

Poly(ADP-ribose) polymerase inhibition: past, present and future

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Abstract | The process of poly(ADP-ribosylation) and the major enzyme that catalyses this reaction, poly(ADP-ribose) polymerase 1 (PARP1), were discovered more than 50 years ago. Since then, advances in our understanding of the roles of PARP1 in cellular processes such as DNA repair, gene transcription and cell death have allowed the investigation of therapeutic PARP inhibition for a variety of diseases — particularly cancers in which defects in DNA repair pathways make tumour cells highly sensitive to the inhibition of PARP activity. Efforts to identify and evaluate potent PARP inhibitors have so far led to the regulatory approval of four PARP inhibitors for the treatment of several types of cancer, and PARP inhibitors have also shown therapeutic potential in treating non-oncological diseases. This Review provides a timeline of PARP biology and medicinal chemistry, summarizes the pathophysiological processes in which PARP plays a role and highlights key opportunities and challenges in the field, such as counteracting PARP inhibitor resistance during cancer therapy and repurposing PARP inhibitors for the treatment of non-oncological diseases.

The nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP1) was first described more than 50 years ago^{1–3} and is now known to play important roles in DNA repair and the maintenance of genome integrity, as well as in the regulation of multiple metabolic and signal transduction processes in health and disease^{1–5}. It catalyses the transfer of ADP-ribose residues from NAD⁺ onto target substrates, building a poly(ADP-ribose) (PAR) chain (FIG. 1a). The building up of PAR chains and the removal of these chains — principally by poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3) — occurs in almost all eukaryotic cells. PARP1 was the first member of a superfamily of ADP-ribosylating enzymes, which consists of proteins that have homology to PARP1 and that, in general, are capable of catalytic ADP-ribosyltransferase reactions (the structure of PARP1 is shown in FIG. 1b). The family now has 17 members, four of which — PARP1, PARP2, PARP5A and PARP5B — are capable of synthesizing PAR chains^{1–3}. Most of the other enzymes in the family build only single ADP-ribose units and are therefore classified as mono(ADP-ribosyl)ases (MARs).

Since the 1970s, the field of PARP biology has expanded dramatically, with more than 20,000 articles published to date. Some of the major milestones in preclinical PARP research relating to the discovery of pharmacological PARP inhibitors and the delineation of their molecular modes of action^{6–63} are shown in FIG. 2. Early milestones in PARP research include the discovery of PAR, the elucidation of its structure and

the discovery that PARP1 produces PAR^{6–8}. Subsequent studies described the purification of PARP1 (REF. 10), showed PARP activation in response to genotoxic agents^{11,12}, linked PARP1 to DNA repair¹⁵ and demonstrated the association of PAR with chromatin, histones and nuclear enzymes including PARP1 itself^{8,9}. The generation and characterization of the *Parp1*-knockout mouse^{24,29,64} was instrumental in the discovery of PARP2, and subsequently additional members of the PARP superfamily were identified⁴⁶.

Specialized aspects of PARP biology have been summarized in other reviews, including PARP biochemistry^{65,66}, PARP molecular biology^{67,68} and the role of PARP in DNA repair^{69,70}, carcinogenesis^{71,72}, metabolism^{73,74}, signalling^{75,76}, cell death^{77,78}, gene transcription^{78,79} and ageing^{80,81}. This Review focuses specifically on the parallel evolution of two therapeutic concepts: the inhibition of PARP in order to interfere with DNA repair and induce tumour cell death for the treatment of oncological diseases and the inhibition of PARP to maintain cellular bioenergetics and suppress proinflammatory signalling for the treatment of non-oncological diseases. The evolution of these two concepts has occurred in parallel with advances with PARP inhibitor medicinal chemistry to produce more potent PARP inhibitors, some of which have entered clinical trials and been approved for treating various cancers (REFS^{82–98}, TABLE 1, reviewed in REFS^{99–101}). In this Review, after briefly summarizing the key advances in PARP biology, we outline the progress made towards therapeutically targeting PARP in

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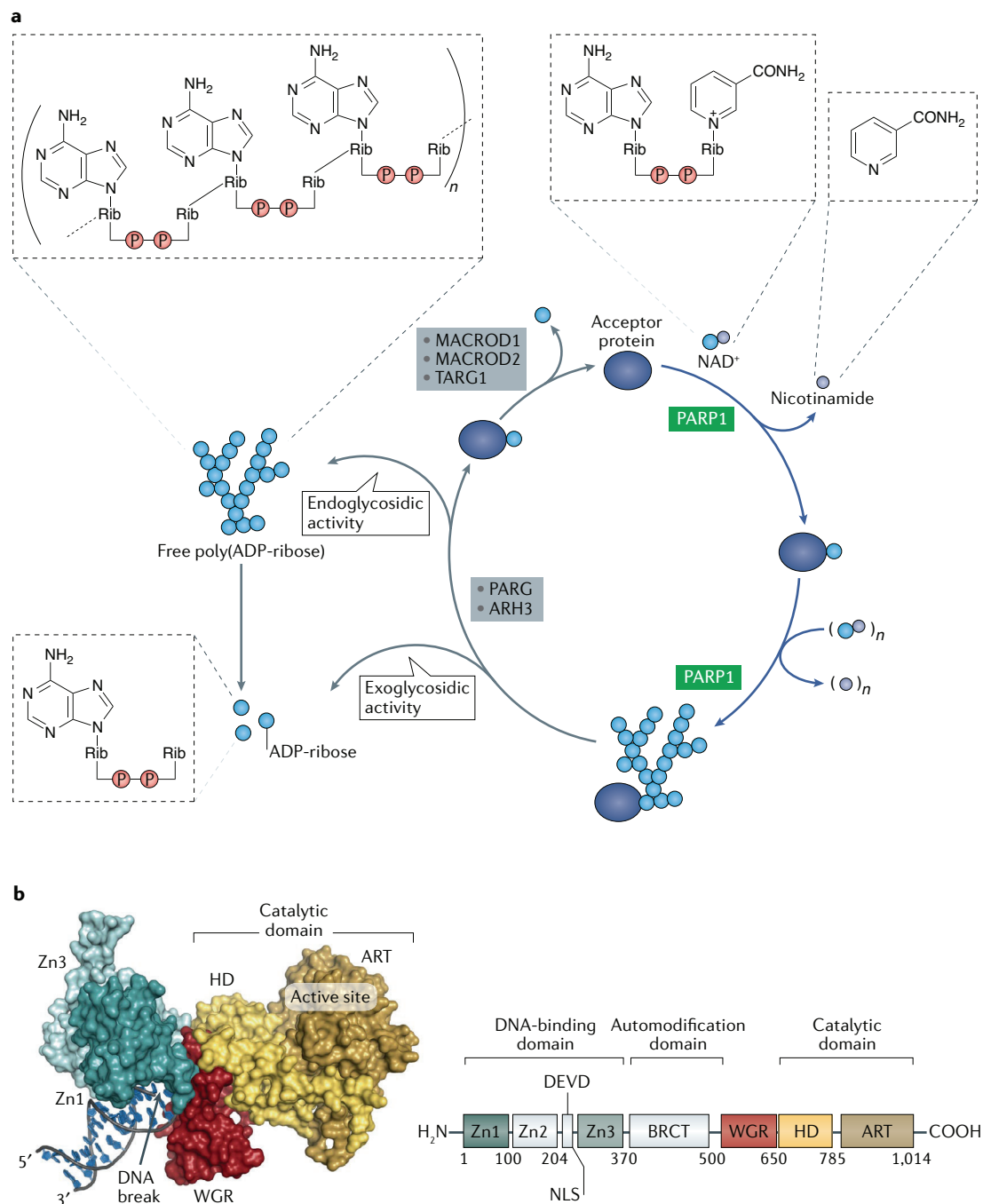


Fig. 1 | PARylation, PAR removal and the structure of PARP1. a | Enzymatic poly(ADP-ribose) (PAR) build-up and PAR degradation processes. Poly(ADP-ribose) polymerase 1 (PARP1) cleaves NAD⁺ to ADP-ribose and nicotinamide, and covalently attaches ADP-ribose to an acceptor protein. Additional NAD⁺ molecules can be cleaved and build up linear and branched forms of PAR. Two enzymes, poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3), play central roles in the degradation of PAR through their exoglycosidic and endoglycosidic activities. The ADP-ribose glycohydrolases MACROD1, MACROD2 and TARG1 act on mono(ADP-ribosyl)ated peptides generated by PARG. Free PAR can also be degraded to mono(ADP-ribose). **b** | A surface representation of PARP1 binding to damaged DNA is shown on the left and the modular organization of human PARP1 is shown on the right. The amino-terminal DNA-binding domain contains three zinc-finger domains, which mediate DNA binding and some protein–protein interactions, and a nuclear-localization signal (NLS) in the caspase cleavage site (DEVD). The central automodification domain contains a breast cancer-susceptibility protein–carboxy terminus (BRCT) motif, which mediates protein–protein interactions. The role of the tryptophan–glycine–arginine-rich (WGR) domain is not fully understood, although it likely represents a nucleic acid-binding domain. The carboxy-terminal catalytic domain contains two subdomains: a helical domain (HD), and the ADP-ribosyltransferase (ART) domain, which is located between residues 785 and 1,014, contains the active NAD⁺-binding site and is conserved in all PARP family members. PARylation, poly(ADP-ribosylation). Part **b** adapted with permission from REF.⁵⁴, AAAS.

cancer and in non-oncological diseases, with a focus on current challenges and emerging opportunities. We finally highlight the potential of targeting other PARP family members, such as PARP5A and PARP5B (also known as tankyrase 1 and tankyrase 2, respectively), PARP3 and PARP7. This Review focuses primarily on the inhibition of PARP1, as this enzyme is responsible for most mammalian PARylation, and inhibition of this enzyme has emerged as a translational and clinical strategy. Throughout this Review, 'PARP' generally refers to PARP1; when we are discussing pharmacological 'PARP inhibitors' these agents can inhibit other PARP isoforms, although the potential contributions of the inhibition of different PARP isoforms to the therapeutic effects of PARP inhibitors are unknown.

Key advances in PARP biology

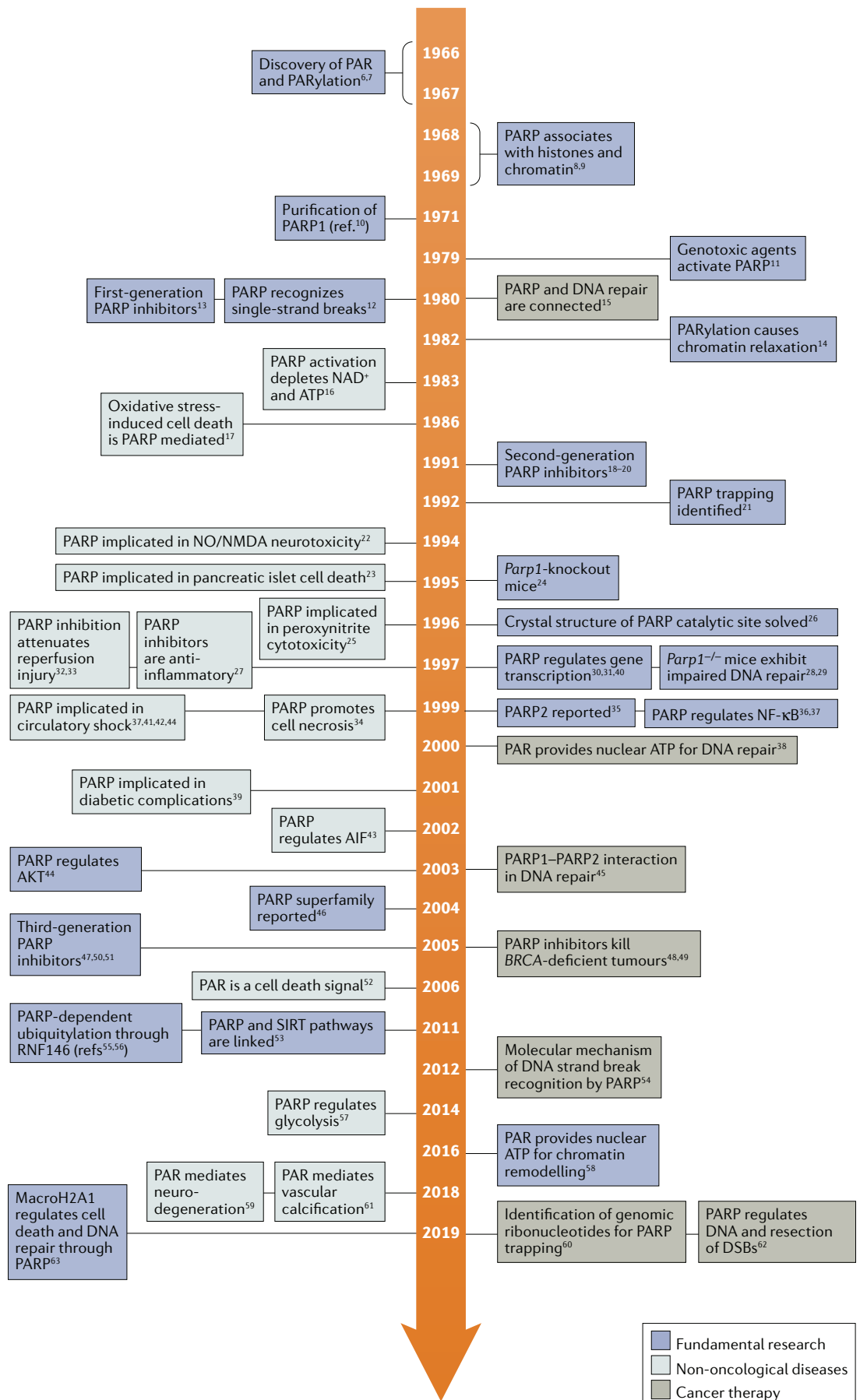
PARP inhibition suppresses DNA repair. The role of PARP1 in relation to DNA repair was first postulated in 1975 (REF.¹⁰²), and DNA-methylating agents and ionizing radiation were indentified as potent activators of PARP1 soon after^{11,103,104}. However, it was not until the discovery of the first PARP inhibitors, in the late 1970s¹⁵, that its function could be explored. These early inhibitors — 3-substituted benzamides, including 3-aminobenzamide (3-AB) — were based on nicotinamide, a weak inhibitor of PARP1 and a by-product of the PARP reaction converting NAD⁺ to PAR (FIG. 1). Virtually all PARP inhibitors described to date contain the nicotinamide pharmacophore (see TABLE 1 for examples). 3-AB allowed further exploration of PARP1's function; a seminal article showed that 3-AB inhibited NAD⁺ depletion, slowed DNA repair and decreased the survival of cells treated with the DNA-methylating agent dimethyl sulfate¹⁵. Subsequently, 3-AB was shown to inhibit the repair of radiation-induced DNA damage and prevent recovery from the effects of potentially lethal ionizing radiation¹⁰⁵, consistent with earlier data showing that DNA-alkylating agents and radiation are potent PARP activators. More potent PARP inhibitors developed in the 1990s were used to show that PARP inhibition slowed the repair of DNA damage caused by topoisomerase 1 poisons — which prevent the re-ligation of DNA single-strand breaks (SSBs) created by topoisomerase 1 — and increased the cytotoxicity of these agents, but not that of topoisomerase 2 poisons or antimetabolites^{85,86}.

PARP1 is best known for its role in DNA SSB repair (SSBR). SSBs can be formed directly through cleavage of the ribose-phosphate backbone by radiation, free radicals or oxidants, or by enzymatic cleavage of DNA by topoisomerase 1 poisons. SSBs can also be formed indirectly following the excision of damaged bases — for example, those that are methylated or oxidized — by glycosylases and cleavage of the resulting abasic site by an endonuclease^{106,107}. PARP1 recognizes the SSB through its DNA-binding domain, which contains three zinc-finger motifs. The binding of PARP1 zinc-finger 2 to the DNA causes a conformational change that activates PARP1 to cleave NAD⁺ into nicotinamide and an ADP-ribose moiety. The ADP-ribose moiety covalently attaches to either PARP1 or other nuclear proteins, such

as histones; other ADP-ribose groups are then added to it to produce long and sometimes branching PAR chains. These negatively charged polymers initially recruit the DNA repair protein XRCC1 to the site of the break, presumably through electrostatic attraction, and cause the PARylated histones and PARP1 to dissociate from the break through electrostatic repulsion, allowing the rest of the SSBR machinery to access the DNA. Digestion of PAR by PARG and ARH3 allows histones to reassociate with DNA and allows PARP1 to attach to other breaks and start the SSBR process again in another location^{108,109}. XRCC1 recruits bifunctional polynucleotide kinase/phosphatase (PNKP) and aprataxin (APTX), which process the DNA ends so that DNA polymerase- β can fill the gap and DNA ligase 3 can join the ends. If several nucleotides are replaced during processing and gap filling, then flap endonuclease 1 (FEN1) is also recruited and DNA ligase 1 may seal the ends (reviewed in REF.¹⁰⁸).

PARP1 has been implicated in DNA repair pathways other than SSBR (as reviewed in REFS¹¹⁰⁻¹¹²), including the repair of DNA double-strand breaks (DSBs) by non-homologous end joining (NHEJ) and alternative end joining, which involves enzymes common to SSBR such as XRCC1, DNA ligase 3 and FEN1, as well as DSB repair protein MRE11 and the DNA repair and telomere maintenance protein NBS1. PARP1 has also been associated with classical NHEJ, which involves the dimeric protein complex Ku70-Ku80 and DNA-dependent protein kinase catalytic subunit (DNA-PKCs). However, the importance of PARP1 to these pathways is not completely clear. Although PARP1 is activated by ionizing radiation — which causes DNA DSBs and SSBs — PARP inhibitors are modest radiosensitizers. Furthermore, PARP inhibitors fail to sensitize cells to topoisomerase 2 poisons, which primarily cause DSBs — suggesting PARP is not involved in DSB repair^{113,114}. There is some suggestion that PARP1 might promote nucleotide excision repair in response to UV radiation and cisplatin, although the evidence for PARP inhibitor-mediated increases in UV or cisplatin cytotoxicity is variable and cell line/experimental system dependent. For cisplatin at least, the enhancement of cytotoxicity by PARP inhibitors may reflect the homologous recombination repair (HRR) status of the cell¹¹⁵. A role for PARP in HRR itself has also been proposed, protecting stalled forks and promoting the restart of DNA synthesis; however, the data are conflicting because in the absence of PARP1, or in the presence of a PARP inhibitor, HRR is increased¹¹⁶⁻¹¹⁸. Further studies have suggested a role for PARP1 and its recruitment of XRCC1 in repairing unligated Okazaki fragments during DNA replication¹¹⁹.

PARP inhibitors inhibit DNA repair through PARP trapping. Inhibition of PARP activity has a greater impact on DNA repair than the lack of the enzyme itself. This was first demonstrated in a 1992 study²¹ that showed that nuclear extracts depleted of PARP1 can repair nicked plasmid DNA, but complete nuclear extracts deprived of NAD⁺ or containing the PARP inhibitor 3-AB cannot. The study authors hypothesized that PARP1 was binding to the nicked DNA, but failed to dissociate in



◀ Fig. 2 | **Selected preclinical PARP research milestones.** Research on poly(ADP-ribose) polymerase (PARP) culminated in the formation of two distinct therapeutic concepts: the targeting of non-transformed cells to preserve cellular energetic pools, produce cytoprotective actions and suppress proinflammatory mediator production in various acute and chronic non-oncological diseases (non-oncological diseases), and the use of PARP inhibitors to suppress DNA repair and tumour growth (cancer therapy). Key basic discoveries, such as the emergence of different classes of PARP inhibitor, are also shown (fundamental research). The timeline of PARP biology was comprehensively reviewed by Kraus in 2015 (REF.⁷). AIF, apoptosis-inducing factor 1; DSB, double-strand break; NF- κ B, nuclear factor- κ B; NMDA, N-methyl-D-aspartate; NO, nitric oxide; PAR, poly(ADP-ribose); PARylation, poly(ADP-ribosyl)ation; SIRT, sirtuin.

the absence of catalytic activity, thereby blocking DNA repair. Additionally, *Parp1*-knockout mice were found to be viable and fertile^{24,29,64}, implying that PARP1 is not necessary for viability and that PARP inhibitors might not be toxic. Further studies confirmed the 1992 data, and the term 'PARP trapping' was coined to refer to the prevention of PARP1 dissociation from the DNA in the presence of a PARP inhibitor^{120,121}. PARP inhibitors differ in their ability to induce PARP trapping; for example, although the clinically approved PARP inhibitors olaparib, rucaparib and talazoparib have similar catalytic inhibitory potencies, talazoparib is around 100-fold more potent at PARP1 trapping than the other inhibitors¹²². Recent studies suggest that whereas talazoparib and olaparib interact allosterically with PARP1 in a way that modestly inhibits the dissociation of PARP1 from DNA, rucaparib, niraparib and veliparib interact in a way that promotes dissociation by a factor of 3–5. However, for all inhibitors, inhibition of the catalytic activity of PARP1 and prevention of autoPARylation are important factors promoting dissociation¹²³. It is likely that the cytotoxic effects of inhibitors are the product of these complex interactions. The capacities of these inhibitors to influence trapping and polymer formation are likely to mediate their cytotoxicity, especially in cases where the inhibitor is used as a single agent in homologous recombination-deficient cells or as a chemosensitizer for DNA-methylating agents. The capacity of inhibitors to influence PARylation is thought to be more important for the sensitization of cells to topoisomerase 1 poisons¹²⁴. The nature of the DNA breaks — for example, whether they are endogenously induced SSBs, unligated Okazaki fragments, downstream SSBs following the removal of a methylated base or an SSB with one end attached to topoisomerase 1 — may inform the relative importance of trapping and catalytic inhibition.

The two faces of PARP inhibition. Following studies demonstrating that PAR can recruit DNA repair enzymes to the site of DNA injury and coordinate their activity, a novel therapeutic concept emerged: by inhibiting PARP, it may be possible to suppress DNA repair and induce the death of cancer cells. In parallel with the growing appreciation of PARP1 as a therapeutic target in cancer, studies described an additional, energetic role for PAR in providing ATP to DNA repair enzymes to ensure successful DNA repair^{38,125}, implicating PARP as an ancient 'stress response' mechanism capable of redirecting cellular energetic pools from the cytosol to the

nuclear compartment. Studies in the early 1980s showed that PARP1 activation in response to DNA-damaging agents is associated with the depletion of cellular NAD⁺ and ATP, which can be prevented by pharmacological inhibition of PARP1 (REF.¹⁶). PARP-mediated bioenergetic defects were subsequently shown to develop in response to endogenous genotoxic agents such as hydroxyl radicals and peroxynitrite, which are produced in a variety of pathophysiological conditions^{17,25}. Further studies showed that prolonged or extensive PARP1 activation can promote a regulated form of cell necrosis³⁴. From these observations, an additional therapeutic concept emerged: by inhibiting PARP, it may be possible to maintain cell viability in oxidatively stressed or nitrosatively stressed cells and thereby induce cytoprotection and organ preservation in various non-oncological diseases (note that PARP-mediated necrosis is not to be confused with the role of PARP1 as a substrate for cleavage during apoptosis^{126,127}; see BOX 1). In the following sections, we separate the distinct pathophysiological mechanisms involved in oncological and non-oncological diseases and review the mechanisms and processes by which PARP inhibition exerts therapeutic effects in these conditions.

PARP inhibition in oncology

PARP inhibitors as chemosensitizers and radiosensitizers. In the 1990s and early 2000s, PARP inhibitors were predominantly developed with the aim of increasing the anticancer activity of ionizing radiation and chemotherapy drugs^{99,128}. PARP inhibitors were first shown to sensitize cancer cells to DNA-methylating agents; chemosensitization was then observed with the topoisomerase 1 poisons camptothecin, topotecan and irinotecan in vitro and in vivo. In advanced preclinical studies, coadministration of the PARP inhibitor AG14361 or AG014699 (now known as rucaparib) with the alkylating agent temozolomide resulted in complete tumour regression for more than 60 days in mice^{50,129}. These data led to the first clinical trial of a PARP inhibitor in patients with cancer in 2003 (REF.¹³⁰), which established that rucaparib at a dose of 12 mg m⁻² could safely be given with a full dose of temozolomide¹³⁰, although myelosuppression — typically seen with temozolomide therapy — was observed in treated patients. Despite promising preclinical data, clinical studies have largely been associated with high toxicity (reviewed in REF.¹³¹). Studies have shown that PARP inhibitors can also sensitize cells to platinum-based agents, although this effect seems to be cell line dependent and as both PARP inhibitors and platinum-based agents alone cause profound cytotoxicity in HRR-defective cells, chemosensitization may be due to an additive toxic effect on defects in HRR¹¹⁵.

Numerous models have shown PARP inhibitors can sensitize cells to ionizing radiation^{131,132}, reflecting the fact that ionizing radiation causes SSBs that are repaired by SSBR. The scientific foundation of PARP inhibitor combination therapy, as well as the current status of PARP inhibitor chemotherapy trials and radiotherapy trials, has been reviewed in more detail elsewhere^{131–134}. So far, no PARP inhibitor has been approved

Table 1 | Notable PARP inhibitors, from first-generation inhibitors to approved drugs

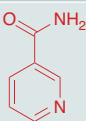
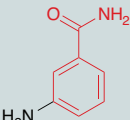
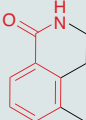
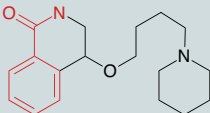
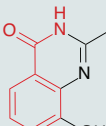
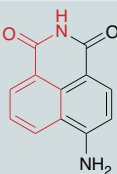
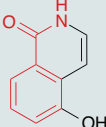
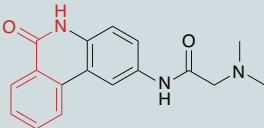
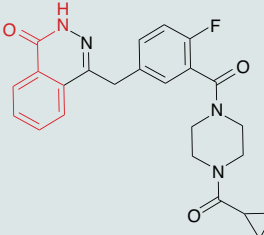
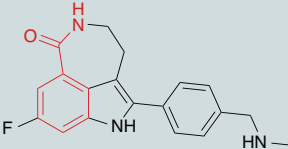
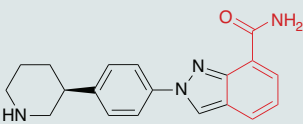
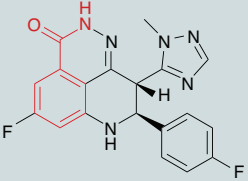
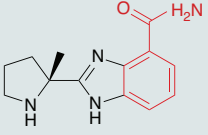
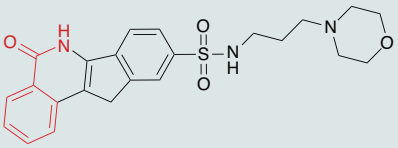
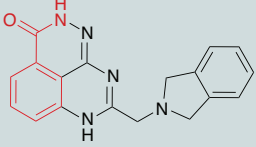
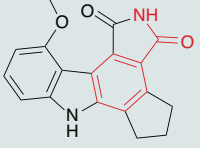
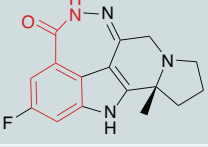
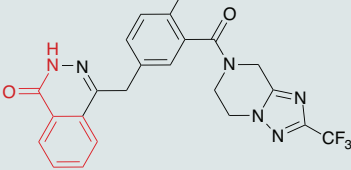
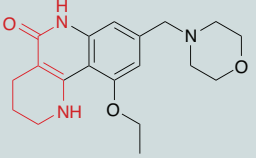
Compound name	IC ₅₀	Structure	Notes
First-generation PARP inhibitors			
Nicotinamide	210 μM		A weak PARP inhibitor and the by-product of the PARP reaction ^{10,11} . In cell-based studies, high (mM) concentrations are required to inhibit PARP. Has many pharmacological actions other than inhibiting PARP ⁸² . Virtually all PARP inhibitors contain the nicotinamide pharmacophore
3-aminobenzamide	33 μM		A weak PARP inhibitor ^{13,20} . In cell-based studies, high (mM) concentrations are required to inhibit PARP. Many other members of the benzamide class are PARP inhibitors. Benzamides have many additional pharmacological actions, including free radical scavenging
Second-generation PARP inhibitors			
PD128763	420 nM		Originally developed by Warner-Lambert ¹⁸ and characterized as a cytoprotective agent ⁸³ and a chemosensitizer and radiosensitizer ¹⁹ . In vivo adverse effects of the compound include hypothermia ⁸⁴
DPO	1 μM		A commonly used Warner-Lambert PARP inhibitor compound based on an isoquinoline core ¹⁸
NU1025	400 nM		An example of a series of quinazoline compounds discovered by a group at Newcastle University that act as potentiators of anticancer agent cytotoxicity ^{85,86}
4-ANI	180 nM		Originally identified by Banasik and colleagues ²⁰ , commonly used as an experimental tool to investigate the role of PARP in DNA repair and cell death
ISO	390 nM		Originally identified by Banasik and colleagues ²⁰ . Commonly used as an experimental tool to investigate the role of PARP in DNA repair and cell death ⁸⁷ . The structurally related, water-soluble compound 5-aminoisoquinoline has also been commonly used in various in vivo experiments ⁸⁷ . Another isoquinoline originally discovered by Guilford Inc. known as GPI 6150 (REF. ⁸⁸) was frequently used in in vitro and in vivo experiments in the 1990s and early 2000s
PJ-34	20 nM		Originally identified and characterized by Jagtap and colleagues ⁴¹ . Commonly used to study the biological roles of PARP in vitro and in vivo. In the high μM range, it also acts as a matrix metalloproteinase inhibitor ⁸⁹
Third generation, FDA-approved PARP inhibitors			
Olaparib (Lynparza)	1 nM		Developed and first used in patients by the UK-based biotechnology company KuDOS Pharmaceuticals ⁴⁹ . The first PARP inhibitor to demonstrate single-agent activity in BRCA-mutated cancers and to be given FDA approval for maintenance therapy for relapsed platinum-sensitive ovarian cancer in December 2014. Received FDA approval in December 2018 for first-line maintenance therapy in ovarian cancer and in December 2019 for first-line maintenance therapy in germ line BRCA-mutant pancreatic cancer. It has FDA breakthrough status in castration-resistant prostate cancer
Rucaparib (Rubraca)	1 nM		Discovered through a collaboration between the Northern Institute of Cancer Research and the Medical School of Newcastle University and Agouron Pharmaceuticals in San Diego, California ⁴⁸ . Accelerated FDA approval in December 2016 with companion diagnostic; subsequent approval in April 2018 independent of companion diagnostic for platinum-sensitive ovarian cancer. Achieved FDA breakthrough status in castration-resistant prostate cancer

Table 1 (cont.) | Notable PARP inhibitors, from first-generation inhibitors to approved drugs

Compound name	IC ₅₀	Structure	Notes
Third generation, FDA-approved PARP inhibitors (cont.)			
Niraparib (Zejula)	4 nM		Discovered and characterized by Merck Inc. ⁹⁰ and licensed to Tesaro in 2012, who obtained regulatory approval. Achieved FDA approval in March 2017 for maintenance therapy in ovarian cancer. Tesaro was acquired by GlaxoSmithKline in 2019
Talazoparib (Talzenna)	0.6 nM		Discovered by LEAD Therapeutics ⁹¹ . FDA approval granted in October 2018 for use in germ line BRCA-mutant, HER2-negative locally advanced or metastatic breast cancer
Veliparib (ABT-888)	2 nM		Discovered by Abbott ⁹² , who, in 2013, moved it to a spin-off company, AbbVie. AbbVie obtained regulatory approval and is responsible for marketing and further development. Received orphan drug status for lung cancer in November 2016
Additional, clinical-stage third-generation PARP inhibitors			
INO-1001	105 nM		Discovered at Inotek Pharmaceuticals ⁴⁷ , who advanced it to clinical trials in 2003. Licensed by Genentech in 2005, who have now terminated its development. Remains a preclinical experimental tool
2x-121	1 nM		Originally discovered by Eisai ⁹³ . Currently being developed by Oncology Venture
CEP-8983	20 nM		Originally discovered by Cephalon ⁹⁴ and currently being clinically evaluated by Checkpoint Therapeutics. A novel 4-methoxycarbazole PARP inhibitor with potential antineoplastic activity. Administered as its prodrug CEP-9722
Pamiparib (BGB-290)	0.83 nM		Currently in clinical trials conducted by BeiGene as a single-agent and combination therapy for ovarian cancer and other solid tumours ⁹⁵
Fluzoparib (SHR-3162)	1.5 nM		Currently in clinical trials conducted by Jiangsu HengRui Medicine in patients with germ line BRCA1/BRCA2 mutations and recurrent, platinum-sensitive ovarian cancer ⁹⁶
Amelparib (JPI-289)	18 nM		Discovered by Jeil Pharmaceuticals ⁹⁷ and currently in clinical trials in patients with stroke

In addition, IMP4297 is in clinical trials conducted by Impact Therapeutics in patients with advanced ovarian, breast, prostate and other solid tumors⁹⁸, and IDX-1197 (formerly NOV-1401) is in early-stage clinical trials conducted by Idience in patients with cancer (structures not available). The half-maximal inhibitory concentrations (IC₅₀ values) presented are not always directly comparable owing to differences in assay conditions. Red coloration of the bond highlights the conserved nicotinamide pharmacophore. Some commercial vendors incorrectly list INO-1001 as 3-aminobenzamide. Iniparib (Sanofi), a late-stage clinical development molecule that was subsequently found not to have significant inhibitory effect on poly(ADP-ribose) polymerase (PARP), is not discussed in this Review. HER2, human epidermal growth factor receptor 2.

in combination with either conventional chemotherapy or radiotherapy.

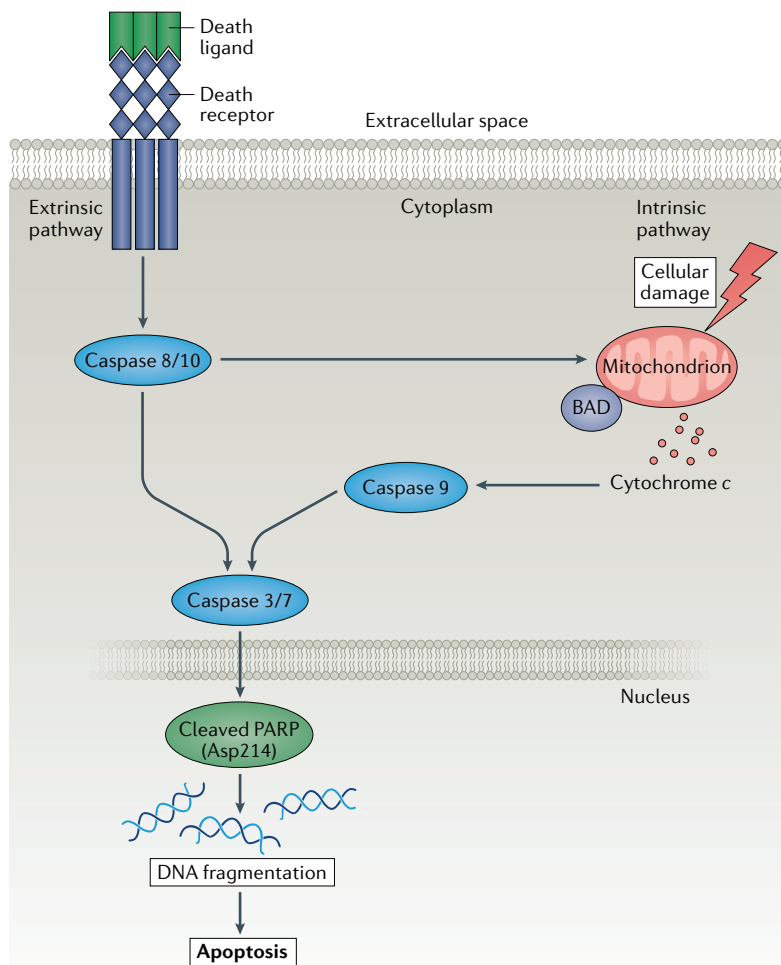
The role of PARP in cancer cells with BRCA mutations and 'synthetic lethality'. In 1995, Lindahl et al. showed that PARP inhibitors cause an increase in homologous recombination events. They suggested that when PARP1 functions normally, the negatively charged ADP-ribose polymer repels negatively charged DNA, preventing unwanted recombination events at sites of DNA breakage¹³⁵. Subsequent studies revealed that cells lacking XRCC1 accumulated more RAD51 foci, which are a marker of HRR, and γ -H2AX foci — a marker of DNA

damage — suggesting that a failure to repair endogenous SSBs leads to replication fork collapse and activation of HRR¹³⁶. This finding led to the hypothesis that cells lacking the ability for SSBR — for example, those lacking PARP1 or XRCC1 — are dependent on HRR for survival and that cells lacking HRR function, known as HRR-defective or homologous recombination-deficient cells, are dependent on PARP1 activity for survival. PARP inhibition in cells lacking HRR function could therefore lead to the death of replicating cells; that is, PARP inhibition and HRR defects are synthetically lethal (FIG. 3). This hypothesis was important for cancer therapy because of the role of the tumour suppressor genes *BRCA1* and *BRCA2*. *BRCA1* and *BRCA2* were first reported in the early 1990s as breast cancer susceptibility genes, and by the beginning of the twenty-first century it was clear that they play key roles in HRR¹³⁷. Two concurrent studies in 2005 demonstrated the synthetic lethality of two different PARP inhibitors in different *BRCA*-knockout and homologous recombination-deficient cell systems^{48,49}. Importantly, both studies showed that a PARP inhibitor given to mice at non-toxic doses could shrink a *BRCA*-defective xenograft. This discovery represented a new paradigm in cancer therapy — exploiting a tumour-specific defect — and prompted the start of a period of intense activity focusing on the development of PARP inhibitors for cancer therapy. The first clinical data showing anticancer activity of PARP inhibitors in patients with *BRCA* mutations appeared only 4 years after these early studies¹³⁸. There was also a large increase in the number of preclinical studies focusing on PARP inhibitor cytotoxicity in cells and tumours with *BRCA* mutations and/or a homologous recombination deficiency (HRD) phenotype (reviewed in REF.¹³⁹), virtually all of which recapitulated the results of the 2005 studies described above.

Studies leading to regulatory approval. In the early 2000s, KuDOS Pharmaceuticals began developing PARP inhibitors and initiated clinical trials with olaparib as a single agent focused on patients with tumours and germ line *BRCA* (*gBRCA*) mutations. A phase I trial initiated in 2005 was the first to show that olaparib suppressed the growth of tumours in patients with *gBRCA* mutations; in this study, approximately 50% of patients with tumours and *gBRCA* mutations who had been heavily pretreated with various other chemotherapeutic agents had a complete or partial response to olaparib, and most of these responding patients had ovarian cancer¹³⁸. Now, several PARP inhibitors have shown success in clinical trials^{140–160}, with some studies leading to regulatory approval; for a list of completed clinical trials on PARP inhibitors, see TABLE 2. Here and in the studies described below 'ovarian cancer' is used as a shorthand term for 'epithelial ovarian, fallopian tube and primary peritoneal cancer' because most of these tumours are now thought to arise from the fallopian tube rather than the ovary. At the end of 2014, olaparib was the first PARP inhibitor to be approved as a maintenance therapy for patients with *gBRCA* mutations and platinum-sensitive ovarian cancer. A randomized phase II trial¹⁴⁵ in patients with platinum-sensitive ovarian cancer receiving olaparib

Box 1 | PARP cleavage and apoptosis

A study in 1994 showed that poly(ADP-ribose) polymerase (PARP) can be cleaved during apoptosis^{126,127}. Apoptosis is an energy-dependent programmed cell death pathway in which the activation of caspases in response to either death signalling molecules or excessive DNA damage leads to the breakdown of cell components (see figure). Apoptosis results in nuclear condensation and blebbing, and proinflammatory cellular contents are retained within membranes to prevent their extracellular release. The cleavage of PARP by activate caspases separates the DNA-binding domain from the catalytic domain, thereby preventing PARP activation. Whether this is to prevent PARP from consuming NAD⁺ and/or limit poly(ADP-ribose) polymer formation is not clear and may depend on the cell or model system investigated. If PARP is not inactivated by cleavage, then there is a massive activation of PARP in response to DNA damage, resulting in NAD⁺ and ATP depletion that may then cause necrosis or facilitate execution of an alternative cell death process known as parthanatos.



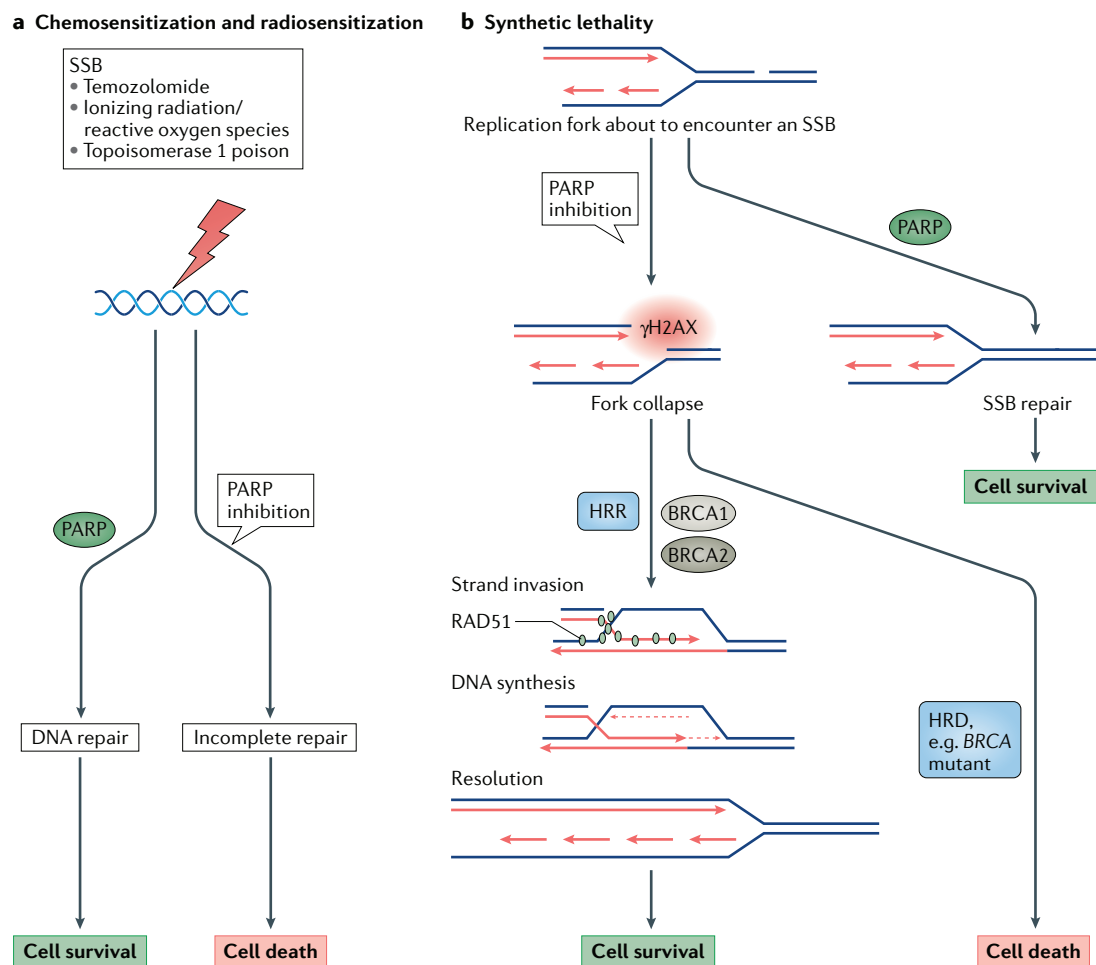


Fig. 3 | **Molecular mechanisms of the anticancer effects of PARP inhibitors.** **a** | The molecular mode of action of poly(ADP-ribose) polymerase (PARP) inhibitors in chemosensitization and radiosensitization. DNA single-strand breaks (SSBs) induced by chemical agents or ionizing radiation may be repaired by PARP-dependent repair, resulting in tumour cell survival. Inhibition of PARP prevents DNA repair, resulting in cell death. **b** | The mechanism of action of PARP inhibitors in synthetic lethality in homologous recombination DNA repair (HRR)-deficient cells. Endogenously induced DNA SSBs are normally repaired by PARP-dependent SSB repair, resulting in cell survival. If PARP is inhibited, SSBs accumulate and cause replication fork collapse. This is usually repaired by HRR, which involves BRCA1, BRCA2 and several other proteins preparing the DNA ends for the loading of RAD51 onto the single-stranded DNA, subsequently enabling strand invasion into the complementary duplex as a template for DNA synthesis and high-fidelity DNA repair. In cases where homologous repair is disrupted — for example in *BRCA*-mutant cells — fork collapse cannot be repaired and the cell undergoes cell death. HRD, homologous recombination deficiency.

capsules orally at a dosage of 400 mg daily showed that those with *BRCA* mutations had a significantly longer median progression-free survival (PFS) (11.2 months) than patients receiving placebo (4.3 months), and even in patients with wild-type *BRCA*, the PFS was extended to 7.4 months compared with 5.5 months for patients who received placebo.

Concurrent studies showed response to olaparib does not perfectly correlate with *BRCA* status¹⁴⁰. The first study to probe the frequency of HRD in ovarian cancer used a functional assay, which showed that although only around 10–15% of ovarian cancers are in carriers of *gBRCA* mutations, 50–60% of tumours lack HRR function¹⁴¹. Further studies confirmed this estimate through mutation screening¹⁴², confirming that *gBRCA* mutations substantially underestimate the frequency of HRD. Several methods can now be used to identify HRD,

including screens for mutations in or epigenetic silencing of genes involved in HRR, copy number variations, signs of chromosomal instability such as loss of heterozygosity, large-scale transitions and telomeric allelic imbalance, and mutation signatures (reviewed in REFS^{143,144}). As a consequence, commercial assays for determining HRD from Myriad Genetics and Foundation Medicine have been used as companion diagnostics in clinical trials that supported approval. A 300 mg tablet formulation of olaparib (Lynparza; AstraZeneca) was approved for treatment of platinum-sensitive ovarian cancer regardless of *BRCA* status in 2017 and in 2018–2019 was approved as a first-line maintenance therapy for ovarian cancer.

The next PARP inhibitor to be approved was rucaparib (Rubraca; Clovis Oncology) in 2016, on the basis of a series of clinical trials^{146–150}, which included a

Table 2 | Selected completed clinical trials for PARP inhibitors in cancer

Drug	Trial phase (start year; registration number; acronym ^a)	Cancer type	Key outcomes and comments	Refs
First-in human and landmark trials				
Rucaparib	I (2003; N/A)	Solid tumours/ melanoma	First trial of a PARP inhibitor in cancer, in combination with temozolomide. Established 12 mg m ⁻² as the RP2D in combination with full-dose temozolomide	130
Olaparib	I (2005; NCT00516373)	Solid tumours, focusing on patients with gBRCA mutations	First trial of a PARP inhibitor as a single agent in patients with BRCA mutations. Responses in 12 of 19 heavily pretreated patients receiving 200 mg twice daily	138
Olaparib	II (2008; NCT00679783)	High-grade ovarian cancer and TNBC with gBRCA mutations	Identified a better response in ovarian cancer than in breast cancer and that BRCA mutations were not the only discriminant associated with platinum sensitivity	140
Trials leading to regulatory approval				
Olaparib	II (2008; NCT00753545)	Relapsed platinum-sensitive ovarian cancer with gBRCA mutations	Patients with BRCA mutations had a longer median PFS in the olaparib (capsules, 400 mg daily) arm (11.2 months) than in the placebo arm (4.3 months); patients with wild-type BRCA receiving olaparib had a PFS of 7.4 months compared with 5.5 months for patients receiving placebo. Data from this study supported FDA approval in 2014, the first for a PARP inhibitor	145
Olaparib	III (2013; NCT01874353; SOLO-2)	BRCA-mutated platinum-sensitive relapsed serous ovarian cancer	PFS in patients receiving olaparib was significantly longer (19.1 months) than in those receiving placebo (5.5 months). Data from this study supported FDA approval for a tablet formulation in 2017	158
Olaparib	III (2013; NCT01844986; SOLO-1)	BRCA-mutated ovarian cancer after platinum-based therapy	Olaparib reduced the risk of disease progression or death by 70% compared with placebo. Data from this study supported FDA approval as first-line maintenance therapy in ovarian cancer in 2018	159
Olaparib	III (2014; NCT02000622)	BRCA-mutated breast cancer	Median PFS in the olaparib-treated patients was 7 months, significantly longer than that of the standard therapy group (4.2 months). Data from this study supported FDA approval in 2018	160
Olaparib	III (2014; NCT02184195; POLO)	BRCA-mutated pancreatic cancer	Median PFS in the olaparib-treated patients (7.4 months) was significantly longer than in the placebo group (3.8 months). These data supported FDA approval as first-line maintenance therapy in 2019	157
Rucaparib	I/II (2011; NCT01482715; Study 10)	Solid tumours/ BRCA-mutated ovarian cancer	Dose escalation established a safe dose of 600 mg orally, twice daily. Patients receiving rucaparib who had gBRCA mutations had an investigator-assessed ORR of 60%. Together with ARIEL2, this study supported accelerated FDA approval in 2016	147
Rucaparib	II (2013; NCT01891344; ARIEL2)	Platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer	Patients receiving rucaparib in a BRCA-mutation group had a median PFS of 12.8 months, patients in a high-LOH group had a median PFS of 5.7 months and patients in a low-LOH group had a median PFS of 5.2 months. Together with Study 10, this supported FDA approval of rucaparib alongside Foundation Medicine's companion diagnostic	148
Rucaparib	III (2014; NCT01968213; ARIEL3)	Platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube or primary peritoneal cancer plus a predictive biomarker	Patients treated with rucaparib had a significant increase in median PFS (10.8 months) compared with patients who received placebo (5.4 months), particularly in the subgroups of patients with BRCA mutations (16.6 months) and HRD (13.6 months). Supported FDA approval in 2018 for second-line therapy without the need for a companion diagnostic	149,150
Niraparib	III (2013; NCT01847274; ENGOT-OV16/NOVA)	Platinum-sensitive ovarian cancer	Niraparib extended the median PFS in patients with BRCA mutations to 21 months compared with 5.5 months in the control arm. In patients without BRCA mutations with or without an HRD signature, the median PFS was 12.3 months and 9.3 months, respectively, in the niraparib arm compared with 3.9 months in the placebo arm. Supported FDA approval as maintenance therapy for platinum-sensitive ovarian cancer in 2017	151
Niraparib	II (2015; NCT02354586; QUADRA)	Ovarian cancer	Patients with tumour BRCA mutations with platinum-sensitive or platinum-resistant disease had an ORR of 39% and 29%, respectively, and those with a positive HRD test (Myriad myChoice CDx) result had an ORR of 24%. These data supported FDA approval as maintenance therapy in ovarian cancer after four previous chemotherapy regimens in 2019	152
Talazoparib	III (2013; NCT01945775; EMBRC)	gBRCA-mutant HER2-negative locally advanced or metastatic breast cancer	Patients receiving talazoparib had a median PFS of 8.6 months compared with 5.6 months in the chemotherapy arm. These data supported FDA approval of talazoparib in 2018	153

Unless otherwise stated, poly(ADP-ribose) polymerase (PARP) inhibitors were evaluated as single agents. gBRCA, germline BRCA; HER2, human epidermal growth factor receptor 2; HRD, homologous recombination deficiency; LOH, loss of heterozygosity; N/A, not applicable; ORR, overall response rate; PFS, progression-free survival; RP2D, recommended phase II dose; TNBC, triple-negative breast cancer. ^aIf available.

phase I/II dose escalation study known as Study 10 (REF.¹⁴⁶), the phase II ARIEL2 trial^{148,149} and the placebo-controlled phase III ARIEL3 trial¹⁵⁰. These trials used Foundation Medicine's CDx HRD test to identify patients with homologous recombination-deficient tumours. There was an increase in PFS in all patients treated with rucaparib (10.8 months) compared with placebo (5.4 months). This increase was most marked in the cohort with *BRCA* mutations (16.6 months) and in those identified as having HRD with wild-type *BRCA* (9.7 months) compared with individuals with no biomarker (6.7 months). As a result of the successful implementation of the CDx HRD test, it was co-approved with rucaparib as a companion diagnostic. Rucaparib was approved in 2018 by the FDA without the need for a companion diagnostic as maintenance therapy for platinum-sensitive ovarian cancer, with 'platinum sensitive' defined as a tumour that does not recur within 6 months or more following platinum-based therapy.

The PARP inhibitor niraparib (Zejula; Tesaro) was first approved in 2017 for platinum-sensitive ovarian cancer, regardless of *BRCA* mutation status, on the basis of the ENGOT-OVA16/NOVA trial¹⁵¹. On the basis of results from the QUADRA trial, approval was subsequently extended to include patients who had undergone three or more prior chemotherapy regimens and were considered to have homologous recombination-deficient tumours, on the basis of results from *BRCA* testing, results from Myriad's myChoice HRD test, or platinum sensitivity¹⁵².

Following the approval of the three aforementioned PARP inhibitors for treatment of ovarian cancer, studies on the next PARP inhibitor — talazoparib (Talzenna; Pfizer) — focused on breast cancer, with the EMBRACA trial¹⁵³ leading to regulatory approval for the treatment of *gBRCA*-mutant, human epidermal growth factor receptor 2 (HER2)-negative breast cancer in 2018. Olaparib was also approved in 2019 for the treatment of HER2-negative locally advanced breast cancer in patients with *gBRCA* mutations. PARP inhibitors also showed efficacy in other tumour types associated with *BRCA* mutations, with rucaparib and olaparib both being approved in 2020 for patients with *BRCA1/BRCA2*-mutated metastatic castration-resistant prostate cancer on the basis of the results of the TOPARP-B (NCT01682772) olaparib trial and the TRITON2 (NCT0295234) rucaparib trial. Niraparib has also shown compelling data for this indication in the GALAHAD (NCT02854436) trial¹⁵⁶. Olaparib was approved in December 2019 for the maintenance of adult patients with deleterious or suspected deleterious *gBRCA*-mutated metastatic pancreatic adenocarcinoma on the basis of the results of the POLO trial¹⁵⁷, in which the median PFS of the olaparib-treated group (7.4 months) was substantially longer than that of the placebo-treated group (3.8 months). Olaparib has also completed several additional phase III trials for use in ovarian and breast cancer^{158–160}.

Ongoing clinical trials. Ongoing phase III trials of PARP inhibitors, including those studying the recently developed inhibitors pamiparib, fluzoparib and IMP4279

both as single agents and in various combinations, are summarized in TABLE 3. Despite entering clinical trials before niraparib or talazoparib, veliparib has failed to obtain regulatory approval, although it does have orphan drug status in the United States in combination with carboplatin and paclitaxel for treatment of non-small-cell lung cancer on the basis of a randomized phase II trial¹⁶¹. Veliparib is weaker than the approved PARP inhibitors and less potent at 'PARP trapping'¹⁶²; this is likely to be a handicap in terms of single-agent activity but may prove an advantage in combination therapies. There are more than 100 studies with veliparib registered on ClinicalTrials.gov, most of which use veliparib in combination with genotoxic agents. Given that veliparib can cross the blood–brain barrier, one would predict it would be effective in patients with glioblastoma in combination with temozolomide and radiotherapy. Indeed, although this combination has been shown to be associated with haematological toxicity¹⁶³, a phase I trial of veliparib in combination with whole brain radiotherapy in patients with brain metastases showed it was well tolerated and gave a better-than-predicted response¹⁶⁴. The combination of veliparib and temozolomide was also well tolerated in paediatric patients with brain tumours¹⁶⁵; however, in combination with radiotherapy, the temozolomide dose could not be escalated without toxicity¹⁶⁶. So far, none of the trials with veliparib have progressed to registration.

The PARP inhibitors pamiparib and fluzoparib are relatively new, and it is not yet clear whether they hold advantages over the approved PARP inhibitors. Pamiparib, formerly BGB-290, was evaluated in a phase I dose escalation trial in patients with advanced solid tumours, which identified 80 mg orally twice daily as the maximum tolerated dosage¹⁶⁷. Pamiparib is reported to be selective for PARP1/2, with potent trapping activity and good brain penetration^{95,168}. The only published clinical study on pamiparib is for its use in combination with an inhibitor of programmed cell death protein 1 (PD1); this combination was well tolerated by patients, with two complete responses and eight partial responses in a cohort of 49 patients with advanced solid tumours¹⁶⁸. Preclinical data on fluzoparib suggest it is also a typical PARP1/2 inhibitor, with target inhibition potency comparable to that of olaparib, as it displays cytotoxicity in homologous recombination-deficient cells and when used in combination with temozolomide and radiotherapy^{96,169}. The maximum tolerated dosage, in combination with apatinib and paclitaxel, was 80 mg twice daily in patients with advanced gastric cancer, with evidence of response¹⁷⁰.

For trials of PARP inhibitors in combination with cytotoxic anticancer agents, glioblastoma is an ideal target because the standard of care is temozolomide treatment and radiotherapy and there are extensive preclinical data showing that the cytotoxicity and anti-tumour activity of both of these treatments is increased in combination with a PARP inhibitor. However, the ability of a PARP inhibitor to cross the blood–brain barrier is likely to be a factor in the effective control of brain tumour growth, with the caveat that the blood–brain barrier at the tumour site may be different from that of

Table 3 | Ongoing phase III clinical trials for PARP inhibitors in cancer

Trial start date	Single agent or combination	Cancer type	Registration number
Olaparib			
Aug. 2013	Combination with paclitaxel	Advanced gastric cancer	NCT01924533
Jan. 2014	Single agent	BRCA-mutated, high-risk HER2-negative primary breast cancer	NCT02032823
Nov. 2014	Single agent	BRCA-mutated ovarian cancer after at least two prior platinum-based treatments	NCT02282020
Nov. 2014	Combination with pembrolizumab	Non-BRCA-mutated advanced epithelial ovarian cancer	NCT03740165
May 2015	Combination with cediranib	Recurrent platinum-sensitive ovarian, fallopian tube or primary peritoneal cancer	NCT02446600
Jun. 2015	Combination with bevacizumab	High-grade serous or endometrioid ovarian, fallopian tube or peritoneal cancer	NCT02477644
Jul. 2015	Combination with cediranib	Recurrent platinum-resistant or platinum-refractory ovarian, fallopian tube or primary peritoneal cancer	NCT02502266
Jun. 2016	Single agent	HER2-negative, BRCA1-like breast cancer	NCT02810743
Dec. 2016	Single agent	Metastatic castration-resistant prostate cancer	NCT02987543
Apr. 2017	Single agent	Non-mucinous epithelial ovarian cancer	NCT03106987
May 2017	Combination with carboplatin and paclitaxel	Neoadjuvant treatment of TNBC and/or gBRCAm breast cancer	NCT03150576
Sep. 2017	Single agent	Metastatic breast cancer	NCT03286842
Sep. 2017	Single agent or combination with cediranib	Ovarian cancer	NCT03278717
Jan. 2018	Single agent	Platinum-sensitive relapsed non-gBRCAm ovarian cancer	NCT03402841
May 2018	Single agent	Ovarian cancer — maintenance therapy after complete or partial response to platinum-based chemotherapy	NCT03534453
Nov. 2018	Combination with abiraterone	Metastatic castration-resistant prostate cancer	NCT03732820
Nov. 2018	Combination with durvalumab, bevacizumab	Advanced ovarian cancer	NCT03737643
Feb. 2019	Combination with pembrolizumab	Metastatic castration-resistant prostate cancer	NCT03834519
Jun. 2019	Combination with pembrolizumab	First-line metastatic squamous non-small-cell lung cancer	NCT03976362
Jun. 2019	Combination with pembrolizumab	Metastatic non-squamous non-small-cell lung cancer	NCT03976323
Dec. 2019	Combination with pembrolizumab	TNBC	NCT04191135
Rucaparib			
Aug. 2016	Single agent	BRCA-mutant ovarian, fallopian tube or primary peritoneal cancer	NCT02855944
Nov. 2016	Single agent	Metastatic castration-resistant prostate cancer and homologous recombination gene deficiency	NCT02975934
May 2018	Combination with nivolumab	Maintenance treatment following response to front-line platinum-based chemotherapy	NCT03522246
Talazoparib			
Jan. 2018	Combination with enzalutamide	Metastatic castration-resistant prostate cancer	NCT03395197
Veliparib			
Jan. 2014	Combination with carboplatin	Early-stage TNBC	NCT02032277
Jun. 2014	Combination with temozolomide	Newly diagnosed glioblastoma	NCT02152982
Jun. 2014	Combination with carboplatin and paclitaxel	HER2-negative metastatic or locally advanced unresectable BRCA-associated breast cancer	NCT02163694
Oct. 2014	Combination with carboplatin and paclitaxel	Metastatic or advanced non-squamous non-small-cell lung cancer	NCT02264990
Jun. 2015	Combination with carboplatin and paclitaxel	Stage III or IV, high-grade serous epithelial ovarian, fallopian tube or primary peritoneal cancer	NCT02470585
Pamiparib			
Feb. 2018	Single agent	Inoperable locally advanced or metastatic gastric cancer that previously responded to platinum-based first-line chemotherapy	NCT03427814
Fluzoparib			
Mar. 2019	Single agent	Platinum-sensitive recurrent ovarian cancer — maintenance therapy	NCT03863860
IMP4297			
Nov. 2019	Single agent	Ovarian cancer — maintenance therapy after first-line chemotherapy	NCT04169997

gBRCAm, germline BRCA mutated; HER2, human epidermal growth factor receptor 2; PARP, poly(ADP-ribose) polymerase; TNBC, triple-negative breast cancer.

healthy brain tissue. In most cases, brain penetration is determined by interaction with drug efflux ATP-binding cassette transporters^{171–173}; olaparib, talazoparib and rucaparib are substrates for the ATP-binding cassette multidrug resistance transporters and are actively exported out of the cell, whereas niraparib and veliparib do not appear to be exported¹⁷².

PARP inhibitors have also been explored as modulators of immune signalling in cancers. The accumulation of DNA damage following PARP inhibition leads to leakage of damaged double-stranded DNA into the cytoplasm in both *BRCA*-mutant and wild-type cells, which can activate innate immune signalling through the cGAS–STING pathway, leading to increased expression and release of type I interferons^{174,175}. Type I interferons drive the infiltration of cytotoxic T cells into the tumour; however, they can also suppress the T cell response by inducing expression of PD-L1 on the tumour surface, such that interfering with PD1/PD-L1 signalling is synergistic with PARP inhibition *in vivo*¹⁷⁶. Several clinical studies of the combination of PARP inhibitors and immune checkpoint inhibitors have been initiated in recent years; it is too early to assess their overall outcome, but early results are promising¹⁷⁷.

Factors affecting PARP inhibitor dosage. The approved dosages of the PARP inhibitors reflect their potencies for PARP inhibition and PARP trapping. The approved dosage of talazoparib, the most potent clinically used PARP inhibitor, is 1 mg once daily, in comparison with much higher dosages for niraparib (300 mg once daily), olaparib (300 mg twice daily) and rucaparib (600 mg twice daily). However, differences in the recommended schedules and doses for niraparib, olaparib and rucaparib may be a reflection of pharmacokinetic data and safe or tolerable dose information rather than potency, as they seem to be similarly potent inhibitors of the catalytic activity of PARP1 in laboratory studies.

Side effects common to PARP inhibitors evaluated in clinical trials so far include fatigue, gastrointestinal effects such as nausea/vomiting, abdominal pain and diarrhoea, and haematological effects such as thrombocytopenia^{154,155}. These effects are usually mild (grade 1 or 2) but can be more serious in rare cases^{154,155}. Differences in side effect profiles between specific PARP inhibitors are likely to reflect differences in the dose and schedule, the half-life and the metabolic route by which the inhibitors are metabolized; for example, olaparib is metabolized by CYP3A4, rucaparib is metabolized by CYP2D6, and niraparib is metabolized by hepatic carboxylesterases. Side effect profiles may also differ because inhibitors have different specificities for PARP family enzymes or may interact with other NAD⁺-dependent enzymes. Niraparib is associated with thrombocytopenia, often necessitating dose reductions — particularly in women with low body weight — whereas with olaparib, rucaparib and talazoparib, anaemia is more likely to occur than thrombocytopenia^{154,155}. An elevation in serum creatinine level has been observed following treatment with rucaparib and olaparib, likely caused by an effect on transporters rather than renal toxicity. Myelodysplastic syndrome and acute myeloid leukaemia

have also been associated with PARP inhibitor therapy; however, as most patients treated with PARP inhibitors have also been treated with chemotherapy drugs known to be associated with myelodysplastic syndrome/acute myeloid leukaemia, it is difficult to attribute this entirely to the inhibition of PARP¹⁵⁵.

Dose, schedule and toxicity are all important considerations when evaluating single-agent PARP inhibitor therapy against combination therapies involving conventional cytotoxic drugs or radiotherapy. Preclinical data demonstrate that only low doses of a PARP inhibitor and short administration schedules are needed to control tumour growth in combination with PARP inhibitor-relevant chemotherapy or radiotherapy, and higher doses can be profoundly toxic. In contrast, much higher doses and continuous schedules are required and tolerated for single agents in HRD xenograft models. The safe dosage of rucaparib in combination with temozolomide was determined as 12 mg m⁻² given intravenously each day for a 5-day period every 28 days¹³⁰. By comparison, the approved dosage for rucaparib when used as a single agent was found to be 600 mg orally twice daily to be taken continuously — equivalent to roughly 20 times the safe dose when used in combination with temozolomide (based on 30% bioavailability and a body surface area of around 1.7 m²). When used in combination with carboplatin, a chemotherapy drug with a mechanism of action that does not influence PARP1 DNA-repair pathways as much as temozolomide, the safe dosage of rucaparib was found to be 240 mg orally daily¹⁷⁸ — only 2.5-fold lower than when used as a single agent. The safe single agent dose of a PARP inhibitor is therefore likely to be at least an order of magnitude too high if used in combination with a chemotherapy drug that acts synergistically with the effects of PARP inhibitors. It is likely that high doses are needed for single-agent therapy as cells have an excess of PARP1 for repairing endogenous DNA damage, so PARP1 must be profoundly inhibited to render these physiological levels of DNA damage cytotoxic in homologous recombination-deficient cells. High doses are well tolerated in single-dose strategies as these high doses will not kill non-tumour, HRR-functional cells. When a massive amount of DNA damage is induced, for example, by temozolomide, there is no longer an overcapacity of PARP1 to repair this damage, so a more modest level of inhibition will render DNA breaks unreparable in both tumour and non-tumour cells.

Counteracting the development of PARP inhibitor resistance. Mutations in cancer cells can render them intrinsically resistant to anticancer drugs, including PARP inhibitors. Cancer cells may also acquire resistance through genomic instability and tumour heterogeneity¹⁷⁹. PARP inhibitor resistance can occur through four different mechanisms: reversal of the HRD phenotype, stabilization of the DNA replication fork, increasing PARYlation activity and removal of the inhibitor from the cell by efflux transporters (reviewed in REF¹⁵⁵). Reversal of the HRD phenotype was the first resistance mechanism to be observed; the restoration of *BRCA* function can occur through the acquisition of

secondary mutations in *BRCA* that negate truncating mutations and restore an open reading frame. This effect was first observed in laboratory studies, then in clinical material from patients after platinum-based therapy^{180,181}. Similar findings have been observed regarding reversion of deleterious mutations in *RAD51C*, *RAD51D* and *PALB2* (REF.¹⁵⁵). Subsequent studies showed that HRR can be restored by other means, most notably by the loss of TP53-binding protein 1 (53BP1). This protein acts in competition with BRCA1 at DNA DSBs, preventing end resection and causing the cell to make a futile attempt at NHEJ^{182,183}. When NHEJ is prevented by deletion of 53BP1, HRR can proceed even in the absence of BRCA1. Similarly, loss of the mitotic spindle assembly checkpoint protein MAD2B, which acts downstream of 53BP1 to antagonize HRR, can also reverse the *BRCA*-related HRD phenotype¹⁸⁴. Loss or inhibition of DNA-PKcs, a pivotal kinase in NHEJ, can also restore HRR function in *BRCA*-mutant cells¹⁸⁵. Increasing replication fork stability by preventing excessive degradation by MRE11 can cause PARP inhibitor resistance even without restoring HRR function¹⁸⁶. It is also possible that inactivation of PARG can restore PAR signalling and cause resistance to PARP inhibitors¹⁸⁷. Loss of PARP1 may also confer PARP inhibitor resistance, as cytotoxicity may be related to PARP trapping rather than inhibition of PARP activity itself⁶².

In an attempt to overcome PARP inhibitor resistance, work has focused on using therapeutic agents to induce the HRD phenotype and/or disrupt replication fork protection. These agents include phosphoinositide 3-kinase inhibitors and the tyrosine kinase inhibitor imatinib, which are reported to downregulate HRR and, in some cases, induce sensitivity to PARP inhibitors^{188,189}; however, in some contexts they can induce protection from PARP inhibitors owing to cell cycle arrest¹⁹⁰. The combination of PARP inhibitors and phosphoinositide 3-kinase inhibitors is currently under clinical evaluation in patients with gynaecological cancers and breast cancer (NCT01623349 and NCT03586661). Inhibitors of histone deacetylase and heat shock protein 90 (HSP90) have also been reported to compromise HRR by destabilizing key HRR proteins such as RAD51 and BRCA2 (REF.¹⁹¹). Currently, there is one trial of olaparib in combination with a histone deacetylase inhibitor (vorinostat) in breast cancer — registered but not yet recruiting (NCT03742245) — and one trial of olaparib and an HSP90 inhibitor (olanespib) in ovarian cancer (NCT02898207).

The DNA damage response can regulate both DNA repair and cell cycle arrest. The ATR–CHK1–WEE1 axis, activated in response to DNA damage, causes S-phase arrest through inhibition of cyclin-dependent kinase 2 (CDK2) and G2/M-phase arrest through inhibition of CDK1. The emerging role of cyclin-dependent kinases in HRR suggests that inhibitors of these cell cycle checkpoint kinases might be synthetically lethal with PARP inhibitors by inducing an HRD phenotype. A prototype small-molecule inhibitor of the serine/threonine-protein kinase ATR known as NU6027 was shown to increase rucaparib cytotoxicity in MCF7 cells and inhibit HRR function, in addition to attenuating cell cycle arrest¹⁹².

Several studies have since shown that ATR, CHK1 and WEE1 inhibitors can sensitize *BRCA*-wild-type and PARP inhibitor-resistant cells to PARP inhibitors^{193–195}. There are currently several ongoing clinical trials of PARP inhibitors in combination with ATR inhibitors (NCT02723864, NCT03462342, NCT03787680, NCT04065269, NCT04149145, NCT03682289, NCT03022409 and NCT03740893) and CHK1 inhibitors (NCT03057145). In addition, CDK1 inhibitors have been shown to induce the HRD phenotype and confer sensitivity to PARP inhibitors preclinically¹⁹⁶ and are undergoing clinical evaluation (NCT01434316).

PARP inhibition beyond oncology

Pathophysiological processes that lead to PARP activation. PARP1 is constitutively expressed in most cells and has a basal activity, as evidenced by PAR formation in normal cells. This basal activity is maintained by the physiological occurrence of DNA strand breakage, likely generated by the endogenous production of oxidants and free radicals as a result of biochemical processes such as mitochondrial electron transfer. It was a surprise when PARP activity was found to be elevated in various non-oncological disease conditions^{32,33,39,42} because until the 1990s it was not commonly accepted that pathophysiological states could produce genotoxic amounts of oxidants or free radicals. PARP activation, characterized by increased PAR accumulation, has now been implicated in a range of pathological conditions, including ischaemia, ischaemia–reperfusion injury, inflammation, neurological injury, vascular disease and diabetes mellitus. In these conditions, DNA-damaging oxidants and free radicals are produced by a variety of sources, including NADPH oxidases, xanthine oxidase, mitochondria and tissue-infiltrating immune cells (reviewed in REFS^{197,198}).

PARP mediates a range of pathological mechanisms.

The use of PARP inhibitors and *Parp1*-knockout mice has revealed that PARP activation plays an active role in cell dysfunction, organ dysfunction and dysregulated inflammatory and immune responses (reviewed in REFS^{199–204}). PARP inhibition therefore represents a potential treatment for a range of diseases caused by these underlying conditions. Early studies showed that PARP overactivation — caused by excessive DNA damage induced by reactive oxygen species — can drive a cellular energetic deficit, inducing cell necrosis and organ dysfunction in various diseases, including stroke, myocardial ischaemia–reperfusion injury, and various forms of critical illness^{200,205,206} (FIG. 4a). Subsequent studies revealed multiple additional mechanisms by which PARP activation can drive pathological states. Free PAR can exit the nucleus and diffuse to various cellular targets. One of the consequences of this process is the mitochondrial release of apoptosis-inducing factor 1 (AIF), which, in turn, induces nuclear DNA fragmentation through a distinct form of cell death called ‘parthanatos’ that is relevant in the pathogenesis of stroke, neuroinflammation and neurodegeneration²⁰⁶ (FIG. 4b). Free PAR also mediates other pathophysiological processes; for example, PAR binding to α -synuclein renders it more neurotoxic

in neurodegenerative processes⁵⁹, and both intracellular and extracellular PAR can drive atherosclerosis by promoting vascular calcification⁶¹ (FIG. 4c).

PARP activation can drive the pathogenesis of various non-oncological diseases through its role as the regulator of signal transduction and gene transcription. PARP regulates the activation of various signalling pathways, including the nuclear factor- κ B and JUN amino-terminal kinase pathways. Studies in various disease models of local and systemic inflammation and autoimmune diseases (arthritis and multiple sclerosis) have demonstrated that PARP inhibitors, or PARP1 deficiency, can suppress the expression of adhesion molecules such as ICAM1, proinflammatory cytokines such as tumour necrosis factor (TNF) and chemokines such as macrophage inflammatory protein 1 α ^{201–203}. It is likely that, in vivo, cell dysfunction and proinflammatory signalling processes form positive-feedback cycles of pathogenesis (reviewed in REF.²⁰¹) (FIG. 4d). PARP acts as a global regulator of gene expression by modulating chromatin folding (FIG. 4e), and similarly, PARP1 interacts with various epigenetic mechanisms to modulate gene expression, including inhibiting the DNA methyltransferase DNMT1, which in turn suppresses DNA methylation (FIG. 4f). PARP activation and direct or indirect PARylation can mediate pathogenic processes by unfavourably regulating the activity of various kinase cascades, including those mediated by AKT/glycogen synthase kinase 3 β , mitogen-activated protein kinases and various protein kinase C isoforms²⁰⁷. In many cardiovascular disease models, pharmacological PARP inhibition induces cytoprotective AKT activation, and this effect underlies some of the beneficial effects of PARP inhibitors^{44,207}. PARP1 PARylates ataxia telangiectasia mutated (ATM), preventing it from interacting with the protein nuclear factor- κ B essential modulator (NEMO). PARP1 inhibition suppresses PARylation of ATM in the nuclear compartment, allowing ATM to form a complex with NEMO that exits the nucleus and combines in the cytosol with mTOR and AKT. Formation of this complex activates the phosphorylation of AKT, which produces a cytoprotective response through the induction of AKT-mediated survival pathways²⁰⁸ (FIG. 4g).

Clinical trials of PARP inhibitors for non-oncological diseases. Studies in the late 1990s demonstrated that pharmacological inhibition of PARP may exert cardioprotective effects; for example, a reduction in infarct size and/or improvements in cardiac contractility and cardiac remodelling in animal models of myocardial infarction, cardiopulmonary bypass and chronic heart failure^{33,209}. Subsequent studies in preclinical samples²¹⁰ and specimens from patients with myocardial infarction²¹¹ confirmed that PARP activation occurs in the heart and in circulating leukocytes after myocardial reperfusion. The third-generation PARP inhibitor INO-1001 reduced infarct size and improved cardiac function in preclinical models of myocardial ischaemia-reperfusion^{212–215} and exerted beneficial effects in models of cell and tissue injury, including improvement of neurological function in models of neuroinjury and improved cardiac, vascular and renal function in models

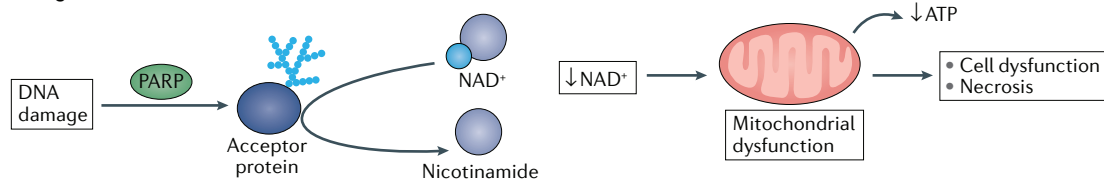
of diabetic complications^{73,216–220}. As a result of these studies, INO-1001 became the first PARP inhibitor to enter human clinical trials in healthy human volunteers in 2003 and patients with myocardial infarction in 2004 (REF.²²¹). The 2004 trial showed that PARP inhibition may suppress inflammation in patients with myocardial infarction, as measured by plasma C-reactive protein and IL-6 levels²²¹; however, the development of INO-1001 for the treatment of cardiovascular disease was not pursued further, and its development as a cancer treatment did not progress beyond an initial safety study in patients with melanoma²²².

There are now renewed efforts to develop PARP inhibitors as cytoprotective agents for various non-oncological diseases. On the basis of preclinical studies in vitro and in murine models^{97,223}, amelparib (formerly JPI-289) entered clinical trials in 2013. Safety and tolerability trials in healthy human volunteers (NCT01983358 and NCT02396069) were completed in 2015, and a multicentre efficacy and safety trial in patients with stroke began in 2017 and is ongoing (NCT03062397). Preclinical data indicated that amelparib treatment is efficacious in reducing infarct size even when treatment with the inhibitor is delayed for 12 hours after stroke onset²²⁴. Although the results of the multicentre stroke trial have not yet been disclosed, an ex vivo study in which leukocytes from patients with stroke were treated with 1–10 μ M amelparib showed that the PARP inhibitor increases regulatory T cell expansion in vitro, reduces the production of TNF and IFN γ , and increases the production of the anti-inflammatory cytokine IL-10 (REF.²²⁴). Mitobridge, a Boston-based biotech company, has disclosed the results of in vitro and in vivo studies with a novel, non-genotoxic PARP inhibitor, showing that the inhibitor has nanomolar potency and improves organ function in a rat model of renal ischaemia-reperfusion²²⁵.

Future potential for PARP inhibitor repurposing. To date, all clinically approved PARP inhibitors have primarily been developed and preclinically evaluated for their anticancer effects in the context of cancer cell lines and tumour-bearing mice. However, these drugs could be repurposed for non-oncological indications (reviewed in REFS^{226–228}). Preclinical studies in various experimental models, including stroke, neuroinjury, acute lung injury and circulatory shock, demonstrate the efficacy of the clinically approved PARP inhibitors^{229–254} (summarized in TABLE 4).

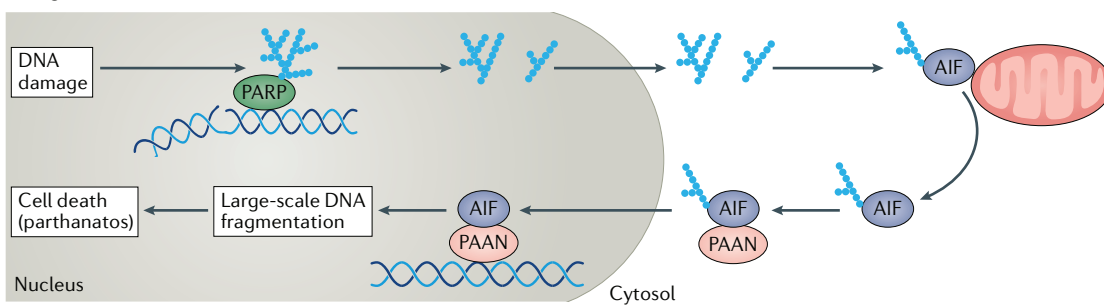
One important common feature of these studies is that the in vivo doses or the in vitro concentrations of the PARP inhibitors that demonstrate efficacy tend to be an order of magnitude lower than those used in preclinical oncology models (reviewed in REF.²²⁷). One possible explanation for this difference is that for the inhibition of DNA repair, a complete or near-complete inhibition of all PARP1 activity is required, while for cytoprotective effects such as the restoration of cellular NAD⁺ levels, the attenuation of free PAR formation or activation of cytoprotective AKT pathways, partial inhibition of PARP1 may be sufficient. This suggests that the effective doses of the PARP inhibitors in repurposing indications will

a Regulation of NAD⁺ and ATP levels



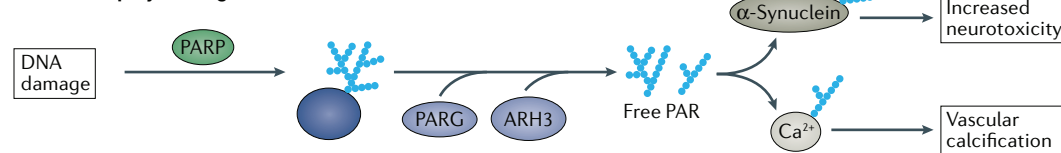
- PARP inhibitors**
- Preserve NAD⁺ and ATP levels
 - Maintain cell function
 - Protect against necrosis

b Signal transduction



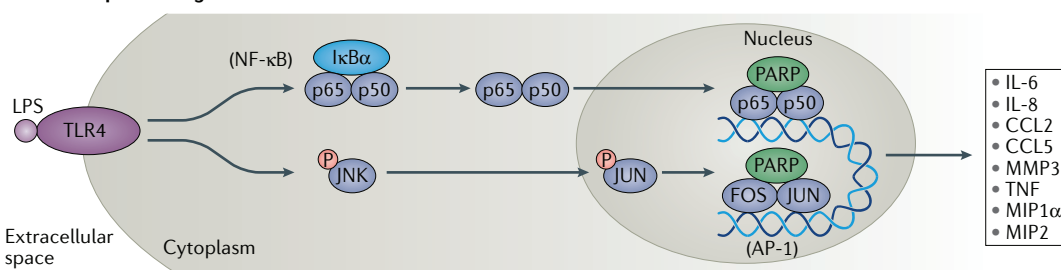
- PARP inhibitors**
- Prevent PAR translocation
 - Inhibit AIF release
 - Suppress parthanatos

c Free PAR polymer regulation



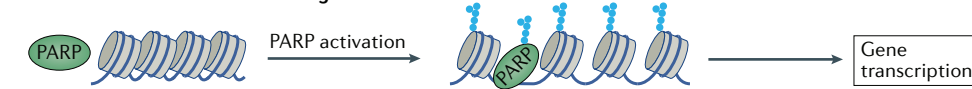
- PARP inhibitors**
- Prevent PAR production
 - Inhibit PAR-related downstream pathophysiological events

d Transcriptional regulation



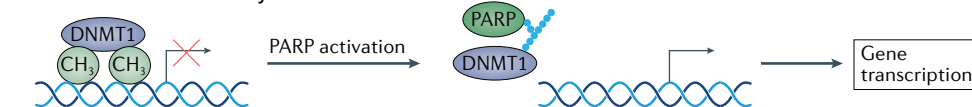
- PARP inhibitors**
- Globally regulate gene expression
 - Suppress the production of many proinflammatory cytokines and chemokines

e Modulation of chromatin folding



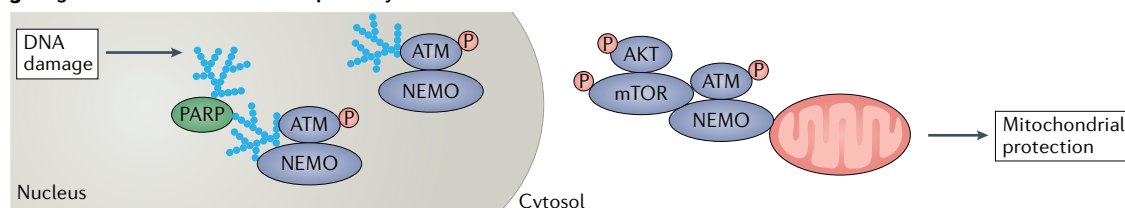
- PARP inhibitors**
- Reduce expression of genes contributing to pathological phenotype

f Modulation of DNA methylation



- PARP inhibitors**
- Reduce expression of genes contributing to pathological phenotype

g Regulation of the AKT survival pathway



- PARP inhibitors**
- Allow the formation of the ATM-NEMO-AKT-mTOR complex
 - Induce mitochondrial cytoprotective pathways

◀ Fig. 4 | **PARP inhibition in treating non-oncological diseases.** **a** | In non-oncological diseases such as reperfusion injury, shock and inflammation, oxidants and free radicals produce DNA single-strand breaks, which activate poly(ADP-ribose) polymerase (PARP). The subsequent cellular energetic deficiency caused by PARP activation produces cell dysfunction and can promote cell death through necrosis, which mediates disorders associated with neuronal or myocardial cell death or vascular dysfunction. PARP inhibitors suppress these processes by attenuating cellular energetic deficit (discussed in REFS^{77,78,199,201}). **b** | Activation of PARP results in poly(ADP-ribose)ylation (PARylation) of acceptor proteins, including PARP itself. Some poly(ADP-ribose) (PAR) translocates to the cytosolic compartment and binds to mitochondrial receptors, resulting in the release of apoptosis-inducing factor 1 (AIF), which diffuses to the nucleus and promotes large-scale nuclear fragmentation and cell death through a process known as parthanatos. The PARP1-dependent AIF-associated nuclease (PAAN) involved in DNA fragmentation was recently identified as macrophage migration inhibitory factor (MIF). PARP inhibitors prevent PARylation and subsequent pathophysiological events (further discussed in REF.²⁰⁶). **c** | Free PAR can post-translationally modify intracellular and extracellular targets, mediating pathophysiological processes such as α -synuclein cytotoxicity in the context of chronic neurodegeneration and vascular calcification in the context of vascular injury. PARP inhibitors prevent PARylation and reduce free PAR formation (further discussed in REFS^{52,59,61,206}). **d** | PARP acts as a transcriptional co-regulator of genes involved in various cellular processes, including genes encoding proinflammatory cytokines and chemokines that are regulated by the JUN amino-terminal kinase (JNK) and nuclear factor- κ B (NF- κ B) pathways. Inhibition of PARP can therefore suppress the production of proinflammatory cytokines that are linked to local inflammatory conditions such as arthritis and systemic inflammatory conditions such as septic shock. **e** | PARylation of histones can regulate their conformation and their interaction with the DNA, loosening the chromatin and allowing transcription of otherwise silenced genes. Inhibition of PARP prevents histone PARylation and access of the gene transcription machinery to the DNA. **f** | PARP can act as an epigenetic regulator through the modulation of DNMT1, a major maintenance methyltransferase. PARP overactivation can lead to the formation of PARP1–DNMT1 complexes through linking of the two proteins by PAR chains; this inhibits the DNA-methylating activity of DNMT1, leading to an increase in gene expression. PARP inhibitors prevent the sequestration of DNMT1, thereby allowing it to methylate DNA and inhibit gene expression. By affecting the processes depicted in parts **d–f**, PARP inhibition can suppress proinflammatory cytokine production and thereby exerts beneficial effects in various local and systemic inflammatory diseases; these processes are further discussed in REFS^{67,68,75,201,216}. **g** | PARP prevents interactions between the proteins phosphorylated ataxia telangiectasia mutated (ATM) and nuclear factor- κ B essential modulator (NEMO). Inhibition of PARP facilitates the translocation of phosphorylated ATM–NEMO from the nucleus to the cytoplasm and formation of the cytoprotective phosphorylated ATM–NEMO–AKT–mechanistic target of rapamycin (mTOR) signalosome, which activates various cell survival pathways. These processes are further discussed in REFS^{208,286}. ARH3, ADP-ribosylhydrolase 3; LPS, bacterial lipopolysaccharide; MIP1 α , macrophage inflammatory protein 1 α ; PARG, poly(ADP-ribose) glycohydrolase; TLR, Toll-like receptor 4; TNF, tumour necrosis factor.

be lower than those used for single-agent treatment of cancer.

Repurposing of PARP inhibitors for non-oncological indications must take into account a number of factors. As discussed in REF.²²⁷, the indications for which repurposing is most promising are those where the preclinical evidence for the pathogenic role of PARP1 is clear, the disease is severe enough and lacks currently available therapies such that novel experimental approaches are justified, and the disease is such that adequately designed and powered trials are practically feasible. Furthermore, to reduce the risk of prolonged exposure to a PARP inhibitor, which could interfere with the maintenance of genomic integrity, indications for which the inhibitor could be administered for a short duration are preferable. Such indications include forms of acute neuroinjury such as stroke and acute neurotrauma, and critical illnesses such as septic shock and acute lung injury.

The risk of PARP inhibitor-associated impairment of DNA repair is mitigated by the fact that patients in the

targeted groups do not have mutations that impair HRR and are not receiving genotoxic drugs. Indeed, it is conceivable that such mutations and the concomitant use of genotoxic drugs would be possible counterindications for the use of PARP inhibitors in non-oncological diseases. In addition, the low dose of the inhibitor compared with oncological doses would be expected to produce only partial inhibition of PARP activity for a relatively short time. One key aspect of PARP-mediated pathogenic mechanisms is that they preferentially affect males; in premenopausal females, the protective effect of PARP inhibitors is less pronounced or non-existent^{245,255–257}. The underlying reason is not clear, but is probably related to the modulation of PARP activation by endogenous androgens or oestrogens²⁵⁸. Consequently, repurposing clinical trials should also consider sex differences and build this factor into the trial design prospectively.

Other PARP family members as targets

Although approximately 90% of cellular PAR formation is catalysed by PARP1 (REF.²⁹), several other PARP family members exert important biological functions and may serve as drug targets. We focus here on those isoforms that can catalyse significant PARylation reactions (as opposed to mono(ADP-ribose)ylation (MARylation) reactions), in particular PARP2, PARP5A and PARP5B. The therapeutic potential of inhibiting various MARYlating enzymes, such as PARP14, has been reviewed elsewhere^{259,260}.

PARP2 was first identified after it was found that cells from *Parp1*-knockout mice were capable of synthesizing PAR after DNA damage³⁵. PARP2 is structurally similar to PARP1, sharing more than 60% sequence homology in its catalytic domain with that of PARP1, although it lacks zinc-finger motifs at its amino terminus⁴⁶. Like PARP1, it is activated by binding to DNA breaks through its tryptophan–glycine–arginine (WGR)-rich domain, and can PARylate itself and histones, leading to the recruitment of XRCC1 and other base excision repair proteins, thereby promoting DNA repair^{261,262}. PARP1 and PARP2 can compensate for the deletion of each other; indeed, deletion of both PARP1 and PARP2 results in embryonic lethality, highlighting the importance of the SSBR pathway for viability⁴⁵.

It is difficult to disentangle the differential effects of inhibitors on PARP1 and PARP2, and most inhibitors have been found to be more promiscuous than first thought, as reviewed in REFS^{263–266}. Several groups have attempted to make PARP1-selective or PARP2-selective inhibitors²⁶⁷ (reviewed in REF.²⁶⁴). There is some discrepancy between publications regarding the potency and selectivity of PARP inhibitors, perhaps reflecting the use of different methods and difficulty in translating in vitro data to in vivo models. For example, talazoparib had similar PARP1 inhibitory potency to olaparib when assayed in a cell-free, in vitro microplate assay that uses streptavidin to detect the incorporation of biotinylated NAD⁺ into polymers²⁶⁶, but in vivo and clinical studies indicate that talazoparib is an order of magnitude more potent than other clinically approved inhibitors, possibly because of talazoparib's greater PARP 'trapping' potency¹⁶².

Table 4 | Clinically approved PARP inhibitors in models of non-oncological diseases

Experimental model	Disease modelled	Drug (concentration or dose/dosage)	Effects	Refs
In vitro				
Endothelial cells subjected to high glucose concentrations	Diabetic vascular dysfunction	Veliparib (5 µM)	Protection against DNA fragmentation	229
AML12 hepatocytes	Fatty liver disease	Olaparib (100 nM)	Increased cellular bioenergetics, induction of mitochondrial biogenesis and induction of lipolysis-related genes	230
C2C12 myotubes subjected to oxidative stress	Oxidative stress-related diseases	Olaparib or veliparib (100 nM)	Maintenance of cellular bioenergetics	231
Human CD4 ⁺ T cells stimulated with anti-CD3 and anti-CD28	Asthma, inflammation	Olaparib (1–5 µM)	Beneficial modulation of cytokine responses and T cell subpopulations	232
Cultured human neurons subjected to NMDA or oxygen/glucose deprivation	Neurotoxicity, neurodegeneration	Olaparib (2 µM)	Neuroprotection	233
Monocytes in co-culture with endothelial cells	Neuroinflammation, stroke	Olaparib or talazoparib (10 µM)	Reduced cell adhesion and migration	234
ATM-deficient primary neurons	Ataxia telangiectasia	Olaparib (500 nM)	Reduced oxidant production, improved cellular bioenergetics	226
Retinal explants from the rd1 or rd10 mouse	Retinitis pigmentosa	Olaparib (0.1–10 µM)	Rescue of photoreceptors and protection against cell death	236,237
ARPE-19 cells subjected to oxidative stress	Retinal degeneration	Olaparib (10 µM)	Protection against cell death and regulation of inflammation-related genes	238,239
C2C12 myotubes subjected to oxidative stress	Myopathies	Olaparib or veliparib (100 nM)	Maintenance of cellular bioenergetics	240
H9c2 cells subjected to oxidative stress	Myocardial infarction	Olaparib (10 µM)	Protection against cell death and mitochondrial dysfunction	241
COS-2 cells subjected to arsenite	ALS	Veliparib (1–5 µM)	Inhibition of stress granule formation	242
Mixed spinal-cord cultures isolated from rat embryos subjected to TDP43 overexpression	ALS	Veliparib (10 µM)	Protection against neuronal degeneration	242
Primary cortical neurons subjected to oxygen/glucose deprivation	Neurotoxicity, neurodegeneration	Veliparib (10 µM)	Neuroprotection and maintenance of NAD ⁺ levels	243
Rat hippocampal-entorhinal cortical slices exposed to ethanol	Ethanol neurotoxicity	Olaparib or veliparib (1 µM)	Neuroprotection and prevention of HMGB1 release	244
U937 cells subjected to oxidative stress	Oxidative stress-related diseases	Olaparib (1–30 µM)	Protection against cell death and maintenance of cellular bioenergetics	245
HPDE cells subjected to oxidative stress	Pancreatitis	Olaparib (1–30 µM)	Protection against cell death and maintenance of cellular bioenergetics	246
Primary mouse cortical neurons subjected to α-synuclein	Neurodegenerative diseases	Veliparib, rucaparib or talazoparib (1 µM)	Protection against cell death	59
HL-1 cardiomyocytes or isolated rat atrial cardiomyocytes subjected to tachypacing	Atrial fibrillation, cardiac arrhythmias	Olaparib (5–20 µM) or veliparib (5–40 µM)	Protection against NAD ⁺ depletion and oxidative protein/DNA damage, prevention of channel remodelling and improved electrophysiology	247
hVSMCs or MC3T3 cells subjected to calcification-inducing conditions	Vascular calcification, atherosclerosis	Olaparib, veliparib or rucaparib (3 µM)	Inhibition of vascular calcification	61
Human adipocytes subjected to differentiation	Obesity	Olaparib (1 µM)	Reduced fat uptake and increased mitochondrial biogenesis	248
In vivo				
Wild-type <i>Caenorhabditis elegans</i>	Ageing	Olaparib (1 µM)	Maintenance of cellular bioenergetics and extension of lifespan	231
<i>C. elegans</i> with ATM mutation	Ataxia telangiectasia	Olaparib (500 nM)	Improvement of movement and memory	235
<i>Drosophila</i> prepupae subjected to tachypacing	Atrial fibrillation	Olaparib or veliparib (200–400 µM)	Protection against NAD ⁺ depletion and improved cardiac contractility	247
Mice subjected to toxic doses of doxorubicin	Side effects of chemotherapy	Rucaparib (10 mg kg ⁻¹)	Prevention of body weight loss and improvement of renal function	112
db/db mice	Type 2 diabetes	Veliparib (15 mg kg ⁻¹ daily)	Improved vascular function ex vivo	229
Dopaminergic neuron loss in AIMP2 transgenic mice	Parkinson disease	Rucaparib (0.125–1.25 mg kg ⁻¹)	Prevention of degeneration of dopaminergic neurons	249

Table 4 (cont.) | Clinically approved PARP inhibitors in models of non-oncological diseases

Experimental model	Disease modelled	Drug (concentration or dose/dosage)	Effects	Refs
<i>In vivo (cont.)</i>				
Mice subjected to MCA occlusion	Acute ischaemic stroke	Olaparib (3–5 mg kg ⁻¹)	Reduced infarct size and improved vascular and neurological function	250
Mice administered with endotoxin intratracheally	ARDS, sepsis, acute kidney injury	Olaparib (5 mg kg ⁻¹)	Attenuation of inflammatory cell infiltration, reduced cytokine production and improved organ function	251
Endotoxin-injected mice	Sepsis, liver failure	Olaparib (50 mg kg ⁻¹)	Improved liver function and reduced inflammatory gene expression	240
Mouse model of ovalbumin-induced or dust mite-induced lung inflammation	Bronchial asthma	Olaparib (1–10 mg kg ⁻¹)	Reduced IgE production, suppression of inflammatory cell mobilization and inhibition of cytokine production	232,252
Mouse fed with high-fat/high-sucrose or methionine/choline-deficient diet	Fatty liver disease	Olaparib (40–50 mg kg ⁻¹ daily)	Reduced obesity, reduced hepatic fat deposition, improved glucose tolerance and improved metabolic function	240,253
Mice subjected to binge alcohol exposure	Alcoholic hepatitis	Olaparib (50 mg kg ⁻¹ daily)	Improved hepatic function and reduced liver inflammation	253
rd1 or rd10 mice	Retinitis pigmentosa	Olaparib (0.1 μM intravitreally)	Rescue of photoreceptors and protection against cell death	236,237
Heterotopic heart transplantation in Lewis rats	Transplant rejection	Olaparib (10 mg kg ⁻¹ daily)	Improved cardiac function and beneficial regulation of gene expression in the transplanted heart	241
Intrastratial injection of α-synuclein in mice	Neurodegeneration	Veliparib (125 mg kg ⁻¹)	Protection against dopaminergic neuron loss	59
Mice subjected to burn injury	Third-degree burns	Olaparib (10 mg kg ⁻¹ daily)	Improved organ function, attenuated inflammatory mediator production and accelerated wound healing	254
Mice subjected to caecal ligation and puncture	Septic shock	Olaparib (9–30 mg kg ⁻¹ daily)	Improved organ function, reduced proinflammatory mediator production and increased survival	245
Cerulein-injected mice	Acute pancreatitis	Olaparib (9–30 mg kg ⁻¹ daily)	Improved organ function and reduced proinflammatory mediator production	246

ALS, amyotrophic lateral sclerosis; ARDS, acute respiratory distress syndrome; ATM, ataxia telangiectasia mutated; HMGB1, high mobility group protein B1; HPDE, human pancreatic duct epithelial; hVSMC, human vascular smooth muscle cell; MCA, middle cerebral artery; NMDA, N-methyl-D-aspartate; PARP, poly(ADP-ribose) polymerase; TDP43, TAR DNA-binding protein 43.

It will be difficult to define the selectivity of the PARP inhibitors until there is a consensus using several different methods. The ultimate confirmation of specificity is the lack of a biological effect in a cell lacking the proposed target. Two recent developments may aid the generation of more selective drugs. First, a novel immunological method for detecting PARylation and PARylation in vitro has recently been described, in which a panel of cell lines were genetically engineered to over-express PARP family members and used to determine the activity of PARP inhibitors against specific PARPs. This approach has been used to identify inhibitors specific for PARP7 (REF.272). Second, non-NAD⁺-based PARP inhibitors could allow greater specificity through allosteric inhibition¹⁰¹.

It is probably advantageous to inhibit both PARP1 and PARP2 when treating cancer owing to their overlapping functions in DNA repair; however, PARP2-selective inhibitors could offer advantages over existing PARP inhibitors. PARP2-deficient mice are highly sensitive to ionizing radiation, which induces chromosomal damage and lethality²⁶⁸. PARP2 has been implicated in DNA repair and in the maintenance of genomic integrity, in proliferative signalling through transforming

growth factor-β and in angiogenesis and the evasion of tumour cells from the host immune response²⁶⁹. These roles suggest that a PARP2 inhibitor might have anti-cancer potential. At the same time, PARP2 has also been implicated in the promotion of various inflammatory responses, including the facilitation of intestinal injury in colitis models²⁷⁰ and improved outcomes in neuro-inflammation models²⁷¹ in mice. These data must be interpreted with caution, because they are all based on studies using PARP2-deficient models, in which both the catalytic and the scaffolding activities of PARP2 are absent. This represents a different scenario to a cell treated with a PARP2 inhibitor, where only the catalytic aspects of the enzyme are inhibited. Nevertheless, the findings with PARP2-deficient models indicate that a selective PARP2 inhibitor may have anti-inflammatory potential. Inhibiting PARP2 specifically might also be safer and more suitable for chronic therapeutic regimens than simultaneous inhibition of PARP1/2 as it would be expected to have less of an inhibitory effect on DNA repair.

The other PARP family members capable of PARylation are the tankyrases PARP5A and PARP5B, which have roles in telomere maintenance, spindle

assembly and WNT signalling (reviewed in REF.²⁷³). PARP5A and PARP5B PARylate telomeric repeat-binding factor 1 (TERF1) — a DNA-binding component of the shelterin protein complex that protects telomeres from DNA repair mechanisms — and inhibit its capacity to bind to DNA²⁷⁴. Limiting tumour immortality by specifically inhibiting tankyrases such as PARP5A and PARP5B is a potentially attractive proposition, particularly since the telomerase inhibitor imetelstat was given a fast-track designation for treatment of relapsed or refractory myelofibrosis by the FDA in late 2019. Even in cases where telomeres are elongated by the alternative lengthening of telomeres mechanism — a repair-based pathway used by cancer cells to maintain telomere length — the role of PARP5A in mitotic spindles is still a potential target to arrest tumour growth. The first reported ‘selective’ tankyrase inhibitors were XAV939 and IWR-1; other compounds identified include WIKI4, JW55, JW74, G007-LK, K-756 and AZ1366 (reviewed in REFS^{275–278}). However, cell-based and in vivo studies with these inhibitors suggest more complex interactions than just tankyrase inhibition and less specificity for tankyrases over PARP1 or PARP2 than first thought, and hence interpretation of the data is difficult. At least one tankyrase inhibitor (E7449 from Oncology Venture) has entered the clinical trial stage²⁷⁹, with two phase II trials currently in the recruitment stage: one in advanced ovarian cancer (NCT03878849) and one in metastatic breast cancer (NCT03562832). Earlier studies demonstrated that E7449 has comparable inhibitory potency among PARP1, PARP2 and the tankyrases²⁸³; therefore, the relative contribution of tankyrase inhibition and the clinical validation of the tankyrases PARP5A and PARP5B as stand-alone oncological targets remain to be defined in future studies using more selective tankyrase inhibitors.

PARP3 plays a role in mediating DNA DSB repair, chromosomal rearrangements, mitotic segregation, mechanistic target of rapamycin complex 2 signalling, transforming growth factor- β -induced epithelial-mesenchymal transition in breast cancer and the maintenance of stem cell traits, making it a prime target for cancer therapy^{280,281}. Although PARP3 was initially thought to have poly(ADP-ribosyl)ation activity, it is now thought to be a mono(ADP-ribosyl)transferase. An inhibitor specific for PARP3 known as ME0328 is in early-stage development²⁸²; this compound has been shown to enhance the mitotic arrest induced by the chemotherapy agent vinorelbine, increasing vinorelbine cytotoxicity 10-fold²⁸³. However, the potential of PARP3 inhibitors as possible future anticancer agents remains to be further elucidated.

Few formal development candidates have emerged that target MAR-producing members of the PARP superfamily. Aside from PARP3, the only notable exception is PARP7, also known as TIPARP — an enzyme implicated in the cellular stress responses and cancer. A small-molecule inhibitor of this enzyme, RBN-237, is in early-stage clinical trials (NCT04053673, Ribbon Therapeutics) in patients with advanced solid tumours; however, no information on RBN-237 has been publicly disclosed thus far.

Outlook

The fundamental biological roles of PARP1 continue to be revealed, with a recent study describing a role for PARP1 in the mitochondria²⁸⁴. Improvements in medicinal chemistry may yield further increases in the potency and selectivity of future generations of PARP inhibitors; for example, mitochondrially targeted PARP inhibitors²⁴³ may yield novel experimental therapeutic approaches and strategies in response to the findings described herein. Further, advances in our understanding of the pathogenesis of various diseases will undoubtedly identify pathways that interact with PARP1, and these advances may yield novel additive or synergistic therapeutic approaches that could be exploited by therapies that simultaneously target PARP1 and the relevant interacting pathway. Indeed, a class of bifunctional PARP inhibitors that also release nitric oxide have been synthesized, with the intention of targeting glutathione *S*-transferase P1-overexpressing cancer cells²⁸⁵. PARP1 and the AKT pathway overlap, and simultaneous modulation of these two systems may have applications in both oncological and non-oncological indications²⁸⁶.

The investigation of novel PARP targets will also lead to novel drug targets. Recent studies revealed that serine is a target for ADP-ribosylation in response to DNA damage, and ADP-ribosylation is dependent on histone PARylation factor 1 (HPF1)^{287,288}. Subsequent studies showed that PARP1 competes with histone acetyltransferases for modification of adjacent serine and lysine residues on histones²⁸⁹, which has implications for the regulation of gene expression by PARP1 (reviewed in REF.⁶⁸). These findings will have implications for basic biology and the role of inhibitors in oncology and non-oncological areas. A large body of foundational work has also been performed in the area of PAR binding by macrodomains and tryptophan- and glutamate-rich (WWE) domains, as some of the proteins containing these domains are considered druggable oncology targets; the scientific background and the translational potential of this field have been discussed in recent comprehensive reviews^{290–293}.

With respect to research and development related to members of the PARP superfamily other than PARP1, basic research unveiling the functional role of these proteins is expected to go hand-in-hand with medicinal chemistry advances producing inhibitors with increased selectivity and potency. These efforts may eventually yield novel first-in-class drug development candidates for oncological and non-oncological indications.

PARP1/2 inhibitors look to become a mainstay as single agents for treating certain classes of cancer. These inhibitors have proved highly effective in platinum-sensitive/homologous recombination-deficient ovarian cancer, although perhaps less so in other tumours associated with *BRCA* mutations and an HRD phenotype such as breast, prostate and pancreatic cancers. Nevertheless, given the mild toxic effects of PARP1/2 inhibitors, these cancers are a worthwhile avenue to pursue. It is clear that *BRCA* mutations cannot predict PARP inhibitor sensitivity in a tumour-agnostic fashion — there is a spectrum

of PARP inhibitor sensitivity across *BRCA*-mutated cancers, with greater sensitivity in *BRCA*-mutant ovarian cancers than *BRCA*-mutant breast, prostate or pancreatic cancers, and *BRCA*-mutated cancers of types not normally associated with *BRCA* carriers are even less sensitive to PARP inhibitor effects^{294,295}. Although platinum sensitivity may be a useful surrogate for PARP inhibitor sensitivity in ovarian cancers, it is not in other cancers. Genomic screens such as the Myriad myChoice assay may help identify homologous recombination-deficient tumours; however, as they also detect genomic changes caused by the inability to perform HRR that persist even when HRR is restored (genomic scarring), they may give false positives by failing to identify tumours that have reversed the HRD phenotype and are therefore resistant to PARP inhibitors. The most reliable predictive biomarkers of HRD are likely to be functional, such as the ability of tumour cells to form RAD51 foci, which has been used to identify that around a third of abdominal tumours and a high frequency of lung tumours are homologous recombination deficient^{296,297}.

The approved single-agent doses of olaparib, rucaparib, niraparib and talazoparib result from early trials that established the maximum tolerated dose, but it is unclear whether the maximum tolerated dose is appropriate for a tumour-selective drug that is not anticipated to damage normal tissue. It would therefore be better to use the optimum biological dose rather than maximum tolerated dose for improved quality of life. Further studies are needed to determine whether less-intense dose schedules that are likely to be less toxic and more affordable will be as effective as approved single-agent dose schedules.

Combinations of PARP inhibitors with genotoxic chemotherapy and radiotherapy remain limited by toxicity issues and the need for careful titration of the cytotoxic agent and the PARP inhibitor. Carefully designed dose schedules will be necessary to optimize the therapeutic index and may have to be considered on an individual basis. It is clear from preclinical and clinical data that compared with their use as single agents, shorter schedules and lower doses of PARP inhibitors are needed for efficacy and tolerability when used in combination with cytotoxic agents. Although the preclinical data in non-HRD cancers look promising for the combination of PARP inhibitors with DNA damage cell cycle checkpoint kinases, this combination may also result in increased toxicity such that there is no improvement in the therapeutic index. Time will tell whether these combinations will live up to their promise clinically. Non-HRD tumours are prime candidates for treatment with PARP inhibitors and immune checkpoint inhibitors, but again it remains to be seen whether this will be a gentler form of therapy compared with conventional cytotoxic agents combined with immuno-oncology agents.

Finally, there have been repeated calls in the literature for trials repurposing PARP inhibitors for the treatment of non-oncological indications such as critical illness, acute lung injury, lung fibrosis and acute and chronic neurological diseases^{205,227,228,245,246,298–302}. To our knowledge, no such trials are ongoing; however, the success of ongoing clinical trials in stroke could reinvigorate pharmaceutical interest in these areas, especially since some of the potential target indications represent significant unmet needs and enormous commercial opportunities.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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