Biochemical Society Transactions (2018) **46** 1681–1695
https://doi.org/10.1042/BST20180418

Review Article

PARPs in genome stability and signal transduction: implications for cancer therapy

Luca Palazzo[1] and **Ivan Ahel**[2]

[1]Institute of Protein Biochemistry, National Research Council, Via Pietro Castellino 111, 80131 Naples, Italy; [2]Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, U.K.

Correspondence: Luca Palazzo (l.palazzo@ibp.cnr.it) or Ivan Ahel (ivan.ahel@path.ox.ac.uk)

The poly(ADP-ribose) polymerase (PARP) superfamily of enzymes catalyses the ADP-ribosylation (ADPr) of target proteins by using nicotinamide adenine dinucleotide (NAD^+) as a donor. ADPr reactions occur either in the form of attachment of a single ADP-ribose nucleotide unit on target proteins or in the form of ADP-ribose chains, with the latter called poly(ADP-ribosyl)ation. PARPs regulate many cellular processes, including the maintenance of genome stability and signal transduction. In this review, we focus on the PARP family members that possess the ability to modify proteins by poly(ADP-ribosyl)ation, namely PARP1, PARP2, Tankyrase-1, and Tankyrase-2. Here, we detail the cellular functions of PARP1 and PARP2 in the regulation of DNA damage response and describe the function of Tankyrases in Wnt-mediated signal transduction. Furthermore, we discuss how the understanding of these pathways has provided some major breakthroughs in the treatment of human cancer.

Introduction

ADP-ribosylation (ADPr) is a post-translational modification (PTM) conserved in bacteria, viruses, and in the majority of eukaryotes [1–3]. ADPr is mainly catalysed by the ADP-ribosyltransferase (ART) superfamily of proteins, which transfer a single or multiple ADP-ribose unit(s) from nicotinamide adenine dinucleotide (NAD^+) onto target substrates [2–4]. Substrates of ADPr can be either proteins or nucleic acids [2–4]. ARTs are widespread in different organisms and regulate diverse cellular processes as the DNA damage response (DDR), transcription, RNA metabolism and antiviral response, cell division, unfolded protein response (UPR), stress granule formation, metabolism, and cell death to cite a few [5–18].

Similarly to other PTMs, ADPr operates by altering the function/localisation/stability of targets. Additionally, ADPr acts as a scaffold for the recruitment of effector proteins ('readers'), which are able to recognise and bind the modification through specialised protein domains, such as the macrodomain, PBZ, and WWE domains [19–23].

ADPr is a 'reversible' PTM; ART activity is indeed counteracted by specific hydrolases (also called 'erasers'). Two evolutionarily unrelated protein domains are known to reverse ART's activity; catalytic macrodomains (e.g. in PARG, MacroD1, MacroD2, and TARG1), and the ARH superfamily proteins (e.g. ARH1 and ARH3) [2,23–25]. Although with possible different substrate specificities, the existence of multiple erasers of ADPr in different cellular compartments (such as the nucleus, cytosol, and membranous organelles for instance mitochondria) ensures the capability of the cells to appropriately limit and fine-tune the ADPr signal when needed [26–32]. Additionally, three classes of enzymes have been described to cleave protein ADPr in a non-canonical manner; some members of NUDIX family (such as the mammal NUDT16) [33,34], members of the Ectonucleotide pyrophosphatase/phosphodiesterase family (such as the Phosphodiesterase I found in the poison glands of rattlesnakes and the vertebrate ENPP1) [35,36], and the *Legionella pneumophila* SdeA protein [37]. Those enzymes

Received: 26 July 2018
Revised: 15 September 2018
Accepted: 21 September 2018

Version of Record published:
12 November 2018

Biochemical Society Transactions (2018) 46 1681–1695
https://doi.org/10.1042/BST20180418

hydrolyse the ADPr phosphodiester bond into adenosine monophosphate (AMP) and a ribose-5′-phosphate moiety linked to the substrate molecule, a protein modification known as phosphoribosylation [2,33–37].

The best-studied ART family are the poly(ADP-ribose) polymerases (PARPs), also called diphtheria toxin-like ADP-ribosyltransferases (ARTDs) [38–40]. In humans, there are 18 genes encoding PARP catalytic domain (CAT) containing proteins [38]. A slightly different classification has been proposed by Vyas et al. [41] that does not include the diverged TpT1 member (see below).

Regarding the amino acid composition of the CAT domain, PARP1 (also called ARTD1), PARP2 (ARTD2), PARP3 (ARTD3), Tankyrase-1 (PARP5a/ARTD5), and Tankyrase-2 PARP5b/ARTD6 are characterised by a histidine–tyrosine–glutamate (HYE) triad in the catalytic pocket [41]. PARP6 (ARTD17), PARP7 (ARTD14), PARP8 (ARTD16), PARP10 (ARTD10), PARP11 (ARTD11), and PARP12 (ARTD12) are characterised by a histidine–tyrosine–isoleucine (HYI) triad [41]. PARP16 (ARTD16) is characterised by a histidine–tyrosine–tyrosine (HYI) motif [41]. PARP14 (ARTD8) and PARP15 (ARTD7) contain a histidine–tyrosine–leucine (HYL) triad [41]. PARP9 (ARTD9) holds a glutamine–tyrosine–threonine (QYT) [41]. The isoform 1 of PARP13 (ARTD13) is characterised by a tyrosine–tyrosine–valine (YYV) motif, while the CAT domain is absent in the isoform 2 of PARP13 (PARP13.2) [41]. Additionally, the highly divergent TpT1 (ARTD18) is also sometimes classified as a PARP-like protein. This protein contains a histidine–histidine–valine (HHV) triad in the CAT and in yeast catalyses a NAD^+-dependent dephosphorylation of tRNA-splicing intermediates, generating ADPr-1-phopshate through a cyclic intermediate [2,38,42]. Most of the PARPs efficiently catalyse the transfer of ADP-ribose onto proteins, albeit with different specificities. PARPs usually transfer ADP-ribose onto aspartic/glutamic acid (via ester linkages; here named as Asp/Glu-ADPr) or serine (via O-glycosylation; here named as Ser-ADPr) residues on target molecules [3,43–45].

Members of the PARP family can be also classified based on their ability to perform mono(ADP-ribosyl)ation (MARylation) or poly(ADP-ribosyl)ation (PARylation) [41]; in the latter case, the ADP-ribose units are linked together through glycosidic ribose–ribose $1'' \rightarrow 2'$ bonds [46]. PARPs capable of synthesis of PARylation include the DNA damage-inducible PARP1 and PARP2, which are known for their ability to produce long (up to 200 ADP-ribose units) and heterogeneous chains of branched PAR [41,46,47], and Tankyrase-1 and Tankyrase-2 [41]. It should be noted that Tankyrases synthesise PAR polymers with an average chain length of 20 units and no detectable branching [41,48]. Tankyrases are involved in multiple cellular processes, such as telomere length maintenance, mitosis, and Wnt signalling regulation [3,4]. Although initially described as able to catalyse ADP-ribose polymers up to 15-mers [49], PARP3 is currently believed to be a MARylating enzyme [3,4,38,41].

In this review, we summarise the recent advances in the understanding of PARPs by focusing on those that carry out PARylation and have functions in genome stability and signal transduction. The targeting of both cellular processes has provided promising strategies for cancer therapy. Indeed, the understanding of the cellular processes regulated by PARPs owes much to the success of PARP inhibitors in preclinical and clinical trials.

Human PARPs

With the exception of TpT1 that appears to act as a RNA phosphotransferase, 17 human PARPs/ARTDs family members have been identified carrying a canonical ART domain [41]. PARPs are located in various cellular compartments and regulate major cellular functions, e.g. DNA damage response, transcription, chromatin structure regulation, UPR, metabolism, mitosis, telomere length maintenance, stress granule formation, antiviral response, and receptor-associated signalling [5–9,11,12,15,16,18].

The better understood PARylating PARPs/ARTDs are the DDR PARPs (PARP1 and PARP2) and Tankyrases 1 and 2 (see extensively below). Conversely, relatively little is known about the mono(ADP-ribosyl)ating (MARylating) PARPs/ARTDs and their physiological function. Recent efforts have sought to assign cellular functions to some MARylating PARPs. For instance, PARP3 is involved in the DDR and mitotic spindle assembly [50]; PARP4 (vPARP or ARTD4) has an unclear function at the mammalian vaults (ribonucleoprotein complexes) and it is possibly involved in antiviral response [51,52]; PARP6 has been proposed to have a role in cell cycle progression and has been associated with the development of colorectal cancer [53]. PARP9 possesses a unique MARylating activity specifically occurring on ubiquitin molecules and it has functions in DDR, transcription in lymphocytes, and antiviral response [52,54–57]. PARP10 is a binding protein and an inhibitor of MYC with inhibitory potential also on the NF-κB signalling pathway [58,59]. Moreover, PARP10 acts as a pro-apoptotic protein [60]. PARP11 is proposed to have a role in nuclear envelope biology [61,62], while PARP12 is a cytosolic protein but preferentially associates with the Golgi apparatus and regulates stress

granule assembly, microRNA activity, and antiviral response [5,16,63]; PARP13 has been so far considered a catalytically inactive ART with functions in the assembly of stress granules [5] and in the regulation of microRNA [64], with implications in immunity and cancer [65]. PARP14 has been linked with multiple cellular functions such as survival of B-cells [66], cell migration [67], assembly of stress granules [5], transcription during inflammation processes [68], DDR [69], and antiviral response [52]. PARP15 is also involved in stress granule formation and antiviral response [5,52]. PARP16 is located at the endoplasmic reticulum and regulates UPR [8,70].

DNA repair PARPs and signalling

The main PARPs with a direct function in the DDR are the PARylating PARP1 and PARP2, and the MARylating PARP3 [71]. PARP1 is the founding member of the PARP/ARTD family and the best-studied one. At the N-terminal end, PARP1 contains three DNA-binding zinc finger domains (ZFI: amino acid (aa) 11–89; ZFII: aa 115–199; ZFIII: aa 233–373) [46,72,73]. The central domain of PARP1 contains the BRCA1 C-terminal (BRCT) domain and the tryptophan–glycine–arginine-rich (WGR) domain [74]. At the carboxyl-terminal region, PARP1 contains the PARP homology domain, comprising of the CAT responsible for ADP-ribosylation [75–77]. PARP1, PARP2, and PARP3 share ∼60% amino acid similarity within their catalytic and WGR domains but diverge at their N-termini. PARP2 and PARP3 lack the ZF and BRCT domains and instead possess shorter unstructured N-terminal regions with poorly understood functions. In the CAT domain, PARP1, PARP2, and PARP3 share a conserved structural feature known as helical domain (HD) [75]. The crystal structure of the essential domains of PARP1 in complex with DNA double-strand breaks revealed that the HD acts as an autoinhibitory domain by blocking the access to the NAD^+-binding site [77,78]. However, in response to the binding of PARP1 with DNA, the HD rapidly unfolds, thus allowing PARP1 catalytic activity [79]. Outside of the CAT domain is the WGR domain, which is vital for the activation of all the three damage-dependent PARPs (PARP1, PARP2, and PARP3) [74,77]. Structural studies showed that the PARP1 WGR domain makes sequence-independent contacts with both DNA backbone, near the 5′-terminus, and the HD within the CAT domain [77]. As a result of the concomitant interaction with damaged DNA and HD, the WGR transfers the information of binding to DNA to the catalytic portion of PARP1. The intramolecular contact between these two domains triggered by damaged DNA structurally destabilises the HD, leading to the catalytic activation of PARP1 [74].

PARP1 and PARP2 have been recognised as central components of the base excision repair/single-strand break repair process (BER/SSBR) [71,80]. Moreover, PARP1 and PARP2 have been found to be activated by double-strand breaks (DSBs) and required for homologous recombination (HR) at stalled or collapsed replication forks as well as for non-homologous end joining (NHEJ) [71,80].

Upon binding to DNA breaks, the catalytic function of PARP1 is activated to generate extensive poly (ADP-ribose) chains (PAR chains) on proteins in the proximity of DNA damage, among which include DNA repair effectors and histones [73,78,81] (Figure 1A). It has also been proposed that PARP2 binds PARP1-neosynthetised PAR chains at DNA damage sites through its unstructured N-terminal region [82]. This interaction facilitates the subsequent activation of PARP2, which seems mainly responsible for the formation of branched chains of PAR [82]. Interestingly, PARP1, PARP2, as well as PARP3 have been recently reported to directly ADP-ribosylate the DNA breaks via free phosphate groups in cellular response to DNA damage; however, how this modification affects DDR is still unclear [83–85]. Overall, the absence or the inhibition of PARP1 or PARP2 in mice or human confers sensitivity to a variety of DNA damaging agents [6,86]. The double *Parp1* and *Parp2* knockout mice show embryonic lethality suggesting significant functional redundancies between the two proteins [87].

One of the main targets of PARP1 is the histone core proteins of nucleosomes (H2A, H2B, H3, and H4) as well as the linker histone H1 [43,88–90]. Although it is not completely clear as to the mechanistic role of the highly abundant histone modification, it has been proposed that it allows the opening of chromatin structure and a better accessibility for DDR factors, thus facilitating DNA repair [91,92] (Figure 1A). The most abundant ADPr histone sites are on the specific serine resides on their tails (Ser-ADPr) [90]. Ser-ADPr fully depends on a protein called histone PARylation factor 1 (HPF1), which was identified as a key protein controlling the DNA damage-inducible PARylation of histone proteins [89,90,93] (Figure 1A). HPF1 is a PARP1-binding protein that confers specificity for substrates to PARP1, allowing specific ADPr of serine residues in histones as well as many other factors involved in genome stability [89,90]. The main target residues of ADPr in histones are Ser6 of H2B and Ser10 of H3 [43,89,90]. Unbiased mass spectrometry studies have identified a common

Biochemical Society Transactions (2018) **46** 1681–1695
https://doi.org/10.1042/BST20180418

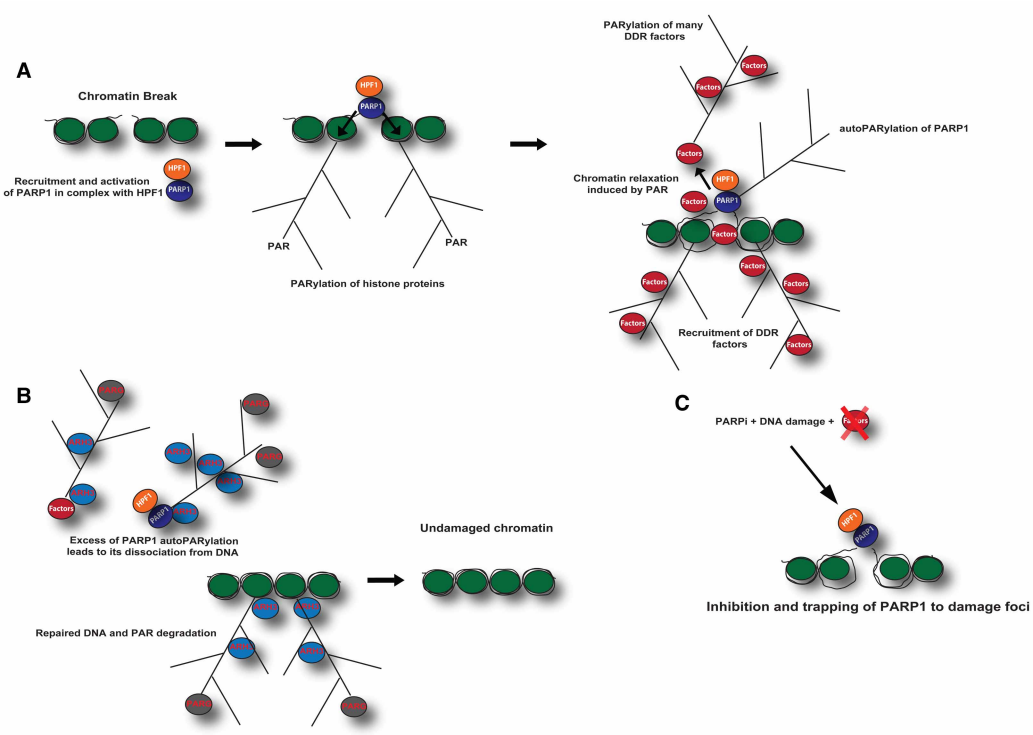

Figure 1. Schematic representation of PARP1-mediated DNA damage repair.

(**A**) PARP1 in complex with accessory proteins, such as HPF1, is recruited to the DNA damage foci and binds damaged DNA. As a consequence of binding to damaged DNA, PARP1 PARylates serine residues on histones as well as many other factors involved in genome stability. PARylation of histone proteins allows the opening of chromatin structure and a better accessibility of DDR factors, thus facilitating DNA repair. ADP-ribosylation (ADPr) also acts as a scaffold for the recruitment of DDR effector proteins ('readers'), which are able to recognise and bind the modification. (**B**) Upon DNA damage repair, the excess of PARP1 automodification induces the release of PARP1 from DNA damage foci and PAR chains are rapidly hydrolysed by ARH3 and PARG. (**C**) The enzymatic inhibition of PARP1 by PARP inhibitors (PARPi) results in suppression of DNA damage repair and in the trapping of PARP1 to damage foci.

Ser-ADPr motif in response to DNA damage. This Ser-ADPr motif is characterised by a lysine (or less frequently an arginine) residue followed by serine, which acts as an acceptor site [89]. These recent results seem to somewhat contradict previous observations, mainly describing ADPr of histones and other DDR proteins on acidic residues [94–97]. While this could be partly due to different cell lines employed and type of stress used to activate the DNA damage, the main reasons lie in the methodology applied to prepare samples and detect the modified amino acids by mass spectrometry. For instance, the majority of studies describing Asp/Glu-ADPr sites in proteins in response to oxidative insults have been conducted by employing the hydroxylamine treatment, a method that enables the sole identification of Asp and Glu modified by ADPr, therefore excluding other types of modification [94].

Importantly, PARP1 PARylates itself (autoPARylation) on multiple acceptor sites that have been characterised by MS *in vitro* and *in vivo* [29,33–36,98–101]. AutoPARylation acts as an important scaffold for recruitment of DDR factors [80]. Excessive PARP1 automodification may also induce the release of PARP1 from DNA damage foci [102,103] (Figure 1A). Extensive automodification of PARP1 is suppressed by HPF1 [93]. HPF1 also changes automodification sites in PARP1 from Asp/Glu-ADPr to Ser-ADP, although the biochemical mechanism is still unknown [89,90]. PARP1- and HPF1-dependent Ser-ADPr also occurs on many proteins involved in the maintenance of genome stability [15,100,104], for instance, the high mobility group proteins [89]. Nevertheless, many other DDR proteins may be not regulated by the PARP1-2/HPF1 complex but instead controlled by distinct PARP complexes, perhaps leading to Asp/Glu-ADPr. Indeed, the absence of HPF1 leads to a reduced ADPr of histone proteins and DNA repair factors, and conversely to the enrichment

of ADPr on Asp/Glu of PARP1 itself and many other proteins [90]. Further efforts will have to clarify what the functional importance/advantage of having Ser-ADPr instead of Asp/Glu-ADPr in response to DNA damage. Deficiency of HPF1 in cells leads to sensitivity to DNA damaging drugs and PARP inhibitors (PARPi; see below) [90,93].

As noted previously, PARP1 automodification as well as the PAR chains on histone core proteins serves as a scaffold for recruitment of critical DNA repair proteins, such as XRCC1 or different chromatin remodelling proteins, to the DNA break, thus facilitating DNA repair [19,20,105–107]. The turnover of longer chains of PAR after damage largely depends on PARG hydrolase function [108–110]. In cells, PARG is inefficient in cleaving short chains of PAR and is not capable of removing MARylation [24,110,111], which are instead a substrate for ARH3 hydrolase when ADP-ribose is linked to serine residues [90,110] (Figure 1B). Glutamate-linked MARylation is hydrolysed by macrodomain-containing proteins TARG1, MacroD1 and MacroD2 [2,4,29], but is a poor substrate for ARH3 [110]. In contrast, TARG1, MacroD1, and MacroD2 are unable to hydrolyse Ser-ADPr [110].

Modulation of DDR PAR signalling for cancer treatment

The enzymatic PARP1 inhibition by small-molecule analogues of NAD^+ results in suppression of both SSB repair and BER and also in trapping PARP1 onto DNA lesions [112–114], which in turn causes the stalling and subsequent collapse of DNA replication forks, further resulting in replication-dependent DNA DSBs and possibly transcription conflicts [115,116]. DSBs are normally repaired by HR; however, in HR-deficient cells, such as BRCA1- and BRCA2-deficient backgrounds, lower-fidelity NHEJ occurs resulting in chromosomal aberration and ultimately cell death [117,118]. The catastrophic scenario induced by PARP inhibition could, therefore, be exploited to sensitise tumour cells to conventional treatments, such as chemotherapy or radiotherapy, which often cause DNA damage. Indeed, two groups in 2005 described the synthetic lethal (SL) interaction between PARP inhibition and BRCA1 or BRCA2 mutation suggesting a novel strategy for treating patients with BRCA mutant tumours [119–123]. Efforts in the last 20 years led to the development of many PARP inhibitors, including several that are already used in the clinics such as Veliparib (Abbvie), Rucaparib (Pfizer/Clovis), Olaparib (KuDOS/AstraZeneca), Niraparib (Merck/Tesaro) [124], Talazoparib (Lead/Biomarin/Medivation/Pfizer) [124,125], and Pamiparib (BeiGene/Merck Serono) [126,127], the latter with significant brain penetration.

All current clinically relevant PARPi are NAD^+-mimetics and bind specifically to the PARP1 and PARP2 catalytic domain. PARPi are particularly effective (1000 times more sensitive) in the treatment of breast, ovarian, and other cancers that are BRCA1 and BRCA2 deficient [120,128–130].

Olaparib was the first PARPi approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients carrying BRCA germline mutations (gBRCAm), whom have advanced ovarian cancer and already received previous lines of therapy [131,132]. The European Medicines Agency (EMA) also approved Olaparib as a maintenance treatment for BRCA mutant patients with platinum-sensitive gynecological cancers, such as high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancers.

SL interactions between PARP inhibition and loss of function BRCA1 or BRCA2 can be expanded to sporadic tumours, a concept called 'BRCAness'. Tumours that have not arisen from a germline gBRCAm are described to partially phenocopy the hereditary cancers in terms of HR defects [133,134]. Several mutations have been described to reproduce the phenotype of gBRCAm, such as somatically occurring mutations in either BRCA1 or BRCA2, somatic hypermethylation of BRCA1 promoter, or mutations in other genes that are involved in DSB repair and the stability of replication forks [133,134]. Beyond BRCA1 and BRCA2 mutations, BRCAness allowed to expand the SL approach to several tumours carrying deficiencies in many tumour suppressor genes involved in HR, such as ATM, ATR, PALB2, and the FANC gene family, which were shown to be sensitive to PARPi [134,135]. Notably, SL interactions between PARP inhibition and loss of function or amplification of other DDR factors, such as PALB2 and ATM, have been demonstrated by *in vitro* and xenograft studies [136–138]. Genome-wide sequencing projects revealed that somatic mutations in many other genes involved in HR occur in a wide spectrum of tumours [134], for instance, in high-grade serous ovarian cancer [139], advanced prostate cancer [140], and pancreatic cancer [141,142]. These and other cancers with HR mutations are therefore candidates for testing PARPi efficacy. Indeed, the use of Olaparib has also been approved for the treatment of metastatic, castration-resistant prostate cancer with genetic defects in DDR-encoding genes [140]. More studies will certainly expand the use of PARPi for the treatment of many other tumours, such as in acute myeloid leukaemia treatment [143]. Nevertheless, it is the obligation of the

Biochemical Society Transactions (2018) **46** 1681–1695
https://doi.org/10.1042/BST20180418

scientific community to improve the selectivity of targeted therapies. This can be achieved by an in-depth understanding of the molecular mechanisms regulated by PARPs and their cell type specificity as well as by a detailed comprehension of PARPi cytotoxic effects and by unveiling new functional interaction, such as with NEDD8/SCF and HDAC inhibitors [144,145].

A relevant topic for the clinic is the side effect of PARP inhibition. PARP1 and PARP2 have multiple important roles beyond the DDR, such as transcription, apoptosis, and immune function; the antitumour efficacy of PARPi might also reflect alterations in those functions [92]. Indeed, dose-limiting myelosuppression and central nervous system side effects were observed in some patients treated with Olaparib [146]. Being able to discriminate between the multiple cellular functions of PARP1 and PARP2 proteins, and specifically target the DDR pathway in a particular genetic background would be the next goal of the basic research-based translational research.

From a biochemical point of view, PARPi acts with different molecular mechanisms. For instance, some PARPi (such as Rucaparib, Olaparib, Niraparib, and Talazoparib) interfere with the catalytic cycle of PARP1. To a different extent, these drugs prevent PARP1 and PARP2 autoPARylation, thus trapping the enzymes on DNA lesions that they recognise [116–148] (Figure 1C); Talazoparib is ∼100 times more potent than Niraparib in trapping PARP1, which in turn traps PARP1 more potently than Olaparib and Rucaparib [147]. In contrast, Veliparib appears to have a limited ability to trap PARP1, despite its ability to inhibit PARylation [147]. These differences in trapping PARP1, rather than simply inhibiting PARylation, may be a better predictor of *in vitro* cytotoxicity in BRCA-deficient cells, with Talazoparib having the most profound cytotoxic effects [116,125,147].

Resistance to PARP inhibition is clinically relevant; multiple potential mechanisms of resistance to PARPi have been described. These include (i) silencing of DDR proteins, such as 53BP1 [149,150] or REV7 [151], which results in the reactivation of HR pathways; (ii) selective loss of PARG [152]; (iii) loss of function of proteins involved in destabilisation of the replication fork [153], such as EZH2 and MUS81 [154]; and (iv) loss of function of PARP1 itself [155]. Additionally, secondary 'revertant' mutations in BRCA1 or BRCA2 that restore sufficient HR function have been also described [156,157]; the same mutations are also responsible for resistance to platinum-based chemotherapy [134,156,158–160].

In the long term, the analysis of the gene expression of those DDR factors could be exploited to predict chemoresistance to PARPi. In addition, an in-depth understanding of cell type-specific PARP function is needed to address and improve patients' stratification for targeted therapies. Indeed, due to different expression of regulatory proteins and therefore with potentially different levels of regulation, the global ADPr in response to DNA damage may differ in a cell-specific manner [95]. For instance, some cell types may prefer Ser-ADPr to Asp/Glu-ADPr or vice versa, or follow alternative DDR pathways. Thus, knowing the origin of tumours and how the specific cell type respond to DNA damage induced by chemotherapy/radiotherapy may be useful to predict whether patients will be sensitive or not to PARPi.

A considerable effort has been made to identify additional SL interactions in ovarian cancers involving BRCA1 and BRCA2, which overcome PARPi resistance. The inhibition of the low-fidelity DNA polymerase-θ (Polθ) [118,161] and RAD52 [162] are synthetic lethal targets for the treatment of BRCA1-mutated cancers. These preclinical observations have led to ongoing efforts in the development of small molecule inhibiting Polθ and RAD52 [163].

In addition to PARP1 and PARP2, multiple players in ADPr regulation can be also targeted for cancer therapy, for instance, the hydrolases. The first study of breast cancer radiosensitisation by a cell-permeable specific inhibitor of PARG (PARGi; PDD00017273) [164] was reported by Gravells et al. [165]. PARGi sensitises cells to ionising radiation (IR) at the same magnitude of PARPi, although with different mechanisms of action. While PARPi radiosensitises to IR through replication-associated DNA breaks, which in turn are repaired at later times by NHEJ [121], inhibition of PARG leads to prolonged activation and persistence of PAR that results in faster DNA damage repair and rapid activation of NHEJ pathways [165]. Additionally, due to the pleiotropic functions of PARG, a severe mitotic phenotype induced by PARGi was observed, possibly due to the mitotic functions of PARG in reverting Tankyrase-mediated PARylation at the mitotic spindle (see below) [165].

The search for novel targets for cancer therapy may possibly open new research lines, perhaps looking for inhibitors of erasers and readers of ADPr (e.g. NUDT16, TARG1, ARH3, and ALC1). The advantage of targeting erasers of ADPr rather than PARPs may rely on the pleiotropic functions of catalytic enzymes, as demonstrated by the mitotic defects induced by PARGi in addition to the DNA repair ones [165]. Indeed, while each

of the multiple PARPs shows a still not well-understood selectivity for certain substrates and regulates a restricted number of cellular functions, few erasers are required to act simultaneously on multiple processes and in different cellular compartments in order to revert any kind of ADPr event. Thus, compared with the inhibition of single PARPs, the inhibition of erasers of ADPr could potentially ensure the strongest impact on the overall physiology of cancer cells, thus affecting the cell survival and reducing the possibility of resistance to the treatment.

Tankyrases

Aside from the DDR, PARPs are recognised master regulators of multiple cellular functions, such as signal transduction, cell aging, and division. In this regard, Tankyrase-1 (also called PARP5a or TNKS1 or ARTD5) and Tankyrase-2 (also called PARP5b or TNKS2 or ARTD6) are poly(ADP-ribose) polymerases with functions in telomere length maintenance [166], HR-mediated DDR [167], mitosis [168–170], and Wnt and Notch-mediated signal transduction [171,172]. Notably, Tankyrases are novel and promising targets for cancer therapy [173].

Structurally, both Tankyrases are equipped with five Ankyrin repeats (ANK), a sterile alpha motif (SAM), and CAT domain [174]. Additionally, Tankyrase-1 contains a histidine, serine, and proline-rich (HPS) region [174]. While the SAM domain is required for multimerisation of Tankyrase molecules [175,176], the ANK domain serves as a binding platform for ADPr substrates [177]. In particular, the consensus hexapeptide motif RxxPDG (where 'x' refers to any amino acid) within the ANK domain was shown to be necessary and sufficient for interaction with protein targets [178]. Several targets of Tankyrases were identified, among them the telomeric-repeat binding factor-1 (TRF1) [168], the insulin-responsive amino-peptidase (IRAP) [178], the 182-kDa tankyrase-binding protein (TAB182) [178], the nuclear mitotic apparatus protein-1 (NuMA1) [178], AXIN1/2 [171], 3BP2 mutated in Cherubism disease [179,180], (Bhardwaj 2017) [172], and the CBP80/CBP20-dependent translation initiation factor (CTIF) [181]. Thus, the interaction with protein substrates can be considered the specificity determinant for Tankyrases. Compared with the knowledge achieved in the understanding of PARP1 activation mechanisms, it should be noted that very little is known about the processes of catalytic activation of Tankyrases, their amino acid specificity, and the modification sites in known Tankyrase substrates (AXIN above all). Recently, new details into the activation mechanism of Tankyrases have been provided. It has been shown that intermolecular SAM–SAM contacts are required to induce polymerisation of Tankyrase molecules, in both *in vitro* and *in cellulo* [175]. Tankyrase polymerisation seems to support the catalytic activity of the enzyme [175]. Formation of Tankyrase polymers also provides a nucleation point for the assembly of non-membranous structures, named signalosomes. The advantage of the formation of signalosomes is the local concentration of proteins, which can be both enzyme and substrates, for an efficient, transient, and spatially confined process [182,183]. Unfortunately, it is still unknown which signal triggers Tankyrase polymerisation and further activation of the catalytic activity.

Notably, Tankyrase-1 and Tankyrase-2 appear to have largely overlapping functions, as deletion of either gene leads to subtle phenotypes, while double knockout of both genes is embryonic lethal [184].

One of the best-characterised substrates of Tankyrases is AXIN, a key regulator of the canonical Wnt signalling pathway [173,185–188] (Figure 2). In the canonical pathway, Wnt regulates the level and subcellular localisation of the β-catenin transcription factor and is thus called a β-catenin-dependent Wnt pathway. In the absence of an activating Wnt signal, glycogen synthase kinase 3β (GSK3β) collaborates with the AXIN and APC (adenomatous polyposis coli) proteins and other factors to phosphorylate β-catenin [185]. The phosphorylated β-catenin is recognised and ubiquitinated by a complex containing a β-transducin repeat-containing protein (βTrCP), then degraded by the proteasome [185] (Figure 2). Wnt binds to a seven-pass transmembrane Frizzled receptor and its co-receptor, low-density lipoprotein receptor-related protein 6 (LRP6) on the cell surface. The resulting phosphorylation cascade inhibits the AXIN/GSK3β complex and stabilises the free pools of β-catenin, which can translocate into the nucleus [185] (Figure 2). In this context, Tankyrases are required to induce AXIN degradation and in turn β-catenin stabilisation. Tankyrases can indeed bind and PARylate AXIN. Tankyrase-mediated PARylation of AXIN acts as a scaffold for the recruitment for the WWE domain-equipped E3 ubiquitin ligase RNF146, which ubiquitinates AXIN leading to its proteasomal degradation [171] (Figure 2). In the nucleus, β-catenin binds to T-cell factor (TCF) transcriptional regulators along with other cofactors and modulates transcription of various genes [185]. Mutational mechanisms activating the WNT pathway and stabilising β-catenin have been found in human cancer, especially colorectal cancer (CRC)

Biochemical Society Transactions (2018) **46** 1681–1695
https://doi.org/10.1042/BST20180418

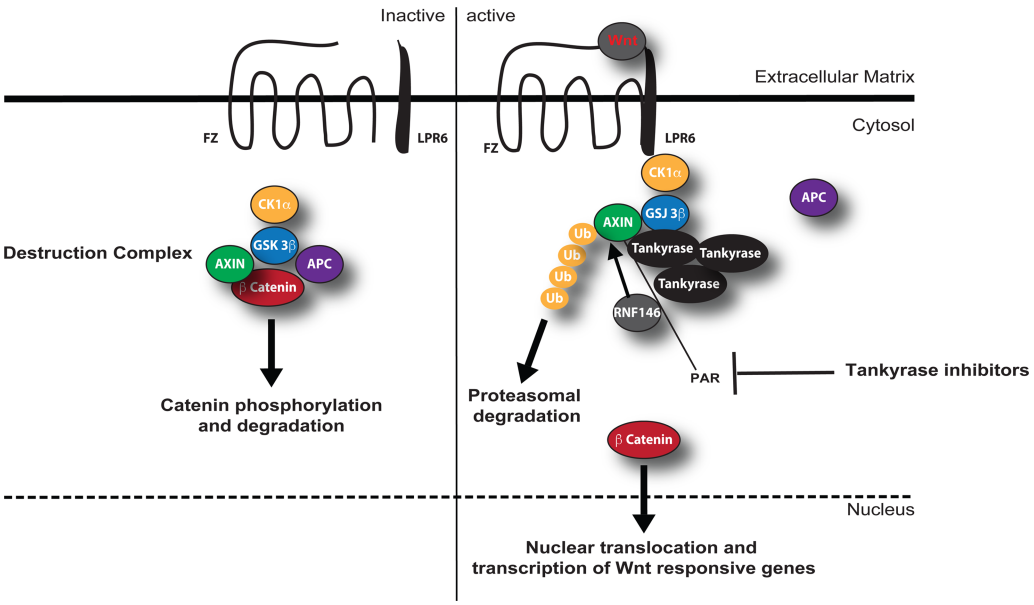

Figure 2. Schematic representation of Tankyrase-mediated Wnt/β-catenin signalling.
The canonical Wnt signalling pathway with its major components in the inactive (left) and active (right) state.

[185–188]. Cancer mutations include inactivation of APC or AXIN or activating mutations in β-catenin, all of which lead to constitutive transcription of β-catenin/TCF-regulated genes [185–188].

Modulation of Tankyrase signalling in cancer

The inhibition of both Tankyrases by the small NAD^+ mimetic XAV939 was shown to inhibit growth of the APC-defective CRCs cell line by stabilising AXIN [171] (Figure 2). Tankyrase inhibitors would be presumably useful to target CRC cells that are characterised by constitutive activation of the Wnt pathway, such as those with upstream ligand-receptor defects or APC defects. In contrast, cancer cells expressing a mutant oncogenic β-catenin protein would presumably be resistant to AXIN stabilisation. Importantly, XAV939 inhibits Tankyrase-1, Tankyrase-2, PARP1, and PARP2 with comparable potency (e.g. IC_{50} values of 95, 5, 74, and 27 nM, respectively) [188]. More specific Tankyrase inhibitors were then developed with no measurable IC_{50} for PARP1 and PARP2, such as IWR-1 and IWR-2 [189–191]. Subsequently, structural studies have allowed structure-based drug design of more selective and potent Tankyrase inhibitors with cytotoxic potential demonstrated in CRC cell lines [184,192], for instance, JW74 [193], JW55 [194], WIKI4 [195,196], K-756 [197], G007-LK [198,199], and NVP-TNKS656 [200]. *In vivo* preclinical studies have demonstrated the antitumour activity of JW55 [194] and G007-LK [199] in xenograft and the $Apc^{-/-}$ mouse models. Major issues reported in preclinical studies are related to the intestinal toxicity of Tankyrase inhibitors *per se* [198,201].

As discussed for inhibitors of PARP1, the multiple functions of PARPs could limit the use of such molecules only in certain clinical conditions. As mentioned, Tankyrase proteins are evolutionarily conserved and function in telomere length regulation and sister telomere cohesion, GLUT4 vesicle translocation, and possibly also mitotic spindle pole regulation [174]. Thus, targeting of Tankyrases is expected to have varied effects on cells. Notably, inhibition of Tankyrase-1 resulted in synthetic lethal effects in cells with BRCA1 or BRCA2 defects, apparently due to exacerbation of the centrosome amplification phenotype associated with BRCA deficiency [202]. Of note, both BRCA1 and Tankyrases have connected functions in the regulation of bipolar mitotic spindle formation [203]. A better understanding of the molecular complexes and additional substrates of Tankyrases as well as knowing whether the expression/function of those proteins changes in tumours originating from specific cancer cells may help patients' stratification and prognosis.

Conclusions

In conclusion, ADPr is a central PTM regulating all the major cellular processes and thus affecting cell pathophysiology. Here, we have highlighted pertinent examples of how our understanding of ADPr signalling, gained

from biochemical, structural, and cell biology studies, has been already exploited for the treatment of human cancer. Nevertheless, many other molecules linked to ADPr, thus not only ARTs, have or may have affect on cancer research; however, in many cases, their link with human pathology still needs to be uncovered.

Abbreviations

ADPr, ADP-ribosylation; ANK, Ankyrin repeats; APC, adenomatous polyposis coli; ART, ADP-ribosyltransferase; ARTD, ADP-ribosyltransferase diphtheria toxin-like; Asp/Glu-ADPr, ADPr on aspartic/glutamic acids; BER, base excision repair; BRCT, BRCA1 C-terminal; CAT, PARP catalytic domain; CRC, colorectal cancer; DDR, DNA damage response; DSBs, double-strand breaks; gBRCAm, BRCA germline mutations; GSK3β, glycogen synthase kinase 3β; HD, helical domain; HPF1, histone PARylation factor 1; HR, homologous recombination; IR, ionising radiation; MARylating, mono(ADP-ribosyl)ating; MARylation, mono(ADP-ribosyl)ation; NAD^+, nicotinamide adenine dinucleotide; NHEJ, non-homologous end joining; PARPs, poly(ADP-ribose) polymerases; PARylation, poly(ADP-ribosyl)ation; PTM, post-translational modification; SAM, sterile alpha motif; Ser-ADPr, ADPr on serine residues; SL, synthetic lethality; SSBR, single-strand break repair; TCF, T-cell factor; UPR, unfolded protein response.

Author Contribution

L.P. and I.A. conceived and co-wrote the manuscript.

Funding

L.P. acknowledges funding from the Italian Foundation/Association for Cancer Research [FIRC/AIRC, Milan, Italy; grant 14895]. I.A. acknowledges funding from the Wellcome Trust [grant 101794], Cancer Research UK [grant C35050/A22284], and the European Research Council [grant 281739].

Acknowledgements

The authors thank Dr Giovanna Grimaldi and Dr Daniela Corda (Institute of Protein Biochemistry, Naples, Italy), Dr Edward Bartlett (Sir William Dunn School of Pathology), and Dr Ian Gibbs-Seymour (Department of Biochemistry, University of Oxford, U.K.) for the helpful comments on the manuscript.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Perina, D., Mikoč, A., Ahel, J., Ćetković, H., Žaja, R. and Ahel, I. (2014) Distribution of protein poly(ADP-ribosyl)ation systems across all domains of life. *DNA Repair* **23**, 4–16 https://doi.org/10.1016/j.dnarep.2014.05.003
2 Palazzo, L., Mikoč, A. and Ahel, I. (2017) ADP-ribosylation: new facets of an ancient modification. *FEBS J.* **284**, 2932–2946 https://doi.org/10.1111/febs.14078
3 Cohen, M.S. and Chang, P. (2018) Insights into the biogenesis, function, and regulation of ADP-ribosylation. *Nat. Chem. Biol.* **14**, 236–243 https://doi.org/10.1038/nchembio.2568
4 Lüscher, B., Bütepage, M., Eckei, L., Krieg, S., Verheugd, P. and Shilton, B.H. (2018) ADP-ribosylation, a multifaceted posttranslational modification involved in the control of cell physiology in health and disease. *Chem. Rev.* **118**, 1092–1136 https://doi.org/10.1021/acs.chemrev.7b00122
5 Leung, A.K., Vyas, S., Rood, J.E., Bhutkar, A., Sharp, P.A. and Chang, P. (2011) Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. *Mol. Cell* **42**, 489–499 https://doi.org/10.1016/j.molcel.2011.04.015
6 Gibson, B.A. and Kraus, W.L. (2012) New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat. Rev. Mol. Cell Biol.* **13**, 411–424 https://doi.org/10.1038/nrm3376
7 Bai, P. and Cantó, C. (2012) The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease. *Cell Metab.* **16**, 290–295 https://doi.org/10.1016/j.cmet.2012.06.016
8 Jwa, M. and Chang, P. (2012) PARP16 is a tail-anchored endoplasmic reticulum protein required for the PERK- and IRE1α-mediated unfolded protein response. *Nat. Cell Biol.* **14**, 1223–1230 https://doi.org/10.1038/ncb2593
9 Bock, F.J., Todorova, T.T. and Chang, P. (2015) RNA regulation by poly(ADP-ribose) polymerases. *Mol. Cell* **58**, 959–969 https://doi.org/10.1016/j.molcel.2015.01.037
10 Rack, J.G., Morra, R., Barkauskaite, E., Kraehenbuehl, R., Ariza, A., Qu, Y. et al. (2015) Identification of a class of protein ADP-ribosylating sirtuins in microbial pathogens. *Mol. Cell* **59**, 309–320 https://doi.org/10.1016/j.molcel.2015.06.013
11 Bai, P. (2015) Biology of poly(ADP-ribose) polymerases: the factotums of cell maintenance. *Mol. Cell* **58**, 947–958 https://doi.org/10.1016/j.molcel.2015.01.034
12 Bock, F.J. and Chang, P. (2016) New directions in poly(ADP-ribose) polymerase biology. *FEBS J.* **283**, 4017–4031 https://doi.org/10.1111/febs.13737
13 Lalić, J., Posavec Marjanović, M., Palazzo, L., Perina, D., Sabljić, I., Žaja, R. et al. (2016) Disruption of macrodomain protein SCO6735 increases antibiotic production in *Streptomyces coelicolor*. *J. Biol. Chem.* **291**, 23175–23187 https://doi.org/10.1074/jbc.M116.721894

14 Jankevicius, G., Ariza, A., Ahel, M. and Ahel, I. (2016) The toxin-antitoxin system DarTG catalyzes reversible ADP-ribosylation of DNA. *Mol. Cell* **64**, 1109–1116 https://doi.org/10.1016/j.molcel.2016.11.014
15 Gupte, R., Liu, Z. and Kraus, W.L. (2017) PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes. *Genes Dev.* **31**, 101–126 https://doi.org/10.1101/gad.291518.116
16 Catara, G., Grimaldi, G., Schembri, L., Spano, D., Turacchio, G., Lo Monte, M. et al. (2017) PARP1-produced poly-ADP-ribose causes the PARP12 translocation to stress granules and impairment of Golgi complex functions. *Sci. Rep.* **7**, Article number: 14035 https://doi.org/10.1038/s41598-017-14156-8
17 Fehr, A.R., Jankevicius, G., Ahel, I. and Perlman, S. (2018) Viral macrodomains: unique mediators of viral replication and pathogenesis. *Trends Microbiol.* **26**, 598–610 https://doi.org/10.1016/j.tim.2017.11.011
18 Di Girolamo, M. and Fabrizio, G. (2018) The ADP-ribosyl-transferases diphtheria toxin-like (ARTDs) family: an overview. *Challenges* **9**, 24 https://doi.org/10.3390/challe9010024
19 Ahel, I., Ahel, D., Matsusaka, T., Clark, A.J., Pines, J., Boulton, S.J. et al. (2008) Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. *Nature* **451**, 81–85 https://doi.org/10.1038/nature06420
20 Ahel, D., Horejsí, Z., Wiechens, N., Polo, S.E., Garcia-Wilson, E., Ahel, I. et al. (2009) Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science* **325**, 1240–1243 https://doi.org/10.1126/science.1177321
21 Kalisch, T., Amé, J.-C., Dantzer, F. and Schreiber, V. (2012) New readers and interpretations of poly(ADP-ribosyl)ation. *Trends Biochem. Sci.* **37**, 381–390 https://doi.org/10.1016/j.tibs.2012.06.001
22 Teloni, F. and Altmeyer, M. (2016) Readers of poly(ADP-ribose): designed to be fit for purpose. *Nucleic Acids Res.* **44**, 993–1006 https://doi.org/10.1093/nar/gkv1383
23 Rack, J.G., Perina, D. and Ahel, I. (2016) Macrodomains: structure, function, evolution, and catalytic activities. *Annu. Rev. Biochem.* **85**, 431–454 https://doi.org/10.1146/annurev-biochem-060815-014935
24 Slade, D., Dunstan, M.S., Barkauskaite, E., Weston, R., Lafite, P., Dixon, N. et al. (2011) The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature* **477**, 616–620 https://doi.org/10.1038/nature10404
25 Mashimo, M., Kato, J. and Moss, J. (2014) Structure and function of the ARH family of ADP-ribosyl-acceptor hydrolases. *DNA Repair* **23**, 88–94 https://doi.org/10.1016/j.dnarep.2014.03.005
26 Winstall, E., Affar, E.B., Shah, R., Bourassa, S., Scovassi, I.A. and Poirier, G.G. (1999) Preferential perinuclear localization of poly(ADP-ribose) glycohydrolase. *Exp. Cell Res.* **251**, 372–378 https://doi.org/10.1006/excr.1999.4594
27 Meyer-Ficca, M.L., Meyer, R.G., Coyle, D.L., Jacobson, E.L. and Jacobson, M.K. (2004) Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp. Cell Res.* **297**, 521–532 https://doi.org/10.1016/j.yexcr.2004.03.050
28 Niere, M., Mashimo, M., Agledal, L., Dölle, C., Kasamatsu, A., Kato, J. et al. (2012) ADP-ribosylhydrolase 3 (ARH3), not poly(ADP-ribose) glycohydrolase (PARG) isoforms, is responsible for degradation of mitochondrial matrix-associated poly(ADP-ribose). *J. Biol. Chem.* **287**, 16088–16102 https://doi.org/10.1074/jbc.M112.349183
29 Sharifi, R., Morra, R., Appel, C.D., Tallis, M., Chioza, B., Jankevicius, G. et al. (2013) Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. *EMBO J.* **32**, 1225–1237 https://doi.org/10.1038/emboj.2013.51
30 Golia, B., Moeller, G.K., Jankevicius, G., Schmidt, A., Hegele, A., Preißer, J. et al. (2017) ATM induces MacroD2 nuclear export upon DNA damage. *Nucleic Acids Res.* **45**, 244–254 https://doi.org/10.1093/nar/gkw904
31 Agnew, T., Munnur, D., Crawford, K., Palazzo, L., Mikoč, A. and Ahel, I. (2018) Macrod1 is a promiscuous ADP-ribosyl hydrolase localized to mitochondria. *Front. Microbiol.* **9**, 20 https://doi.org/10.3389/fmicb.2018.00020
32 Bütepage, M., Preisinger, C., von Kriegsheim, A., Scheufen, A., Lausberg, E., Li, J. et al. (2018) Nucleolar-nucleoplasmic shuttling of TARG1 and its control by DNA damage-induced poly-ADP-ribosylation and by nucleolar transcription. *Sci. Rep.* **8**, 6748 https://doi.org/10.1038/s41598-018-25137-w
33 Palazzo, L., Thomas, B., Jemth, A.-S., Colby, T., Leidecker, O., Feijs, K.L. et al. (2015) Processing of protein ADP-ribosylation by Nudix hydrolases. *Biochem. J.* **468**, 293–301 https://doi.org/10.1042/BJ20141554
34 Daniels, C.M., Thirawatananond, P., Ong, S.-E., Gabelli, S.B. and Leung, A.K. (2016) Nudix hydrolases degrade protein-conjugated ADP-ribose. *Sci. Rep.* **5**, 18271 PMID: 26669448
35 Chapman, J.D., Gagné, J.-P., Poirier, G.G. and Goodlett, D.R. (2013) Mapping PARP-1 auto-ADP-ribosylation sites by liquid chromatography-tandem mass spectrometry. *J. Proteome Res.* **12**, 1868–1880 https://doi.org/10.1021/pr301219h
36 Palazzo, L., Daniels, C.M., Nettleship, J.E., Rahman, N., McPherson, R.L., Ong, S.-E. et al. (2016) ENPP1 processes protein ADP-ribosylation *in vitro*. *FEBS J.* **283**, 3371–3388 https://doi.org/10.1111/febs.13811
37 Bhogaraju, S., Kalayil, S., Liu, Y., Bonn, F., Colby, T., Matic, I. et al. (2016) Phosphoribosylation of ubiquitin promotes serine ubiquitination and impairs conventional ubiquitination. *Cell* **167**, 1636–1649.e13 https://doi.org/10.1016/j.cell.2016.11.019
38 Hottiger, M.O., Hassa, P.O., Lüscher, B., Schüler, H. and Koch-Nolte, F. (2010) Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends Biochem. Sci.* **35**, 208–219 https://doi.org/10.1016/j.tibs.2009.12.003
39 Pascal, J.M. and Ellenberger, T. (2015) The rise and fall of poly(ADP-ribose): An enzymatic perspective. *DNA Repair* **32**, 10–16 https://doi.org/10.1016/j.dnarep.2015.04.008
40 Barkauskaite, E., Jankevicius, G. and Ahel, I. (2015) Structures and mechanisms of enzymes employed in the synthesis and degradation of PARP-dependent protein ADP-ribosylation. *Mol. Cell* **58**, 935–946 https://doi.org/10.1016/j.molcel.2015.05.007
41 Vyas, S., Matic, I., Uchima, L., Rood, J., Zaja, R., Hay, R.T. et al. (2014) Family-wide analysis of poly(ADP-ribose) polymerase activity. *Nat. Commun.* **5**, 4426 https://doi.org/10.1038/ncomms5426
42 Lopes, R.R., Kessler, A.C., Polycarpo, C. and Alfonzo, J.D. (2015) Cutting, dicing, healing and sealing: the molecular surgery of tRNA. *Wiley Interdiscip. Rev. RNA* **6**, 337–349 https://doi.org/10.1002/wrna.1279
43 Leidecker, O., Bonfiglio, J.J., Colby, T., Zhang, Q., Atanassov, I., Zaja, R. et al. (2016) Serine is a new target residue for endogenous ADP-ribosylation on histones. *Nat. Chem. Biol.* **12**, 998–1000 https://doi.org/10.1038/nchembio.2180

44 Crawford, K., Bonfiglio, J.J., Mikoč, A., Matic, I. and Ahel, I. (2018) Specificity of reversible ADP-ribosylation and regulation of cellular processes. *Crit. Rev. Biochem. Mol. Biol.* **53**, 64–82 https://doi.org/10.1080/10409238.2017.1394265
45 Voorneveld, J., Rack, J.G.M., Ahel, I., Overkleeft, H.S., van der Marel, G.A. and Filippov, D.V. (2018) Synthetic α- and β-Ser-ADP-ribosylated peptides reveal α-Ser-ADPr as the native epimer. *Org. Lett.* **20**, 4140–4143 https://doi.org/10.1021/acs.orglett.8b01742
46 D'Amours, D., Desnoyers, S., D'Silva, I. and Poirier, G.G. (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem. J.* **342**, 249–268 https://doi.org/10.1042/bj3420249
47 de Murcia, G. and Ménissier de Murcia, J. (1994) Poly(ADP-ribose) polymerase: a molecular nick-sensor. *Trends Biochem. Sci.* **19**, 172–176 https://doi.org/10.1016/0968-0004(94)90280-1
48 Rippmann, J.F., Damm, K. and Schnapp, A. (2002) Functional characterization of the poly(ADP-ribose) polymerase activity of tankyrase 1, a potential regulator of telomere length. *J. Mol. Biol.* **323**, 217–224 https://doi.org/10.1016/S0022-2836(02)00946-4
49 Rulten, S.L., Fisher, A.E., Robert, I., Zuma, M.C., Rouleau, M., Ju, L. et al. (2011) PARP-3 and APLF function together to accelerate nonhomologous end-joining. *Mol. Cell* **41**, 33–45 https://doi.org/10.1016/j.molcel.2010.12.006
50 Boehler, C., Gauthier, L.R., Mortusewicz, O., Biard, D.S., Saliou, J.-M., Bresson, A. et al. (2011) Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression. *Proc. Natl Acad. Sci. U.S.A.* **108**, 2783–2788 https://doi.org/10.1073/pnas.1016574108
51 Kickhoefer, V.A., Siva, A.C., Kedersha, N.L., Inman, E.M., Ruland, C., Streuli, M. et al. (1999) The 193-kD vault protein, VPARP, is a novel poly (ADP-ribose) polymerase. *J. Cell. Biol.* **146**, 917–928 https://doi.org/10.1083/jcb.146.5.917
52 Daugherty, M.D., Young, J.M., Kerns, J.A. and Malik, H.S. (2014) Rapid evolution of PARP genes suggests a broad role for ADP-ribosylation in host-virus conflicts. *PLoS Genet.* **10**, e1004403 https://doi.org/10.1371/journal.pgen.1004403
53 Tuncel, H., Tanaka, S., Oka, S., Nakai, S., Fukutomi, R., Okamoto, M. et al. (2012) PARP6, a mono(ADP-ribosyl) transferase and a negative regulator of cell proliferation, is involved in colorectal cancer development. *Int. J. Oncol.* **41**, 2079–2086 https://doi.org/10.3892/ijo.2012.1652
54 Yan, Q., Xu, R., Zhu, L., Cheng, X., Wang, Z., Manis, J. et al. (2013) BAL1 and its partner E3 ligase, BBAP, link poly(ADP-ribose) activation, ubiquitylation, and double-strand DNA repair independent of ATM, MDC1, and RNF8. *Mol. Cell. Biol.* **33**, 845–857 https://doi.org/10.1128/MCB.00990-12
55 Yang, C.-S., Jividen, K., Spencer, A., Dworak, N., Ni, L., Oostdyk, L.T. et al. (2017) Ubiquitin modification by the E3 ligase/ADP-ribosyltransferase Dtx3L/Parp9. *Mol. Cell* **66**, 503–516.e5 https://doi.org/10.1016/j.molcel.2017.04.028
56 Juszczynski, P., Kutok, J.L., Li, C., Mitra, J., Aguiar, R.C. and Shipp, M.A. (2006) BAL1 and BBAP are regulated by a gamma interferon-responsive bidirectional promoter and are overexpressed in diffuse large B-cell lymphomas with a prominent inflammatory infiltrate. *Mol. Cell. Biol.* **26**, 5348–5359 https://doi.org/10.1128/MCB.02351-05
57 Camicia, R., Bachmann, S.B., Winkler, H.C., Beer, M., Tinguely, M., Haralambieva, E. et al. (2013) BAL1/ARTD9 represses the anti-proliferative and pro-apoptotic IFNγ-STAT1-IRF1-p53 axis in diffuse large B-cell lymphoma. *J. Cell Sci.* **126**(Pt 9), 1969–1980 https://doi.org/10.1242/jcs.118174
58 Yu, M., Schreek, S., Cerni, C., Schamberger, C., Lesniewicz, K., Poreba, E. et al. (2005) PARP-10, a novel Myc-interacting protein with poly(ADP-ribose) polymerase activity, inhibits transformation. *Oncogene* **24**, 1982–1993 https://doi.org/10.1038/sj.onc.1208410
59 Verheugd, P., Forst, A.H., Milke, L., Herzog, N., Feijs, K.L., Kremmer, E. et al. (2013) Regulation of NF-κB signalling by the mono-ADP-ribosyltransferase ARTD10. *Nat. Commun.* **4**, 1683 https://doi.org/10.1038/ncomms2672
60 Herzog, N., Hartkamp, J.D., Verheugd, P., Treude, F., Forst, A.H., Feijs, K.L. et al. (2013) Caspase-dependent cleavage of the mono-ADP-ribosyltransferase ARTD10 interferes with its pro-apoptotic function. *FEBS J.* **280**, 1330–1343 https://doi.org/10.1111/febs.12124
61 Meyer-Ficca, M.L., Ihara, M., Bader, J.J., Leu, N.A., Beneke, S. and Meyer, R.G. (2015) Spermatid head elongation with normal nuclear shaping requires ADP-ribosyltransferase PARP11 (ARTD11) in mice. *Biol. Reprod.* **92**, 80 PMID: 25673562
62 Carter-O'Connell, I., Jin, H., Morgan, R.K., Zaja, R., David, L.L., Ahel, I. et al. (2016) Identifying family-member-specific targets of mono-ARTDs by using a chemical genetics approach. *Cell Rep.* **14**, 621–631 https://doi.org/10.1016/j.celrep.2015.12.045
63 Li, L., Zhao, H., Liu, P., Li, C., Quanquin, N., Ji, X. et al. (2018) *PARP12* suppresses Zika virus infection through PARP-dependent degradation of NS1 and NS3 viral proteins. *Sci. Signal.* **11**, pii: eaas9332 https://doi.org/10.1126/scisignal.aas9332
64 Seo, G.J., Kincaid, R.P., Phanaksri, T., Burke, J.M., Pare, J.M., Cox, J.E. et al. (2013) Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. *Cell Host Microbe* **14**, 435–445 https://doi.org/10.1016/j.chom.2013.09.002
65 Todorova, T., Bock, F.J. and Chang, P. (2015) Poly(ADP-ribose) polymerase-13 and RNA regulation in immunity and cancer. *Trends Mol. Med.* **21**, 373–384 https://doi.org/10.1016/j.molmed.2015.03.002
66 Cho, S.H., Goenka, S., Henttinen, T., Gudapati, P., Reinikainen, A., Eischen, C.M. et al. (2009) PARP-14, a member of the B aggressive lymphoma family, transduces survival signals in primary B cells. *Blood* **113**, 2416–2425 https://doi.org/10.1182/blood-2008-03-144121
67 Vyas, S., Chesarone-Cataldo, M., Todorova, T., Huang, Y.-H. and Chang, P. (2013) A systematic analysis of the PARP protein family identifies new functions critical for cell physiology. *Nat. Commun.* **4**, 2240 https://doi.org/10.1038/ncomms3240
68 Caprara, G., Prosperini, E., Piccolo, V., Sigismondo, G., Melacarne, A., Cuomo, A. et al. (2018) PARP14 controls the nuclear accumulation of a subset of type I IFN-inducible proteins. *J. Immunol.* **200**, 2439–2454 https://doi.org/10.4049/jimmunol.1701117
69 Schuller, M., Riedel, K., Gibbs-Seymour, I., Uth, K., Sieg, C., Gehring, A.P. et al. (2017) Discovery of a selective allosteric inhibitor targeting macrodomain 2 of polyadenosine-diphosphate-ribose polymerase 14. *ACS Chem. Biol.* **12**, 2866–2874 https://doi.org/10.1021/acschembio.7b00445
70 Di Paola, S., Micaroni, M., Di Tullio, G., Buccione, R. and Di Girolamo, M. (2012) PARP16/ARTD15 is a novel endoplasmic-reticulum-associated mono-ADP-ribosyltransferase that interacts with, and modifies karyopherin-β1. *PLoS ONE* **7**, e37352 https://doi.org/10.1371/journal.pone.0037352
71 Beck, C., Robert, I., Reina-San-Martin, B., Schreiber, V. and Dantzer, F. (2014) Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. *Exp. Cell Res.* **329**, 18–25 https://doi.org/10.1016/j.yexcr.2014.07.003
72 Tao, Z., Gao, P., Hoffman, D.W. and Liu, H.-W. (2008) Domain C of human poly(ADP-ribose) polymerase-1 is important for enzyme activity and contains a novel zinc-ribbon motif. *Biochemistry* **47**, 5804–5813 https://doi.org/10.1021/bi800018a
73 Langelier, M.-F., Servent, K.M., Rogers, E.E. and Pascal, J.M. (2008) A third zinc-binding domain of human poly(ADP-ribose) polymerase-1 coordinates DNA-dependent enzyme activation. *J. Biol. Chem.* **283**, 4105–4114 https://doi.org/10.1074/jbc.M708558200
74 Langelier, M.-F. and Pascal, J.M. (2013) PARP-1 mechanism for coupling DNA damage detection to poly(ADP-ribose) synthesis. *Curr. Opin. Struct. Biol.* **23**, 134–143 https://doi.org/10.1016/j.sbi.2013.01.003

75 Ruf, A., Mennissier de Murcia, J., de Murcia, G. and Schulz, G.E. (1996) Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken. *Proc. Natl Acad. Sci. U.S.A.* **93**, 7481–7485 https://doi.org/10.1073/pnas.93.15.7481
76 Altmeyer, M., Messner, S., Hassa, P.O., Fey, M. and Hottiger, M.O. (2009) Molecular mechanism of poly(ADP-ribosyl)ation by PARP1 and identification of lysine residues as ADP-ribose acceptor sites. *Nucleic Acids Res.* **37**, 3723–3738 https://doi.org/10.1093/nar/gkp229
77 Langelier, M.-F., Planck, J.L., Roy, S. and Pascal, J.M. (2012) Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. *Science* **336**, 728–732 https://doi.org/10.1126/science.1216338
78 Dawicki-McKenna, J.M., Langelier, M.-F., DeNizio, J.E., Riccio, A.A., Cao, C.D., Karch, K.R. et al. (2015) PARP-1 activation requires local unfolding of an autoinhibitory domain. *Mol. Cell* **60**, 755–768 https://doi.org/10.1016/j.molcel.2015.10.013
79 Langelier, M.-F., Zandarashvili, L., Aguiar, P.M., Black, B.E. and Pascal, J.M. (2018) NAD^+ analog reveals PARP-1 substrate-blocking mechanism and allosteric communication from catalytic center to DNA-binding domains. *Nat. Commun.* **9**, 844 https://doi.org/10.1038/s41467-018-03234-8
80 Liu, C., Vyas, A., Kassab, M.A., Singh, A.K. and Yu, X. (2017) The role of poly ADP-ribosylation in the first wave of DNA damage response. *Nucleic Acids Res.* **45**, 8129–8141 https://doi.org/10.1093/nar/gkx565
81 Eustermann, S., Wu, W.-F., Langelier, M.-F., Yang, J.-C., Easton, L.E., Riccio, A.A. et al. (2015) Structural basis of detection and signaling of DNA single-strand breaks by human PARP-1. *Mol. Cell* **60**, 742–754 https://doi.org/10.1016/j.molcel.2015.10.032
82 Chen, Q., Kassab, M.A., Dantzer, F. and Yu, X. (2018) PARP2 mediates branched poly ADP-ribosylation in response to DNA damage. *Nat. Commun.* **9**, 3233 https://doi.org/10.1038/s41467-018-05588-5
83 Talhaoui, I., Lebedeva, N.A., Zarkovic, G., Saint-Pierre, C., Kutuzov, M.M., Sukhanova, M.V. et al. (2016) Poly(ADP-ribose) polymerases covalently modify strand break termini in DNA fragments in vitro. *Nucleic Acids Res.* **44**, 9279–9295
84 Munnur, D. and Ahel, I. (2017) Reversible mono-ADP-ribosylation of DNA breaks. *FEBS J.* **284**, 4002–4016 https://doi.org/10.1111/febs.14297
85 Zarkovic, G., Belousova, E.A., Talhaoui, I., Saint-Pierre, C., Kutuzov, M.M., Matkarimov, B.T. et al. (2018) Characterization of DNA ADP-ribosyltransferase activities of PARP2 and PARP3: new insights into DNA ADP-ribosylation. *Nucleic Acids Res.* **46**, 2417–2431 https://doi.org/10.1093/nar/gkx1318
86 Schreiber, V., Dantzer, F., Ame, J.-C. and de Murcia, G. (2006) Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell. Biol.* **7**, 517–528 https://doi.org/10.1038/nrm1963
87 Ménissier de Murcia, J., Ricoul, M., Tartier, L., Niedergang, C., Huber, A., Dantzer, F. et al. (2003) Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J.* **22**, 2255–2263 https://doi.org/10.1093/emboj/cdg206
88 Poirier, G.G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C. and Mandel, P. (1982) Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. *Proc. Natl Acad. Sci. U.S.A.* **79**, 3423–3427 https://doi.org/10.1073/pnas.79.11.3423
89 Bonfiglio, J.J., Fontana, P., Zhang, Q., Colby, T., Gibbs-Seymour, I., Atanassov, I. et al. (2017) Serine ADP-ribosylation depends on HPF1. *Mol. Cell* **65**, 932–940.e6 https://doi.org/10.1016/j.molcel.2017.01.003
90 Palazzo, L., Leidecker, O., Prokhorova, E., Dauben, H., Matic, I. and Ahel, I. (2018) Serine is the major residue for ADP-ribosylation upon DNA damage. *eLife* **7**, pii: e34334 https://doi.org/10.7554/eLife.34334
91 Satoh, M.S. and Lindahl, T. (1992) Role of poly(ADP-ribose) formation in DNA repair. *Nature* **356**, 356–358 https://doi.org/10.1038/356356a0
92 Krishnakumar, R. and Kraus, W.L. (2010) The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol. Cell* **39**, 8–24 https://doi.org/10.1016/j.molcel.2010.06.017
93 Gibbs-Seymour, I., Fontana, P., Rack, J.G.M. and Ahel, I. (2016) HPF1/C4orf27 is a PARP-1-interacting protein that regulates PARP-1 ADP-ribosylation activity. *Mol. Cell* **62**, 432–442 https://doi.org/10.1016/j.molcel.2016.03.008
94 Zhang, Y., Wang, J., Ding, M. and Yu, Y. (2013) Site-specific characterization of the Asp- and Glu-ADP-ribosylated proteome. *Nat. Methods* **10**, 981–984 https://doi.org/10.1038/nmeth.2603
95 Zhen, Y., Zhang, Y. and Yu, Y. (2017) A cell-line-specific atlas of PARP-mediated protein Asp/Glu-ADP-ribosylation in breast cancer. *Cell Rep.* **21**, 2326–2337 https://doi.org/10.1016/j.celrep.2017.10.106
96 Karch, K.R., Langelier, M.-F., Pascal, J.M. and Garcia, B.A. (2017) The nucleosomal surface is the main target of histone ADP-ribosylation in response to DNA damage. *Mol. Biosyst.* **13**, 2660–2671 https://doi.org/10.1039/C7MB00498B
97 Gibson, B.A., Conrad, L.B., Huang, D. and Kraus, W.L. (2017) Generation and characterization of recombinant antibody-like ADP-ribose binding proteins. *Biochemistry* **56**, 6305–6316 https://doi.org/10.1021/acs.biochem.7b00670
98 Daniels, C.M., Ong, S.-E. and Leung, A.K. (2014) Phosphoproteomic approach to characterize protein mono- and poly(ADP-ribosyl)ation sites from cells. *J. Proteome Res.* **13**, 3510–3522 https://doi.org/10.1021/pr401032q
99 Daniels, C.M., Ong, S.-E. and Leung, A.K. (2015) The promise of proteomics for the study of ADP-ribosylation. *Mol. Cell* **58**, 911–924 https://doi.org/10.1016/j.molcel.2015.06.012
100 Martello, R., Leutert, M., Jungmichel, S., Bilan, V., Larsen, S.C., Young, C. et al. (2016) Proteome-wide identification of the endogenous ADP-ribosylome of mammalian cells and tissue. *Nat. Commun.* **7**, 12917 https://doi.org/10.1038/ncomms12917
101 Gagné, J.-P., Langelier, M.-F., Pascal, J.M. and Poirier, G.G. (2018) Hydrofluoric acid-based derivatization strategy to profile PARP-1 ADP-ribosylation by LC-MS/MS. *J. Proteome Res.* **17**, 2542–2551 https://doi.org/10.1021/acs.jproteome.8b00146
102 Ferro, A.M. and Olivera, B.M. (1982) Poly(ADP-ribosylation) in vitro: reaction parameters and enzyme mechanism. *J. Biol. Chem.* **257**, 7808–7813 PMID: 6282854
103 Zahradka, P. and Ebisuzaki, K. (1982) A shuttle mechanism for DNA-protein interactions: the regulation of poly(ADP-ribose) polymerase. *Eur. J. Biochem.* **127**, 579–585 https://doi.org/10.1111/j.1432-1033.1982.tb06912.x
104 Tallis, M., Morra, R., Barkauskaite, E. and Ahel, I. (2014) Poly(ADP-ribosyl)ation in regulation of chromatin structure and the DNA damage response. *Chromosoma* **123**, 79–90 https://doi.org/10.1007/s00412-013-0442-9
105 Caldecott, K.W. (2014) DNA single-strand break repair. *Exp. Cell Res.* **329**, 2–8 https://doi.org/10.1016/j.yexcr.2014.08.027
106 Timinszky, G., Till, S., Hassa, P.O., Hothorn, M., Kustatscher, G., Nijmeijer, B. et al. (2009) A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. *Nat. Struct. Mol. Biol.* **16**, 923–929 https://doi.org/10.1038/nsmb.1664
107 Li, M. and Yu, X. (2015) The role of poly(ADP-ribosyl)ation in DNA damage response and cancer chemotherapy. *Oncogene* **34**, 3349–3356 https://doi.org/10.1038/onc.2014.295

108 Brochu, G., Duchaine, C., Thibeault, L., Lagueux, J., Shah, G.M. and Poirier, G.G. (1994) Mode of action of poly(ADP-ribose) glycohydrolase. *Biochim. Biophys. Acta* **1219**, 342–350 https://doi.org/10.1016/0167-4781(94)90058-2
109 Lambrecht, M.J., Brichacek, M., Barkauskaite, E., Ariza, A., Ahel, I. and Hergenrother, P.J. (2015) Synthesis of dimeric ADP-ribose and its structure with human poly(ADP-ribose) glycohydrolase. *J. Am. Chem. Soc.* **137**, 3558–3564 https://doi.org/10.1021/ja512528p
110 Fontana, P., Bonfiglio, J.J., Palazzo, L., Bartlett, E., Matic, I. and Ahel, I. (2017) Serine ADP-ribosylation reversal by the hydrolase ARH3. *eLife* **6**, pii: e28533 https://doi.org/10.7554/eLife.28533
111 Barkauskaite, E., Brassington, A., Tan, E.S., Warwicker, J., Dunstan, M.S., Banos, B. et al. (2013) Visualization of poly(ADP-ribose) bound to PARG reveals inherent balance between exo- and endo-glycohydrolase activities. *Nat. Commun.* **4**, 2164 https://doi.org/10.1038/ncomms3164
112 Shall, S. (1975) Seminar on poly(ADP-ribose) and ADP-ribosylation of proceedings: experimental manipulation of the specific activity of poly(ADP-ribose) polymerase. *J. Biochem.* **77**, 2 https://doi.org/10.1093/oxfordjournals.jbchem.a130859
113 Purnell, M.R. and Whish, W.J. (1980) Novel inhibitors of poly(ADP-ribose) synthetase. *Biochem. J.* **185**, 775–777 https://doi.org/10.1042/bj1850775
114 Terada, M., Fujiki, H., Marks, P.A. and Sugimura, T. (1979) Induction of erythroid differentiation of murine erythroleukemia cells by nicotinamide and related compounds. *Proc. Natl Acad. Sci. U.S.A.* **76**, 6411–6414 https://doi.org/10.1073/pnas.76.12.6411
115 Helleday, T. (2011) The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings. *Mol. Oncol.* **5**, 387–393 https://doi.org/10.1016/j.molonc.2011.07.001
116 Murai, J., Huang, S.-Y., Das, B.B., Renaud, A., Zhang, Y., Doroshow, J.H. et al. (2012) Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Res.* **72**, 5588–5599 https://doi.org/10.1158/0008-5472.CAN-12-2753
117 Ceccaldi, R., Rondinelli, B. and D'Andrea, A.D. (2016) Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol.* **26**, 52–64 https://doi.org/10.1016/j.tcb.2015.07.009
118 Mateos-Gomez, P.A., Gong, F., Nair, N., Miller, K.M., Lazzerini-Denchi, E. and Sfeir, A. (2015) Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. *Nature* **518**, 254–257 https://doi.org/10.1038/nature14157
119 Ashworth, A., Lord, C.J. and Reis-Filho, J.S. (2011) Genetic interactions in cancer progression and treatment. *Cell* **145**, 30–38 https://doi.org/10.1016/j.cell.2011.03.020
120 Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B. et al. (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 https://doi.org/10.1038/nature03445
121 Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E. et al. (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434**, 913–917 https://doi.org/10.1038/nature03443
122 Bryant, H.E., Petermann, E., Schultz, N., Jemth, A.-S., Loseva, O., Issaeva, N. et al. (2009) PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. *EMBO J.* **28**, 2601–2615 https://doi.org/10.1038/emboj.2009.206
123 Zaremba, T. and Curtin, N.J. (2007) PARP inhibitor development for systemic cancer targeting. *Anticancer Agents Med. Chem.* **7**, 515–523 https://doi.org/10.2174/187152007781668715
124 Lord, C.J. and Ashworth, A. (2017) PARP inhibitors: synthetic lethality in the clinic. *Science* **355**, 1152–1158 https://doi.org/10.1126/science.aam7344
125 Shen, Y., Rehman, F.L., Feng, Y., Boshuizen, J., Bajrami, I., Elliott, R. et al. (2013) BMN 673, a novel and highly potent PARP1/2 inhibitor for the treatment of human cancers with DNA repair deficiency. *Clin. Cancer Res.* **19**, 5003–5015 https://doi.org/10.1158/1078-0432.CCR-13-1391
126 Tang, Z., Jiang, B., Shi, Z., Gong, W., Liu, Y., Wang, X. et al. (2015) BGB-290, a novel PARP inhibitor with unique brain penetration ability, demonstrated strong synergism with temozolomide in subcutaneous and intracranial xenograft models. *Cancer Res.* **75**, Abstract no. 1651 https://doi.org/10.1158/1538-7445.AM2015-1651
127 Tang, Z., Liu, Y., Zhen, Q., Ren, B., Wang, H., Shi, Z. et al. (2015) BGB-290: A highly potent and specific PARP1/2 inhibitor potentiates anti-tumor activity of chemotherapeutics in patient biopsy derived SCLC models. *Cancer Res.* **75**, Abstract no. 1653 https://doi.org/10.1158/1538-7445.AM2015-1653
128 King, M.-C. (2014) 'The race' to clone BRCA1. *Science* **343**, 1462–1465 https://doi.org/10.1126/science.1251900
129 Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66–71 https://doi.org/10.1126/science.7545954
130 Wooster, R., Bignell, G., Lancaster, J., Swift, S., Seal, S., Mangion, J. et al. (1995) Identification of the breast cancer susceptibility gene BRCA2. *Nature* **378**, 789–792 https://doi.org/10.1038/378789a0
131 Kaufman, B., Shapira-Frommer, R., Schmutzler, R.K., Audeh, M.W., Friedlander, M., Balmaña, J. et al. (2015) Olaparib monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation. *J. Clin. Oncol.* **33**, 244–250 https://doi.org/10.1200/JCO.2014.56.2728
132 Kim, G., Ison, G., McKee, A.E., Zhang, H., Tang, S., Gwise, T. et al. (2015) FDA approval summary: olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. *Clin. Cancer Res.* **21**, 4257–4261 https://doi.org/10.1158/1078-0432.CCR-15-0887
133 Turner, N., Tutt, A. and Ashworth, A. (2004) Hallmarks of 'BRCAness' in sporadic cancers. *Nat. Rev. Cancer* **4**, 814–819 https://doi.org/10.1038/nrc1457
134 Lord, C.J. and Ashworth, A. (2016) BRCAness revisited. *Nat. Rev. Cancer* **16**, 110–120 https://doi.org/10.1038/nrc.2015.21
135 McCabe, N., Turner, N.C., Lord, C.J., Kluzek, K., Białkowska, A., Swift, S. et al. (2006) Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res.* **66**, 8109–8115 https://doi.org/10.1158/0008-5472.CAN-06-0140
136 Buisson, R., Dion-Côté, A.-M., Coulombe, Y., Launay, H., Cai, H., Stasiak, A.Z. et al. (2010) Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. *Nat. Struct. Mol. Biol.* **17**, 1247–1254 https://doi.org/10.1038/nsmb.1915
137 Williamson, C.T., Muzik, H., Turhan, A.G., Zamò, A., O'Connor, M.J., Bebb, D.G. et al. (2010) ATM deficiency sensitizes mantle cell lymphoma cells to poly(ADP-ribose) polymerase-1 inhibitors. *Mol. Cancer Ther.* **9**, 347–357 https://doi.org/10.1158/1535-7163.MCT-09-0872
138 Weston, V.J., Oldreive, C.E., Skowronska, A., Oscier, D.G., Pratt, G., Dyer, M.J. et al. (2010) The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells in vitro and in vivo. *Blood* **116**, 4578–4587 https://doi.org/10.1182/blood-2010-01-265769
139 Bell, D., Berchuck, A., Birrer, M., Chien, J., Cramer, D., Dao, F., et al. (2011) Integrated genomic analyses of ovarian carcinoma. *Nature* **474**, 609–615 https://doi.org/10.1038/nature10166

Biochemical Society Transactions (2018) **46** 1681–1695
https://doi.org/10.1042/BST20180418

140 Mateo, J., Carreira, S., Sandhu, S., Miranda, S., Mossop, H., Perez-Lopez, R., et al. (2015) DNA-repair defects and olaparib in metastatic prostate cancer. *N. Engl. J. Med.* **373**, 1697–1708 https://doi.org/10.1056/NEJMoa1506859

141 Waddell, N., Pajic, M., Patch, A.-M., Chang, D.K., Kassahn, K.S., Bailey, P., et al. (2015) Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* **518**, 495–501 https://doi.org/10.1038/nature14169

142 Carnevale, J. and Ashworth, A. (2015) Assessing the significance of *BRCA1* and *BRCA2* mutations in pancreatic cancer. *J. Clin. Oncol.* **33**, 3080–3081 https://doi.org/10.1200/JCO.2015.61.6961

143 Zhao, L. and So, C.W. (2016) PARP-inhibitor-induced synthetic lethality for acute myeloid leukemia treatment. *Exp. Hematol.* **44**, 902–907 https://doi.org/10.1016/j.exphem.2016.07.007

144 Michelena, J., Lezaja, A., Teloni, F., Schmid, T., Imhof, R. and Altmeyer, M. (2018) Analysis of PARP inhibitor toxicity by multidimensional fluorescence microscopy reveals mechanisms of sensitivity and resistance. *Nat. Commun.* **9**, 2678 https://doi.org/10.1038/s41467-018-05031-9

145 Liszczak, G., Diehl, K.L., Dann, G.P. and Muir, T.W. (2018) Acetylation blocks DNA damage-induced chromatin ADP-ribosylation. *Nat. Chem. Biol.* **14**, 837–840 https://doi.org/10.1038/s41589-018-0097-1

146 Fong, P.C., Boss, D.S., Yap, T.A., Tutt, A., Wu, P., Mergui-Roelvink, M. et al. (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. *N. Engl. J. Med.* **361**, 123–134 https://doi.org/10.1056/NEJMoa0900212

147 Murai, J., Huang, S.-Y.N., Renaud, A., Zhang, Y., Ji, J., Takeda, S. et al. (2014) Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. *Mol. Cancer Ther.* **13**, 433–443 https://doi.org/10.1158/1535-7163.MCT-13-0803

148 Pommier, Y., O'Connor, M.J. and de Bono, J. (2016) Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. *Sci. Transl. Med.* **8**, 362ps17 https://doi.org/10.1126/scitranslmed.aaf9246

149 Bunting, S.F., Callén, E., Wong, N., Chen, H.-T., Polato, F., Gunn, A. et al. (2010) 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* **141**, 243–254 https://doi.org/10.1016/j.cell.2010.03.012

150 Jaspers, J.E., Kersbergen, A., Boon, U., Sol, W., van Deemter, L., Zander, S.A. et al. (2013) Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. *Cancer Discov.* **3**, 68–81 https://doi.org/10.1158/2159-8290.CD-12-0049

151 Xu, G., Chapman, J.R., Brandsma, I., Yuan, J., Mistrik, M., Bouwman, P. et al. (2015) REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* **521**, 541–544 https://doi.org/10.1038/nature14328

152 Gogola, E., Duarte, A.A., de Ruiter, J.R., Wiegant, W.W., Schmid, J.A., de Bruijn, R. et al. (2018) Selective loss of PARG restores PARylation and counteracts PARP inhibitor-Mediated synthetic lethality. *Cancer Cell* **33**, 1078–1093.e12 https://doi.org/10.1016/j.ccell.2018.05.008

153 Chaudhuri, A.R., Callen, E., Ding, X., Gogola, E., Duarte, A.A., Lee, J.-E. et al. (2016) Replication fork stability confers chemoresistance in BRCA-deficient cells. *Nature* **535**, 382–387 https://doi.org/10.1038/nature18325

154 Rondinelli, B., Gogola, E., Yücel, H., Duarte, A.A., van de Ven, M., van der Sluijs, R. et al. (2017) EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. *Nat. Cell Biol.* **19**, 1371–1378 https://doi.org/10.1038/ncb3626

155 Pettitt, S.J., Rehman, F.L., Bajrami, I., Brough, R., Wallberg, F., Kozarewa, I. et al. (2013) A genetic screen using the PiggyBac transposon in haploid cells identifies Parp1 as a mediator of olaparib toxicity. *PLoS ONE* **8**, e61520 https://doi.org/10.1371/journal.pone.0061520

156 Edwards, S.L., Brough, R., Lord, C.J., Natrajan, R., Vatcheva, R., Levine, D.A. et al. (2008) Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* **451**, 1111–1115 https://doi.org/10.1038/nature06548

157 Barber, L.J., Sandhu, S., Chen, L., Campbell, J., Kozarewa, I., Fenwick, K. et al. (2013) Secondary mutations in *BRCA2* associated with clinical resistance to a PARP inhibitor. *J. Pathol.* **229**, 422–429 https://doi.org/10.1002/path.4140

158 Sakai, W., Swisher, E.M., Karlan, B.Y., Agarwal, M.K., Higgins, J., Friedman, C. et al. (2008) Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* **451**, 1116–1120 https://doi.org/10.1038/nature06633

159 Norquist, B., Wurz, K.A., Pennil, C.C., Garcia, R., Gross, J., Sakai, W. et al. (2011) Secondary somatic mutations restoring *BRCA1/2* predict chemotherapy resistance in hereditary ovarian carcinomas. *J. Clin. Oncol.* **29**, 3008–3015 https://doi.org/10.1200/JCO.2010.34.2980

160 Lord, C.J. and Ashworth, A. (2013) Mechanisms of resistance to therapies targeting BRCA-mutant cancers. *Nat. Med.* **19**, 1381–1388 https://doi.org/10.1038/nm.3369

161 Ceccaldi, R., Liu, J.C., Amunugama, R., Hajdu, I., Primack, B. and Petalcorin, M.I. (2015) O'connor KW, Konstantinopoulos PA, Elledge SJ, Boulton SJ, Yusufzai T, D'Andrea AD. Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. *Nature* **518**, 258–262 https://doi.org/10.1038/nature14184

162 Murfuni, I., Basile, G., Subramanyam, S., Malacaria, E., Bignami, M., Spies, M. et al. (2013) Survival of the replication checkpoint deficient cells requires MUS81-RAD52 function. *PLoS Genet.* **9**, e1003910 https://doi.org/10.1371/journal.pgen.1003910

163 Hengel, S.R., Spies, M.A. and Spies, M. (2017) Small-molecule inhibitors targeting DNA repair and DNA repair deficiency in research and cancer therapy. *Cell Chem. Biol.* **24**, 1101–1119 https://doi.org/10.1016/j.chembiol.2017.08.027

164 James, D.I., Smith, K.M., Jordan, A.M., Fairweather, E.E., Griffiths, L.A., Hamilton, N.S. et al. (2016) First-in-class chemical probes against poly (ADP-ribose) glycohydrolase (PARG) inhibit DNA repair with differential pharmacology to olaparib. *ACS Chem. Biol.* **11**, 3179–3190 https://doi.org/10.1021/acschembio.6b00609

165 Gravells, P., Neale, J., Grant, E., Nathubhai, A., Smith, K.M., James, D.I. et al. (2018) Radiosensitization with an inhibitor of poly(ADP-ribose) glycohydrolase: a comparison with the PARP1/2/3 inhibitor olaparib. *DNA Repair* **61**, 25–36 https://doi.org/10.1016/j.dnarep.2017.11.004

166 Smith, S., Giriat, I., Schmitt, A. and de Lange, T. (1998) Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science* **282**, 1484–1487 https://doi.org/10.1126/science.282.5393.1484

167 Nagy, Z., Kalousi, A., Furst, A., Koch, M., Fischer, B. and Soutoglou, E. (2016) Tankyrases promote homologous recombination and check point activation in response to DSBs. *PLoS Genet.* **12**, e1005791 https://doi.org/10.1371/journal.pgen.1005791

168 Smith, S. and de Lange, T. (1999) Cell cycle dependent localization of the telomeric PARP, tankyrase, to nuclear pore complexes and centrosomes. *J. Cell Sci.* **112**, 3649–3656

169 Dynek, J.N. and Smith, S. (2004) Resolution of sister telomere association is required for progression through mitosis. *Science* **304**, 97–100 https://doi.org/10.1126/science.1094754

170 Chang, P., Coughlin, M. and Mitchison, T.J. (2005) Tankyrase-1 polymerization of poly(ADP-ribose) is required for spindle structure and function. *Nat. Cell Biol.* **7**, 1133–1139 https://doi.org/10.1038/ncb1322

171 Huang, S.-M.A., Mishina, Y.M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G.A. et al. (2009) Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* **461**, 614–620 https://doi.org/10.1038/nature08356
172 Bhardwaj, A., Yang, Y., Ueberheide, B. and Smith, S. (2017) Whole proteome analysis of human tankyrase knockout cells reveals targets of tankyrase-mediated degradation. *Nat. Commun.* **8**, 2214 https://doi.org/10.1038/s41467-017-02363-w
173 Riffell, J.L., Lord, C.J. and Ashworth, A. (2012) Tankyrase-targeted therapeutics: expanding opportunities in the PARP family. *Nat. Rev. Drug Discov.* **11**, 923–936 https://doi.org/10.1038/nrd3868
174 Hsiao, S.J. and Smith, S. (2008) Tankyrase function at telomeres, spindle poles, and beyond. *Biochimie* **90**, 83–92 https://doi.org/10.1016/j.biochi.2007.07.012
175 Mariotti, L., Templeton, C.M., Ranes, M., Paracuellos, P., Cronin, N., Beuron, F. et al. (2016) Tankyrase requires SAM domain-dependent polymerization to support Wnt-β-catenin signaling. *Mol. Cell* **63**, 498–513 https://doi.org/10.1016/j.molcel.2016.06.019
176 Riccio, A.A., McCauley, M., Langelier, M.-F. and Pascal, J.M. (2016) Tankyrase sterile α motif domain polymerization Is required for Its role in Wnt signaling. *Structure* **24**, 1573–1581 https://doi.org/10.1016/j.str.2016.06.022
177 Eisemann, T., McCauley, M., Langelier, M.-F., Gupta, K., Roy, S., Van Duyne, G.D. et al. (2016) Tankyrase-1 ankyrin repeats form an adaptable binding platform for targets of ADP-ribose modification. *Structure* **24**, 1679–1692 https://doi.org/10.1016/j.str.2016.07.014
178 Sbodio, J.I. and Chi, N.-W. (2002) Identification of a tankyrase-binding motif shared by IRAP, TAB182, and human TRF1 but not mouse TRF1. NuMA contains this RXXPDG motif and is a novel tankyrase partner. *J. Biol. Chem.* **277**, 31887–31892 https://doi.org/10.1074/jbc.M203916200
179 Guettler, S., LaRose, J., Petsalaki, E., Gish, G., Scotter, A., Pawson, T. et al. (2011) Structural basis and sequence rules for substrate recognition by Tankyrase explain the basis for cherubism disease. *Cell* **147**, 1340–1354 https://doi.org/10.1016/j.cell.2011.10.046
180 Levaot, N., Voytyuk, O., Dimitriou, I., Sircoulomb, F., Chandrakumar, A., Deckert, M. et al. (2011) Loss of Tankyrase-mediated destruction of 3BP2 is the underlying pathogenic mechanism of cherubism. *Cell* **147**, 1324–1339 https://doi.org/10.1016/j.cell.2011.10.045
181 Krastev, D.B., Pettitt, S.J., Campbell, J., Song, F., Tanos, B.E., Stoynov, S.S. et al. (2018) Coupling bimolecular PARylation biosensors with genetic screens to identify PARylation targets. *Nat. Commun.* **9**, 2016 https://doi.org/10.1038/s41467-018-04466-4
182 Wu, H. (2013) Higher-order assemblies in a new paradigm of signal transduction. *Cell* **153**, 287–292 https://doi.org/10.1016/j.cell.2013.03.013
183 Bienz, M. (2014) Signalosome assembly by domains undergoing dynamic head-to-tail polymerization. *Trends Biochem. Sci.* **39**, 487–495 https://doi.org/10.1016/j.tibs.2014.08.006
184 Lehtiö, L., Chi, N.-W. and Krauss, S. (2013) Tankyrases as drug targets. *FEBS J.* **280**, 3576–3593 https://doi.org/10.1111/febs.12320
185 MacDonald, B.T., Tamai, K. and He, X. (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell* **17**, 9–26 https://doi.org/10.1016/j.devcel.2009.06.016
186 Clevers, H. and Nusse, R. (2012) Wnt/β-catenin signaling and disease. *Cell* **149**, 1192–1205 https://doi.org/10.1016/j.cell.2012.05.012
187 Zhan, T., Rindtorff, N. and Boutros, M. (2017) Wnt signaling in cancer. *Oncogene* **36**, 1461–1473 https://doi.org/10.1038/onc.2016.304
188 Nusse, R. and Clevers, H. (2017) Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. *Cell* **169**, 985–999 https://doi.org/10.1016/j.cell.2017.05.016
189 Thorsell, A.-G., Ekblad, T., Karlberg, T., Löw, M., Pinto, A.F., Trésaugues, L. et al. (2017) Structural basis for potency and promiscuity in poly (ADP-ribose) polymerase (PARP) and tankyrase inhibitors. *J. Med. Chem.* **60**, 1262–1271 https://doi.org/10.1021/acs.jmedchem.6b00990
190 Chen, B., Dodge, M.E., Tang, W., Lu, J., Ma, Z., Fan, C.-W. et al. (2009) Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. *Nat. Chem. Biol.* **5**, 100–107 https://doi.org/10.1038/nchembio.137
191 Gunaydin, H., Gu, Y. and Huang, X. (2012) Novel binding mode of a potent and selective tankyrase inhibitor. *PLoS ONE* **7**, e33740 https://doi.org/10.1371/journal.pone.0033740
192 Steffen, J.D., Brody, J.R., Armen, R.S. and Pascal, J.M. (2013) Structural implications for selective targeting of PARPs. *Front. Oncol.* **3**, 301 https://doi.org/10.3389/fonc.2013.00301
193 Waaler, J., Machon, O., von Kries, J.P., Wilson, S.R., Lundenes, E., Wedlich, D. et al. (2011) Novel synthetic antagonists of canonical Wnt signaling inhibit colorectal cancer cell growth. *Cancer Res.* **71**, 197–205 https://doi.org/10.1158/0008-5472.CAN-10-1282
194 Waaler, J., Machon, O., Tumova, L., Dinh, H., Korinek, V., Wilson, S.R. et al. (2012) A novel tankyrase inhibitor decreases canonical Wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice. *Cancer Res.* **72**, 2822–2832 https://doi.org/10.1158/0008-5472.CAN-11-3336
195 James, R.G., Davidson, K.C., Bosch, K.A., Biechele, T.L., Robin, N.C., Taylor, R.J. et al. (2012) WIKI4, a novel inhibitor of tankyrase and Wnt/β-catenin signaling. *PLoS ONE* **7**, e50457 https://doi.org/10.1371/journal.pone.0050457
196 Haikarainen, T., Venkannagari, H., Narwal, M., Obaji, E., Lee, H.-W., Nkizinkiko, Y. et al. (2013) Structural basis and selectivity of tankyrase inhibition by a Wnt signaling inhibitor WIKI4. *PLoS ONE* **8**, e65404 https://doi.org/10.1371/journal.pone.0065404
197 Okada-Iwasaki, R., Takahashi, Y., Watanabe, Y., Ishida, H., Saito, J., Nakai, R. et al. (2016) The discovery and characterization of K-756, a novel Wnt/β-Catenin pathway inhibitor targeting tankyrase. *Mol. Cancer Ther.* **15**, 1525–1534 https://doi.org/10.1158/1535-7163.MCT-15-0938
198 Lau, T., Chan, E., Callow, M., Waaler, J., Boggs, J., Blake, R.A. et al. (2013) A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth. *Cancer Res.* **73**, 3132–3144 https://doi.org/10.1158/0008-5472.CAN-12-4562
199 Voronkov, A., Holsworth, D.D., Waaler, J., Wilson, S.R., Ekblad, B., Perdreau-Dahl, H. et al. (2013) Structural basis and SAR for G007-LK, a lead stage 1,2,4-triazole based specific tankyrase 1/2 inhibitor. *J. Med. Chem.* **56**, 3012–3023 https://doi.org/10.1021/jm4000566
200 Shultz, M.D., Cheung, A.K., Kirby, C.A., Firestone, B., Fan, J., Chen, C.H. et al. (2013) Identification of NVP-TNKS656: the use of structure-efficiency relationships to generate a highly potent, selective, and orally active tankyrase inhibitor. *J. Med. Chem.* **56**, 6495–6511 https://doi.org/10.1021/jm400807n
201 Zhong, Y., Katavolos, P., Nguyen, T., Lau, T., Boggs, J., Sambrone, A. et al. (2016) Tankyrase inhibition causes reversible intestinal toxicity in mice with a therapeutic index< 1. *Toxicol. Pathol.* **44**, 267–278 https://doi.org/10.1177/0192623315621192
202 McCabe, N., Cerone, M.A., Ohishi, T., Seimiya, H., Lord, C.J. and Ashworth, A. (2009) Targeting tankyrase 1 as a therapeutic strategy for BRCA-associated cancer. *Oncogene* **28**, 1465–1470 https://doi.org/10.1038/onc.2008.483
203 Palazzo, L., Della Monica, R., Visconti, R., Costanzo, V. and Grieco, D. (2014) ATM controls proper mitotic spindle structure. *Cell Cycle* **13**, 1091–1091 https://doi.org/10.4161/cc.27945