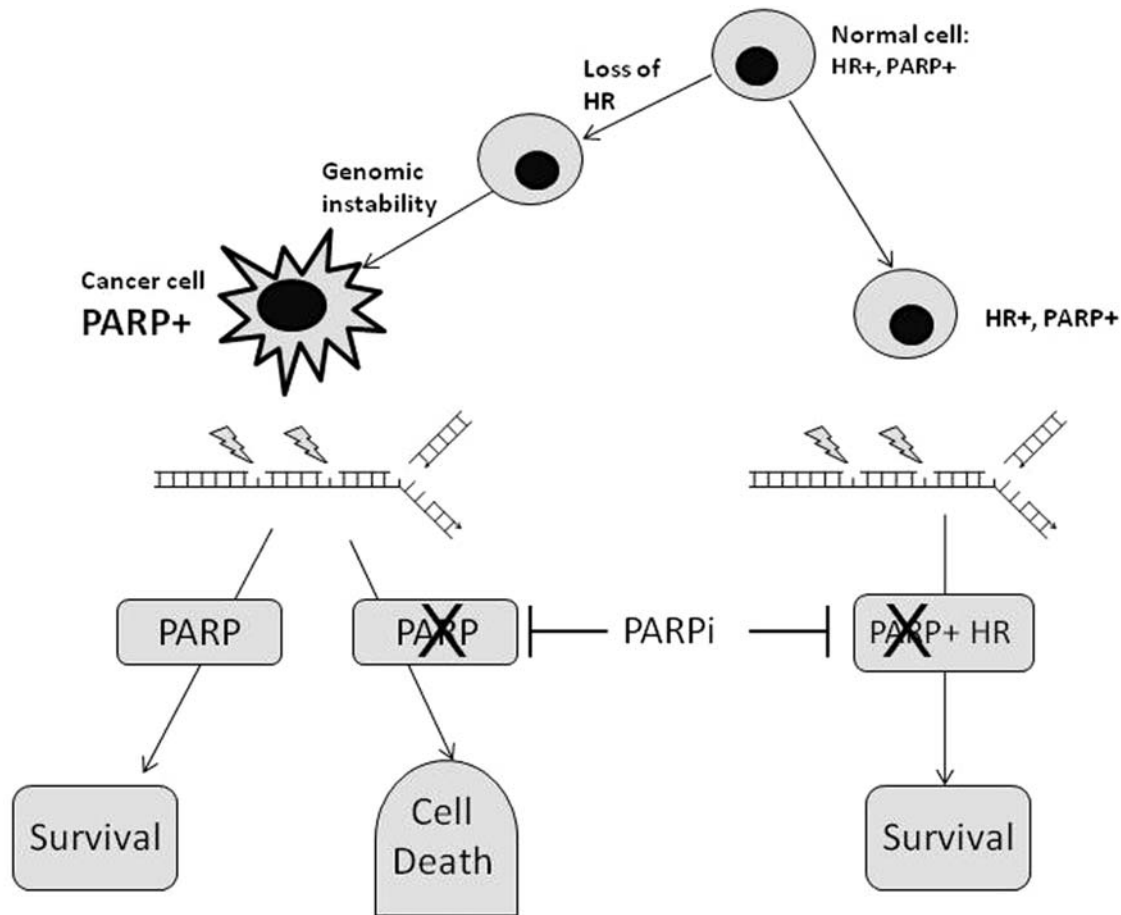


Figure 1. Poly (ADP-ribose) polymerase (PARP) is upregulated in conditions causing genotoxic stress, leading to increased single-strand break repair. In cases of homologous recombination (HR) deficiency, this becomes the main pathway for DNA repair and therefore its inhibition leads to synthetic lethality. PARPi, PARP inhibitor.

recruit other proteins that are critical in BER [De Murcia *et al.* 1994] and chromatin remodeling [Ahel *et al.* 2009; Timinszky *et al.* 2009].

PARP activity also promotes the activation of mitotic recombination 11 (MRE11) and Nijmegen breakage syndrome (NBS), members of the DNA damage-sensing MRN complex which activates ATM to sites of double-strand DNA damage [Haince *et al.* 2007]. Thus, the role of PARP-1 in DNA repair extends beyond the repair of DNA single-strand breaks. PARP-1 not only plays a critical role in genomic maintenance but is also involved in transcriptional regulation, energy metabolism and cell death and these roles are discussed below.

PARP-1 has three distinct domains: an amino terminal DNA-binding domain, a nuclear

localization signal, an automodification domain and a carboxy-terminal catalytic 'PARP-signature' domain that is responsible for PAR formation [De Murcia *et al.* 1994]. The DNA-binding domain also contains two zinc fingers that are required for the detection of DNA strand breaks resulting eventually in PARP-1 activation while a third zinc finger motif coordinates DNA-dependent enzyme activation.

The baseline activity of PARP-1 is low, but is stimulated by DNA strand breaks. PARP is upregulated in several cancers, implying its possible role in cancer growth and survival [Virag and Szabo, 2002]. In colorectal cancer, for instance, PARP-1 mRNA overexpression was detected in over 70% of colorectal cancers and correlated with the expression of beta-catenin, c-myc, cyclin D1 and MMP-7 [Nosho *et al.* 2006].

Inhibition of PARP is detrimental to cancer cells. However, PARP inhibition may not result in critical injury to normal cells. PARP-1 knockout mice have been reported to grow normally [Shall and De Murcia, 2000], however, the inactivation of both PARP-1 and PARP-2, confers embryonic lethality [Schreiber *et al.* 2002]. Owing to the very close structural homology of the catalytic domains of PARP-1 and PARP-2, it is thought that most PARPi inhibit both enzymes. Therefore, PARP inhibition in the clinical setting could potentially cause serious adverse effects but the experience to date suggests that profound PARP inhibition is associated with very mild toxicity. The clinical application of PARPi is therefore an active area of research and development.

Development of PARP Inhibitors

The first-generation PARPi included nicotinamide, benzamides and substituted benzamides such as 3-aminobenzamide (3-AB). These agents had relatively low potency and specificity and, therefore, second-generation benzamides and more recently, third-generation inhibitors, most of which are competitive NAD⁺ inhibitors and based on 3-AB structure, such as the nicotinamide pharmacophore have been developed (Figure 2). The preclinical/clinical development of PARPi has been as: (a) single agents in cases of deficient DNA repair mechanisms such as *BRCA1* or *BRCA2* mutations; (b) combined with cytotoxics (PARPi sensitize tumor cells to chemotherapeutic anticancer agents that induce DNA damage through BER; these agents include temozolomide, platinum analogues and topoisomerase-1 inhibitors); or (c) as radiation sensitizers [Calabrese *et al.* 2004]. Radiosensitization by PARP inhibition is enhanced in the presence of defective DNA repair and is more pronounced in rapidly dividing cancer tissues in the S phase as compared with normal, noncycling cells and may thus result in an enhanced safety ratio [Dungey *et al.* 2008]. *PARP* knockout models have been utilized to confirm the chemo- and radio-potential of the PARPi. Both *PARP-1* knockout and *PARP-2* knockout mice are hypersensitive to ionizing radiation (IR) and DNA alkylating agents (as reviewed by Rouleau *et al.* [2010]). In preclinical cancer models, Tentori and colleagues noted that melanoma models with stably silenced PARP-1 expression were highly sensitive to temozolomide. In the same study, decreased tumorigenicity and angiogenesis were noted in the PARP-1^{-/-} melanoma models [Tentori *et al.* 2008]. On the other hand, Chalmers and

colleagues, noted that while chemical inhibition of PARP-1 markedly enhanced the efficacy of low-dose radiation, such an effect was lacking in PARP-1 knockout models. The latter may be explainable on the basis of PARP-2 upregulation, which may compensate for the absence of PARP-1 [Chalmers *et al.* 2004]. Thus PARPi, by inhibiting both PARP-1 and PARP-2, are likely to have more profound effects than indicated by genetic knockout of only one of the two enzymes. Select PARP-1 inhibitors in clinical trials are discussed below.

AG014699 (PF-01367338)

AG014699 is a phosphate salt of AG14447 with aqueous solubility and was selected as suitable for clinical trial from a panel of 42 potential PARPi based on its chemo- and radio-potentiating effect [Thomas *et al.* 2007]. This PARPi, and its fore-runner AG14361, showed spectacular activity in xenograft models in combination with temozolomide, resulting in complete and durable tumor regression [Thomas *et al.* 2007; Calabrese *et al.* 2004]. AG14361 also caused a twofold to threefold enhancement of irinotecan-induced and radiation-induced tumor growth delay. AG014699 was the first PARPi to enter clinical trial for cancer therapy and has been studied in phase I and phase II clinical trials in combination with temozolomide for the treatment of metastatic melanoma. In the phase I study, dose escalation was driven by pharmacodynamic measurement of PARP inhibition and the PARP inhibitory dose (PID) was estimated at 12 mg/m² based on 74–97% inhibition of PARP activity in peripheral lymphocytes and a >50% PARP inhibition in tumor biopsies posttreatment. AG014699 showed linear pharmacokinetics with no interaction with temozolomide [Plummer *et al.* 2008]. The recommended phase II dose was 200 mg/m² of temozolomide with 12 mg/m² of AG014699. In the phase II study, a doubling of response rate and time to tumor progression were noted as compared with temozolomide alone, but at the cost of significantly higher myelosuppression in the combination arm. At the present time, single-agent studies in ovarian or breast cancers in BRCA mutation carriers and combination studies with cisplatin, pemetrexed and epirubicin are being conducted. The combination of AG014699 with these latter drugs not classically associated with PARP may be based on the observation that AG014699 is vasoactive, potentially increasing drug delivery to the tumor [Ali *et al.* 2009].

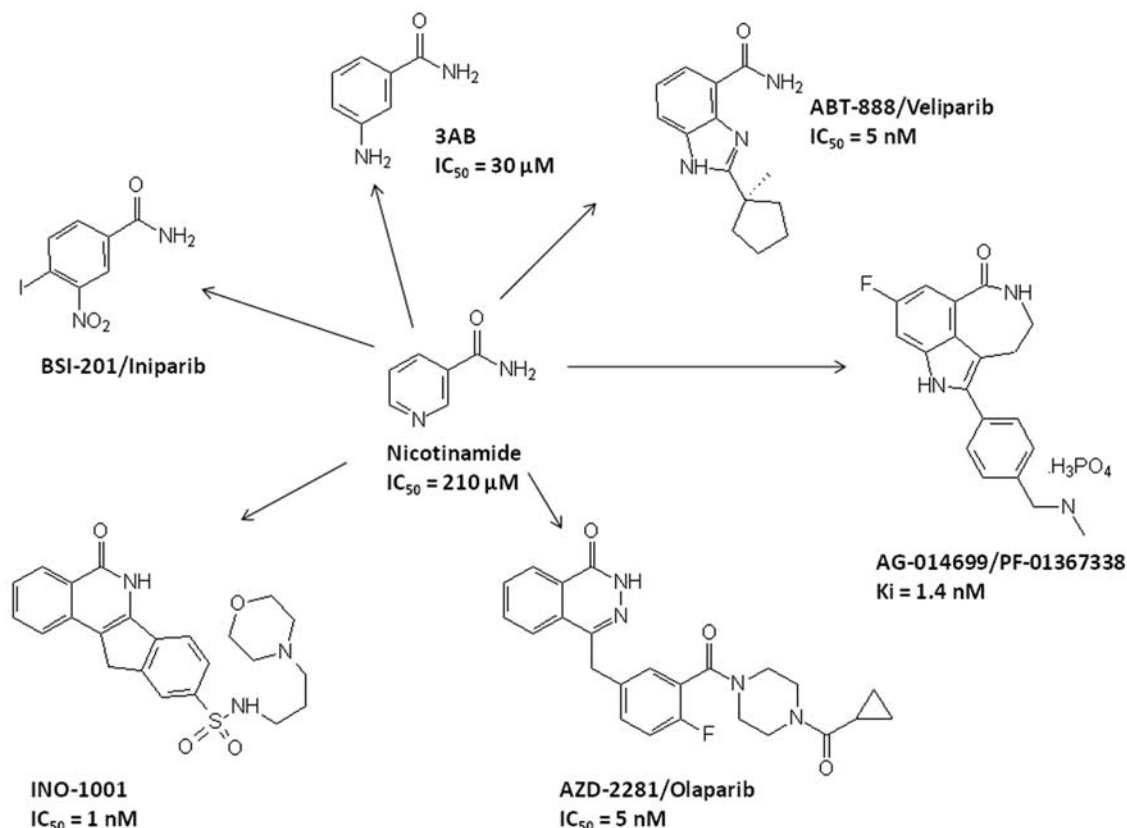


Figure 2. Structure of 3-aminobenzamide (3-AB) and the recent poly (ADP-ribose) polymerase inhibitors (PARPi) developed from the nicotinamide pharmacophore. The K_i of AG014699/PF-01367388 and the IC_{50} values for most other inhibitors are presented to show potency, but it should be noted that these values are not directly comparable due to different experimental conditions, e.g. substrate concentrations.

Veliparib (ABT-888)

Veliparib has been developed as a PARP-1 and PARP-2 inhibitor with $K(i)s$ of 5.2 and 2.9 nmol/l, respectively [Penning *et al.* 2009]. It is orally bioavailable and crosses the blood–brain barrier. ABT-888 potentiated the cytotoxic effect of temozolomide in several human tumor models and IR in HCT116 human colon cancers [Clarke *et al.* 2009; Palma *et al.* 2009; Donawho *et al.* 2007]. The activity of platinum analogues and cyclophosphamide were also enhanced by ABT-888 in the BRCA1 and 2 defective MX-1 xenografts but ABT-888 had no single-agent activity in this model in the schedule used [Donawho *et al.* 2007]. Veliparib was investigated in an innovative phase 0 trial, the first such study in oncology [Kummar *et al.* 2009]. The primary study endpoint was target modulation by the PARPi. PARP activity, when measured after a single dose of veliparib was significantly inhibited at the 25 and 50 mg doses. There is an extensive clinical trial program

associated with this agent with 32 ongoing clinical trials of veliparib in combination with cytotoxics in ovarian, breast, colorectal, prostate, liver cancers, neurologic malignancies and leukemias.

Olaparib (AZD2281)

Olaparib (AZD2281) also inhibits PARP-1 and PARP-2 at nanomolar concentrations [Menear *et al.* 2008]. Preclinical studies have largely concentrated on investigations of synthetic lethality in BRCA1 or BRCA2 defective models or combinations with platinum in these models [Rottenberg *et al.* 2008]. Radiosensitization in a glioma model has also been demonstrated [Dungey *et al.* 2008]. Studies with human ovarian cancer xenografts demonstrated that olaparib had single-agent activity and increased the efficacy of carboplatin in xenografts defective in BRCA2 but not those with normal BRCA function [Kortmann *et al.* 2011]. Olaparib was observed to increase the toxicity of topotecan in animal models [Zander *et al.* 2010]. The first

clinical study of PARP inhibition in BRCA mutant cancers was with this agent. In this phase I study which enrolled 60 patients, olaparib doses were escalated from 10 mg daily for 2 of every 3 weeks to 600 mg twice daily [Fong *et al.* 2009a]. The dose of 200 mg twice daily was selected for further study in a select cohort of 23 patients with BRCA mutations. In this group, nine had partial responses according to the NCI response evaluation criteria (RECIST). A total of 19 of the 23 patients had BRCA-associated tumors, including breast, ovarian, and prostate cancers.

Given these interesting preliminary data, two multicenter, international phase II studies of olaparib in patients with breast or ovarian cancers having BRCA1 or BRCA2 mutations were conducted [Fong *et al.* 2009b; Tutt *et al.* 2010]. Patients enrolled were refractory to standard chemotherapeutic regimens. A total of 27 patients in the first cohort received 400 mg of olaparib twice daily for 28 days, and 27 patients in the second cohort received 100 mg of olaparib twice daily. The overall response rate was 41% with 400 mg, and 22% with 100 mg olaparib. The median time to progression was 5.7 and 3.8 months, respectively. The common adverse effects were mild, including fatigue, nausea and vomiting. A parallel study using the two dosage regimens in 55 BRCA-mutated carriers with ovarian cancer confirmed an overall response rate of 33% in the 400 mg group, and 12.5% in the 100 mg group. These proof-of-concept studies confirmed that BRCA1 or BRCA2 mutational status serves as a predictive marker for PARPi.

Iniparib (BSI-201)

Unlike other PARPi that compete with NAD⁺ for the PARP catalytic site, iniparib (4-iodo, 3-nitrobenzamide) is unique in that it targets the zinc finger domain and prevents PARP-1 activation by DNA breaks [Mendeleyev *et al.* 1995]. Therefore, it may have differential effects as compared with other synthetic catalytic PARPi. Moreover, as this inhibitor has also been shown to inhibit other enzymes such as GAPDH [Bauer *et al.* 2002], it would be dangerous to conclude that its anticancer effects are solely attributable to PARP inhibition. This agent has been extensively investigated in triple-negative (TN) breast cancers.

TN breast cancers are believed to share the molecular characteristics of *BRCA1*-associated

cancers [Vona-Davis *et al.* 2008]. Both, *BRCA1*-associated cancers and sporadic TN tumors share a high degree of genomic instability, implying an impaired ability to repair DNA damage. HR defects seen in TN breast cancer include BRCA1 methylation, overexpression of deregulators including ID4 and HMG as well as aberrations of MRE11, ATM and PALB2 [Alli *et al.* 2009; Alexander *et al.* 2010]. Iniparib (BSI-201), when combined with gemcitabine and carboplatin for the treatment of TN breast cancer, has been studied in a randomized phase II trial compared with the same chemotherapy alone. The addition of iniparib increased disease control rate (56% to 34%), response rate (52% to 32%), progression-free survival (5.9 to 3.6 months) and overall survival (12.3 to 7.7 months) without increasing toxicity [O'shaughnessy *et al.* 2011]. A follow-up phase III study, however, was negative as it did not meet the prespecified criteria for significance for coprimary endpoints of overall survival and progression-free survival. Given the structural and mechanistic differences between iniparib and other PARPi, these negative results do not necessarily imply a 'class effect' and further study of TN breast cancer with other PARPi should be encouraged.

INO-1001

This agent is an isoindolinone derivative and is being developed for both oncological and cardiovascular indications. Preclinical studies demonstrate its protective effect in models of cardiac dysfunction [Pacher *et al.* 2006] and reversal of temozolomide resistance in MMR-defective xenografts [Cheng *et al.* 2005]. This was the first PARP-1 inhibitor to be investigated for cardiovascular disease and has been granted orphan drug status by the US Food and Drug Administration for the prevention of postoperative aortic aneurysm repair complications. In this phase II study, INO-1001 may have reduced the plasma levels of C-reactive protein and the inflammatory marker interleukin-6, without reducing plasma markers of myocardial injury. No serious toxic events ensued in this trial [Morrow *et al.* 2009]. This agent is being developed in oncology in melanoma and glioma and as a single agent in cancer for BRCA1- and BRCA2-deficient tumors. Phase I studies of INO-001 at 100, 200 and 400 mg/m² in combination with temozolomide indicated a short terminal half life and the dose-limiting toxicities observed at the highest dose were

myelosuppression and elevated liver enzymes [Bedikian *et al.* 2009].

Other PARPi in preclinical and phase I trial stages include GPI21016 (MGI/Eisai), MK-4827 (Merck), BMN-673 (Biomarin) and CEP-9722 (Cephalon). Additional information on these inhibitors can be found in a review by Ferraris [Ferraris, 2010].

Resistance mechanisms for PARPi

Acquired resistance to targeted agents is common and PARPi are no exception in this regard. As the clinical development of PARPi is still at an early stage, the underlying resistance mechanisms have not yet been elucidated. However, preclinical studies offer interesting possibilities. CAPAN-1 pancreatic cancer cells lines are BRCA2 deficient secondary to a 6174delT frameshift mutation, which makes them exquisitely sensitive to PARPi. CAPAN-1 cells cannot form damage-induced RAD51 foci, as they are HR-defective. PARPi-resistant clones were highly resistant to the drug (over 1000-fold), and were also cross-resistant to the DNA crosslinking agent, cisplatin. Interestingly, these resistant clones acquired the ability to form RAD51 foci after PARPi treatment or exposure to irradiation suggesting that re-acquisition of HR capability may be the mechanism of acquired resistance. In support of this, DNA sequencing of PARP inhibitor-resistant clones revealed new *BRCA2* isoforms as a result of intragenic deletion of the c.6174delT mutation and restoration of the open reading frame [Edwards *et al.* 2008; Sakai *et al.* 2008; Swisher *et al.* 2008]. Recently, 53BP1 has been shown to promote error-prone NHEJ in *BRCA1* mutant cells and that loss of 53BP1 partially restores HR function and can rescue from DNA damaging agent and PARPi sensitivity [Bouwman *et al.* 2010; Bunting *et al.* 2010]. Loss of 53BP1 appears to be relatively common in TN and *BRCA1*-mutant breast cancer samples [Bouwman *et al.* 2010].

An alternative mechanism was described with olaparib [Rottenberg *et al.* 2008]. In this case, resistance may be related to the upregulation of the *ABCB1a/b* genes, which encode P-glycoprotein multidrug resistance drug efflux pumps; this effect could be reversed with the P-glycoprotein inhibitor, tariquidar. A recent study investigated the role of 6-thioguanine in reversing this resistance mechanism. Issaeva and colleagues first noted that *BRCA1*, *BRCA2* or *XRCC3* tumors

are very sensitive to 6-thioguanine as HR is involved in the repair of 6-thioguanine-induced DSBs [Issaeva *et al.* 2010]. 6-thioguanine is not a substrate for p glycoprotein and is a potent cytotoxic in PARP-resistant tumors. Furthermore, these investigators noted that genetically reverted *BRCA2* defective tumors also retain sensitivity to 6-thioguanine. Altogether, these findings suggest that 6-thioguanine may be efficient in also killing advanced and drug-resistant *BRCA1* or *BRCA2* defective tumors.

Patient selection for PARPi trials

A major challenge is to identify predictive markers for PARPi therapy for those sporadic cancers that may benefit due to functional defects in the HR pathways. Several surrogate markers have been described, none of which are widely available in the clinical setting at the current time. Gene-expression arrays have been investigated for their predictive value [Jazaeri *et al.* 2002]. Turner and colleagues hypothesized that a gene and protein expression signature may have the ability to identify the BRCA-ness profile in patients [Turner *et al.* 2004]. Konstantinopoulos and colleagues defined a BRCA-like gene expression profile that correlated with PARPi and platinum sensitivity [Konstantinopoulos *et al.* 2010]. Phosphorylation of the Ser-139 residue of the histone variant H2AX, forming γ H2AX, is an early cellular response to the induction of DNA DSBs. Detection of this phosphorylation event has emerged as a highly specific and sensitive molecular marker for monitoring DNA damage initiation and resolution. This accumulation is detectable by immunofluorescence using an antibody to γ H2AX. Rad51 is a crucial downstream protein involved in HR repair, which is relocalized within the nucleus in response to DNA damage. Rad51 foci can also be visualized by immunofluorescent microscopy and are thought to represent assemblies of proteins at these sites of HR repair. Combination γ H2AX/RAD51 immunofluorescence has been investigated in primary ovarian cancer cell cultures [Mukhopadhyay *et al.* 2010] and primary acute myeloid leukemia (AML) cultures [Gaymes *et al.* 2009]. Both studies demonstrated that raised γ H2AX and decreased RAD51 foci expression predicts PARPi sensitivity. Graeser and colleagues investigated RAD51 immunofluorescence in replicating (geminin positive) cells in breast cancer biopsy specimens from women receiving neoadjuvant anthracycline therapy.

The RAD51 score was predictive of complete response in women receiving neoadjuvant therapy for breast cancer [Graeser *et al.* 2010]. Although cumbersome, these assays currently represent the most reliable way to identify HR defects, particularly in light of the recent studies showing that even in BRCA1-mutant tumors, coincident loss of 53BP1 can restore HR function and PARPi resistance [Bouwman *et al.* 2010; Bunting *et al.* 2010].

Conclusions

Genomic instability in cancer may result from an imbalance of DNA damage signaling and repair pathways; upregulated pathways may confer resistance to certain types of genotoxic agent but may also be necessary for the survival of the cancer cell. Targeting these pathways may result in single-agent activity specifically in the cancer cell where repair of endogenously generated DNA damage is inhibited as well as tumor-selective chemo and radio-sensitization. PARPi increase the anticancer activity of temozolomide, topoisomerase I poisons and IR in a wide range of tumor models, an approach that has been validated by genetic knockdown of PARP-1 and PARP-2. However, the most exciting aspect is the discovery that PARPi alone selectively kill cancer cells that lack HR without affecting repair competent cells. This observation has rapidly translated into clinical trials where PARPi have shown good anticancer activity in BRCA1 and BRCA2 patients with breast, ovarian and prostate cancer with only mild toxicities. HR is a complex and multicomponent pathway and preclinical data indicates that PARPi will be useful in tumors lacking any one of a number of these key proteins. Identification of these potentially PARPi-responsive tumors is the next challenge. Gene expression signatures and assays of HR function can fulfill this function but they are currently too expensive and cumbersome to become routine clinical practice.

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Conflict of interest statement

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