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PARP inhibition: PARP1 and beyond

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Abstract

Recent findings have thrust poly(ADP-ribose) polymerases (PARPs) into the limelight as potential chemotherapeutic targets. To provide a framework for understanding these recent observations, we review what is known about the structures and functions of the family of PARP enzymes, and then outline a series of questions that should be addressed to guide the rational development of PARP inhibitors as anticancer agents.

Current efforts to develop poly(ADP-ribose) polymerase (PARP) inhibitors as anticancer drugs represent the culmination of over 40 years of research. After Paul Mandel's research group first described a nuclear enzymatic activity that synthesizes an adenine-containing RNA-like polymer¹, independent studies by French and Japanese teams demonstrated that this polymer, designated poly(ADP-ribose) (pADPr), is composed of two ribose moieties and two phosphates per unit polymer^{2–5}. The purification of an enzyme that could generate large amounts of pADPr, PARP1 (REFS ^{6,7}), led to the discovery that PARP1 is activated by DNA strand breaks^{8–10}. Seminal work by Sydney Shall's group showed that PARP1 is involved in DNA repair and also suggested the potential use of PARP inhibitors to enhance the cytotoxic effects

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/gene>

Ku80 | *Trp53*

National cancer institute Drug Dictionary: <http://www.cancer.gov/drugdictionary>

AG014699 | *Bsi-201* | *carboplatin* | *gemcitabine* | *olaparib* | *temozolomide*

OMIM: <http://www.ncbi.nlm.nih.gov/omim>

Protein Data Base: <http://www.pdb.org>

UniProtKB: <http://www.uniprot.org>

ARH3 | *BRCA1* | *BRCA2* | *ER* | *ERBB2* | *MRE11* | *PARG* | *PARP1* | *PARP2* | *PARP3* | *PARP4* | *PR* | *PTEN* | *RAD51* | *TNKS* | *TNKS2* | *TRF1* | *XRCC1*

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of alkylating agents¹⁰. Examination of knockout mouse models¹¹ strengthened the hypothesis that PARP1 participates in DNA repair and simultaneously provided the first evidence for the existence of PARP2 (REF. ¹²). A parallel set of experiments demonstrated that PARP1 hyperactivation leads to nicotinamide adenine dinucleotide (NAD⁺) and ATP depletion after various types of DNA damage^{13,14} (BOX 1), potentially contributing to a unique form of metabolic cell death, which is now termed parthanatos¹⁵. PARP was thrust into the limelight by the discovery that PARP inhibition is particularly toxic in cancer cell lines^{16,17} and human tumours¹⁸ that lack BRCA1 or BRCA2. Despite this progress, there is still much that we do not understand about the biology of the PARP family and pADPr, as detailed below.

Box 1

PARP1 hyperactivation and cell death

Nicotinamide adenine dinucleotide (NAD⁺) is the source of ADP-ribose used by poly(ADP-ribose) polymerases (PARPs) to produce poly(ADP-ribose) (pADPr). Because hyperactivation of PARP1 consumes the cytosolic and nuclear pools of NAD⁺ to generate pADPr, pADPr synthesis translates DNA damage intensity into changes in cellular energy. Low to moderate DNA damage triggers pADPr-dependent DNA repair. In the context of excessive DNA damage, however, PARP1 hyperactivation leads to extended pADPr synthesis and extensive NAD⁺ consumption^{8,13,14}. Depending on the cellular context, this intense pADPr synthesis can induce cell death through several mechanisms. Long and branched pADPr (60mers and longer) can directly trigger a form of cell death termed parthanatos^{15,107}. Moreover, because several cellular metabolic pathways are NAD⁺ dependent, the cell may attempt to replenish the cytosolic NAD⁺ pool, a reaction that exhausts cellular ATP and induces necrotic cell death^{13,14,68}. In addition to this energy depletion, pADPr degradation by poly(ADP-ribose) glycohydrolase generates high levels of ADP-ribose, which may be further hydrolysed by the pyrophosphohydrolase NUDIX enzymes NUDT5 and NUDT9 to phospho-ribose and AMP^{99,108}. The net consequence of NAD⁺ depletion and pADPr hydrolysis is a tremendous increase in the cellular AMP:ATP ratio, which can activate AMP-activated protein kinase and induce an autophagic state through the inhibition of mTORC1-regulated cell growth^{45,109,110}.

PARP1 and DNA damage responses

PARP1 is a nuclear protein comprised of three functional domains (FIG. 1). The amino-terminal DNA-binding domain contains two zinc fingers that are important for the binding of PARP1 to single-strand breaks and double-strand breaks (DSBs)^{19,20}. A third zinc finger was recently described and found to be dispensable for DNA binding, but is important for coupling damage-induced changes in the DNA-binding domain to alterations in PARP1 catalytic activity^{21,22}. In the central automodification domain, specific glutamate and lysine residues serve as acceptors of ADP-ribose moieties, thereby allowing the enzyme to poly(ADP-ribosyl)ate itself^{23,24}. Interestingly, this domain also comprises a BRCA1 carboxy-terminal (BRCT) repeat motif, a protein–protein interaction domain that is found in other components of the DNA damage response pathway. The presence of this motif raises the possibility that unexplored protein–protein interactions involving this domain might also play an important part in PARP1 biology. Finally, the C-terminal catalytic domain sequentially transfers ADP-ribose subunits from NAD⁺ to protein acceptors, thereby forming pADPr²⁵.

Studies in various model systems have implicated PARP1 in multiple processes, all of which involve DNA-related transactions. After the induction of certain types of DNA damage, including nicks and DNA DSBs, PARP1 is rapidly recruited to the altered DNA and its catalytic activity increases 10- to 500-fold, resulting in the synthesis of protein-conjugated long

branched pADPr chains 15 to 30 sec after damage^{20,26}. Owing to the size and large negative charge of pADPr (which is twice the charge density of DNA), the addition of pADPr interferes with the functions of modified proteins, such as histones, topoisomerase I and DNA protein kinase (DNA-PK) (reviewed in REF. ²⁷). Notably, however, the bulk of pADPr is attached to PARP1. Once formed, this polymer could recruit hundreds of other proteins^{28–32}. Some of these recruited proteins — typified by XRCC1, the scaffolding protein that assembles and activates the DNA base excision repair (BER) machinery^{33,34} — bind directly to pADPr, whereas others are indirectly recruited because they interact with pADPr-binding proteins. At the same time, formation of pADPr diminishes the affinity of PARP1 and histones for DNA, providing a mechanism for removing PARP1 from damaged DNA and for the local modulation of chromatin compaction^{29,35–37}. *In vitro* studies suggest that removal of PARP1 provides access for repair proteins³⁸ and suppresses further pADPr synthesis³⁹. Further polymer growth is also antagonized by two enzymes that hydrolyse pADPr, poly(ADP-ribose) glycohydrolase (PARG) and, possibly, the ADP-ribose hydrolase ARH3 (REFS ^{40,41}). ADP-ribosyl protein lyase, which cleaves the link between the first ADP-ribose and modified amino acids, has been described in rat tissues^{42,43} and might also function in human cells. The concerted action of these enzymes removes pADPr from PARP1, restoring its ability to recognize DNA strand breaks and initiate a new round of damage signalling.

Although pADPr has a half-life of seconds to minutes, the consequences of pADPr metabolism on cellular homeostasis can persist long after PARP1 and the hydrolases have acted. Polymer synthesis consumes substantial amounts of NAD⁺ and pADPr cleavage generates large amounts of AMP, leading to activation of the bioenergetic sensor AMP-activated protein kinase (AMPK)^{44,45} (BOX 1). Therefore, the various consequences of PARP1 activation reflect the collective effects of pADPr synthesis on PARP1 substrates, binding of various proteins to pADPr, changes in cellular NAD⁺ (and ATP) levels during pADPr synthesis and changes in AMP levels owing to pADPr degradation (FIG. 1). In conditions that cause excessive DNA damage, such as post-ischaemic damage in the heart or brain, PARP1 hyperactivation produces high levels of pADPr at the expense of NAD⁺ and ATP, which become depleted¹⁴ and induce death by necrosis or apoptosis (BOX 1).

In addition to its role in BER described above, PARP1 is involved in several other nuclear processes. The observation that rapid recruitment of mitotic recombination 11 (MRE11) and ataxia telangiectasia-mutated (ATM), crucial components of the homologous recombination machinery, to DNA DSBs is dependent on pADPr synthesis^{26,46} suggests that PARP1 acts as a facilitator of homologous recombination. Studies in rodent and chicken cells indicate that recruitment of MRE11 to help restart stalled replication forks is also dependent on PARP1 (REFS ^{47–49}). Additional *in vitro* studies in rodent and human cells have implicated PARP1 in non-homologous end joining (NHEJ)^{50–52}. Consistent with these various roles in DNA damage responses, *Parp1*^{−/−} mice demonstrate heightened sensitivity to DNA-damaging agents, particularly alkylating agents and ionizing radiation¹¹ (TABLE 1). PARP1 might also regulate transcription by modulating chromatin structure, altering DNA methylation patterns, acting as a co-regulator of transcription factors and interacting with chromatin insulators (reviewed in REFS ^{53,54}). Chromatin immunoprecipitation experiments demonstrate that PARP1 is generally associated with actively transcribed genes, at which it is postulated to regulate histone H1 binding to chromatin⁵⁵. Moreover, gene expression profiling in *Parp1*^{−/−} mouse cells⁵⁶ and human breast cancer cells treated with *PARP1* short-hairpin RNAs (shRNAs)⁵⁷ reveal that PARP1 loss or downregulation alters the expression of many genes involved in cell cycle control and stress response, including p53. Collectively, these observations implicate PARP1 in transcription as well as in multiple aspects of the DNA damage response.

Extending the family

Against this backdrop of extensive structural and functional studies on PARP1, genes encoding 16 structurally related proteins (members of the so-called PARP family) have been identified on the basis of sequence similarity with the PARP1 catalytic domain⁵⁸. If stringent structural and functional criteria are applied, however, it now seems that only six of these may actually be poly(ADP-ribose) polymerases (BOX 2), whereas the remainder are probably mono-ADP-ribosyltransferases^{20,59}. Based on structural considerations, these six PARPs are subdivided in three groups, with PARP1, PARP2 and PARP3 in the first group, PARP4 (also known as vault PARP) in the second, and tankyrase 1 (TNKS) and TNKS2 in the third²⁰. Among this handful of true PARPs, structures have been determined for the catalytic domain of human PARP1, PARP2, PARP3 and TNKS^{60–62} (see Databases for a link to the [Protein Data Bank](#)). Although the structures of several other domains of human PARP1 and the WGR domain of human PARP3 have also been determined, the structure of a full-length PARP has not been reported.

Box 2

What is a true PARP?

A true poly(ADP-ribose) polymerase (PARP) can transfer the first ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD⁺) to an acceptor protein (preferably to glutamate or lysine residues) and can sequentially add multiple ADP-ribose units to the preceding ones to form poly(ADP-ribose) (pADPr) chains. Although 17 open reading frames in the human genome encode a region with sequence similarity to the 'PARP signature' in the catalytic domain of PARP1 (REF. ⁵⁸) (FIG. 1), further analysis suggests that these are unlikely to all code for PARPs. The catalytic domain of PARP1 contains three crucial residues: a histidine and a tyrosine that are important for NAD⁺ binding, and a glutamic acid that is essential for the polymerase activity^{59,111}. Although most of the so-called PARPs seem to have the residues required for NAD⁺ binding, the presence of isoleucine, leucine or tyrosine in place of the crucial glutamic acid residue in PARPs 6–16 suggests that they are mono-ADP-ribosyltransferases (see REF. ⁵⁹ for a detailed discussion).

The only way to determine whether a molecule is truly a PARP is to analyse the product of its catalysis as described¹¹² to show that pADPr has been synthesized. Based on this criterion, PARP1, PARP2 and tankyrase 1 (TNKS) are true PARPs. Human PARP1 and mouse PARP2 can synthesize long branched polymers^{12,112}, and human TNKS synthesizes long linear poly(ADP-ribose) chains¹¹³. Partial characterization of human TNKS2 activity suggests that it also produces long polymers¹¹⁴. Although PARP3 and PARP4 (also known as vault PARP) contain all of the structural features of a true PARP, their ability to synthesize pADPr remains to be established. A recent study of human PARP3 produced in bacteria raised the possibility that this enzyme might be a mono-ADP-ribosyl-transferase¹⁰⁰. Although this work and those of others^{23,115} suggest that PARP3 displays little PARP activity, a thorough study of the human enzyme is still needed.

In addition to *Parp1*, the mouse genes encoding PARP2, PARP4, TNKS and TNKS2 have been interrupted (TABLE 1). These studies revealed substantial redundancy among related PARPs. Mice can survive in the absence of *Parp1* or *Parp2*, for example, but not when both are deleted⁶³. Likewise, mice can survive in the absence of *Tnks* or *Tnks2* but not both⁶⁴. These results simultaneously indicate the essential functions of these PARPs, at least during development, and demonstrate the functional redundancy among PARPs of the same subgroups. It would be extremely useful to generate conditional knockout *Parp1*^{-/-}; *Parp2*^{-/-} and *Tnks*^{-/-}; *Tnks2*^{-/-} mice to overcome the embryonic lethality phenotype. Such mice could be used to study the roles of these PARPs in tumorigenesis in adult mice.

Additional studies in mice have shown that PARP2 can be activated by DNA damage¹². Consistent with this view, *Parp2*^{-/-} mice displayed many similarities to *Parp1*^{-/-} mice, including hypersensitivity to ionizing radiation and alkylating agents, as well as increased genomic instability^{63,65} (TABLE 1). In addition, defects in spermatogenesis, adipogenesis and T cell development are seen in *Parp2*^{-/-} but not *Parp1*^{-/-} mice (reviewed in REF. ⁶⁵), suggesting tissue-specific requirements for *Parp2*. Based on observations in *Parp1*^{-/-} mouse cells, PARP2 is thought to be responsible for no more than 15% of the total pADPr synthesis stimulated by DNA strand breaks^{66,67}, which is likely to reflect a lower abundance and/or lower catalytic activity of this enzyme. The observation that PARP2 consumes much less NAD⁺ than PARP1 provides an explanation for the observation that NAD⁺ levels are preserved and necrosis is diminished when *Parp1*^{-/-} cells sustain DNA damage⁶⁸.

Gene-targeting experiments and careful studies in human cell lines have also begun to provide valuable information about some of the other PARPs. Although *Parp4*^{-/-} mice develop normally, they develop more colon tumours when treated with the carcinogen dimethylhydrazine^{69,70}, raising the possibility that PARP4 has a tumour suppressive role. Studies in human cell lines have demonstrated that TNKS and TNKS2 both contribute to the maintenance of normal telomere length through a process that involves interaction with and poly(ADP-ribosylation) of the telomeric protein TRF1 (REF. ⁷¹). By contrast, deletion of *Tnks* or *Tnks2* has no effect on telomere length in mouse cells⁷¹. Although this could be explained by functional redundancy between the two mouse tankyrases, mouse TRF1 also lacks the tankyrase interaction motif, suggesting that there are substantial differences between the functions of mouse and human tankyrases⁷². In human cells, TNKS seems to be essential for mitotic spindle function through interactions with nuclear mitotic apparatus protein 1 (NuMA) and for resolving sister telomeres during mitosis, which are functions that cannot be assumed by human TNKS2 (REFS ^{71,73,74}). Human TNKS and TNKS2 have also recently been shown to bind and poly(ADP-ribosyl)ate axin in the β -catenin degradation complex, thereby regulating Wnt signalling⁷⁵. Because the study of these other PARPs is in its infancy, much remains to be learned about their roles in the DNA damage response, other facets of NAD⁺ and pADPr metabolism and cancer, as described below.

How to best target PARPs for therapy?

The observation that PARP1 is dramatically activated by ionizing radiation and DNA-methylating agents provided the original impetus for examining the effects of PARP inhibitors in combination with DNA-damaging agents. Although one could theoretically target PARP1 by depleting its substrate NAD⁺ or by using catalytic inhibitors^{10,76}, the latter approach has been much more extensively explored. Initial studies performed with 3-aminobenzamide, an agent that is neither selective enough nor potent enough by current standards, demonstrated enhanced radiation sensitivity when poly(ADP-ribosylation) was inhibited, fuelling a structure-based search for more potent competitive inhibitors (reviewed in REF. ⁷⁷). After extensive medical chemistry studies and preclinical development, third generation PARP inhibitors have now entered the clinic (TABLE 2). These agents are designed to compete with NAD⁺ at the enzyme active site. They universally inhibit PARP1 and, because they are largely based on benzamide or purine structures, have the potential to inhibit other enzymes that use NAD⁺, including other members of the PARP family, mono-ADP-ribosyl-transferases and sirtuins, although the extent to which they do so is largely unknown.

These new agents exhibit increased potency and specificity relative to earlier inhibitors⁷⁷. The need for highly potent and specific PARP inhibitors stems from the observation that PARP activity must be inhibited by >90% to detectably impair DNA repair⁷⁸. Defects in differentiation of lymphocytes and muscle cells observed with first generation PARP inhibitors^{79,80} have not been reported with the current inhibitors, raising the possibility that

the impaired differentiation observed earlier might have been a result of an off-target effect that has been engineered out of the new inhibitors. As noted below, however, additional studies are required to further assess the long-term effects of new PARP inhibitors.

Clinical development of PARP inhibitors follows two distinct approaches: targeting cells that are genetically predisposed to die when PARP activity is lost; and combining PARP inhibition with DNA-damaging therapy to derive additional therapeutic benefit from DNA damage. Both approaches are being pursued vigorously.

The development of PARP inhibitors as agents to treat tumours with certain sensitizing genetic lesions is based on the notion that cells with defects in DSB repair such as BRCA-deficient cells are more dependent on PARP1 and BER to maintain genomic integrity⁸¹. This synthetic lethal approach has been validated in studies that show striking single-agent activity of PARP inhibitors in preclinical models of BRCA1 and BRCA2 inactivation *in vitro* and *in vivo*^{16,17} (BOX 3). Consistent with these results, the PARP inhibitor olaparib (previously known as AZD2281) has shown promising single-agent activity against *BRCA1*- or *BRCA2*-mutant tumours in early clinical testing¹⁸. Because triple-negative (oestrogen receptor (*ER*)-negative, progesterone receptor (*PR*)-negative and *ERBB2*-negative) breast cancer and sporadic serous ovarian cancer exhibit some of the properties of BRCA1- or BRCA2-deficient cells^{82,83}, PARP inhibitors are also being tested against these tumours. Likewise, the observation that cells deficient in other crucial homologous recombination proteins are sensitive to PARP inhibitors⁸⁴ provides the rationale for testing PARP inhibitors in other cancers. For instance, with the recent finding that the tumour suppressor *PTEN* is important for expression of the repair protein *RAD51*, it was shown that *PTEN*-deficient cells are exquisitely sensitive to PARP inhibitors⁸⁵, providing a rationale for ongoing studies of PARP inhibitors in *PTEN*-deficient tumours.

Box 3

PARP inhibition and BRCA-deficient cells

Recent findings that poly(ADP-ribose) polymerase (PARP) inhibitors have cytotoxic effects on BRCA1- or BRCA2-deficient cells^{16,17} and human tumours¹⁸ have generated intense interest in moving PARP inhibitors into clinical practice (TABLE 2). The prevailing explanation for these findings centres on a phenomenon called synthetic lethality, in which the individual deletion of either of two genes has no effect but the combined deletion of both genes is cytotoxic. Each day, normal cells repair thousands of DNA lesions that result from various genotoxic insults such as oxidative damage. PARP1 and PARP2 promote the repair of these lesions by base excision repair (BER)³³ and contribute to the restarting of replication forks arrested at damaged sites^{48,49}. When PARP1 and PARP2 are inhibited, these lesions are unresolved and result in increased DNA double-strand breaks (DSBs)⁸¹. Because BRCA1- or BRCA2-deficient cells are unable to efficiently complete homologous recombination, the most faithful mechanism of DNA DSB repair, PARP inhibition in these cells causes a high degree of genomic instability and eventual cell death. In patients with hereditary BRCA mutations, PARP inhibitors would be highly selective for tumour tissues (expected to be completely BRCA deficient) compared with normal tissues (expected to be heterozygous at the BRCA locus). Moreover, these observations lay the groundwork for targeting other homologous recombination-deficient genetic lesions. Genetic screens have identified a host of homologous recombination-related genes that, on deletion, render cells hypersensitive to PARP inhibitors⁸⁴. Moreover, because of loss of expression of *RAD51*, *PTEN*-deficient cells are also sensitive to PARP inhibitors⁸⁵.

Whether this is the complete explanation for the hypersensitivity of BRCA1- or BRCA2-deficient tumours to PARP inhibitors remains unclear. Current models of synthetic lethality

largely ignore other roles of PARP1 in cellular survival, particularly its involvement in non-BER repair modalities (FIG. 1). Moreover, recent work has identified synthetic lethality between tankyrase 1 and the BRCA genes¹⁰¹, suggesting that successful targeting of BRCA-deficient tumours might be accomplished independently of PARP1 and PARP2 inactivation.

As trials of PARP inhibitors in repair-deficient tumours progress, it will be interesting to see how durable the responses are. Resistance to PARP inhibitors and carboplatin can arise in *BRCA1*- or *BRCA2*-deficient cancer cells concomitant with the reactivation of these genes by secondary mutations^{86–88}. This is unfortunate but somewhat predictable in view of the deficiency of error-free homologous recombination in *BRCA2*^{−/−} cells, which limits them to lower fidelity repair mechanisms and the subsequent accumulation of mutations. Hopefully, further elucidation of the finer details of the DNA damage response pathways will provide clues to circumvent this resistance.

The alternative approach of pairing PARP inhibitors with DNA-damaging agents to achieve chemosensitization is based on extensive preclinical studies showing that PARP inhibitors enhance the action of methylating agents, topoisomerase I poisons and ionizing radiation in tumour cell lines *in vitro* and in human tumour xenografts *in vivo*^{77,89} (TABLE 2). These observations were first translated into a clinical trial of the PARP inhibitor AG014699 in combination with the methylating agent temozolomide⁹⁰. This trial demonstrated that this PARP inhibitor could safely be administered with standard doses of temozolomide and that marrow suppression, a known toxicity of the alkylating agent, was dose-limiting when pADPr synthesis was inhibited by >90%. In additional studies, the PARP inhibitor BSI-201 has shown tantalizing activity in triple-negative breast cancer in combination with gemcitabine and carboplatin^{91,92}. Phase I and Phase II trials of several PARP inhibitors in combination with DNA-damaging agents are ongoing (TABLE 2).

As these clinical trials mature, it will be crucial to examine potential long-term effects of PARP inhibition. Because PARP1 plays a part in protection of the cardiovascular system (reviewed in REF. ⁹³) and development of memory⁹⁴, it will be important to assess cardiovascular and mental health in patients who receive long-term PARP inhibitor therapy. Moreover, in light of studies that have shown a higher incidence of cancers in mice when *Parp1* is knocked out in combination with *Trp53*, *Prkdc* or *Ku80*, implicating PARP1 in tumour suppression^{95–97}, the possibility of secondary malignancies will need to be examined. Indeed, the therapeutic benefit of inhibiting PARP1 will need to be weighed against any deleterious effect that results from loss of the postulated PARP1 tumour suppressive effect.

Unanswered questions

For the full potential of PARP inhibitors to be realized, it is our view that several gaps in current knowledge need attention. Some of these gaps relate to the biology of PARP1 itself.

First, how DNA damage is translated into a 10- to 500-fold increase in PARP1 enzymatic activity is incompletely understood. Recent reports not only indicate that an N-terminal fragment of PARP1 undergoes a conformational change after interaction with damaged DNA⁹⁸, but also implicate the third zinc finger in the interaction between the PARP1 DNA-binding domain and the catalytic domain²³. Because these studies were carried out with truncated fragments of PARP1, however, they provide somewhat limited insight into the activation process. Hopefully, the structure of the full-length enzyme will soon be solved and will provide additional information about PARP1 activation. Indeed, it is possible that a more complete understanding of the structural changes involved in PARP1 activation might lead to alternative and more selective methods of inhibiting this enzyme.

Second, further study of PARP1 in normal and tumour cells is required. If a kinase inhibitor were being discussed, one would immediately enquire about activating mutations and/or overexpression in tumour cells. Relatively little is known about PARP1 protein levels and activity in normal cells versus tumour cells and how this contributes (if at all) to a therapeutic index when PARP inhibitors are used in conjunction with DNA-damaging agents in cells with normal repair pathways.

Third, the roles of PARP1 in DNA damage responses require further study. In view of its BRCT motif, the possibility that PARP1 functions as a scaffold protein independently of its catalytic function after some types of DNA damage needs further investigation. Further studies are also required to distinguish between several potentially separable effects of PARP1 activation. Activated PARP1 attaches pADPr to various other polypeptides, altering their functions in ways that are still being elucidated. Moreover, PARP1 activation results in the synthesis of large amounts of the polymer attached to PARP1 itself, potentially facilitating the binding of hundreds of polypeptides that have pADPr-binding motifs and altering their functions. Finally, PARP1 activation consumes NAD^+ and alters AMP:ATP ratios through the actions of PARG, which hydrolyses pADPr into ADP-ribose, and of the ADP-ribose pyrophosphohydrolase NUDIX enzymes, which cleave ADP-ribose into AMP and phospho-ribose⁹⁹ (FIG. 1). Further evaluation of these different effects of PARP1 activation should improve our understanding of how PARP1 can affect so many different cellular pathways.

Additional gaps in our current knowledge relate to the biology of PARPs other than PARP1. In particular, we know little about the effects of inhibiting most of the other PARPs. In some cases, gene-targeted mice still need to be created to study the functions of these enzymes (TABLE 1). In other cases, the consequences of gene deletion are known, but it is unclear whether various PARP inhibitors affect these enzymes^{17,59,75,100} (TABLE 2). Recently, synthetic lethality between loss of the BRCA genes and *TNKS* has been reported in human cell lines¹⁰¹. A potentially specific tankyrase inhibitor, XAV-939, has been identified⁷⁵, raising the possibility that *BRCA1*- or *BRCA2*-mutant tumours might be successfully targeted without inhibiting PARP1. In addition, the demonstration that human tankyrases participate in Wnt- β -catenin signalling suggests that cancers with misregulated Wnt signalling might also benefit from tankyrase inhibition⁷⁵. These observations highlight the importance of further study of non-PARP1 PARPs.

There are also important gaps in our understanding of PARP inhibitors and their effects. Several recent studies of PARP inhibitors have concluded that the observed results reflect PARP1 inhibition without considering effects on other enzymes, including other PARPs. In our opinion, conclusions drawn from studies using PARP inhibitors should, whenever possible, be validated using *PARP1*^{-/-} cells or PARP1 short-interfering RNAs (siRNAs) to confirm that the observations are dependent on the presence of PARP1. In addition, understanding of current PARP inhibitors (TABLE 1) would be enhanced by a careful analysis of the effects of these agents on the catalytic activity of other NAD^+ -utilizing enzymes, including other PARPs, mono-ADP-ribosyltransferases and sirtuins. Although many of these agents are described as PARP1 inhibitors or PARP1 and PARP2 inhibitors, little documentation of their selectivity for these enzymes is currently available.

Additional studies might also be required to better understand how PARP inhibitors exert their beneficial effects in tumour cells. Previous studies have demonstrated that PARP1 siRNAs or shRNAs are toxic to BRCA-deficient cells *in vitro*^{16,17}, leaving little doubt that PARP inhibitors kill these cells by diminishing the catalytic activity of PARP1. Some of the preclinical data, however, raise the possibility that *BRCA2*^{-/-} cells might respond better to PARP inhibition than *BRCA1*^{-/-} cells^{17,102}. If this effect is confirmed in additional studies, it is not completely explained by current models of PARP inhibitor-induced killing. Whether PARP

inhibitors also potentiate the chemo-therapeutic effects of DNA-damaging therapy in the same fashion remains unclear, as earlier studies performed under cell-free conditions raised the possibility that catalytically inactive PARP1 will bind to DNA lesions and prevent their repair³⁸. Whether this mechanism contributes to chemosensitization in intact cells or in a clinical setting remains to be investigated. Finally, studies are also needed to improve our understanding of why PARP inhibitors sensitize tumour cells more to some DNA-damaging agents (for example, temozolomide and DNA topoisomerase I inhibitors) than others (DNA topoisomerase II inhibitors)^{77,89}.

It will also be important to determine whether different PARP inhibitors are equivalent in terms of suppression of PARP activity in cells and inhibition of polymer synthesis in patients. One of the inhibitors currently undergoing clinical testing (BSI-201) covalently and irreversibly inhibits PARP1 and possibly other enzymes^{103,104}. It is tempting to speculate that the ability to inhibit other enzymes might explain the relatively unique ability of BSI-201 to synergize with certain other agents such as gemcitabine⁹¹. Moreover, the ability of BSI-201 to covalently inhibit PARP1, thereby necessitating *de novo* synthesis to replenish PARP1 activity, might contribute to the clinical activity of BSI-201 in triple-negative breast cancer, which reportedly has high PARP1 levels⁹¹. By contrast, the other PARP inhibitors currently in clinical trials were designed as reversible inhibitors. Based on the law of mass action, these other PARP inhibitors would be predicted to suppress pADPr synthesis more completely in tumours with lower PARP1 expression. In addition, as mentioned above, the various inhibitors might also differ in their abilities to inhibit other PARPs, mono-ADP-ribosyltransferases, sirtuins and other NAD⁺-dependent enzymes. Whether any of these differences between the various inhibitors translate into important differences in clinical efficacy and/or side effects remain to be explored.

Finally, there are gaps in our understanding of the effects of long-term PARP inhibition. PARP1 is known to have effects on transcription and a wide variety of DNA repair pathways. Whether long-term PARP1 inhibition will have any deleterious effects such as secondary malignancies requires careful investigation, particularly when inhibitors are administered with DNA-damaging agents. Given the high potency of third generation PARP inhibitors and the reported tumour suppressor functions of PARP1, the long-term effects of near-complete PARP inhibition should be tested in animal models, as was done for earlier generations of inhibitors^{105,106}. It will be important, however, to weigh the risk of secondary malignancies — if they occur — against the benefits of improved treatment of currently intractable tumours. In view of the postulated roles of PARP1 in cardiovascular physiology⁹³ and memory⁹⁴, any long-term effects of PARP inhibitors on cardiovascular or mental well being, either beneficial or deleterious, should also be monitored.

In summary, more than 40 years of research have culminated in the recent demonstration that PARP inhibitors are active anticancer agents in *BRCA1*- and *BRCA2*-mutant tumours. Although these results are exciting, there is still much work to be done. As indicated above, fairly basic biological questions — ranging from the structure of full-length PARP1 and the molecular basis for its activation by damaged DNA to the biological roles of other PARPs — remain to be answered. On the clinical side, it will be important to determine whether preclinical models have accurately predicted the activity of PARP inhibitors in settings beyond *BRCA1*- and *BRCA2*-deficient tumours. Moreover, it will be interesting to see whether all of the PARP inhibitors are equivalent or not. Although there is still much to be learnt about PARPs and PARP inhibitors, the recent tantalizing results suggest that further basic and translational studies are likely to be informative and rewarding.

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References

1. Chambon P, Weill JD, Mandel P. Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. *Biochem Biophys Res Commun* 1963;11:39–43. [PubMed: 14019961]
2. Doly J, Petek F. Etude de la structure d'un composé "poly(ADP-ribose)" synthétisé par des extraits nucléaires de foie de poulet. *C R Hebd Seances Acad Sci Ser D Sci Nat* 1966;263:1341–1344.
3. Chambon P, Weill JD, Doly J, Strosser MT, Mandel P. On the formation of a novel adelylic compound by enzymatic extracts of liver nuclei. *Biochem Biophys Res Commun* 1966;25:638–643.
4. Nishizuka Y, Ueda K, Nakazawa K, Hayaishi O. Studies on the polymer of adenosine diphosphate ribose. I. Enzymic formation from nicotinamide adenine dinucleotide in mammalian nuclei. *J Biol Chem* 1967;242:3164–3171. [PubMed: 4291072]
5. Sugimura T, Fujimura S, Hasegawa S, Kawamura Y. Polymerization of the adenosine 5'-diphosphate ribose moiety of NAD by rat liver nuclear enzyme. *Biochim Biophys Acta* 1967;138:438–441. [PubMed: 4292779]
6. Yamada M, Miwa M, Sugimura T. Studies on poly (adenosine diphosphate-ribose): X. Properties of a partially purified poly (adenosine diphosphate-ribose) polymerase. *Arch Biochem Biophys* 1971;146:579–586. [PubMed: 5114920]
7. Okayama H, Edson CM, Fukushima M, Ueda K, Hayaishi O. Purification and properties of poly (adenosine diphosphate ribose) synthetase. *J Biol Chem* 1977;252:7000–7005. [PubMed: 198398]
8. Juarez-Salinas H, Sims JL, Jacobson MK. Poly(ADP-ribose) levels in carcinogen-treated cells. *Nature* 1979;282:740–741. [PubMed: 229416]
9. Benjamin RC, Gill DM. ADP-ribosylation in mammalian cell ghosts. Dependence of poly(ADP-ribose) synthesis on strand breakage in DNA. *J Biol Chem* 1980;255:10493–10501. [PubMed: 7430132]
10. Durkacz BW, Omidiji O, Gray DA, Shall S. (ADP-ribose)_n participates in DNA excision repair. *Nature* 1980;283:593–596. [PubMed: 6243744]
11. Shall S, de Murcia G. Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? *Mutat Res* 2000;460:1–15. [PubMed: 10856830]
12. Amé JC, et al. PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. *J Biol Chem* 1999;274:17860–17868. [PubMed: 10364231]
13. Berger SJ, Sudar DC, Berger NA. Metabolic consequences of DNA damage: DNA damage induces alterations in glucose metabolism by activation of poly (ADP-ribose) polymerase. *Biochem Biophys Res Commun* 1986;134:227–232. [PubMed: 3080986]
14. Carson DA, Carrera CJ, Wasson DB, Yamanaka H. Programmed cell death and adenine deoxynucleotide metabolism in human lymphocytes. *Adv Enzyme Regul* 1988;27:395–404. [PubMed: 2907967]
15. David KK, Andrabi SA, Dawson TM, Dawson VL. Parthanatos, a messenger of death. *Front Biosci* 2009;14:1116–1128. [PubMed: 19273119]
16. Bryant HE, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005;434:913–917. [PubMed: 15829966]
17. Farmer H, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434:917–921. [PubMed: 15829967]
18. Fong PC, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–134. [PubMed: 19553641]
19. Gradwohl G, et al. The second zinc-finger domain of poly(ADP-ribose) polymerase determines specificity for single-stranded breaks in DNA. *Proc Natl Acad Sci USA* 1990;87:2990–2994. [PubMed: 2109322]

20. Hassa PO, Hottiger MO. The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. *Front Biosci* 2008;13:3046–3082. [PubMed: 17981777]
21. Langelier MF, Servent KM, Rogers EE, Pascal JM. A third zinc-binding domain of human poly(ADP-ribose) polymerase-1 coordinates DNA-dependent enzyme activation. *J Biol Chem* 2008;283:4105–4114. [PubMed: 18055453]
22. Tao Z, Gao P, Hoffman DW, Liu HW. Domain C of human poly(ADP-ribose) polymerase-1 is important for enzyme activity and contains a novel zinc-ribbon motif. *Biochemistry* 2008;47:5804–5813. [PubMed: 18452307]
23. Altmeyer M, Messner S, Hassa PO, Fey M, Hottiger MO. Molecular mechanism of poly(ADP-ribosyl)ation by PARP1 and identification of lysine residues as ADP-ribose acceptor sites. *Nucleic Acids Res* 2009;37:3723–3738. [PubMed: 19372272]
24. Tao Z, Gao P, Liu HW. Identification of the ADP-ribosylation sites in the PARP-1 automodification domain: analysis and implications. *J Am Chem Soc* 2009;131:14258–14260. [PubMed: 19764761]
25. Kameshita I, Matsuda Z, Taniguchi T, Shizuta Y. Poly (ADP-ribose) synthetase. Separation and identification of three proteolytic fragments as the substrate-binding domain, the DNA-binding domain, and the automodification domain. *J Biol Chem* 1984;259:4770–4776. [PubMed: 6325408]
26. Haince JF, et al. PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *J Biol Chem* 2008;283:1197–1208. [PubMed: 18025084]
27. D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem J* 1999;342:249–268. [PubMed: 10455009]
28. Gagné JP, et al. Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes. *Nucleic Acids Res* 2008;36:6959–6976. [PubMed: 18981049]
29. Timinszky G, et al. A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. *Nature Struct Mol Biol* 2009;16:923–929. [PubMed: 19680243]
30. Kraus WL. New functions for an ancient domain. *Nature Struct Mol Biol* 2009;16:904–907. [PubMed: 19739287]
31. Ahel D, et al. Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science* 2009;325:1240–1243. [PubMed: 19661379]
32. Gottschalk AJ, et al. Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. *Proc Natl Acad Sci USA* 2009;106:13770–13774. [PubMed: 19666485]
33. Masson M, et al. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol Cell Biol* 1998;18:3563–3571. [PubMed: 9584196]
34. El-Khamisy SF, Masutani M, Suzuki H, Caldecott KW. A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. *Nucleic Acids Res* 2003;31:5526–5533. [PubMed: 14500814]
35. Ogata N, Ueda K, Kagamiyama H, Hayaishi O. ADP-ribosylation of histone H1. Identification of glutamic acid residues 2, 14, and the COOH-terminal lysine residue as modification sites. *J Biol Chem* 1980;255:7616–7620. [PubMed: 6772638]
36. Poirier GG, de Murcia G, Jongstra-Bilen J, Niedergang C, Mandel P. Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. *Proc Natl Acad Sci USA* 1982;79:3423–3427. [PubMed: 6808510]
37. Tulin A, Spradling A. Chromatin loosening by poly(ADP-ribose) polymerase (PARP) at *Drosophila* puff loci. *Science* 2003;299:560–562. [PubMed: 12543974]
38. Satoh MS, Lindahl T. Role of poly(ADP-ribose) formation in DNA repair. *Nature* 1992;356:356–358. [PubMed: 1549180]
39. Zahradka P, Ebisuzaki K. A shuttle mechanism for DNA–protein interactions. The regulation of poly (ADP-ribose) polymerase. *Eur J Biochem* 1982;127:579–585. [PubMed: 6293817]
40. Meyer-Ficca ML, Meyer RG, Coyle DL, Jacobson EL, Jacobson MK. Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp Cell Res* 2004;297:521–532. [PubMed: 15212953]
41. Oka S, Kato J, Moss J. Identification and characterization of a mammalian 39-kDa poly(ADP-ribose) glycohydrolase. *J Biol Chem* 2006;281:705–713. [PubMed: 16278211]

42. Oka J, Ueda K, Hayaishi O, Komura H, Nakanishi K. ADP-ribosyl protein lyase. Purification, properties, and identification of the product. *J Biol Chem* 1984;259:986–995. [PubMed: 6693407]
43. Okayama H, Honda M, Hayaishi O. Novel enzyme from rat liver that cleaves an ADP-ribosyl histone linkage. *Proc Natl Acad Sci USA* 1978;75:2254–2257. [PubMed: 276865]
44. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nature Rev Mol Cell Biol* 2007;8:774–785. [PubMed: 17712357]
45. Huang Q, Wu YT, Tan HL, Ong CN, Shen HM. A novel function of poly(ADP-ribose) polymerase-1 in modulation of autophagy and necrosis under oxidative stress. *Cell Death Differ* 2009;16:264–277. [PubMed: 18974775]
46. Haince JF, et al. Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. *J Biol Chem* 2007;282:16441–16453. [PubMed: 17428792]
47. Yang YG, Cortes U, Patnaik S, Jasin M, Wang ZQ. Ablation of PARP-1 does not interfere with the repair of DNA double-strand breaks, but compromises the reactivation of stalled replication forks. *Oncogene* 2004;23:3872–3882. [PubMed: 15021907]
48. Sugimura K, Takebayashi S, Taguchi H, Takeda S, Okumura K. PARP-1 ensures regulation of replication fork progression by homologous recombination on damaged DNA. *J Cell Biol* 2008;183:1203–1212. [PubMed: 19103807]
49. Bryant HE, et al. PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. *EMBO J* 2009;28:2601–2615. [PubMed: 19629035]
50. Audebert M, Salles B, Calsou P. Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. *J Biol Chem* 2004;279:55117–55126. [PubMed: 15498778]
51. Veuger SJ, Curtin NJ, Smith GC, Durkacz BW. Effects of novel inhibitors of poly(ADP-ribose) polymerase-1 and the DNA-dependent protein kinase on enzyme activities and DNA repair. *Oncogene* 2004;23:7322–7329. [PubMed: 15286704]
52. Wang M, et al. PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. *Nucleic Acids Res* 2006;34:6170–6182. [PubMed: 17088286]
53. Caiafa P, Guastafierro T, Zampieri M. Epigenetics: poly(ADP-ribosyl)ation of PARP-1 regulates genomic methylation patterns. *FASEB J* 2009;23:672–678. [PubMed: 19001527]
54. Kraus WL. Transcriptional control by PARP-1: chromatin modulation, enhancer-binding, coregulation, and insulation. *Curr Opin Cell Biol* 2008;20:294–302. [PubMed: 18450439]
55. Krishnakumar R, et al. Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes. *Science* 2008;319:819–821. [PubMed: 18258916]
56. Simbulan-Rosenthal CM, et al. Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase. *Proc Natl Acad Sci USA* 2000;97:11274–11279. [PubMed: 11016956]
57. Frizzell KM, et al. Global analysis of transcriptional regulation by poly(ADP-ribose) polymerase-1 and poly(ADP-ribose) glycohydrolase in MCF-7 human breast cancer cells. *J Biol Chem* 2009;284:33926–33938. [PubMed: 19812418]
58. Amé JC, Spenlehauer C, de Murcia G. The PARP superfamily. *Bioessays* 2004;26:882–893. [PubMed: 15273990]
59. Kleine H, et al. Substrate-assisted catalysis by PARP10 limits its activity to mono-ADP-ribosylation. *Mol Cell* 2008;32:57–69. [PubMed: 18851833]
60. Karlberg T, Hammarstrom M, Schutz P, Svensson L, Schuler H. Crystal structure of the catalytic domain of human PARP2 in complex with PARP inhibitor ABT-888. *Biochemistry* 2010;49:1056–1058. [PubMed: 20092359]
61. Lehtio L, et al. Zinc binding catalytic domain of human tankyrase 1. *J Mol Biol* 2008;379:136–145. [PubMed: 18436240]
62. Lehtio L, et al. Structural basis for inhibitor specificity in human poly(ADP-ribose) polymerase-3. *J Med Chem* 2009;52:3108–3111. [PubMed: 19354255]
63. Ménissier de Murcia J, et al. Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J* 2003;22:2255–2263. [PubMed: 12727891]

64. Chiang YJ, et al. Tankyrase 1 and tankyrase 2 are essential but redundant for mouse embryonic development. *PLoS ONE* 2008;3:e2639. [PubMed: 18612384]
65. Yelamos J, Schreiber V, Dantzer F. Toward specific functions of poly(ADP-ribose) polymerase-2. *Trends Mol Med* 2008;14:169–178. [PubMed: 18353725]
66. Shieh WM, et al. Poly(ADP-ribose) polymerase null mouse cells synthesize ADP-ribose polymers. *J Biol Chem* 1998;273:30069–30072. [PubMed: 9804757]
67. Sallmann FR, Vodenicharov MD, Wang ZQ, Poirier GG. Characterization of sPARP-1. An alternative product of PARP-1 gene with poly(ADP-ribose) polymerase activity independent of DNA strand breaks. *J Biol Chem* 2000;275:15504–15511. [PubMed: 10809783]
68. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes Dev* 2004;18:1272–1282. [PubMed: 15145826]
69. Liu Y, et al. Vault poly(ADP-ribose) polymerase is associated with mammalian telomerase and is dispensable for telomerase function and vault structure *in vivo*. *Mol Cell Biol* 2004;24:5314–5323. [PubMed: 15169895]
70. Raval-Fernandes S, Kickhoefer VA, Kitchen C, Rome LH. Increased susceptibility of vault poly(ADP-ribose) polymerase-deficient mice to carcinogen-induced tumorigenesis. *Cancer Res* 2005;65:8846–8852. [PubMed: 16204055]
71. Hsiao SJ, Smith S. Tankyrase function at telomeres, spindle poles, and beyond. *Biochimie* 2008;90:83–92. [PubMed: 17825467]
72. Sbdio JI, Chi NW. Identification of a tankyrase-binding motif shared by IRAP, TAB182, and human TRF1 but not mouse TRF1. NuMA contains this RXXPDG motif and is a novel tankyrase partner. *J Biol Chem* 2002;277:31887–31892. [PubMed: 12080061]
73. Canudas S, et al. Protein requirements for sister telomere association in human cells. *EMBO J* 2007;26:4867–4878. [PubMed: 17962804]
74. Hsiao SJ, Smith S. Sister telomeres rendered dysfunctional by persistent cohesion are fused by NHEJ. *J Cell Biol* 2009;184:515–526. [PubMed: 19221198]
75. Huang SM, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 2009;461:614–620. [PubMed: 19759537]
76. Nobori T, Yamanaka H, Carson DA. Poly(ADP-ribose) polymerase inhibits DNA synthesis initiation in the absence of NAD. *Biochem Biophys Res Commun* 1989;163:1113–1118. [PubMed: 2506850]
77. Zaremba T, Curtin NJ. PARP inhibitor development for systemic cancer targeting. *Anticancer Agents Med Chem* 2007;7:515–523. [PubMed: 17896912]
78. Satoh MS, Poirier GG, Lindahl T. Dual function for poly(ADP-ribose) synthesis in response to DNA strand breakage. *Biochemistry* 1994;33:7099–7106. [PubMed: 8003475]
79. Farzaneh F, Zalin R, Brill D, Shall S. DNA strand breaks and ADP-ribosyl transferase activation during cell differentiation. *Nature* 1982;300:362–366. [PubMed: 6292727]
80. Johnstone AP, Williams GT. Role of DNA breaks and ADP-ribosyl transferase activity in eukaryotic differentiation demonstrated in human lymphocytes. *Nature* 1982;300:368–370. [PubMed: 6292728]
81. Saleh-Gohari N, et al. Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks. *Mol Cell Biol* 2005;25:7158–7169. [PubMed: 16055725]
82. Anders C, Carey LA. Understanding and treating triple-negative breast cancer. *Oncology* 2008;22:1233–1239. [PubMed: 18980022]
83. Turner N, Tutt A, Ashworth A. Hallmarks of ‘BRCAness’ in sporadic cancers. *Nature Rev Cancer* 2004;4:814–819. [PubMed: 15510162]
84. McCabe N, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res* 2006;66:8109–8115. [PubMed: 16912188]
85. Mendes-Pereira AM, et al. Synthetic lethal targeting of *PTEN* mutant cells with PARP inhibitors. *EMBO Mol Med* 2009;1:315–322. [PubMed: 20049735]
86. Ashworth A. Drug resistance caused by reversion mutation. *Cancer Res* 2008;68:10021–10023. [PubMed: 19074863]

87. Sakai W, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* 2008;451:1116–1120. [PubMed: 18264087]
88. Edwards SL, et al. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 2008;451:1111–1115. [PubMed: 18264088]
89. Ratnam K, Low JA. Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. *Clin Cancer Res* 2007;13:1383–1388. [PubMed: 17332279]
90. Plummer R, et al. Phase I study of the poly(ADP-ribose) polymerase inhibitor, AG014699, in combination with temozolomide in patients with advanced solid tumors. *Clin Cancer Res* 2008;14:7917–7923. [PubMed: 19047122]
91. O'Shaughnessy J, et al. Efficacy of BSI-201, a poly (ADP-ribose) polymerase-1 (PARP1) inhibitor, in combination with gemcitabine/carboplatin (G/C) in patients with metastatic triple-negative breast cancer (TNBC): results of a randomized Phase II trial. *J Clin Oncol* 2009;27:3.
92. Ossovskaya V, et al. BSI-201 enhances the activity of multiple classes of cytotoxic agents and irradiation in triple negative breast cancer. Abstract 5552. *Proc Annu Meet Am Assoc Cancer Res.* 2009
93. Pacher P, Szabo C. Role of poly(ADP-ribose) polymerase 1 (PARP-1) in cardiovascular diseases: the therapeutic potential of PARP inhibitors. *Cardiovasc Drug Rev* 2007;25:235–260. [PubMed: 17919258]
94. Goldberg S, Visochek L, Giladi E, Gozes I, Cohen-Armon M. PolyADP-ribosylation is required for long-term memory formation in mammals. *J Neurochem* 2009;111:72–79. [PubMed: 19645746]
95. Morrison C, et al. Genetic interaction between PARP and DNA-PK in V(D)J. recombination and tumorigenesis. *Nature Genet* 1997;17:479–482. [PubMed: 9398855]
96. Tong WM, et al. Synergistic role of Ku80 and poly(ADP-ribose) polymerase in suppressing chromosomal aberrations and liver cancer formation. *Cancer Res* 2002;62:6990–6996. [PubMed: 12460917]
97. Tong WM, et al. Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53^{-/-} mice. *Am J Pathol* 2003;162:343–352. [PubMed: 12507917]
98. Lilyestrom W, van der Woerd MJ, Clark N, Luger K. Structural and biophysical studies of human PARP-1 in complex with damaged DNA. *J Mol Biol* 2010;395:983–994. [PubMed: 19962992]
99. Formentini L, et al. Poly(ADP-ribose) catabolism triggers AMP-dependent mitochondrial energy failure. *J Biol Chem* 2009;284:17668–17676. [PubMed: 19411252]
100. Loseva O, et al. Poly(ADP-ribose) polymerase-3 (PARP-3) is a mono-ADP ribosylase that activates PARP-1 in absence of DNA. *J Biol Chem.* Jan 11;2010 10.1074/jbc.M109.077834
101. McCabe N, et al. Targeting tankyrase 1 as a therapeutic strategy for BRCA-associated cancer. *Oncogene* 2009;28:1465–1470. [PubMed: 19182824]
102. Turner NC, et al. A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor. *EMBO J* 2008;27:1368–1377. [PubMed: 18388863]
103. Mendeleyev J, Kirsten E, Hakam A, Buki KG, Kun E. Potential chemotherapeutic activity of 4-iodo-3-nitrobenzamide. Metabolic reduction to the 3-nitroso derivative and induction of cell death in tumor cells in culture. *Biochem Pharmacol* 1995;50:705–714. [PubMed: 7669074]
104. Moore, J., et al. Treatment of cancer. US Patent Application Publication. US2008/0103104 A1. 2008.
105. Konishi Y, et al. Possible model of liver carcinogenesis using inhibitors of NAD⁺ ADP ribosyl transferase in rats. *Toxicol Pathol* 1986;14:483–488. [PubMed: 3101158]
106. Takahashi S, et al. Enhancement of DEN initiation of liver carcinogenesis by inhibitors of NAD⁺ ADP ribosyl transferase in rats. *Carcinogenesis* 1984;5:901–906. [PubMed: 6145526]
107. Andrabi SA, et al. Poly(ADP-ribose) (PAR) polymer is a death signal. *Proc Natl Acad Sci USA* 2006;103:18308–18313. [PubMed: 17116882]
108. McLennan AG. The Nudix hydrolase superfamily. *Cell Mol Life Sci* 2006;63:123–143. [PubMed: 16378245]
109. Huang Q, Shen HM. To die or to live: the dual role of poly(ADP-ribose) polymerase-1 in autophagy and necrosis under oxidative stress and DNA damage. *Autophagy* 2009;5:273–276. [PubMed: 19139632]

110. Munoz-Gamez JA, et al. PARP-1 is involved in autophagy induced by DNA damage. *Autophagy* 2009;5:61–74. [PubMed: 19001878]
111. Otto H, et al. *In silico* characterization of the family of PARP-like poly(ADP-ribosyl)transferases (pARTs). *BMC Genomics* 2005;6:139. [PubMed: 16202152]
112. Alvarez-Gonzalez R, Jacobson MK. Characterization of polymers of adenosine diphosphate ribose generated *in vitro* and *in vivo*. *Biochemistry* 1987;26:3218–3224. [PubMed: 3038179]
113. Rippmann JF, Damm K, Schnapp A. Functional characterization of the poly(ADP-ribose) polymerase activity of tankyrase 1, a potential regulator of telomere length. *J Mol Biol* 2002;323:217–224. [PubMed: 12381316]
114. Sbodio JI, Lodish HF, Chi NW. Tankyrase-2 oligomerizes with tankyrase-1 and binds to both TRF1 (telomere-repeat-binding factor 1) and IRAP (insulin-responsive aminopeptidase). *Biochem J* 2002;361:451–459. [PubMed: 11802774]
115. Augustin A, et al. PARP-3 localizes preferentially to the daughter centriole and interferes with the G1/S cell cycle progression. *J Cell Sci* 2003;116:1551–1562. [PubMed: 12640039]
116. Bauer PI, Buki KG, Hakam A, Kun E. Macromolecular association of ADP-ribosyltransferase and its correlation with enzymic activity. *Biochem J* 1990;270:17–26. [PubMed: 2144419]
117. Mendoza-Alvarez H, Alvarez-Gonzalez R. Poly(ADP-ribose) polymerase is a catalytic dimer and the automodification reaction is intermolecular. *J Biol Chem* 1993;268:22575–22580. [PubMed: 8226768]
118. de Murcia JM, et al. Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *Proc Natl Acad Sci USA* 1997;94:7303–7307. [PubMed: 9207086]
119. Masutani M, et al. Function of poly(ADP-ribose) polymerase in response to DNA damage: gene-disruption study in mice. *Mol Cell Biochem* 1999;193:149–152. [PubMed: 10331651]
120. Wang ZQ, et al. Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. *Genes Dev* 1995;9:509–520. [PubMed: 7698643]
121. Yelamos J, et al. PARP-2 deficiency affects the survival of CD4⁺CD8⁺ double-positive thymocytes. *EMBO J* 2006;25:4350–4360. [PubMed: 16946705]
122. Yeh TY, et al. Hypermetabolism, hyperphagia, and reduced adiposity in tankyrase-deficient mice. *Diabetes* 2009;58:2476–2485. [PubMed: 19651815]
123. Chiang YJ, et al. Generation and characterization of telomere length maintenance in tankyrase 2-deficient mice. *Mol Cell Biol* 2006;26:2037–2043. [PubMed: 16507984]
124. Hsiao SJ, Poitras MF, Cook BD, Liu Y, Smith S. Tankyrase 2 poly(ADP-ribose) polymerase domain-deleted mice exhibit growth defects but have normal telomere length and capping. *Mol Cell Biol* 2006;26:2044–2054. [PubMed: 16507985]
125. Albert JM, et al. Inhibition of poly(ADP-ribose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models. *Clin Cancer Res* 2007;13:3033–3042. [PubMed: 17505006]
126. Clarke MJ, et al. Effective sensitization of temozolomide by ABT-888 is lost with development of temozolomide resistance in glioblastoma xenograft lines. *Mol Cancer Ther* 2009;8:407–414. [PubMed: 19174557]
127. Donawho CK, et al. ABT-888, an orally active poly(ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. *Clin Cancer Res* 2007;13:2728–2737. [PubMed: 17473206]
128. Horton TM, et al. Poly(ADP-ribose) polymerase inhibitor ABT-888 potentiates the cytotoxic activity of temozolomide in leukemia cells: influence of mismatch repair status and O6-methylguanine-DNA methyltransferase activity. *Mol Cancer Ther* 2009;8:2232–2242. [PubMed: 19671751]
129. Liu SK, et al. A novel poly(ADP-ribose) polymerase inhibitor, ABT-888, radiosensitizes malignant human cell lines under hypoxia. *Radiother Oncol* 2008;88:258–268. [PubMed: 18456354]
130. Liu X, et al. Potentiation of temozolomide cytotoxicity by poly(ADP)ribose polymerase inhibitor ABT-888 requires a conversion of single-stranded DNA damages to double-stranded DNA breaks. *Mol Cancer Res* 2008;6:1621–1629. [PubMed: 18922977]
131. Penning TD, et al. Discovery of the poly(ADP-ribose) polymerase (PARP) inhibitor 2-[(R.)-2-methylpyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide (ABT-888) for the treatment of cancer. *J Med Chem* 2009;52:514–523. [PubMed: 19143569]

132. Daniel RA, et al. Inhibition of poly(ADP-ribose) polymerase-1 enhances temozolomide and topotecan activity against childhood neuroblastoma. *Clin Cancer Res* 2009;15:1241–1249. [PubMed: 19174487]
133. Thomas HD, et al. Preclinical selection of a novel poly(ADP-ribose) polymerase inhibitor for clinical trial. *Mol Cancer Ther* 2007;6:945–956. [PubMed: 17363489]
134. Dungey FA, Caldecott KW, Chalmers AJ. Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90. *Mol Cancer Ther* 2009;8:2243–2254. [PubMed: 19671736]
135. Dungey FA, Loser DA, Chalmers AJ. Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-ribose) polymerase: mechanisms and therapeutic potential. *Int J Radiat Oncol Biol Phys* 2008;72:1188–1197. [PubMed: 18954712]
136. Evers B, et al. Selective inhibition of *BRCA2*-deficient mammary tumor cell growth by AZD2281 and cisplatin. *Clin Cancer Res* 2008;14:3916–3925. [PubMed: 18559613]
137. Hay T, et al. Poly(ADP-ribose) polymerase-1 inhibitor treatment regresses autochthonous *Brca2/p53*-mutant mammary tumors *in vivo* and delays tumor relapse in combination with carboplatin. *Cancer Res* 2009;69:3850–3855. [PubMed: 19383921]
138. Menear KA, et al. 4-[3-(4-cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one: a novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. *J Med Chem* 2008;51:6581–6591. [PubMed: 18800822]
139. Rottenberg S, et al. High sensitivity of *BRCA1*-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. *Proc Natl Acad Sci USA* 2008;105:17079–17084. [PubMed: 18971340]
140. Miknyoczki S, et al. The selective poly(ADP-ribose) polymerase-1(2) inhibitor, CEP-8983, increases the sensitivity of chemoresistant tumor cells to temozolomide and irinotecan but does not potentiate myelotoxicity. *Mol Cancer Ther* 2007;6:2290–2302. [PubMed: 17699724]
141. Jones P, et al. Discovery of 2-{4-[(3S)-Piperidin-3-yl] phenyl}-2H-indazole-7-carboxamide (MK-4827): a novel oral poly(ADP-ribose)polymerase (PARP) inhibitor+ efficacious in *BRCA-1* and -2 mutant tumors. *J Med Chem* 2009;52:7170–7185. [PubMed: 19873981]

Figure 1. Structural and functional characteristics of PARP1

a | Poly(ADP-ribose) polymerase 1 (PARP1) is shown with its DNA-binding (DBD), automodification (AD) and catalytic domains. The PARP signature sequence (yellow box within the catalytic domain) comprises the sequence most conserved among PARPs. Crucial residues for nicotinamide adenine dinucleotide (NAD^+) binding (histidine; H and tyrosine; Y) and for polymerase activity (glutamic acid; E) are indicated. **b** | Consequences of PARP1 activation by DNA damage. Although not shown to simplify the scheme, PARP1 is active in a homodimeric form^{116,117}. PARP1 detects DNA damage through its DBD. This activates PARP1 to synthesize poly(ADP) ribose (pADPr; yellow beads) on acceptor proteins, including histones and PARP1. Owing to the dense negative charge of pADPr, PARP1 loses affinity for DNA, allowing the recruitment of repair proteins by pADPr to the damaged DNA (blue and purple circles). Poly(ADP-ribose) glycohydrolase (PARG) and possibly ADP-ribose hydrolase 3 (ARH3) hydrolyse pADPr into ADP-ribose molecules and free pADPr. ADP-ribose is further metabolized by the pyrophosphohydrolase NUDIX enzymes into AMP, raising AMP:ATP ratios, which in turn activate the metabolic sensor AMP-activated protein kinase (AMPK). NAD^+ is replenished by the enzymatic conversion of nicotinamide into NAD^+ at the expense

of phosphoribosylpyrophosphate (PRPP) and ATP. Examples of proteins non-covalently (pADPr-binding proteins) or covalently poly(ADP-ribosyl)ated are shown with the functional consequences of modification (reviewed in REF. ²⁰). It is important to note that many potential protein acceptors of pADPr remain to be identified owing to the difficulty of purifying pADPr-binding proteins *in vivo*. PARP inhibitors prevent the synthesis of pADPr and hinder subsequent downstream repair processes, lengthening the lifetime of DNA lesions. ATM, ataxia telangiectasia-mutated; BER, base excision repair; BRCT, BRCA1 carboxy-terminal repeat motif; DNA-PKcs, DNA-protein kinase catalytic subunit; DSB, double-strand break; HR, homologous recombination; NHEJ, non-homologous end joining; NLS, nuclear localisation signal; PP_i, inorganic pyrophosphate; SSB, single-strand break; Zn, zinc finger.

Table 1

Mouse models of the PARP family

Deletion	Summary of phenotypes relevant to cancer	Refs
<i>Parp1</i>	<ul style="list-style-type: none"> Three mouse models showed hypersensitivity to γ-irradiation and alkylating agents, high genomic instability (high rate of sister chromatid exchange and micronuclei), accumulation of DNA strand breaks and impaired DNA repair No defects in viability, fertility, development or tissue differentiation 	118–120
<i>Parp2</i>	<ul style="list-style-type: none"> Hypersensitivity to γ-irradiation and alkylating agents High genomic instability (mis-segregation of chromosomes and high rate of sister chromatid exchange) Impaired thymopoiesis, adipogenesis and spermatogenesis No defects in viability, fertility, development or tissue differentiation 	63, 121
<i>Parp1; Parp2</i>	<ul style="list-style-type: none"> Embryonic lethality at onset of gastrulation 	63
<i>Parp4</i>	<ul style="list-style-type: none"> Increased colon tumour incidence after dimethylhydrazine treatment 	70
<i>Tnks</i>	<ul style="list-style-type: none"> Increased energy expenditure and decreased adiposity No defects in telomere maintenance or telomere capping 	64, 122
<i>Tnks2</i>	<ul style="list-style-type: none"> Growth retardation No defects in telomere maintenance or telomere capping 	123
PARP catalytic domain of <i>Tnks2</i>	<ul style="list-style-type: none"> Growth retardation No defects in telomere maintenance or telomere capping 	124
<i>Tnks; Tnks2</i>	<ul style="list-style-type: none"> Embryonic lethality at day 10 	64

PARP, poly(ADP-ribose) polymerase; TNKS, tankyrase.

Table 2

Clinical cancer studies involving PARP inhibitors

Drug	Company	Biophysical parameters	Synergizes with (<i>in vitro</i>)	Clinical trials*	Phase*	Refs
ABT-888	Abbott	K _i = 5.2 nM (PARP1) K _i = 2.9 nM (PARP2) EC ₅₀ = 2 nM (C41 cells)	Temozolomide Platin Cyclophosphamide Ionizing radiation MNNG Topoisomerase I poisons	Glioblastoma multiforme (with temozolomide)	Phase II	125-131
				Solid tumours and leukaemia (various combinations)	Phase I	
				BRCA1- or BRCA2-mutant tumours	Phase I	
AG014699	Pfizer	K _i = 1.4 nM (PARP1)	Temozolomide Ionizing radiation Topotecan	BRCA1- or BRCA2-mutant tumours	Phase II	90, 132, 133
AZD2281 (olaparib)	AstraZeneca	IC ₅₀ = 5 nM (PARP1) IC ₅₀ = 1 nM (PARP2) IC ₅₀ = 1.5 μM (tankyrase 1)	Temozolomide Platin MMS Ionizing radiation (with and without 17-AAG)	Platin-sensitive ovarian cancer	Phase II	134-139
				BRCA1- or BRCA2-mutant tumours (with carboplatin)	Phase II	
				Triple-negative breast cancer (single-agent or with carboplatin)	Phase II	
BSI-201	SanoFi-Aventis	ND	Ionizing radiation Oxaliplatin Gemcitabine and carboplatin Topotecan	Other solid tumours	Phase I/II	91, 92, 103
				Triple-negative breast cancer (with gemcitabine and carboplatin)	Phase III	
				Ovarian cancer, glioblastoma multiforme and uterine cancer (various combinations)	Phase II	
				BRCA2-mutant pancreatic cancer (various combinations)	Phase Ib	
CEP-8983/CEP-9722 (produg)	Cephalon	IC ₅₀ = 20 nM (PARP1) IC ₅₀ = 6 nM (PARP2)	Temozolomide Topoisomerase I poisons	Other solid tumours	Phase I/II	140
MK-4827	Merck	IC ₅₀ = 3.2 nM (PARP1) IC ₅₀ = 4 nM (PARP2)		Solid tumours (with temozolomide)	Phase I	
				Solid tumours and ovarian cancer	Phase I	141

* Based on information obtained from <http://www.clinicaltrials.gov>. 17-AAG, 17-allylamino-17-demethoxygelatinamycin; EC₅₀, half maximal effective concentration; IC₅₀, half maximal inhibitory concentration; K_i, binding affinity of inhibitor; MMS, methyl methanesulfonate; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; ND, not determined; PARP, poly(ADP-ribose) polymerase.