Poly(ADP-Ribose) Polymerase-1 Inhibition: Preclinical and Clinical Development of Synthetic Lethality

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The hereditary forms of breast cancer identified by *BRCA1* and *BRCA2* genes have a defect in homologous DNA repair and demonstrate a dependence on alternate DNA repair processes by base excision repair, which requires poly(ADP-ribose) polymerase 1 (PARP-1). siRNA and deletion mutations demonstrate that interference with PARP-1 function results in enhanced cell death when the malignancy has a defect in homologous recombination. These findings resulted in a plethora of agents in clinical trials that interfere with DNA repair, and these agents offer the potential of being more selective in their effects than classic chemotherapeutic drugs. An electronic search of the National Library of Medicine for published articles written in English used the terms "PARP inhibitors" and "breast cancer" to find prospective, retrospective and review articles. Additional searches were done for articles dealing with mechanism of action. A total of 152 articles dealing with breast cancer and PARP inhibition were identified. PARP inhibition not only affects nonhomologous repair, but also has several other nongenomic functions. Mutational resistance to these agents was seen in preclinical studies. To date, PARP-1 inhibitors were shown to enhance cytotoxic effects of some chemotherapy agents. This new class of agents may offer more therapeutic specificity by exploiting a DNA repair defect seen in some human tumors with initial clinical trials demonstrating antitumor activity. Although PARP inhibitors may offer a therapeutic option for selected malignancies, the long-term effects of these agents have not yet been defined.

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DEFECTS IN DNA REPAIR MAY OFFER A THERAPEUTIC APPROACH

In hereditary cancers, genomic instability results from mutations in DNA repair and mitotic checkpoint genes that drive cancer progression. In sporadic (nonhereditary) cancers, the molecular bases of genomic instability remain unclear but has been attributed to oncogene-induced DNA damage caused by mutations in *ataxia telangiectasia mutated* (*ATM*) and *p53* genes (1) among others. Specific defects in hereditary breast cancer have led to the development of potentially more selective agents.

The cell cycle requires a series of events that ensures faithful, error-free duplication of the cellular genome and subsequent physical division into two daughter cells. Tight regulation of this process ensures that the DNA in a dividing cell is copied correctly, any damage in the DNA is repaired and that each daughter cell receives a full set of intact chromosomes. A variety of genes are involved in the control of cell growth and division. For a mammalian cell, DNA damage resulting in either single-strand or double-strand breaks due to exogenous or endogenous insults is estimated to occur about 10,000 times per day (2).

To maintain the genomic integrity, all cells are equipped with several DNA repair mechanisms that have partially overlapping pathways (3). The main DNA repair pathways include base-excision repair (BER), nucleotide-excision repair, homologous recombination (HR), nonhomologous end joining (NHEJ), mismatch repair, and translesion synthesis (3,4). NHEJ is the "error-prone" pathway with higher tendencies to compromise genomic integrity (5). Defects in these processes or incorrect repair can result in tumorigenesis (6).

Patients with either *BRCA1*- or *BRCA2*-defective genes (involved in HR) have genomic instability (7). Both *BRCA* genes are the most common causes of hereditary breast cancer and hereditary ovarian cancer, with a potential lifetime risk as high as 50% and 40%, respectively (8,9). *BRCA1* or *BRCA2* mutation increases the lifetime risk of male breast cancer more than 50-fold (10). The majority of *BRCA1*-defective breast tumors are estrogen-receptor and progesterone-receptor poor with no evidence of overexpression of Her2/neu. In addition, they exhibit a basal phenotype and poor prognosis (11).

Defective HR has been implicated in genomic instability in other tumors such as myeloma, myeloid leukemia and

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myelodysplastic syndromes (12–14). Aurora-A kinase overexpression, which is seen in many breast tumors, especially basal subtypes (15,16), has also been shown to downregulate HR by interfering with RAD51 localization at double-stranded DNA breaks (17). Aurora-A kinase also phosphorylates BRCA1, abrogating its inhibition of centrosome microtubule nucleation (18). Recently, miR-182 (microRNA) overexpression caused the downregulation of BRCA1 in human breast cancer cell lines (19). These findings suggest that functionally defective HR can be caused by deregulation of multiple independent components affecting the HR pathway and that defective or inefficient HR occurs in tumors without BRCA mutations (20).

Conversely, intact DNA repair pathways in tumor cells contribute to drug resistance (21). Overexpression of DNA repair pathway molecules is associated with more aggressive cancers (22–24). Inhibiting DNA repair mechanisms may then enhance cytotoxic chemotherapeutic agents. Inhibition of the poly(ADP-ribose) polymerase (PARP) protein, involved in multiple DNA repair pathways including BER, has reached the clinic (25,26).

SYNTHETIC LETHALITY

The concept that interference with tumor-specific pathways could enhance therapeutic index is not new (27). Normal cellular function has redundant and interconnecting pathways to prevent a single mutation from causing lethality. In contrast, tumor growth and viability may depend on a nonredundant pathway. Therefore, in tumors with defects in normal control or in reparative pathways, interference with the active pathway could potentially lead to therapeutic effects (28–30). Kaelin (30) defined this process as "synthetic lethality" when an alternation in one normal pathway does not cause lethality, whereas a tumor lacking the essential redundant pathway is killed by inhibition of the specific pathway. This concept allows the screening of candidate genes or proteins as targets by using knockout models or by siRNA in tumors with defective pathways (22,31,32). In some cases, inhibition of a protein or pathway with siRNA is not equivalent to knocking out the gene (33). This finding may suggest that a gene dosage effect is functional in some tumors. "Synthetic sickness" was defined to explain a dosage effect wherein interference with a pathway critical for tumor viability results in selective impairment of tumor growth (29).

Table 1. Known functions of PARPs.

PARP	Presence of DNA damage	Function
PARP-1	+ + +	Cell death via necrosis or apoptosis
PARP-1	+	DNA damage detection, DNA repair (BER, SSB, DSB)
PARP-2	+	BER
PARP-1	+/–	Regulation of chromatin structure (compaction versus decondensation), gene-specific enhancer/promoter-binding factor, poly(ADP)ribosylation at transcriptional insulators
PARP-1, PARP-2, PARP-3, VPARP, tankyrase 1, tankyrase 2	–	Involved in different components of the mitotic apparatus for segregation of chromosomes in cell division

PARPs were shown to be involved in various molecular and cellular processes (reviewed in (12)). DSB, double-strand breaks; SSB, single-strand breaks.

PARP: STRUCTURE AND FUNCTION

PARPs are a family of nuclear enzymes with 16 members (34). Recent criteria suggested that only six of these proteins can polymerize ADP-ribose, whereas others can only transfer a single ADP-ribose moiety (35). The most abundant and the best characterized enzyme, PARP-1, was first described over 40 years ago (36). PARPs have several cellular functions including DNA recombination and repair, cellular proliferation, apoptosis in ischemic conditions and necrotic cell death (Table 1) (37–39). Only PARP-1 and PARP-2 are known to be activated by DNA strand breaks and participate in the single-strand repair via the base excision pathway (40). Single-site nucleotide nicks in DNA are known to be an active stimulus of this enzyme (41).

PARP-1 is a 116-kDA protein that has three principal domains (Figure 1). Detection of single-strand breaks in DNA increases PARP-1 activity by several hundred-fold. The DNA-binding domain at the NH_2-terminal region with two zinc finger motifs recognizes and binds DNA single-strand breaks, whereas a third zinc finger motif coordinates DNA-

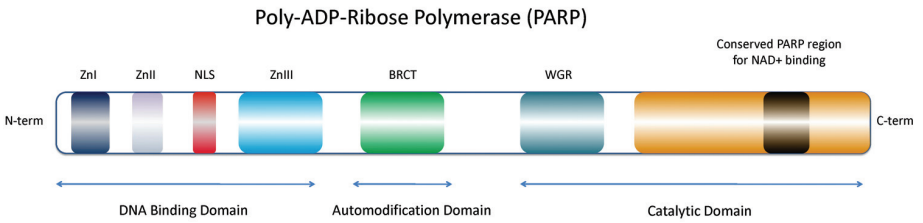

Figure 1. PARP structure and function. PARP contains an NH_2-terminal (*N*-term) DNA binding domain, an automodification domain and a COOH-terminal (C-term) catalytic domain. Zinc finger motifs (Zn) recognize and bind damaged DNA, activating the catalytic function that polymerizes poly(ADP-ribose) (PAR) from NAD^+. PARP first polymerizes PAR moieties to itself in the automodification domain. NLS, nuclear localization sequence; BRCT, BRCA1 carboxy-terminal repeat motif; WGR, WGR motif.

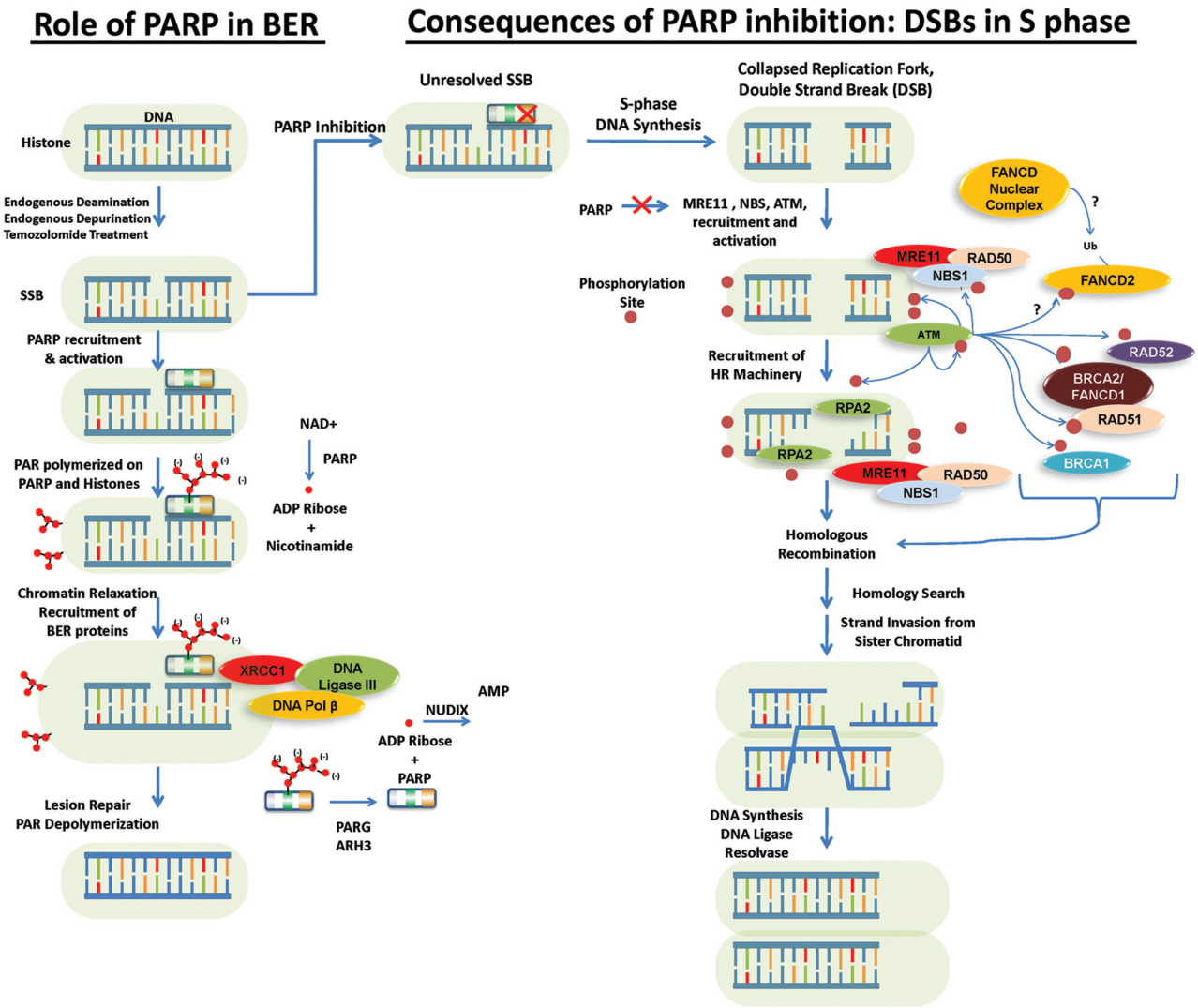

Figure 2. Model of PARP function in base excision repair and consequences of PARP inhibition. PARP is recruited and activated to sites of spontaneous or drug-induced single-strand DNA damage through its DNA binding domain. ADP-ribose is then polymerized from its substrate NAD^+ to PARP-1 itself at the automodification domain and to histone (H1 and H2B) tails, forming linear and branched poly(ADP-ribose) chains. Histone poly(ADP-ribosyl)ation causes relaxation of the chromatin to accommodate more DNA repair enzymes. The auto-poly(ADP-ribosyl)ation of PARP-1 creates a negatively charged region that mediates the recruitment of base excision repair proteins, including XRCC1 (X-ray repair cross-complementing 1), DNA polymerase β and DNA ligase III. After repair of damaged DNA, PARP-1 dissociates and the poly(ADP-ribose) chains are degraded by poly(ADP-ribose) glycohydrolase (PARG) and possibly the ADP-ribose hydrolase ARH3 to produce PARP and ADP-ribose, which is converted to AMP by NUDIX. PARP hyperactivation can cause cell death by mechanisms involving NAD^+ depletion and AMP accumulation. Consequences of PARP inhibition are as follows: When PARP is inhibited, unresolved single-strand breaks will accumulate and convert into double-strand DNA legions in the S-phase. The MRN complex and ATM are recruited to sites of double-strand DNA damage and damage repair signaling is initiated. Of note, MRN and ATM will likely be less efficiently recruited because of PARP inhibition, resulting in prolonged double-strand breaks. During the S-phase, ATM activation will result in activation of homologous recombination repair (HR) proteins such as BRCA1, BRCA2, RPA2, RAD51, RAD52 and potentially FANCD2 proteins. Functional Rad51-mediated HR may then repair DNA breaks through homology search, strand invasion, DNA synthesis and ligation. In the absence of a functional HR pathway, dsDNA breaks may persist or be repaired by the error-prone NHEJ pathway.

dependent enzyme activation (42,43) (see Figure 1). The automodification domain, which is at the central region of PARP-1, is a site for auto-poly(ADP-ribosyl)ation (44). The COOH-terminal region contains the catalytic domain that binds NAD^+. ADP-ribose is then repeatedly transferred from its substrate NAD^+ to PARP-1 itself at the automodification domain and to histone (H1 and H2B) tails, forming linear and branched poly(ADP-ribose) chains (Figure 2). Histone

poly(ADP-ribosyl)ation causes relaxation of the DNA to accommodate more DNA repair enzymes. The auto-poly(ADP-ribosyl)ation of PARP-1 creates a negatively charged region that mediates the recruitment of base excision repair proteins, including XRCC1 (X-ray repair cross-complementing 1), DNA polymerase β and DNA ligase III (see Figure 2). PARP-1 then dissociates from the DNA, and the poly(ADP-ribose) chains are degraded by poly(ADP-ribose) glycohydrolase (PARG) and possibly the ADP-ribose hydrolase ARH3, after ligation of the DNA break (34,45).

PARP activity also promotes recruitment and activation of mitotic recombination 11 (MRE11) and Nijmegen breakage syndrome (NBS), members of the DNA damage-sensing MRN complex that activates ATM, to sites of double-strand DNA damage (46,47). PARP activity promotes activation of ataxia mutated kinase (ATM kinase) (46,47). This, along with PARP's proposed role in NHEJ (48–50), suggests that PARP functions in response to multiple types of DNA damage, including DNA crosslinks, stalled replication forks and double strand break repair, in addition to its established role in single-strand break repair. Therefore, patients with alterations in repair pathways other than homologous recombination might also be sensitive to PARP inhibitors. In addition to a role in detecting DNA damage, PARP may also function to regulate gene expression through various mechanisms including control of chromatin condensation, DNA methylation and regulation of transcriptional repressors/enhancers (51,52).

Although PARP-1 plays a critical role in DNA single-strand break repair, it is not vital to life. Preclinical studies have shown that PARP-1 knockout mice develop normally (53) and are viable and fertile, but have increased susceptibility to carcinogenic insults (44,54,55). Nitrosamine exposure is a particularly potent carcinogen in this model system (56) and may have implications on the use of PARP inhibitors in humans, since this is a common carcinogen in the food supply. Without PARP-1, other DNA repair mechanisms are required to maintain genomic stability.

Because of cross-talk, other pathways may be perturbed when PARP is deficient. Disruption of PARP, either by small molecules or by siRNA, can result in suppression of HR thru inhibition of the BRCA1 and the RAD51 promoters in a model system (RKO colon cancer) (57). In addition, inhibition of PARP-1 results in indirect activation of AKT, which leads to resistance to taxane-induced apoptosis (58). Additional experiments are required to clarify whether phosphatidylinositol 3-kinase itself is activated on PARP inhibition or whether another phosphatidylinositol 3-kinase family member such as DNA-PK, a component of NHEJ, may be responsible for activation of AKT when PARP is inhibited (59).

PARP may play a role in nonmalignant disease, since excessive activation occurs in diabetes, renal disease, inflammatory states and preeclampsia and is associated with loss of cellular NAD^+ (60–63). Diabetic neuropathy is a particularly attractive target for PARP inhibitors (64,65). Cytoskeletal function can also be perturbed by overexpression of PARP (66).

SPECIFIC CASE OF *BRCA* MUTANT TUMORS

In *BRCA1* or *BRCA2* carriers, both copies of either wild-type gene are mutant only in tumor cells, whereas the rest of the somatic cells contain one wild-type copy of the gene. Therefore, those tumor cells have defective HR mechanisms and are particularly sensitive to additional inhibition of DNA repair machinery. With PARP inhibition, unresolved single-strand DNA breaks convert to double-strand lesions during the S-phase. In this fashion, PARP inhibition in HR-defective $BRCA^{-/-}$ cells leads to lethality.

"BRCAness" in which HR is defective may be seen in sporadic cancers. These tumors are apt to be highly proliferative, commonly having P53 and RB loss (67). BRCA1 was found to have reduced expression in sporadic breast cancers and predicted progression of disease (68). Tumors that are receptor negative and Her2/neu non-overexpressing are more apt to exhibit a BRCA defect (67). Overexpression of ID4, a negative regulator protein of *BRCA1*, was also shown to decrease BRCA1 expression (11), as does overexpression of Aurora-A kinase (17). BRCA1 is inactivated by methylation (69). BRCA2 is inactivated by other pathways in some sporadic breast and ovarian cancers (70). Overexpression of the *EMSY* gene was found to result in the suppression of *BRCA2* function, which was noted in 13% sporadic breast cancers and 17% sporadic ovarian cancers (71).

PARP INHIBITION

First-generation PARP inhibitors were nicotinamide analogs, including nicotinamide, benzamide and substituted benzamides, such as 3-aminobenzamide (72). These compounds lacked potency and specificity. Second-generation benzamide analogs were developed in the 1990s (62). Compounds in preclinical and clinical studies are third-generation PARP inhibitors. Many are derived from the 3-aminobenzamide structure, and most are competitive inhibitors (62). Recently, rapamycin, an immunosuppressive agent affecting M-TOR, was reported to downregulate PARP-1 (73).

In 1980, 3-aminobenzamide was found to compromise the repair of DNA damage caused by dimethyl sulfate in murine leukemic cells (74). In 2005, two simultaneous publications demonstrated synthetic lethality in BRCA1- and BRCA2-deficient cells when exposed to PARP inhibitors (75,76). The BRCA2-deficient cell line V-C8 had decreased survival when exposed to PARP inhibitors NU1025 and AG14361. siRNA inhibition in the human breast cancer cell lines MCF-7 and MD-MB-231 demonstrated that cytotoxicity seen with PARP inhibitors was associated with BRCA2 deficiency, regardless of p53 mutation status (75). By using embryonic stem cells deficient in BRCA1 or BRCA2, Farmer *et al.* (76) reported that PARP inhibition de-

creased survival in BRCA1- or BRCA2-depleted cells and blocked tumor growth *in vivo* in BRCA2-deficient cells. Inhibition of the HR mechanism by a defect in the CRCC3 protein also makes cells sensitive to PARP inhibition (77).

Inhibiting PARP, in HR-deficient tumors, makes the tumor more sensitive to the effects of DNA-damaging agents such as alkylating agents, topoisomerase I inhibitors, platinum and ionizing radiation (78–81). Whether cells deficient in DNA repair pathways other than HR are sensitive to PARP inhibitors is unknown.

Resistance to PARP inhibitors was reported in preclinical models. The human CAPAN1 pancreatic cell line, which has a frame shift mutation, becomes resistant to PARP inhibitor and also to cisplatinum with continuous drug exposure (82). The mechanism of resistance involves intragenic deletion with the production of a functional truncated BRCA protein. This drug-induced mutation of the defective *BRCA* gene with reversion to a functional protein was also observed in samples of tumors from patients with ovarian carcinoma treated with cisplatinum (83). In preclinical models, 6-thioguanine selectively kills BRCA2-defective tumors resistant to the PARP inhibitor AG014699 by induction of double-strand breaks, which require homologous repair for survival (84). 6-Thioguanine is also active in BRCA1 cells resistant to PARP inhibitors by virtue of overexpression of p-glycoprotein (84).

CLINICAL DATA

The first clinical study of PARP inhibition as monotherapy for BRCA-null patients was presented in 2007 and subsequently published (85). This phase I study with olaparib (AZD2281, formerly known as KU0059436) studied 60 patients with advanced solid tumors. Twenty-two patients had *BRCA1* or *BRCA2* mutations. One woman had a strong family history indicating *BRCA* mutation but declined genetic testing. Of those 23 patients, 9 had partial responses according to the National Cancer Institute (NCI) Response Evaluation Criteria in Solid Tumors (RECIST). Of the 23 patients, 19 had *BRCA*-associated tumors, including breast, ovarian and prostate cancers. A total of 12 of the 19 patients were found to have clinical benefit. Adverse side effects were mild and reversible, including grade 1 or 2 nausea, vomiting and fatigue. The maximum tolerated dose was determined to be 400 mg, twice daily (85).

A phase II multicenter international study was conducted for advanced breast cancer *BRCA* mutation carriers (86). Two sequential cohorts were studied in refractory patients with a median number of three prior treatments. A total of 27 patients in the first cohort received 400 mg olaparib twice daily for 28 days, and 27 patients in the second cohort received 100 mg olaparib twice daily. The overall response rate was 41% (11 patients) with 400 mg and 22% (6 patients) with 100 mg. The median time to progression was 5.7 and 3.8 months, respectively. The common adverse effects were mild, including fatigue, nausea and vomiting (86). A similar study using the two dosage regimens in 55 *BRCA*-mutated carriers with ovarian cancer confirmed an overall response rate of 33% (11 patients) in the 400 mg group and 12.5% (3 patients) in the 100 mg group (87). These studies support the use of PARP inhibitors in tumors with defective HR mechanisms. Additional clinical trials are under way using olaparib combined with chemotherapeutic agents in *BRCA*-mutated patients and in patients with sporadic tumors (Table 2). Abstract reports suggest the enhancement of neutropenia with the combination. Olaparib is also being investigated in tumors with other defects in DNA repair pathways, such as the defective mismatch repair pathway (see Table 2).

Iniparib (BSI-201) is another PARP inhibitor in clinical trials. A phase I trial with iniparib as monotherapy was presented at the 2008 American Society of Clinical Oncology Annual Meeting (88). Twenty-three patients with advanced solid tumors were treated with doses between 0.5 and 8 mg/kg. All doses were well tolerated, and no maximum tolerated dose was identified. The most common adverse events were gastrointestinal. To determine the safety and maximum tolerated dose of BSI-201 in combination with other chemotherapeutic agents, a phase Ib study was performed (89). The secondary objective was to determine clinical response. A total of 55 patients with advanced solid tumors were treated with BSI-201 in doses ranging from 1.1 to 8 mg/kg given twice weekly, combined with topotecan, gemcitabine, temozolomide or carboplatin/paclitaxel. None of the 21 adverse events were related to the test drug. One patient with ovarian cancer had a complete response at 6 months, whereas five other patients with tumors including breast cancer, renal carcinoma, sarcoma and uterine cancer had partial responses. Nineteen other patients had stable disease for ≥2 months.

A randomized phase II trial of iniparib with carboplatin/gemcitabine versus carboplatin/gemcitabine alone determined the safety and clinical benefit of adding the PARP inhibitor to standard chemotherapy in patients with metastatic triple-negative breast cancer (90). Secondary endpoints were overall response rate, progression-free survival and overall survival. Of the 123 patients analyzed, the clinical benefit rate was 56% with iniparib compared with 34% (P = 0.01) in the standard arm. Adding iniparib did not increase the number of adverse events. About 20% of patients in both arms experienced grade 3 or 4 hematologic toxicities. No grade 4 nonhematologic toxicities were reported. The overall response rate (52% versus 32%, P = 0.002), median progression-free survival (5.9 versus 3.6 months; $P < 0.01$) and median overall survival (12.3 versus 7.7 months, P = 0.01) were all superior in the iniparib arm compared with the standard therapy (90). A phase III multicenter, open-label, randomized trial of breast cancer patients with triple-negative (ER^-, PR^-, $Her2/neu^-$) tumors treated with iniparib did not reach its goals according to the sponsor. Further information is awaited. Other phase II

Table 2. PARP inhibitors under active investigation.

Name	Route	Regimen	Tumors	Phase
Olaparib, AZD2281, KU0059436 (AZ), AstraZeneca, KuDOS Pharm	Oral	AZ	BRCA1- or BRCA2-associated advanced ovarian cancer	I,II
			Colorectal cancer stratified by microsatellite instability	II
		AZ + carboplatin	BRCA1- or BRCA2-associated breast or ovarian cancer, sporadic TNBC and ovarian cancer	I
		AZ + cisplatinum	Solid tumors, TNBC for neoadjuvant therapy	I,II
		AZ + gemcitabine	Pancreatic cancer	I
		AZ + cisplatinum + gemcitabine	Solid tumors	II
		AZ + liposomal doxorubicin	BRCA1- or BRCA2-associated advanced ovarian cancer	I
		AZ + irinotecan	Colorectal cancer	I
		AZ + carboplatin, paclitaxel, or carboplatin/paclitaxel	TNBC, advanced ovarian cancer	II
		AZ ± paclitaxel	Gastric cancer	II
Iniparib, BSI-201 (B), BiPar Sciences, Sanofi-Aventis	Intravenous	B	Solid tumors	I
			BRCA1- or BRCA2-associated primary peritoneal cancer, advanced epithelial ovarian cancer	II
				II
		B + gemcitabine/carboplatin	TNBC for neoadjuvant therapy	II
			Recurrent ovarian cancer	II
			Solid tumors	I
		Gemcitabine/carboplatin ± B	TNBC	II,III
		B + topotecan, B + temozolomide, B + gemcitabine, B + carboplatin/paclitaxel	Solid tumors	I
		B + irinotecan	Solid tumors	II
		B + paclitaxel/carboplatin	Uterine carcinosarcoma	II
		B + temozolomide	Glioblastoma	I,II
AG 014699, PF-01367338, Pfizer	Intravenous	AG 014699	BRCA1 or BRCA2 mutation with breast or ovarian cancer	II
Veliparib, ABT-888 (AB), Abbott	Oral	AB	BRCA1 or BRCA2 mutation with any BRCA-associated solid tumors	I
		AB + temozolomide	Solid tumors	I
			Glioblastoma multiforme	I,II
			Breast cancer	II
			Colorectal cancer	II
		AB ± temozolomide	Metastatic melanoma	II
		AB + carboplatin + gemcitabine	Solid tumors	I
		AB + topotecan	Solid tumors	I
			Refractory solid tumors, lymphoma, chronic lymphocytic leukemia	II
		AB + mitomycin C	Refractory ovarian cancer or peritoneal cancer	I
		AB + paclitaxel + carboplatin + bevacizumab	Untreated epithelial ovarian, fallopian tube or peritoneal cancer	I
		AB + cyclophosphamide	Solid tumors, leukemia, lymphoma	I,II
		AB + cyclophosphamide ± doxorubicin	Metastatic solid tumors and non-Hodgkin lymphoma	I
		AB + whole brain radiation therapy	Non–central nervous system primary with brain metastases	I
		AB + vinorelbine + cisplatinum	Refractory tumors	I
MK-4827, Merck	Oral	MK-4827	Locally advanced or metastatic solid tumors	I
CEP-9722, Cephalon	Oral	CEP-9722 and CEP-9722 + temozolomide	Solid tumors with temozolamide	I
INO1001, Inotek, Genentech	Intravenous	INO1001	Heart diseases, heart surgeries	II

More information can be found at www.clinicaltrials.gov. TNBC, triple negative breast cancer.

trials with iniparib are also underway (see Table 2).

AG014699 was the first PARP inhibitor to undergo a phase I trial as an enhancing agent for chemotherapy (60). In preclinical models, the drug enhanced uptake of the cytotoxic agent into tumor (91). AG14699 enhanced cytotoxic effects of both topotecan and temazolamide in a neuroblastoma model (92). The drug is specifically toxic for human tumor cell lines with mutated or methylated *BRCA1* or *BRCA2*, causing double-strand breaks in DNA (77). Phase I results of the combination of AG014699 with temozolamide are published (93). The PARP inhibitory dose was 12 mg/m^2 with 74% to 97% inhibition of PARP in peripheral blood lymphocytes and linear pharmacokinetics. No dose-limiting toxicity occurred. Doses of 12 mg/m^2 AG014699 and 200 mg/m^2 temozolomide were combined in malignant melanoma patients. The combination regimen was well tolerated. On the basis of these results, a phase II study was conducted with the established doses of AG014699 with temozolomide in chemonaïve metastatic melanoma patients (94). Of 40 patients treated, greater bone marrow suppression was observed than in the phase I study. Four patients demonstrated partial responses and four others demonstrated stable disease. A phase II study using AG014699 as monotherapy in *BRCA1/2* mutation patients with breast or ovarian cancers is ongoing.

Preclinical combination studies of veliparib (ABT-888) demonstrated chemopotentiation with temozolomide, platinum, cyclophosphamide and ionizing radiation (95). A phase 0 trial conducted in 13 patients demonstrated good bioavailability of the drug (96). There are currently approximately 20 phase I or II trials assessing veliparib as monotherapy or combination therapies in various cancers, including *BRCA* mutation carriers.

MK4827 is currently in phase I study for patients with advanced solid tumors. CEP-9722 is now in phase I study to be used as a single agent or combined with temozolomide in patients with advanced solid tumors.

As PARPs are implicated in other cellular processes such as inflammation and cell death, INO-1001 is being investigated in myocardial ischemia and cardiovascular diseases. In a pig model, INO-1001 improved functional recovery after ischemia but did not affect infarct size (97). In humans, this agent reduced C-reactive and interleukin-6 levels in myocardial infarction (98) and caused myelosuppression and transaminitis when combined with temozolamide in patients with advanced melanoma (99).

FUTURE DIRECTIONS

Breast cancer is a heterogeneous disease. Chemotherapy remains the standard of care for basal-like and receptor-poor tumors, whereas none of the traditional agents demonstrate curative potential in patients with metastatic disease. Synthetic lethality induced by PARP inhibitors was demonstrated to date in *BRCA*-associated breast cancers. The rationale for targeting a specific DNA repair defect in tumor cells has strong preclinical evidence and represents a new clinical approach. This area of therapeutics is in rapid evolution.

The use of PARP inhibition may be expanded to other types of cancers on the basis of preclinical and early clinical data. Evidence that Aurora-A kinase and other pathways perturb HR, thus making the tumor cells sensitive to synthetic lethality, suggest that this group of drugs may have a larger role in treating malignant disease. Phosphatase and tensin homolog (*PTEN*) is a tumor suppressor gene that, when mutated, enhances sensitivity of malignant cells to PARP inhibition (100). Additionally, because of the multifaceted role of PARP in multiple DNA repair processes, it is plausible that tumors defective in DNA repair pathways other than HR may also be sensitive to PARP inhibition.

Clinical trials to date have shown that PARP inhibitors in combination with chemotherapeutic agents have manageable side effects. Because most trials reported to date were done on cancer patients who were refractory to several regimens and even then demonstrated anticancer activity, a question remains as to what effect PARP inhibition has in front-line use. However, caution is needed for the long-term use of PARP inhibitors. Fong *et al.* (85) reported in the phase I study of olaparib that PARP inhibition is associated with the accumulation of γH2AX, an indicator for DNA breaks, in eyebrow hair follicles. Enhanced mutation frequency by blocking a DNA repair pathway remains a major concern. In addition, early evidence suggests that tumor cells can become resistant to PARP inhibition by developing mutations that restore HR.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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