


REVIEW ARTICLE

Opportunities for the repurposing of PARP inhibitors for the therapy of non-oncological diseases

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The recent clinical availability of the PARP inhibitor olaparib (Lynparza) opens the door for potential therapeutic repurposing for non-oncological indications. Considering (a) the preclinical efficacy data with PARP inhibitors in non-oncological diseases and (b) the risk–benefit ratio of treating patients with a compound that inhibits an enzyme that has physiological roles in the regulation of DNA repair, we have selected indications, where (a) the severity of the disease is high, (b) the available therapeutic options are limited, and (c) the duration of PARP inhibitor administration could be short, to provide first-line options for therapeutic repurposing. These indications are as follows: acute ischaemic stroke; traumatic brain injury; septic shock; acute pancreatitis; and severe asthma and severe acute lung injury. In addition, chronic, devastating diseases, where alternative therapeutic options cannot halt disease development (e.g. Parkinson's disease, progressive multiple sclerosis or severe fibrotic diseases), should also be considered. We present a preclinical and clinical action plan for the repurposing of PARP inhibitors.

LINKED ARTICLES

This article is part of a themed section on Inventing New Therapies Without Reinventing the Wheel: The Power of Drug Repurposing. To view the other articles in this section visit <http://onlinelibrary.wiley.com/doi/10.1111/bph.v175.2/issuetoc>

Abbreviations

HRR, homologous recombination DNA repair; PBMC, peripheral blood mononuclear cells; TBI, traumatic brain injury

Tables of Links

TARGETS
Enzymes
PARP, poly(ADP-ribose) polymerase

LIGANDS
Olaparib
Rucaparib
Veliparib

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

PARP inhibitors, a novel class of anticancer therapeutics

Activation of PARP (also termed ARTD) catalyses the cleavage of NAD⁺ into nicotinamide and ADP-ribose. Initially, this response was linked to DNA damage (DNA single strand breakage) in response to genotoxic agents (Chambon *et al.*, 1963; Durkacz *et al.*, 1980; Shall, 1983; Hottiger *et al.*, 2010). PARylation – the modification of a growing number of cellular proteins by PAR units (Pic *et al.*, 2011; Gibson and Kraus, 2012; Krietsch *et al.*, 2013; Ji and Tulin, 2013; Li and Chen, 2014; Gibson *et al.*, 2016) – is now recognized as an important posttranslational modification that extends beyond DNA damage responses and transcends many aspects of cell signalling. PAR, in fact, is now viewed as an anionic matrix for trapping, recruiting and scaffolding proteins, and thereby affecting many key cellular functions. Some of these functions depend on the catalytic activity of the enzyme, while others depend on its physical presence and consequent protein–protein interactions (Thomas and Tulin, 2013) (Figure 1).

Identification of the role of PARP in DNA repair provided the rationale for inhibitor development, based on the hypothesis that by inhibiting PARP, the repair of chemotherapy and radiotherapy-inflicted DNA damage would be suppressed and cancer cell death promoted (see Shall, 1983, Berger *et al.*, 1987; Griffin *et al.*, 1995; Jagtap and Szabo, 2005; Lupo and Trusolino, 2014). A decade ago, it was recognized that cells deficient in the homologous recombination DNA repair (HRR) system – due, for example, to BRCA1 or BRCA2 mutations – are exquisitely sensitive to the cytotoxic effects of PARP inhibition (Bryant *et al.*, 2005; Farmer *et al.*, 2005). As reviewed elsewhere (Curtin and Szabo, 2013; Drew, 2015), in BRCA-proficient cancer cells, their ‘baseline’ single strand breakage (that occurs as part of endogenous DNA damaging agents, e.g. oxidants) is being repaired by the base excision repair (BER) system, which is recruited by PARP activation. In the absence of efficient BER, however, these single strand breaks persist, collapsing replication forks that are then repaired by the HRR pathway.

In BRCA-deficient cells, the HRR pathway is genetically impaired, and therefore, the collapsed replication forks remain unrepaired, which, ultimately, results in the death of the PARP-inhibitor-treated cancer cells. This unique constellation of events, which offers the prospect of selective tumour targeting via induction of synthetic lethality, has vitalized the interest of numerous pharmaceutical companies for PARP inhibitors, to be used in monotherapy, and stimulated extensive drug development that culminated, after dozens of clinical trials, in the approval of the first PARP inhibitor, olaparib (Lynparza) for the therapy of ovarian cancer (Deeks, 2015). In addition, rucaparib has recently been also approved by the FDA for ovarian cancer (<https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm533891.htm>). Several other PARP inhibitors are currently in various late-stage clinical trials and clinicaltrials.gov lists over 200 clinical trials with PARP inhibitors, examples of which are shown in Table 1. Some of these trials focus on PARP inhibitor monotherapy and some of them extend beyond BRCA deficiency, because – in addition to the BRCA-related cytotoxicity mechanism outlined above – there are additional mechanisms through which PARP inhibition can induce cancer cell toxicity (Figure 2, lower section). The mechanistic aspects of the anticancer effects of PARP inhibitors and the clinical activities and therapeutic perspectives related to the oncological uses of PARP inhibitors are covered in separate review articles (Penning, 2010; Ratner *et al.*, 2012; Lee *et al.*, 2014; O’Sullivan Coyne *et al.*, 2015; Buege and Mahajan, 2015; Sistigu *et al.*, 2015; Sonnenblick *et al.*, 2015; Bao *et al.*, 2016; Crafton *et al.*, 2016; Konecny and Kristelit, 2016; Parkes and Kennedy, 2016). PARP inhibition in patients has now been achieved with two approved drugs, olaparib (Lynparza) and rucaparib (Rubraca). In addition, it is expected that several other PARP inhibitors will emerge, in coming years, from the pharmaceutical pipelines.

Although the currently on-going clinical development focuses on oncology, the goal of the current article is to review the potential opportunities of using PARP inhibitors for non-oncological indications (a repurposing approach), especially for those diseases where DNA damage constitutes

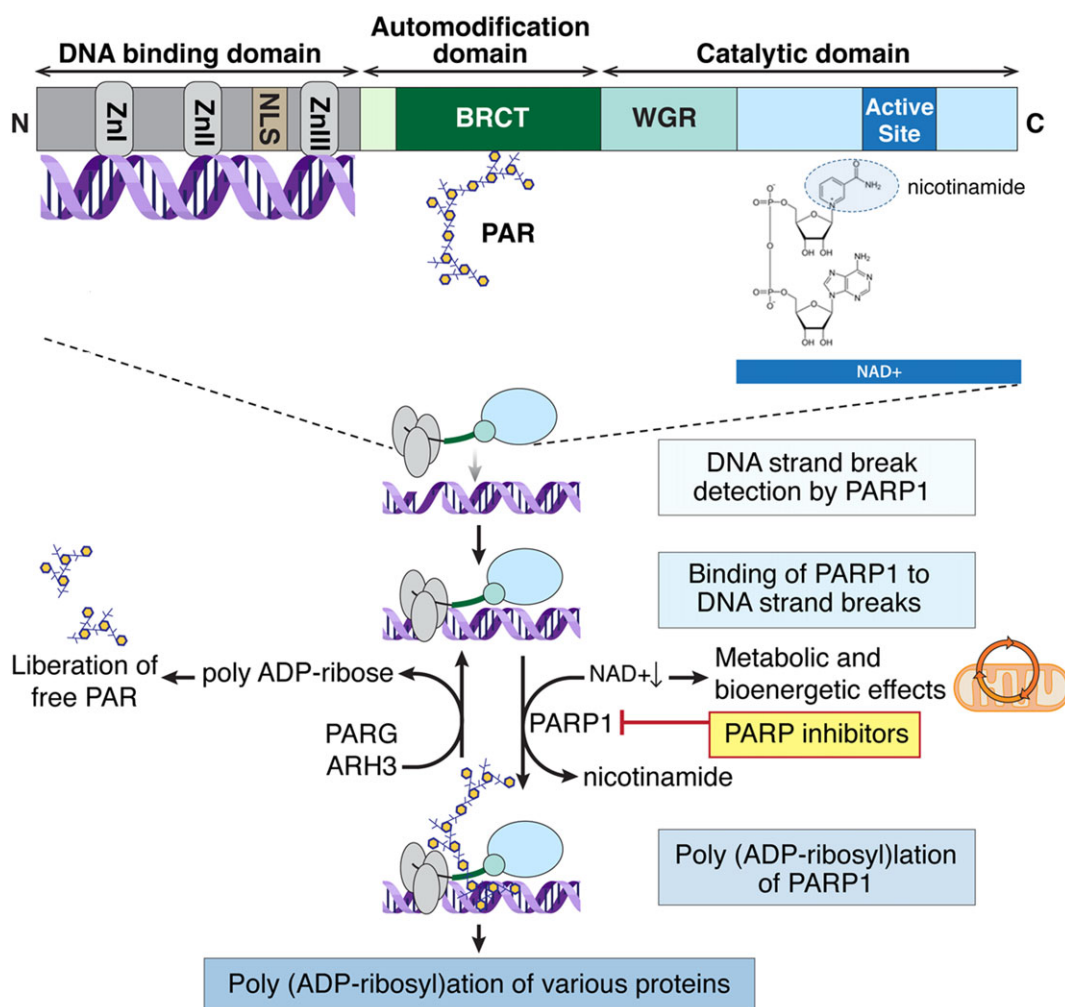


Figure 1

Overview of key biological functions of PARP1. The top section shows the various domains of PARP, including its DNA-binding domain, with its zinc fingers (ZnI, ZnII, ZnIII) that are essential for recognition of DNA strand breaks. This domain also contains the nuclear localization signal (NLS). The auto-modification domain contains the conserved BRCT fold that serves an important protein : protein interaction module in DNA repair and cell signalling. This domain accepts PARP in the context of auto-PARylation of PARP1. The catalytic domain contains the active site of the enzyme, where binding and cleavage of NAD⁺ takes place. It also contains the WGR domain, which is one of the domains involved in the RNA-dependent activation of PARP1. Below the domains, on the right side, the structure of NAD⁺ is presented, with the nicotinamide part highlighted. The middle part of the figure shows the sequences of the PARylation process catalysed by PARP, starting with recognition of the DNA strand breaks by the DNA-binding domain (grey ovals depicting the zinc fingers binding to the DNA breaks), followed by the catalytic activation of the enzyme and the cleavage of NAD⁺ the production of nicotinamide and the generation of PARP, which, in turn, PARylates various acceptor proteins as well as PARP itself. The consumption of NAD⁺ has metabolic and bioenergetic effects. PARP inhibitors prevent the binding of NAD⁺ to the active site of PARP and inhibit the catalytic activity of the enzyme. On the left side, the effect of PAR glycohydrolases and ARH3 is shown; these enzymes break down the PARP, leading to the liberation of free PAR.

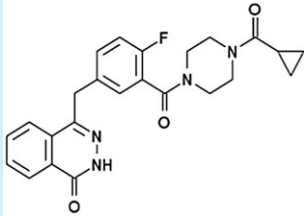
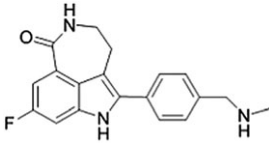
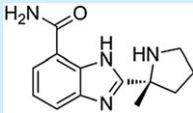
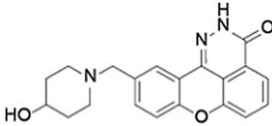
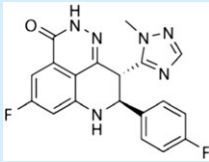
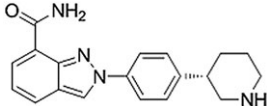
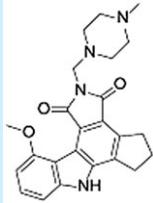
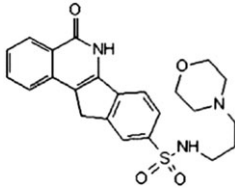
part of the pathophysiological response. We will outline the pathomechanisms that make PARP inhibitors attractive for a variety of non-oncological diseases, review the pros and cons of a repurposing approach and we will recommend a set of indications [including acute ischaemic stroke, severe acute traumatic brain injury (TBI), septic shock, acute pancreatitis, severe acute lung injury (ALI), Parkinson's disease or severe fibrotic diseases] where the expected benefits outweigh the potential risks and where, therefore, clinical trials aimed at repurposing PARP inhibitors are scientifically and medically indicated.

Five decades of advances in the field of PARP

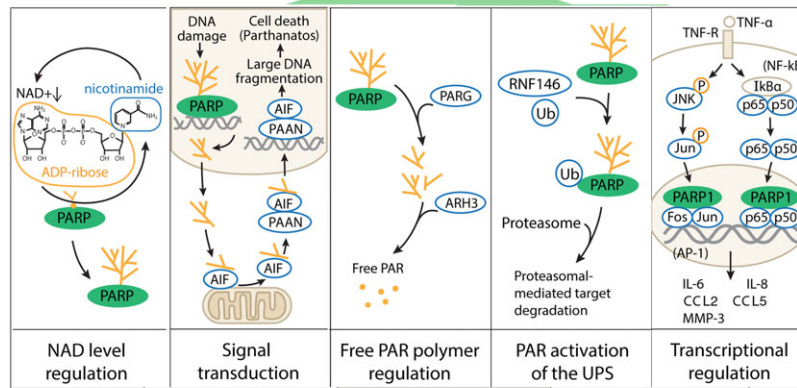
Five decades of intensive work on the biology of PARP have produced thousands of articles, which cannot be reviewed here in detail. PARP (or, as it is now known, PARP1) is now recognized as the first (and most abundant) example of an extensive superfamily of enzymes (reviewed in Amé *et al.*, 2004; Schreiber *et al.*, 2006; Riffell *et al.*, 2012). The physiological role of PARP in the regulation of DNA repair and in the maintenance of genomic integrity has expanded

Table 1

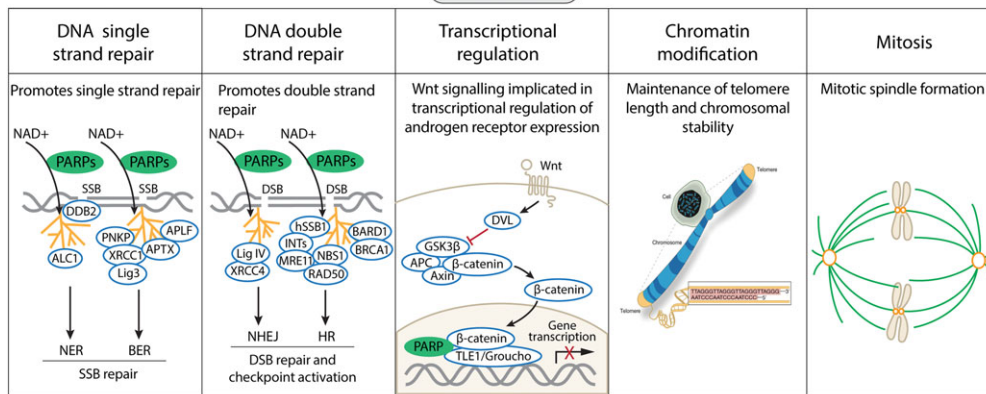
Examples of clinical-stage PARP inhibitors

Inhibitor	Structure	Clinical development examples
Olaparib (KU59436/AZD2281) <i>AstraZeneca</i>		Oncology Approved in the US and Europe for the therapy of ovarian cancer as a single agent
Rucaparib (AG-014699/PF0367338) <i>Clovis</i>		Oncology FDA priority review commenced in 2016 for ovarian cancer as a single agent; decision expected Q1 2017
Veliparib (ABT-888) <i>Abbvie</i>		Oncology In 2016, the FDA granted Fast Track designation for veliparib, in combination with chemotherapies, for example, carboplatin and paclitaxel, or radiation in advanced squamous non-small cell lung cancer
E7016 <i>Eisai</i>		Oncology Phases I–II trials in combination with temozolomide melanoma and advanced solid tumours on-going
Talazoparib (BMN-673) <i>Biomarin</i>		Oncology Phase III trial in breast cancer as a single agent on-going
Niraparib (MK4827) <i>Tesaro</i>		Oncology In 2016, the FDA granted Fast Track designation for the treatment of patients with recurrent platinum-sensitive ovarian, fallopian tube or primary peritoneal cancer as a single agent. Rolling submission under way
CEP-9722 <i>Checkpoint Therapeutics</i>		Oncology Phase I trials in combination with temozolomide and with gemcitabine + cisplatin in solid tumours
INO-1001 <i>Inotek/Genentech</i>		Cardiovascular (myocardial infarction) and oncology No longer in development

Inhibition of PARP in cells exposed to oxidative/nitrative stress and pro-inflammatory stimuli leads to downregulation of inflammatory mediator production, improved metabolic status and protection from cell death



PARP functions



Inhibition of PARP leads to impairment of cancer cell DNA repair inducing cancer cell death and enhancing the effects of chemotherapeutic agents

Figure 2

Top section: Mechanisms responsible for the cytoprotective and anti-inflammatory effects of PARP inhibitors on non-oncological diseases. From left to right: First subpanel shows PARP activation and consequent NAD⁺ depletion. These processes can lead to cellular energetic deficit and cell dysfunction; inhibition of PARP prevents these processes and exerts cytoprotective effects (inhibition of cell necrosis). Second subpanel shows the role of PARP activation and free PAR polymers in inducing mitochondrial release of apoptosis-inducing factor (AIF), which in turn induces cell death (parthanatos). Inhibition of PARP suppresses these processes and inhibits parthanatos. Third subpanel shows the role of PARP in liberating free PAR polymers, which on their own exert cytotoxic effects; inhibition of PARP prevents free PAR polymer formation and suppresses cell death. Fourth subpanel shows that PARylation contributes to activation of the proteasome through an interaction with RNF146; PARP inhibitors suppress these processes. Fifth subpanel shows the role of PARP in contributing to pro-inflammatory signal transduction via enhancing JNK-mediated (left sequence) and NF-κB-mediated (right sequence) activation of multiple genes and gene products. By inhibiting PARP, these processes are attenuated and inflammatory signalling can be attenuated. The five scenarios shown here can either be cell-type and stimulus- and context-dependent or can also occur simultaneously, depending on the pathophysiological condition. Taken together, PARP inhibitors, by blocking these processes, protect against cell death and suppress inflammatory responses. **Bottom section:** Mechanisms responsible for the cytotoxic effects of PARP inhibitors on oncological diseases. From left to right: The left side of the first subpanel shows that PARP contributes to single strand break repair, either through facilitating nucleotide excision repair (NER) via interactions with the WD40-repeat protein DDB2 and the chromatin remodelling enzyme ALC1. The right side of the first subpanel shows that PARP contributes to BER through interaction with a variety of proteins including polynucleotide kinase 3'-phosphatase (PNKP), X-ray repair cross-complementing 1 (XRCC1), aprataxin (APTX), Lig3 (DNA ligase 3) and APLF (a human protein putatively involved in DNA damage response). The second subpanel shows the role of PARP in DNA strand repair; the left side of this subpanel depicts the interactions of PARP with Lig IV (DNA ligase IV) and XRCC4 in the context of NHEJ (nonhomologous end joining); the right side of this subpanel depicts the interactions of PARP with components of the homologous repair (HR). In this context, PAR is recognized by several repair machineries, such as the BRCA1–BARD1 complex, the MRN complex and the hSSB1–INTS complex. The third subpanel depicts the role of PARP in the context of transcriptional regulation of WNT signalling, a pathway implicated in the process of androgen receptor expression. The fourth subpanel depicts the role of PARP in the maintenance of telomere length and chromatin stability, and the fifth subpanel shows the role of PARP in mitotic spindle formation. By inhibiting these processes, PARP inhibitors exert antiproliferative effects and cytotoxic effects, which can be exploited, with beneficial effects, in the therapy of various forms of cancers.

into a field of its own (see Tong *et al.*, 2001; De Vos *et al.*, 2012; Pears *et al.*, 2012; Tallis *et al.*, 2014; Kraus, 2015; Wei and Yu, 2016; Martin-Hernandez *et al.*, 2017), with novel discoveries showing that nuclear PAR may serve as a substrate for nuclear ATP synthesis required for chromatin remodelling (Wright *et al.*, 2016). PARP is now viewed as a broad regulator of a wide variety of nuclear events, including the regulation of protein-nucleic acid interactions by means of protein shuttling (Thomas and Tulin, 2013). In addition to nuclear PARP1, the mitochondrial isoform of PARP is receiving more attention (Du *et al.*, 2003; Lai *et al.*, 2008; Bai *et al.*, 2015; Brunyanski *et al.*, 2016). PAR homeostasis is now viewed as a balance between PAR formation (by PARP superfamily members) and PAR degradation – the latter process being catalysed by PAR glycohydrolases as well as other enzymes such as ARH3 (Gagné *et al.*, 2006; Mashimo *et al.*, 2014; Barkauskaite *et al.*, 2015; Pascal and Ellenberger, 2015).

Another area of intensive research relates to the epigenetic role of PARP as a regulator of gene expression via (1) regulation of chromatin remodelling, (2) functioning as a transcriptional co-regulator, (3) modulating DNA methylation, (4) poly(ADP-ribose)ylation of target proteins involved in gene transcription and/or (5) regulation of RNA metabolism and function (Kraus and Hottiger, 2013; Schiewer and Knudsen, 2014; Bock *et al.*, 2015; Ryu *et al.*, 2015; Jubin *et al.*, 2017; Posavec Marjanović *et al.*, 2017). PARP inhibitors have been found to affect the activation of many transcription factors and the expression of many gene products (Figure 2, top section). Several levels of crosstalk have been demonstrated between PARP and the sirtuin system (Cantó *et al.*, 2013; Imai and Guarente, 2014; Gueguen *et al.*, 2014; Faraone-Mennella, 2015; Ješko and Strosznajder, 2016). In addition to the role of PARP in cell death and in various disease conditions (discussed in the next section), the roles of PARP have been recognized in carcinogenesis (Masutani and Fujimori, 2013) and ageing (Bürkle *et al.*, 2005; Beneke and Bürkle, 2007; Mangerich and Bürkle, 2012; Shilovsky *et al.*, 2013; Imai and Guarente, 2014).

In parallel with the progress made in the field of basic science, the field of pharmacological inhibitors has also advanced significantly. The first generation of inhibitors (3-aminobenzamide, nicotinamide – active at millimolar concentrations) were followed by second-generation compounds (e.g. 1,5-dihydroisoquinoline, 2-nitro-6[5H]phenanthridinone, 4-amino-1,8-naphthalimide – active at mid-micromolar concentrations) and, finally, the third generation, ultrapotent class of inhibitors, (active at low micromolar to high nanomolar concentrations) many of which have progressed into clinical development (see Curtin and Szabo, 2013). Structures of clinical-stage PARP inhibitors, together with representative examples of their clinical trials, are shown in Table 1.

PARP, an executor of cell necrosis

In parallel with the recognition that PARP activation consumes its substrate, NAD⁺, Berger put forward the hypothesis that cellular depletion of NAD⁺, and, secondarily, ATP, in cells exposed to DNA-damaging agents, may be deleterious to cell viability. Thus, it was proposed that

inhibitors of PARP, by preventing the activation of a deleterious bioenergetic cycle, have the potential to sustain vital cellular functions, through the maintenance of cellular bioenergetics and protein synthesis (Sims *et al.*, 1983). The original hypothesis developed in cells exposed to genotoxic agents has subsequently extended to include a diverse set of triggers of DNA damage, such as reactive oxygen species (Schraufstatter *et al.*, 1986), nitric oxide (Heller *et al.*, 1994; Zhang *et al.*, 1994), peroxynitrite (Szabo *et al.*, 1996) and various pathophysiological relevant triggers, where the oxidative/nitrative stress is produced by NMDA receptor activation in neurons (Zhang *et al.*, 1994), hypoxia/reoxygenation in cardiac myocytes (Gilad *et al.*, 1997), endotoxin stimulation in macrophages (Zingarelli *et al.*, 1996) or elevated extracellular glucose in endothelial cells (Soriano *et al.*, 2001). The mode of cell death triggered by PARP overactivation was found to include mitochondrial dysfunction and is now typically viewed as a regulated (active) form of cell necrosis (Virág *et al.*, 1998; Ha and Snyder, 1999). While the PARP-mediated cell death processes are diverse, and – depending on the cell type, stimulus and experimental context – range from processes that are more dependent on the energetic deficit, and therefore can be rescued not only by PARP inhibition but also by NAD⁺ supplementation (Alano *et al.*, 2010; Weidele *et al.*, 2010) to processes that are dependent on the intracellular toxic action of free PAR polymers, a separate mode of cell death termed parthanatos (Andrabi *et al.*, 2006; Fatokun *et al.*, 2014). PARP activation in neurons, in addition to being a downstream effector of NMDA-receptor activation-induced neurotoxicity, also increases the expression of calcium-permeable calcium channels that are responsible for a delayed type of neuronal death (Gerace *et al.*, 2015). By inhibiting PARP, the viability of cells subjected to oxidative/nitrative stress can be improved. In the context of neuroinjury, thus, a therapeutic opportunity exists not only to reduce the delayed loss of neurons associated with brain ischaemia (stroke, cardiac arrest or trauma) but also to decrease the late dementia frequently occurring within a few months of brain ischaemic events. The cytoprotective effects of pharmacological inhibition of PARP are schematically depicted in Figure 2, top section. One of the key aspects that determines whether PARP inhibition has positive or negative effects on cell viability is time. Generally, PARP inhibitors are protective in postmitotic (i.e. non-replicating) or slowly replicating cells that have sufficient time to repair DNA before it has deleterious biological consequences. On the other hand, in rapidly replicating cells, the impact of PARP inhibitors on cell survival is generally negative. The PARP-mediated active form of cell death has been reviewed, in detail, in recent articles (Virág and Szabo, 2002; Virág *et al.*, 2013; Baxter *et al.*, 2014; Fatokun *et al.*, 2014).

PARP activation, a facilitator of inflammation and a pathogenetic factor in various non-oncological diseases

The activators of PARP described in the previous section (e.g. oxidants, free radicals) are produced in a variety of

pathophysiological conditions. PARP is not only involved in an active, regulated forms of cell necrosis (see above) but is also involved in signal transduction, including the promotion of various pro-inflammatory signalling pathways. The processes of cell necrosis and the processes of inflammation are intricately interlinked and form a self-amplifying positive feedforward cycle (Jagtap and Szabo, 2005) that promote more chronic pathophysiological processes, for example, neurodegeneration and fibrosis, which, in turn, may induce further PARP activation (Strosznajder *et al.*, 2010). Starting with studies in stroke (Zhang *et al.*, 1994; Eliasson *et al.*, 1997) and myocardial infarction (Zingarelli *et al.*, 1997), in the mid 90's, the concept was formulated that PARP inhibitors can be used as cytoprotective and/or anti-inflammatory agents. As reviewed elsewhere for various specific disease conditions (Pieper *et al.*, 1999; Chiarugi, 2002; Liaudet *et al.*, 2003; Evgenov and Liaudet, 2005; Jagtap and Szabo, 2005; Koh *et al.*, 2005; Komjáti *et al.*, 2005; Kauppinen and Swanson, 2007; Moroni, 2008; Pacher and Szabo, 2008; Besson, 2009; Giansanti *et al.*, 2010; Strosznajder *et al.*, 2010; Szabó and Módis, 2010; Ba and Garg, 2011; Laudisi *et al.*, 2011; Cavone and Chiarugi, 2012; Curtin and Szabo, 2013; Sriram *et al.*, 2014), the list of diseases where preclinical studies demonstrate significant beneficial effects of PARP inhibition includes neurological diseases (e.g. stroke, neurotrauma, neurodegeneration), various forms of critical illness (e.g. septic shock, ALI, acute liver failure), reperfusion injury (e.g. myocardial infarction), inflammatory diseases (arthritis, colitis, asthma) and vascular diseases (diabetic complications, atherosclerosis). In all of these diseases, the likely trigger of PARP activation is DNA strand breakage, which develops, at least in part, due to the formation of reactive species (oxidants and free radicals) produced, as part of disease pathophysiology (reviewed in Pacher and Szabo, 2008). However, other non-DNA-damage-dependent mechanisms of PARP activation are also known (Cohen-Armon *et al.*, 2007); and it may also be conceivable that basal PARP activity may also have pathophysiological roles as well.

Several PARP inhibitors – including olaparib and veliparib – demonstrated cytoprotective efficacy in an *in vitro* model of human cortical neuronal death induced by either NMDA receptor activation or oxygen–glucose deprivation (Xu *et al.*, 2016). The body of preclinical data with olaparib in various *in vitro* and *in vivo* models of non-oncological diseases is summarized in Table 2 and includes models of stroke, acute lung, liver and kidney injury, lung inflammation and chronic liver disease (Kapoor *et al.*, 2015; Ghonim *et al.*, 2015a,b; Rom *et al.*, 2016; Teng *et al.*, 2016; Gariani *et al.*, 2017; Mukhopadhyay *et al.*, 2017). With rucaparib – the second PARP inhibitor compound that has been recently approved – other than *in vitro* data showing its protective effect in human neuronal cultures exposed to NMDA or oxygen/glucose deprivation (Xu *et al.*, 2016), there are no publications available in non-oncological indications. Although the dose of the PARP inhibitor in the context of cancer therapy can be variable (depending whether it is used as a single agent or in combination with chemo- or radiotherapy), generally, in non-oncological models, the effective doses of the PARP

inhibitors (1–5 mg·kg⁻¹ i.p.) are lower than the doses of olaparib used in cancer models (50–200 mg·kg⁻¹·day⁻¹ i.p.) (e.g. To *et al.*, 2014; Henneman *et al.*, 2015; Ter Brugge *et al.*, 2016). This difference in the dose has mechanistic explanations and has clear advantages for therapeutic repurposing (see below).

Expression and activity of PARP is dynamically regulated

Considering pharmacological modulation of PARP for non-oncological indications, another factor to discuss is the dynamic regulation of PARP activity (as well as PARP expression) by environmental factors or by physiological conditions. If PARP is subject to positive or negative regulation by physiological factors, one can predict that the cells are more likely to tolerate its pharmacological modulation as well. In this respect, several endogenous or natural-product-based inhibitors of PARP activity have been described, including nicotinamide, caffeine, hypoxanthine, taurine, xanthurenic acid and kynurenic acid, various naturally occurring diadenosine polyphosphates, the active form of Vitamin D (1 α ,25-dihydroxyvitamin D3), Vitamin A and its precursors, 3,5,3'-triiodothyronine, as well as natural products, like ginsenosides (Banasik *et al.*, 2012; Nabavi *et al.*, 2015). It must be emphasized that most of the above studies are based on *in vitro* observations only and may have used supra-physiological concentrations and that the potency of most of these natural/non-conventional PARP inhibitors is lower than the potency of the state-of-the-art third-generation PARP inhibitors.

The issue of antibiotic-mediated PARP inhibition deserves further discussion. *In vitro*, several antibiotics, including actinomycin D, coumermycin A1, formycin B, novobiocin and showdomycin have been shown to exhibit PARP inhibitory effects (Banasik *et al.*, 2012). In addition, tetracyclines, with the rank order of minocycline > doxycycline > demeclocycline > chlortetracycline, have also been identified as PARP inhibitors. Minocycline also prevented neuronal NAD⁺ depletion *in vitro* and neurotoxicity *in vivo* and exerted the type of anti-inflammatory action that is expected from a PARP inhibitor (Alano *et al.*, 2006). Minocycline also exerted protective effects in re-oxygenated cardiac myocytes *in vitro*, on a cardiopulmonary bypass model *in vivo* and in a diabetic retinopathy model *in vivo*. These effects were associated with a suppression of PAR polymer formation (Tao *et al.*, 2010; Dhein *et al.*, 2015; Wu *et al.*, 2015). However, in other models (e.g. in a model of asthma), the PARP inhibitory effects of minocycline were found to involve indirect (i.e. antioxidant) actions, rather than direct effects on the PARP enzyme (Naura *et al.*, 2013).

In addition to regulation at the level of PARP catalytic activity, the *expression* of PARP1 may also be dynamically regulated. For instance, the expression of PARP1 is regulated during skeletal muscle cell maturation: as myoblasts differentiate into myotubes, PARP1 expression is reduced; this change confers resistance to oxidative stress (Oláh *et al.*, 2015). Also, PARP expression is highly up-regulated during

Table 2Examples of the protective actions of olaparib in various *in vitro* and *in vivo* models of non-oncological diseases

Experimental model	Human disease(s) modelled	Dose or concentration of olaparib	Effects of olaparib	Reference
Differentiated human neurons in culture exposed to NMDA receptor ligands or oxygen–glucose deprivation (OGD)	Stroke, neurodegeneration, neuroinflammation	2 μM (<i>in vitro</i>)	Olaparib protected against NMDA and OGD-induced neuronal cell death.	Xu <i>et al.</i> , 2016
CD3/CD28-stimulated human CD4 + T-cells	Asthma, inflammation	1–5 μM (<i>in vitro</i>)	Olaparib reduced Th2 cytokine production while moderately affecting T-cell proliferation. It also increased IL-17 production and increased the T-reg cell population.	Ghonim <i>et al.</i> , 2015b
Models of monocyte adhesion and migration across blood brain barrier (BBB)	Blood brain barrier function (relevant for stroke, TBI, neuroinflammation)	Leukocytes were treated <i>ex vivo</i> with olaparib 10 μM for various durations	Olaparib in human primary monocytes diminished their adhesion to and migration across BBB (human primary microvascular endothelium) in <i>in vitro</i> models and prevented barrier injury. <i>In vivo</i> olaparib diminished leukocyte-endothelial interactions and protected against the deterioration of BBB integrity.	Rom <i>et al.</i> 2016
C2C12 myotubes exposed to H_2O_2	Skeletal muscle disorders	100 nM <i>in vitro</i>	Olaparib rescued the oxidant-induced decline in cellular NAD^+ levels and improved mitochondrial function in myotubes.	Pirinen <i>et al.</i> 2014
Hepatocyte cell line AML12	Ageing	100 nM <i>in vitro</i>	Olaparib rescued the oxidant-induced decline in cellular NAD^+ levels and increased mitochondrial biogenesis.	Mouchiroud <i>et al.</i> , 2013
<i>Caenorhabditis elegans</i>	Ageing	1 μM <i>in vitro</i>	Olaparib prevented age-associated metabolic decline and promoted longevity. It increased mitochondrial biogenesis.	Mouchiroud <i>et al.</i> , 2013
Mouse model of transient middle cerebral artery occlusion (2 h ischaemia, 24 h reperfusion)	Acute ischaemic stroke	1, 3, 5 10 and 25 $\text{mg}\cdot\text{kg}^{-1}$, single intraperitoneal dosing	At 3 and 5 $\text{mg}\cdot\text{kg}^{-1}$ (but not doses higher or lower) olaparib reduced infarct size, IgG extravasation, and improved neurological scores in some (but not all) tests used.	Teng <i>et al.</i> , 2016
Intratracheal administration of endotoxin to mice	ALI, sepsis, acute renal failure	5 $\text{mg}\cdot\text{kg}^{-1}$, single intraperitoneal dosing	Olaparib reduced inflammatory cell (in particular neutrophil) infiltration into the lungs and attenuated pulmonary oedema. It also protected against the endotoxin-induced secondary kidney injury, shown by improvements in serum urea and creatinine levels. These effects were associated with a reduction in tissue oxidative stress markers	Kapoor <i>et al.</i> , 2015

continues

Table 2 (Continued)

Experimental model	Human disease(s) modelled	Dose or concentration of olaparib	Effects of olaparib	Reference
Intraperitoneal administration of endotoxin to mice	Acute hepatitis, sepsis	50 mg·kg ⁻¹ , oral dosing	and reduced production of various pro-inflammatory factors (e.g. TNF- α , IL-1 β and VCAM-1). Olaparib reduced plasma levels of hepatic injury markers and improved hepatic NAD ⁺ levels. It also reduced the LPS-induced hepatic proinflammatory genes IL-1 β and IL-6. These effects were associated with a reduction of the infiltration of the liver with inflammatory cells.	Gariani <i>et al.</i> , 2017
Ovalbumin exposure in ovalbumin-sensitized mice: pulmonary inflammation and airway hyperreactivity in mice	Asthma bronchiale	1, 5 and 10 mg·kg ⁻¹ , single intraperitoneal dosing	At all doses tested, olaparib afforded a dose-dependent reduction in ovalbumin-specific IgE production in bronchoalveolar lavage fluid. It also reduced inflammatory cell numbers in bronchoalveolar lavage, and reduced mucus production in the lungs. It improved airway hyperreactivity response to metacholine. Finally, olaparib suppressed several inflammatory mediators including eotaxin, IL-2, IL-4, IL-5, IL-6, IL-13 and M-CSF.	Ghonim <i>et al.</i> , 2015a
Mice chronically exposed to house dust mite: pulmonary inflammation and airway hyperreactivity	Asthma bronchiale	5 mg·kg ⁻¹ , single intraperitoneal dosing	Olaparib prevented the dust mite-induced increase in overall cellularity, weight and CD4 ⁺ T-cell population in spleens. It also inhibited eotaxin, IL-4, IL-5 and IL-13 production. It reduced IL-2 and IP-10 levels and increased the T-regulatory cell population. In addition, olaparib improved the airway hyperreactivity response to methacholine.	Ghonim <i>et al.</i> , 2015b
Mice subjected to a high fat high-sucrose diet for 18 weeks	Non-alcoholic fatty liver disease (NASH)	50 mg·kg ⁻¹ ·day ⁻¹ orally, either in a preventive mode (starting at 7 weeks of age), or 9 weeks after the start of the diet, in a therapeutic mode (starting at 16 weeks of age)	Olaparib reduced obesity without changes in food intake. It also improved triglyceride and cholesterol status. In post-treatment mode it reversed hepatic fat deposition, improved hepatic histopathological changes and reduced liver fibrosis. These effects were associated with increased mitochondrial biogenesis and β -oxidation in the liver; reduction in reactive oxygen species levels and suppression of endoplasmic reticulum stress. Olaparib also improved insulin sensitivity in a glucose tolerance test.	Gariani <i>et al.</i> , 2017
Mice subjected to methionine and choline deficient diet (MCD) for 5 weeks	Non-alcoholic fatty liver disease (NASH)	50 mg·kg ⁻¹ ·day ⁻¹ , oral dosing	Olaparib improved hepatic fat deposition, improvement of hepatic histopathological changes and reduction in liver fibrosis. It also reduced plasma levels of	Gariani <i>et al.</i> , 2017

continues

Table 2 (Continued)

Experimental model	Human disease(s) modelled	Dose or concentration of olaparib	Effects of olaparib	Reference
			hepatic injury markers. Olaparib improved hepatic NAD ⁺ levels and suppressed the expression of multiple pro-inflammatory genes.	
Mice subjected to MCD diet for 5 weeks	Non-alcoholic fatty liver disease (NASH)	50 mg·kg ⁻¹ ·day ⁻¹ , oral dosing	Olaparib improved hepatocellular injury, attenuated steatosis and metabolic dysregulation, decreased hepatic inflammation (Ly6G, F4/80, Cxcl2, TNFalpha mRNA expression) and fibrosis.	Mukhopadhyay <i>et al.</i> 2017
Mice subjected to high fat diet for 12 weeks	Non-alcoholic fatty liver disease (NASH)	40 mg·kg ⁻¹ ·day ⁻¹ , oral dosing for 8 days	Olaparib reduced body weight, suppressed liver steatosis and dysregulated hepatic fatty acid metabolism without changing food intake and ambulatory activity. Olaparib also increased O ₂ consumption, CO ₂ production and total energy expenditure associated with significantly increased fat oxidation.	Mukhopadhyay <i>et al.</i> 2017
Mice subjected to high fat diet for 12 weeks and a single binge of alcohol	Acute alcoholic hepatitis	40 mg·kg ⁻¹ ·day ⁻¹ , oral dosing for 8 days	Olaparib attenuated hepatocellular injury (ALT) and inflammation (decreased hepatic neutrophil infiltration, mRNA expression of Ly6G, ICAM1, Cxcl2, CCR2, IL1β, TNFα).	Mukhopadhyay <i>et al.</i> 2017

dendritic cell maturation and contributes to immunocompetence and T cell proliferation (Aldinucci *et al.*, 2007). In other conditions – rodent models of chronic heart failure, renal transplantation or unilateral ureteral obstruction and in the brain of patients with ALS – up-regulation of PARP1 protein has been demonstrated (Kim *et al.*, 2004; Pillai *et al.*, 2005; O'Valle *et al.*, 2007; Vagnerova *et al.*, 2010; Kim and Padanilam, 2011).

It should be also noted that the expression and activity of PARP shows marked cell-type differences. For instance, PARP levels are low in resting lymphocytes, high in mitogenically activated lymphocytes and virtually absent in mature granulocytes (Berger *et al.*, 1987). There is also a wide (up to 200-fold) range in PARP activity between various human subjects (Zaremba *et al.*, 2011). These differences may indicate relative differences in the importance of PARP-dependent regulation in different cell types or perhaps even in different individual human subjects.

Taken together, PARP activity and expression appears to be regulated by physiological and pathophysiological factors, in response to many commonly used drugs, as well as by gender, as highlighted below. These data, indicating that negative modulation of PARP activity occurs under different physiological settings, indirectly support the notion that pharmacological targeting of PARP may be a therapeutically acceptable strategy in carefully selected non-oncological indications.

Many of the protective effects of PARP inhibitors are gender-dependent

The issue of gender differences in the therapeutic effects of PARP inhibitors requires special emphasis. Several studies showed that the efficacy of various PARP inhibitors in rodent models of stroke is preferentially observed in male mice and is also dependent on the age of the animals (Eliasson *et al.*, 1997; Hagberg *et al.*, 2004; McCullough *et al.*, 2005; Yuan *et al.*, 2009); similarly, the protective effect of minocycline is primarily observed in male animals subjected to stroke (Li and McCullough, 2009). However, the gender difference was not observed in all studies. In some studies, PARP inhibitors maintain partial efficacy in female animals (Moroni *et al.*, 2012), and in a primate study of stroke, the PARP inhibitor MP-124 showed comparable protective effects in both sexes (Matsuura *et al.*, 2011). PARP inhibition was equally protective in sex-segregated primary cortical neurons exposed to peroxynitrite, but neurons from males were more vulnerable than their female counterparts to nitrosative stress (Du *et al.*, 2004). In addition, in a recent study, olaparib exerted neuroprotective effects on human cortical neurons derived from the human ESC H9 cell line, which is female, as well as in human ESC H1 cell line-derived cortical cultures, which is male (Xu *et al.*, 2016). Although the mechanism behind the gender effect of PARP inhibitors and the reason why some studies/some PARP inhibitors exhibit sex-

specificity, while others do not, remains unclear, it is interesting that PARP1 appears to become activated in response to stroke in both sexes, but this is associated with a more pronounced decrease of brain NAD⁺ levels in males than in females (Hagberg *et al.*, 2004).

The sex differences with respect to PARP may also apply to rodent models of shock and inflammation (Mabley *et al.*, 2005). For example, the PARP inhibitor PJ34 decreases the endotoxin-induced production of TNF- α and protects against endotoxin-induced mortality in male, but not female mice. However, the protective effect of PARP inhibitors is restored in female mice by ovariectomy. Moreover, in a porcine model of thoracoabdominal aortic ischaemia–reperfusion injury, PARP inhibitors protect against cardiovascular collapse in male, but not in female animals (Hauser *et al.*, 2006). In addition, PARP inhibition protects in a murine model of autoimmune nephritis in male, but female mice (Jog *et al.*, 2009). Although oestrogen does not appear to inhibit PARP activation in a direct manner, an interaction has been characterized between PARP1 and oestrogen receptor α , whereby a stable complex may sequester PARP1 to specific regions on the DNA making it difficult for its zinc fingers to access and recognize DNA breakpoints (which is essential for the enzymic activation of PARP) (Mabley *et al.*, 2005). In cells generated from male (but not from female) mice, oestrogen was found to inhibit oxidant-induced PARP activation *in vitro* (Jog and Caricchio, 2013). One possible explanation for the greater effects of PARP inhibitors in males than females, is the finding that PARP activity in peripheral blood mononuclear cells (PBMCs) and liver tissue was significantly higher in male than female mice (Zaremba *et al.*, 2010). These authors also showed that this effect may be driven by male sex hormones as PARP activity in PBMCs isolated from castrated male mice was lower than in intact males and similar to that in females, and unaffected by oestrogen supplementation. Studies in healthy volunteer humans and cancer patients also revealed that PARP activity in PBMCs isolated from women had significantly lower PARP activity than PMBCs isolated from men. Moreover, women <45 years old (pre-menopausal) tended to have a lower PARP activity than older women and men (Zaremba *et al.*, 2010). Taken together, PARP activation is influenced by sex and sex hormones, but whether this is androgen driven (in males) or oestrogen suppressed (in females) or both – and the underlying molecular mechanisms – remains to be better characterized. In addition, even though PARP activation is lower in females than in males, the clinical data from olaparib in ovarian cancer clearly indicate that PARP remains functionally relevant in women and can be pharmacologically targeted with clinically relevant therapeutic effects.

On the adverse effect profile of PARP inhibitors

One of the most common objections against the use of PARP inhibitors for non-oncological indications relates to the question as to whether by ‘artificially’ preventing cell necrosis, do we prolong the life of cells that are ‘doomed’ due to their extensive degree of DNA damage? And if so, what

is the potential that these cells later become subject to malignant transformation?

PARP1 is not a DNA repair enzyme *per se*; it is an enzyme that helps with the recruitment of DNA repair enzymes that execute the DNA repair. PARP1 deficient mice are viable and fertile, although cells from PARP1^{-/-} mice exhibit an increased degree of genomic instability in response to genotoxic agents and PARP1^{-/-} mice exhibit increased mortality in response to irradiation (Shall and de Murcia, 2000). In other studies, BER efficiency was found comparable in wild-type and PARP1 deficient cells (Vodenicharov *et al.*, 2000). One must keep in mind that in PARP1-deficient systems, not only the catalytic activity of PARP1 is completely removed but also its structural (scaffolding) function. Furthermore, in PARP1-deficient systems, the enzyme is absent for the life of the cell, which is not the same as acute treatment of the cells with intermittent or short-term dosing with PARP inhibitors (which doses perhaps do not induce a 100% inhibition of the catalytic activity of the enzyme).

Many *in vitro* studies have examined the effect of PARP inhibitors on cellular DNA integrity. Studies with early generation PARP inhibitors (e.g. 3-aminobenzamide) produced equivocal results, as in some studies, sister chromatid exchange rates and malignant transformations were reduced (Borek *et al.*, 1984), while in other studies, they remained unaffected (D'Souza *et al.*, 1992), and in yet other studies, increases were reported (Shiraishi *et al.*, 1983; Schwartz *et al.*, 1984; D'Souza *et al.*, 1992). In line with the concept that PARP1 deficiency does not equal PARP catalytic inhibition (as already discussed above), Smulson and colleagues compared the tetraploidy inducing potential of PARP1 deficiency with GPI 6150 and found that while PARP1 deficiency increased the percentage of a genetically unstable tetraploid cell population in fibroblasts, the PARP inhibitor (20 μ M, 3 weeks of continuous exposure) did not (Simbulan-Rosenthal *et al.*, 2001). In a number of different cell lines, 24 h exposure to rucaparib (10 μ M) resulted in a similar number of H2AX foci as were induced by 2 Gy irradiation (Drew *et al.*, 2011). Ito and colleagues have recently examined the effect of olaparib and veliparib in several human cells, including primary lymphoid cells and non-tumorigenic and tumorigenic epithelial cell lines. Both olaparib and veliparib, at respective concentrations of 0.1–1 and 1.5–80 μ M, induced concentration-dependent increases in sister chromatid exchange rates and chromatid aberrations (Ito *et al.*, 2016). Similarly, in an *in vitro* assay where whole chromosomal instability was assessed by using a nonessential human artificial chromosome in the HT1080 human fibrosarcoma cell line, olaparib and veliparib – at concentrations of 5–10 μ M (where they inhibited cell proliferation by 50%) – induced significant increases in the rate of human artificial chromosome loss (Lee *et al.*, 2016). In summary, although the above studies did not examine oxidatively stressed cells, and only evaluated a limited number of PARP inhibitors, the data demonstrate that PARP inhibitors can, indeed, exert genotoxic effects *in vitro*. The predictive power (or lack thereof) of the various *in vitro* genotoxicity and genetic stability studies on the *in vivo* tumorigenic potential remains unclear (e.g. Snyder and Green, 2001; Brambilla and Martelli, 2009). As far as direct studies on PARP inhibitors or PARP deficiency on tumourigenesis are concerned, the data are

mixed (see Masutani *et al.*, 2003; Masutani and Fujimori, 2013). For instance, in an azoxymethane-induced colon and liver carcinogenesis model in mice, PARP1 deficiency increased tumour incidence (Nozaki *et al.*, 2003). In a study of age-related spontaneous carcinogenesis in mice, the incidence of spontaneous tumours was similar in both wild-type and PARP1-deficient mice, but the incidence of some malignant tumours (uterine tumours, lung adenocarcinomas and hepatocellular carcinomas) was higher in PARP1-deficient mice (Piskunova *et al.*, 2008). However, in other models (e.g. nitrosamine-induced oral and hepatic carcinogenesis) in mice, PARP1 deficiency failed to affect tumour incidence (Gunji *et al.*, 2006; Ogawa *et al.*, 2006). Surprisingly, in some studies, PARP inhibition was even found to *reduce* carcinogenesis. For instance, when Ela-myc-driven pancreatic tumour development in mice was analysed on a PARP1 knockout background, increased tumour necrosis and decreased proliferation, apoptosis and angiogenesis were noted (Martínez-Bosch *et al.*, 2014). Moreover, inhibition of PARP with PJ34 was found to prevent *Helicobacter*-induced gastritis and precancerous lesions in IL-10^{-/-} mice (Toller *et al.*, 2010). In addition, PARP1-deficient male mice and mice treated with the PARP inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone showed a marked *delay* in tumour formation, as well as a dramatic reduction in tumour size and multiplicity in a model of chemically induced skin carcinogenesis, most likely due to its suppressive effect on the expression of several pro-inflammatory genes (Martin-Oliva *et al.*, 2004; Martin-Oliva *et al.*, 2006). Most relevant to the current article is a carcinogenesis study with olaparib and veliparib in a BRCA-deficient mouse model of breast cancer development. In this model, both veliparib diet (100 mg·kg⁻¹·day⁻¹), or olaparib diet (25, 50, 100 or 200 mg·kg⁻¹·day⁻¹), when administered continuously for up to 43 weeks, exerted antitumor effects. The highest dose of the PARP inhibitor delayed the average age of the first detectable tumour by 2.4 and 6.5 weeks, respectively, compared with controls. Olaparib also increased the average lifespan of these mice by 7 weeks (To *et al.*, 2014). Interestingly, several of the above studies (e.g. the studies by To and the studies by Piskunova and colleagues) utilized female mice only; given the gender-differences in PARP inhibitors' effects discussed above, it remains to be further investigated whether the effects of PARP inhibitors on carcinogenicity may also be gender-dependent.

There are additional published studies where animals were treated with PARP inhibitors for extended time periods, in some cases up to 6–9 months (e.g. Bartha *et al.*, 2009; Gariani *et al.*, 2017); these studies have not reported any tumorigenic effects of the PARP inhibitors, although it should be emphasized that these studies did not specifically investigate this issue as part of their original trial design.

Many *in vitro* studies have been conducted to investigate the effect of PARP inhibitors on different cell populations (normal, necrotic, apoptotic), exposed to various concentrations of genotoxic oxidants (e.g. hydrogen peroxide or peroxyxynitrite). These oxidants trigger DNA damage, PARP activation, and shift cells from the normal to the necrotic and apoptotic populations (Virág *et al.*, 1998; Ha and Snyder, 1999; Ye, 2008). When cells are treated with PARP inhibitors, the necrotic cell population decreases, while

the apoptotic and the normal populations increase. In other words, by inhibiting PARP, necrosis can be prevented and/or shifted to apoptosis, a distinct form of cell death, where the cellular content is not spilled into the extracellular space and, therefore, does not induce further cytotoxicity or inflammation to bystander cells. Through pharmacological suppression of cell necrosis, one can, therefore, not only maintain functional parenchymal cells but also down-regulate the inflammatory process and prevent self-amplifying cycles of injury. By pharmacological control of inflammation (either by the direct transcriptional effect of PARP inhibitors discussed above) or by reducing the spillage of necrotic cells into the environment, as well as by interrupting various positive feedback cycles of injury, the degree of genotoxic burden to the cells would be expected to be attenuated (as discussed in Jagtap and Szabo, 2005). Indeed, several studies demonstrate that genetic or pharmacological inactivation of PARP1 reduces tissue myeloperoxidase levels (indicating reduced infiltration of inflammatory cells) and tissue malondialdehyde levels (a marker of tissue oxidative burden) and tissue nitrotyrosine levels (a marker of tissue nitrosative burden) (Soriano *et al.*, 2002; Chatterjee *et al.*, 2003; Esposito *et al.*, 2011; Kapoor *et al.*, 2015). It is, therefore, conceivable that a diseased organism – which is exposed to endogenous genotoxic free radicals and oxidants and exhibits signs of a 'baseline' damage to the genetic material (e.g. Bao *et al.*, 2015) – when treated with a PARP inhibitor, may ultimately encounter a reduced 'burden' of genotoxic oxidative/nitrative damage than an untreated one. Whether this type of 'indirect' genoprotective effect may be able to counteract or compensate for any 'direct' genotoxic effect of a PARP inhibitor remains to be determined. In any case, one must emphasize that the effect of a PARP inhibitor on a normal (unperturbed) organism may be drastically different from the effect of the same inhibitor under pathophysiological conditions. For instance, PARP inhibition in normally cultured fibroblasts accelerates the rate of telomere shortening (Cohausz *et al.*, 2008; Boesten *et al.*, 2013). However, under conditions of chronic oxidative stress, PARP inhibition did not accelerate telomere shortening (Boesten *et al.*, 2013). Unfortunately, the vast majority of published studies focusing on the therapeutic effect of PARP inhibitors on various experimental models of disease *in vivo* do not measure parameters of DNA integrity. However, it must be noted that in a porcine study of thoracic aortic cross-clamping induced ischaemia–reperfusion model, DNA integrity was monitored in isolated leukocytes *ex vivo*, and the beneficial cardiovascular effects of the PARP inhibitor INO-1001 were not associated with increases in DNA damage (Hauser *et al.*, 2006).

Risk/benefit analysis: selection of prime non-oncological PARP indications

The evidence reviewed above and the list of indications where preclinical data show that PARP inhibitors can exert beneficial therapeutic effects are substantial, both in acute (Table 3) and chronic (Table 4) diseases (Figure 3). Data overviewed in this table (and further discussed in review articles (Virág and Szabo, 2002; Szabó, 2005; Pacher and

Table 3

The therapeutic potential of PARP inhibitors in acute non-oncological diseases. Overview of acute non-oncological indications where PARP inhibitors or PARP1 deficiency demonstrated benefit in *in vivo* preclinical studies

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
Myocardial infarction	Many classes of PARP inhibitors, as well as PARP1 deficiency exert protective effects, mainly in transient ischaemia models. The time window of intervention is substantial; the beneficial effects are multiple (reduction of infarct size, suppression of inflammation, prevention of later remodelling) is long-lasting. Efficacy data in large animals also support this indication.	PARP activation was demonstrated in circulating leukocytes in patients with myocardial infarction and therapeutic revascularization. PARP inhibition with INO-1001 in an exploratory Phase I trial showed a tendency to reduce biomarkers (CRP, IL-6).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> PARP inhibitors may be administered in single bolus or short infusion at the time of revascularization.	<input checked="" type="checkbox"/> Revascularization therapy improved acute outcomes (as long as the patient is rapidly transferred into a hospital). However, development of chronic heart failure later on represents a substantial challenge.	<input checked="" type="checkbox"/> Revascularization is effective. Although there were many preclinical and clinical efforts to combine revascularization with specific therapies to counteract reperfusion injury, no approved or widely used anti-reperfusion therapy is available.	<input checked="" type="checkbox"/> Small, exploratory trials, focusing on infarct size and indicators of reperfusion injury trials are feasible. However, large trials (registration endpoints, e.g. survival) require large number of patients and multicenter efforts.
Circulatory shock	Multiple classes of PARP inhibitors, as well as PARP1 deficiency exert protective effects, in models of shock induced by bacterial LPS, live bacteria, haemorrhage, burn injury and others. The time window of intervention is substantial; the beneficial effects include improvements in organ function and extension of survival. Efficacy data in large animal models also support this indication. Olaparib protects against renal and pulmonary dysfunction in a rodent model of circulatory shock.	In myocardial sections and circulating leukocytes of patients with circulatory shock, significant PARP activation was observed. The degree of PARP activation showed a close correlation with the degree of sepsis-induced myocardial dysfunction. There is also evidence of PARP activation in endothelial cells and leukocytes of burn patients.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> PARP inhibitors can be administered orally or parenterally at the time of diagnosis and to continue for several weeks.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Mortality rates of septic shock remain very high (30–50%).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> The current state-of-the-art is purely supportive; the only specific therapy (activated protein C) has been withdrawn for lack of efficacy.	<input checked="" type="checkbox"/> Small, exploratory trials, focusing on biomarkers are feasible. However, large trials (registration endpoints, e.g. survival) are expensive, complex and require large number of patients and multicenter efforts.
Stroke	Multiple classes of PARP inhibitors, as well as PARP1 deficiency exert protective	Activation of PARP was shown in brain sections from patients dying	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> PARP inhibitors may be administered in	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Revascularization therapy improved	<input checked="" type="checkbox"/> No approved or widely used anti-reperfusion or	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Small, exploratory trials are feasible. However,

continues

Table 3 (Continued)

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
Brain trauma	effects, both in transient and permanent ischaemia models. The time window of intervention is substantial; the beneficial effect is long-lasting. Efficacy data in non-human primates also support this indication. Olaparib and veliparib protect against NMDA-receptor activation induced neurotoxicity in an in vitro model of human neuron injury.	from stroke, brain ischaemia due to cardiac arrest.	single bolus or short infusion at the time of revascularization.	acute outcomes, but significant morbidity and mortality remains even with state-of-the-art therapy and rehabilitation.	neuroprotective therapy is available.	large trials (registration endpoint) are complex and expensive.
Brain trauma	Multiple classes of PARP inhibitors, as well as PARP1 deficiency exert protective effects in various brain trauma models. The therapeutic window is substantial. The beneficial effect is long-lasting as it also protects against the breakdown of the blood-brain barrier and prevents the later-onset neuro-inflammatory processes. Efficacy data in non-human primates also support this indication. Olaparib and veliparib protect against NMDA-receptor activation induced neurotoxicity in an in vitro model of human neuron injury.	Activation of PARP was shown in brain sections from patients dying from brain trauma. Increased PARylated proteins were also found in cerebrospinal fluid samples from adults and children with brain trauma. PARP1 polymorphisms were also identified: a PARP-1 polymorphism within the automodification-catalytic domain was associated with neurological outcome, while a polymorphism within the promoter region was associated with cerebrospinal fluid PAR-modified protein levels.	PARP inhibitors may be administered in single bolus or short infusion at the time of diagnosis or hospitalization.	Significant morbidity and mortality remains even with state-of-the-art therapy and rehabilitation.	No widely used or highly effective neuroprotective therapy is available.	Small, exploratory trials are feasible. However, large trials (registration endpoint) are complex and expensive.
Pancreatitis	The PARP inhibitor 3-aminobenzamide improves outcomes in several rodent models	No human data so far	PARP inhibitors can be administered orally or parenterally	Mortality rates of remain very high (30–50%).	The current state-of-the-art is largely supportive; antibiotics	Small, exploratory trials, focusing on biomarkers are readily

continues

Table 3 (Continued)

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
	of pancreatitis. PJ34 was tested in one study (cerulein model) and showed protective effects. PARP1 deficient mice are not protected in one cerulein-model of pancreatitis, but not in another one. The PARP inhibitor KU0058684 (which is structurally closely related to olaparib) has also demonstrated protective effects in a cerulein model. The therapeutic window of intervention has not yet been carefully explored.		at the time of diagnosis and to continue for several weeks.		may offer some therapeutic benefit.	feasible. Larger trials (registration endpoints) are more involved but nevertheless feasible.
Transplant rejection	Treatment of the donor organs and/or the recipient with PARP inhibitors of various structural classes improves organ function, extends transplant life and in some studies also reduced immune-mediated rejection.	Increased expression of PARP-1 in transplanted kidneys was detected: it was found to correlate with worsened outcome after transplantation.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered as part of the storage fluid or to the recipient (acutely or for longer duration to limit rejection).	<input checked="" type="checkbox"/> Outcomes have substantially improved in recent years; the main problem is the lack of availability of donor organs. If PARP inhibitors could extend the donor pool (e.g. render marginal transplants viable), this would be considered a substantial advance.	<input checked="" type="checkbox"/> Many effective therapeutic options are available for transplant rejection; less so for organ preservation.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Small, and larger trials are both feasible as single-centre or multi-centre efforts.
ALI	Multiple classes of PARP inhibitors, as well as PARP1 deficiency exert protective effects in a variety of ALI models (endotoxin, smoke inhalation, ischaemia-reperfusion, bacterial pneumonia, paraquat). The beneficial effect are multiple (suppression of	No human evidence so far.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> PARP inhibitors can be administered orally or parenterally at the time of diagnosis and to continue for several weeks.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Outcomes have improved in recent years with changes in the way supportive care is applied. Nevertheless, morbidity and mortality rates remain high.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> No specific, approved pharmaceutical therapies are available.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Small, exploratory trials, focusing on physiological parameters and/or biomarkers are feasible. Larger trials (registration endpoints) are more involved but nevertheless feasible.

continues

Table 3 (Continued)

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
	inflammation, improvement of gas exchange). Efficacy data in large animals also support this indication. Olaparib protects against pulmonary dysfunction in a rodent model of endotoxin-induced lung injury.					

In the right 4 columns, attractiveness/feasibility is shown in a graded fashion: indicates least attractive/feasible and indicates most attractive/feasible.

Table 4

The therapeutic potential of PARP inhibitors in chronic non-oncological diseases. Overview of chronic non-oncological indications where PARP inhibitors or PARP1 deficiency demonstrated benefit in *in vivo* preclinical studies

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
Chronic neuro-inflammatory diseases	Several studies, using several different models of neuroinflammation (including EAE) show the protective effect of PARP inhibitors, although some of the data are conflicting; in one study PARP1 deficiency was found to increase, rather than decrease disease severity. Olaparib and	In isolated PBMC relapsing–remitting untreated MS patients, increased PARP1 expression and increased PARylation was found.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> In many neuroinflammatory indications (e.g. MS) significant morbidity and mortality remains.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> In many neuroinflammatory indications (e.g. MS) therapeutic options are limited.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> The complexity and the duration of the trials is substantial.

continues

Table 4 (Continued)

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
	veliparib protect against NMDA-receptor activation induced neurotoxicity in an <i>in vitro</i> model of human neuron injury.					
Chronic neurodegenerative diseases	Several studies, using several different models of neurodegeneration (including the MPTP model of Parkinson's disease) show the protective effect of PARP inhibitors. PARP1 deficiency is also protective. PARP inhibition protects against dopamine neuron death in AIMP2 transgenic mice. In a Drosophila model of PARKIN induced neurodegeneration, mutation of PARP1 protects against dopaminergic neurodegeneration.	Poly(ADP-ribose)ylation in brain sections from patients with Alzheimer's disease, Parkinson's disease and ALS. A PARP1-410C/T polymorphism was detected in Sicilian patients with Parkinson's disease and several other PARP1 polymorphisms have also been detected in other patient populations with Parkinson's disease.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Significant morbidity and mortality remains.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> In many neurodegenerative indications (e.g. Parkinson's) therapeutic options are limited.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> The complexity and the overall design of the trials does not represent a substantial hurdle, but the duration of the trials is substantial.
Chronic inflammatory and autoimmune diseases	PARP inhibitors exert protective effects in various models of arthritis, colitis, uveitis, and other models of local inflammation. In some cases, PARP1	Auto-antibodies against PARP in arthritis, SLE and other autoimmune diseases. Increased poly(ADP-ribose)ylation was found in biopsies from patients with colitis.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> In many local inflammatory diseases (e.g. arthritis) outcomes have substantially improved in recent years.	<input checked="" type="checkbox"/> In many local inflammatory diseases, state-of-the-art and several therapeutic options are available (e.g. arthritis).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> In many local inflammatory diseases, trial designs are well established and feasible.

continues

Table 4 (Continued)

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
	deficient systems have also been evaluated and they also afford protective effects. Olaparib protects against pulmonary inflammation in an allergen-induced pulmonary inflammation model.					
Vascular diseases	PARP inhibitors and/or PARP1 deficiency has been shown to improve vascular contractile and/or relaxant function in vascular dysfunction induced by circulatory shock, diabetes mellitus, atherosclerosis, and physiological ageing.	Activation of PARP has been shown in human atherosclerotic samples and in microvessels present in skin biopsies from type 2 diabetic patients.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> Outcomes have substantially improved in recent years.	<input checked="" type="checkbox"/> Many effective therapeutic options are available.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Small, exploratory trials, focusing on markers or functional endpoints are readily feasible. Larger trials are more involved but nevertheless feasible.
Chronic heart failure	Multiple classes of PARP inhibitors, as well as PARP1 deficiency exert protective effects in multiple models of heart failure (mechanically induced, chemically induced, diabetes-associated). PARP inhibitors suppress pathological myocardial remodelling.	Increased poly(ADP-ribose)ation, as well as increased PARP1 expression was demonstrated in human heart samples in end-stage heart failure and in heart failure followed by left ventricular heart assist device implantation.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> 5-year mortality rates remain very high.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> None of the therapies used currently are efficacious to extend survival.	<input checked="" type="checkbox"/> Large trials (registration endpoints, e.g. survival) require large number of patients and multicenter efforts.
Type I diabetes mellitus	Various PARP inhibitors suppress the onset and severity of streptozotocin-induced Type I diabetes by preventing pancreatic beta cell necrosis. PARP inhibition also prevents spontaneous and recurrent autoimmune diabetes in NOD mice.	No human evidence so far.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Outcomes have not improved in recent years.	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> No therapies are available to prevent or delay the autoimmune beta-cell destruction.	<input checked="" type="checkbox"/> Large trials are necessary to seek demonstration of efficacy; such trials are complex, lengthy and expensive.

continues

Table 4 (Continued)

Disease	Effect of PARP inhibitors in preclinical models	Human evidence for PARP activation	Short duration of drug therapy?	Indication severe?	Therapeutic options limited?	Trial design feasible?
Chronic liver diseases	PARP inhibitors protect against various forms of chronic liver injury (alcohol-induced, steatosis-induced). PARP inhibition suppresses fatty liver disease. PARP inhibition protects against liver fibrosis development.	Increased PARP activation and/or PARP1 expression in human livers from hep B and alcohol-induced cirrhosis, NASH, and ASH.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> This is a heterogeneous category, but there are many diseases in this category (e.g. liver fibrosis) where morbidity and mortality remains high.	<input checked="" type="checkbox"/> This is a heterogeneous category, but in some cases (e.g. liver fibrosis) all of the existing therapeutic options remain only marginally effective.	<input checked="" type="checkbox"/> Both smaller and larger trials are feasible. Trials with meaningful endpoints are long in duration.
Chronic pulmonary diseases	In multiple models of asthma, PARP inhibitors (including olaparib) exert protective effects, including functional parameters and reduced inflammatory mediator production. Hyperoxia-induced lung injury and bleomycin-induced fibrosis have also been shown to be attenuated by PARP inhibitors.	Increased poly(ADPribose)ylation was found in peripheral blood lymphocytes from patients with chronic obstructive pulmonary disease (COPD). Increased poly(ADPribose)ylation was also demonstrated in PBMCs and lung tissues from asthmatic patients.	<input checked="" type="checkbox"/> PARP inhibitors would be expected to be administered chronically (years – life-long).	<input checked="" type="checkbox"/> This is a heterogeneous category, but there are many diseases in this category (e.g. lung fibrosis) where morbidity and mortality remains high.	<input checked="" type="checkbox"/> This is a heterogeneous category, but in some cases (e.g. lung fibrosis) all of the existing therapeutic options remain only marginally effective.	<input checked="" type="checkbox"/> Both smaller and larger trials are feasible. Trials with meaningful endpoints are long in duration.

In the right 4 columns, attractiveness/feasibility is shown in a graded fashion: indicates least attractive/feasible and indicates most attractive/feasible.

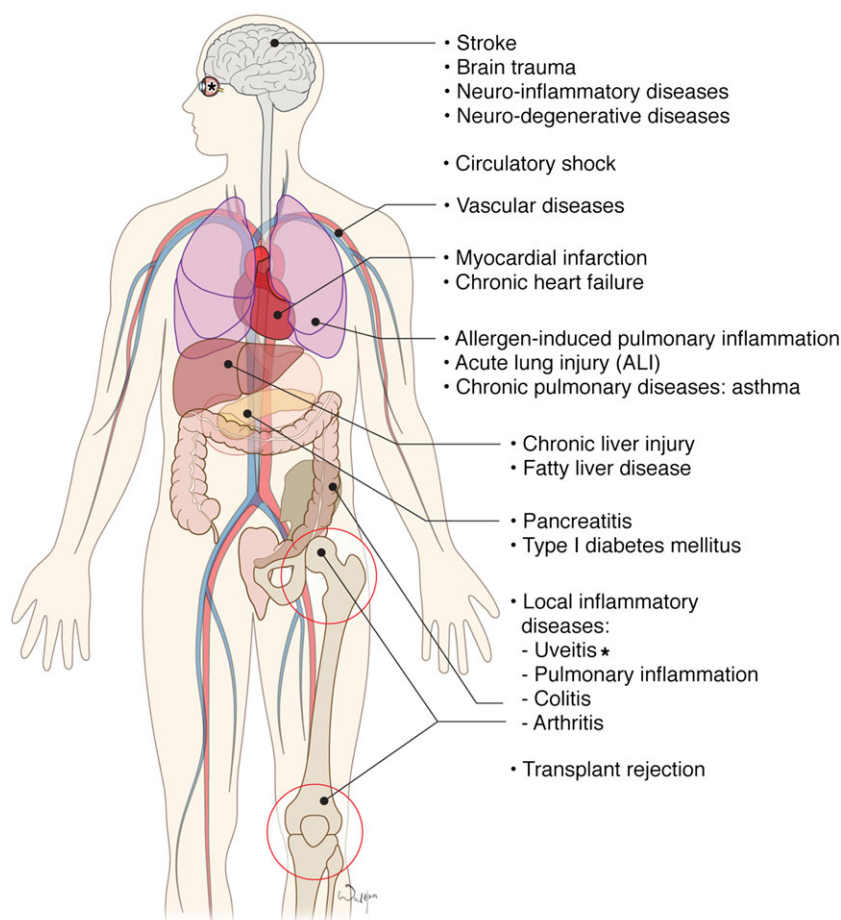


Figure 3

Pathogenic role of PARP1 in various non-oncological diseases. See Tables 3 and 4 for additional details.

Szabo, 2008; Curtin and Szabo, 2013; García and Conde, 2015) and other papers (Mota *et al.*, 2005, 2007; Yu *et al.*, 2012; Lee *et al.*, 2013; Li *et al.*, 2013; Mukhopadhyay *et al.*, 2014, 2017; Stoica *et al.*, 2014; Ghonim *et al.*, 2015a,b; Rom *et al.*, 2015; Tao *et al.*, 2015; Ibba *et al.*, 2016; Lehmann *et al.*, 2016; Lucarini *et al.*, 2016; Salluzzo *et al.*, 2016; Wang *et al.*, 2016; Yu *et al.*, 2016; Zhang *et al.*, 2016; Gariani *et al.*, 2017) support the view that PARP inhibitors may be therapeutically advantageous for a variety of non-oncological indications, if the selection of these indications is supported by appropriate risk/benefit analysis. The ancient Hippocratic oath (*Primum nil nocere*) calls, first and foremost, for doing no harm. While the risk of increased chromosomal instability – as predicted by the various *in vitro* assays discussed earlier (e.g. Ito *et al.*, 2016; Lee *et al.*, 2016) – must be kept in mind, below, we outline our method aimed at striking a balance between the expected benefits and potential risks associated with PARP inhibitor therapy for non-oncological indications.

Olaparib (Lynparza) – similar to a host of other PARP inhibitors that are currently in late-stage clinical trials – has gone through the expected battery of preclinical safety studies, as well as the early stage human safety studies, even though the required preclinical ‘safety package’ for oncological indications is smaller than for many other

disease indications. The product label for Lynparza states that the drug ‘was clastogenic in an *in vitro* chromosomal aberration assay in mammalian CHO cells and in an *in vivo* rat bone marrow micronucleus assay’ and that it ‘was teratogenic and caused embryo-fetal toxicity in rats at exposures below those in patients receiving the recommended human dose of 400 mg twice daily’. Nevertheless, even with the above preclinical toxicity profile, olaparib appears to be well tolerated in cancer patients, nausea and occasional neutropenia being some of the most common side effects (e.g. Kaye *et al.*, 2012; Bixel and Hays, 2015; Bao *et al.*, 2016; Domchek *et al.*, 2016; Ledermann *et al.*, 2016; Leichman *et al.*, 2016; Yonemori *et al.*, 2016). Nevertheless, it must be noted that – to our knowledge – no studies have examined the effect of olaparib on DNA integrity or chromosomal stability in humans. In addition, to our knowledge, the PARP1-dependent versus PARP1-independent cellular actions of olaparib (e.g. on DNA stability or cellular viability) have not yet been thoroughly investigated, e.g. by comparing the effect of the drug in wild-type vs. PARP1-deficient cell systems.

One way to put the potential side effects of PARP inhibitors into perspective is by comparing their effects with other drugs currently used for non-oncological indications.

For instance, Lee *et al.* (2016) compared the effect of olaparib and veliparib on chromosomal stability with several classes of drugs, including methotrexate (currently used, in a chronic regimen, in the therapy of arthritis and psoriasis). Methotrexate (tested at 20 μM) increased chromosomal instability to a comparable degree as olaparib and veliparib (tested at 5–10 μM) (Lee *et al.*, 2016). It should also be mentioned that many commonly used drugs have genotoxic effects on various standard toxicological tests (e.g. prazoles, salazines, olmesartan, theobromine) (Brambilla and Martelli, 2006; Brambilla *et al.*, 2010; Brambilla *et al.*, 2013). Examples where many of the currently used drugs (including methotrexate, but also some of the immunotherapies) are known to increase the risk of oncological diseases are chronic rheumatoid arthritis and psoriasis (Solomon *et al.*, 2014; Van Lümig *et al.*, 2015).

Based on the above considerations, one can conclude that genotoxicity (or a risk of genotoxicity) alone should not be a reason to exclude a study drug for testing in a non-oncological indication. Another way of looking at the question is to consider the alternative therapeutic options. Would one be willing to test a novel, potentially life-saving therapeutic intervention – even when there is some risk of potential adverse events down the line – if no other drugs were available for the therapy of that particular disease? Consider the parallel with cancer therapy, where therapeutic successes come at a price of increased incidence of malignant diseases in later life (Bhatia and Sklar, 2002; Armstrong *et al.*, 2014).

Based on the above considerations, we conclude that the risk of genotoxicity, alone, should *not* be sufficient reason to exclude a study drug such as a PARP inhibitor for testing in a non-oncological indication. On the other hand, it is imperative to minimize the risks and maximize the benefits by identifying the most suitable indications. What common-sense criteria, then, should be established for our selection process? First of all, there should be preclinical data demonstrating the efficacy of PARP inhibition in clinically relevant preclinical models of disease (see also below). Second, it would be preferred if the selected indication had *human* data to confirm the activation of PARP in the target organ. Third, the duration of treatment with the PARP inhibitor should be as short as possible, in order to limit potential side effects. Fourth, the selected indication should be severe enough to justify an attempt for novel therapies, especially in light of the potential genotoxic ‘baggage’ that comes with PARP inhibition. Fifth, the existing therapeutic alternatives should be insufficient. Finally, the proposed clinical trial(s) should be logistically feasible.

Based on all of the above criteria (Tables 3 and 4), indications where the PARP inhibition would be given chronically and where various therapies (albeit often of insufficient efficacy) already exist (e.g. chronic local inflammatory diseases, vascular diseases, many forms of liver and pulmonary diseases) should be deprioritized. Myocardial infarction – even though it is supported by a vast body of preclinical data and even a small-scale clinical study with INO-1001 (Morrow *et al.*, 2009) – should also be deprioritized, given the fact that most patients who are transported in time into qualified clinical centres are doing reasonably well with the current standard of therapy.

Transplant rejection should also be initially excluded, because of the availability of drugs, which are effective in controlling transplant rejection. Type I diabetes should be excluded at the present stage: the logistics of diabetes prevention trials is very challenging; also, there was a lack of efficacy (Skyler and Type 1 Diabetes TrialNet Study Group, 2008; Simmons and Michels, 2014) of the nicotinamide-based prevention trials conducted over the last two decades.

Thus, the acute indications, where we recommend repurposing and where the duration of the PARP inhibitor therapy would be expected to be short, are the following: acute ischaemic stroke and acute severe TBI (two acute neurological indications), septic shock, haemorrhagic shock, acute pancreatitis and severe ALI (also termed ARDS). In all of these conditions, many lines of preclinical data support the efficacy of PARP inhibition; the duration of treatment would be expected to be relatively short (days to weeks), the severity of the disease is high, the available specific therapeutic options are limited (stroke, ALI) or, in essence, non-existent (circulatory shock, pancreatitis). The indication of acute brain injury may be expanded to also include acute spinal cord injury (e.g. in the context of trauma or thoracoabdominal aortic cross-clamping); the indication of circulatory shock may be expanded to other forms of shock, where PARP inhibition has shown significant benefit in preclinical studies (e.g. haemorrhagic shock, a critical condition with high mortality, and so far, no treatment except from transfusions, and damage control). Haemorrhagic shock – in addition to stroke and TBI – may be prototypical indications for first-responder/in-ambulance trials, because these events occur outside the hospital, and the condition of the patients rapidly deteriorates during their transit time to hospital care. One final group of indications relates to the potential use of these agents to attenuate the organ damage induced by chemotherapeutic agents (Ali *et al.*, 2011; Kim and Padanilam, 2011; Mukhopadhyay *et al.*, 2011).

In addition to the acute indications discussed above, there are also a number of severe, debilitating, chronic indications that represent a high unmet need with no alternative therapeutic options (e.g. Parkinson’s disease, multiple sclerosis or severe, untreatable forms of metabolic diseases including fibrotic liver, lung and kidney disease), which remain candidates for urgent repurposing, even though the administration of the PARP inhibitor would have to be chronic, and this may be associated with a risk of genotoxicity. In these indications, the currently available therapeutic options are extremely limited and of marginal efficacy. For indications of this type, treatment with PARP inhibitors may be considered in an intermittent fashion (i.e. with ‘drug holidays’). Alternatively, a lower dose of the PARP inhibitor may be attempted. In chronic studies, evaluation of the effects of the PARP inhibitor on DNA integrity may be incorporated into the trials and/or may be incorporated into the routine (e.g. annual) check-up of these patients. Perhaps chronic PARP inhibitor trials may be delayed until more clinical data are available with PARP inhibitors in oncological indications and in acute non-oncological indications. Such data (including, for instance, the presence or absence of secondary cancer development in cancer survivors treated with PARP inhibitors) may guide the decision with respect

to chronic PARP inhibitor trials. Once sufficient safety data are available in the most severely affected patient populations, additional indications may be considered as well. For instance, one may consider testing low-dose PARP inhibitor therapy in patients with mild-to-moderate or repeat TBI, in order to prevent the onset of subsequent neuroinflammatory or neurodegenerative processes.

We are well aware of the fact that many of disease indications proposed above are viewed as 'pharmaceutical graveyard' indications where some very spectacular therapeutic failures (e.g. Opal and Cross, 1999; Moretti *et al.*, 2015; Gruenbaum *et al.*, 2016; Standiford and Ward, 2016) have been encountered over recent decades, but there are also important lessons learned from these failed trials that could be used in the design of future trials. We are also aware of the fact that many of these diseases represent significant unmet medical needs, as well as considerable markets that should make them attractive for development by large pharmaceutical companies. As all PARP inhibitors currently in clinical development are novel chemical entities, the intellectual property status covers both oncological and non-oncological diseases. In addition, with positive data in non-oncological indications, label extension as well as additional intellectual property may be created.

The next steps

What, then, are the logical next steps in the field? Are there any activities necessary prior to embarking on clinical trials (with olaparib or rucaparib, or with other PARP inhibitors that are expected to be approved for oncological indications in the future)? First of all, it would be advantageous to generate preclinical efficacy data with the PARP inhibitor that is selected for the clinical trial (rather than relying on data with earlier classes of PARP inhibitors), because one must appreciate the fact that these compounds were primarily designed (and optimized) for cancer therapy, where cytotoxicity (mechanism-based or perhaps mechanism-independent, or a combination of the two) may be acceptable. In the context of repurposing them for non-oncological indications, however, we expect to use the same drugs to elicit cytoprotective and/or anti-inflammatory therapeutic actions. The preclinical studies should utilize clinically relevant models (typically, large animal models are considered more clinically predictable than rodent models), should include several dose levels (that are reflective of the human doses) and should include animals from both sexes and – if it reflects better the targeted patient population in the planned clinical studies – even aged animals or animals on the background of pre-existing cardiovascular disease. Such animals are known to be more sensitive to injury than young and healthy animals, and it is likely that the pathophysiological pathways involved in their organ injury processes are not only quantitatively but also qualitatively different (Coletta *et al.*, 2014; Starr and Saito, 2014; Ungvari and Sonntag, 2014).

Currently, the clinically approved PARP inhibitors are olaparib (Lynparza) and rucaparib (Rubraca), but once additional inhibitors are available, the choice of the

compound will become important. Although the primary mode of all PARP inhibitors is identical (binding into the active site of PARP1 and thus inhibiting the binding of NAD^+ , preventing its cleavage to an ADP-ribosyl unit and nicotinamide plus a proton, thereby blocking the formation of polyADP-ribose), not all PARP inhibitors are 'created equal'. They can have variable inhibitory effects on the PARP isoforms other than PARP1, some of them may be more prone to trapping PARP1 at the DNA replication forks than others, some of them may have mechanism-independent actions (e.g. cytotoxicity), some of them have more off-target effects on kinases than others, some of them have more inhibitory activity on members of the PARP enzyme family in addition to PARP1 (Wahlberg *et al.*, 2012; Antolín and Mestres, 2014; Pommier *et al.*, 2016). For non-oncological indications, the safety profile of PARP inhibitor would be expected to be better if it had less PARP trapping activity and less mechanism-independent cytotoxicity.

The issue of PARP1 inhibition versus effects on other PARP isoforms requires some additional discussion. Olaparib inhibits PARP1 with the highest potency, but it also inhibits PARP2 and other PARP isoforms, while veliparib is a selective inhibitor of PARP1 and PARP2 (and not of other PARP isoforms) (Knezevic *et al.*, 2016; Thorsell *et al.*, 2017). Although PARP1 plays a major role in the pathogenesis of the various non-oncological diseases discussed in the current review (shown by the protection seen in PARP1-deficient cells or animals), other PARP isoforms (most notably PARP2) may also contribute to the pathogenesis of some non-oncological diseases (Popoff *et al.*, 2002; Kofler *et al.*, 2006; Li *et al.*, 2010; Kamboj *et al.*, 2013). Thus, inhibition of PARP2 (and perhaps other PARP isoforms) is likely to contribute to the efficacy of PARP inhibitors. At the same time, effects on PARP2 (or other PARP isoforms) may also affect the safety profile of the PARP inhibitor. As there are still many unknowns with respect to the roles of the minor PARP isoforms in the pathogenesis of both oncological and non-oncological diseases, from the (practical) standpoint of repurposing, we recommend that we view this issue empirically and should focus on the relevant endpoints (i.e. safety of efficacy of olaparib and veliparib) in various non-oncological diseases.

Not only the presence of the therapeutic effect of a PARP inhibitor should be established in non-oncological indications, but the magnitude of this effect and the time window of administration are also very important, in comparison with, or on the background of state-of-the-art therapies (when available). The efficacy of the PARP inhibitor should be comparable or better than any other therapies (or potential development candidates), targeting different pathways or mechanisms of the same disease. All of these issues should be carefully considered in relevant preclinical models of disease, which should be performed to the highest scientific rigour including state-of-the-art blinding and randomization.

In this respect, it is encouraging that the existing body of data (with PARP1-deficient mice and with various classes of PARP inhibitors) shows marked efficacy in the indications we recommend for repurposing (Tables 3 and 4); the preclinical body of efficacy data include large animal data in

state-of-the-art, translationally relevant models of stroke (Matsuura *et al.*, 2011), acute neurological injury (Maier *et al.*, 2007), circulatory shock (Goldfarb *et al.*, 2002) and ALI (Iványi *et al.*, 2003; Shimoda *et al.*, 2003; Murakami *et al.*, 2004). However, there are currently no reports with PARP inhibitors in large animal models of acute brain injury, pancreatitis or Parkinson's disease. Large animal studies – or whatever animal model is considered the *most clinically predictable* for the disease indication in question – remain to be confirmed, *specifically with the choice compound* selected for human therapeutic repurposing.

In addition to efficacy endpoints, the preclinical studies with the PARP inhibitor(s) selected for therapeutic repurposing should also incorporate evaluation of multiple parameters of DNA injury and chromosomal integrity, for instance, in circulating leukocytes and in relevant parenchymal tissues.

Another, more long-term line of thought – in analogy with the BRCA/cancer indications for PARP inhibitors – relates to the identification of potential subsets of patients, where PARP inhibitors would be expected to be most efficacious. For instance, Daemen and colleagues have recently developed an algorithm to predict responsiveness of cancer cells to olaparib therapy, based on the transcriptional levels of seven genes involved in DNA repair pathways (Daemen *et al.*, 2012) and development of similar predictive algorithms may also be feasible for non-oncological diseases.

Once the clinical indication selection has been finalized, optimal clinical trial design should be implemented, considering issues such as dose selection, timing of administration and concomitant therapies. In addition to the carefully selected endpoints, pharmacokinetic and pharmacodynamic endpoints (to confirm target engagement) should be incorporated. Earlier trials have used measurement of PARP activity (Wang *et al.*, 2015) and/or measurement of PARP-dependent phosphorylation of histone H2AX in PBMCs *ex vivo* (Gojo *et al.*, 2017) or the ability of patient plasma to inhibit PARP activity *ex vivo* (Morrow *et al.*, 2009) to demonstrate that an effective dose of the inhibitor had been achieved. Instead of PBMCs, PARP activity in plucked eyebrow-hair follicles has also been measured (Fong *et al.*, 2009). When patient tissues are accessible, lowering of cellular NAD⁺ levels may also be potentially suitable to assess the degree of PARP activation and the effect of PARP inhibitors to restore NAD⁺. As with to the proposed preclinical studies, the clinical studies should also evaluate the effects of the PARP inhibitor on DNA and chromosome integrity.

In view of the importance of the BRCA/PARP interrelationship in DNA repair, should we consider excluding patients who harbour BRCA mutations (meaning that all potential study subjects would have to undergo rapid BRCA testing prior to the first dosing with the PARP inhibitor)? Considering that BRCA mutant subjects in their somatic cells are heterozygote BRCA mutants (which means that their DNA repair is not compromised) and the BRCA homozygote genotype, which can only be found in cancer cells, produces a loss-of function mutation, heterozygous BRCA mutation does not confer HRR deficiency and sensitivity to PARP inhibitors – as demonstrated in several studies including experiments in BRCA2^{+/-} mice treated with

olaparib (Drew *et al.*, 2011; Kobayashi *et al.*, 2013; Lord and Ashworth, 2016), exclusion of BRCA mutant subjects for PARP inhibitor trials for non-oncological diseases does not appear to be scientifically justified. At the same time, one should consider excluding patients with other (BRCA-unrelated) known forms of hyperresponsiveness to DNA damage. What should also be examined, in future clinical trials, is whether the DNA integrity in the BRCA mutant subjects was affected differently from that in the rest of the patient population. However, meaningful data on this question can only be expected from fairly large trials, because the incidence of this mutation in the general population is only about 0.3%.

As far as additional inclusion and exclusion criteria are concerned: females of childbearing potential enrolled into the trial would have to undertake contraception. Male subjects in the trial should also use rigorous contraception. In the acute indications we propose for the first wave of repurposing, the severity of the indications is such that sexual activity of the subjects is unlikely. This issue would be more relevant in some of the first-line chronic indications such as Parkinson's disease or fibrotic diseases. For safety reasons, initially, the trials should be conducted in adult populations, rather than paediatric or mixed populations.

The issue of sex difference of the protective effect of PARP inhibitors (as discussed earlier) presents an additional challenge of trial design. Because sex difference has not been consistently seen in *all* preclinical studies, and because the protective effect of PARP inhibitors can be reconstituted in ovariectomized animals (Mabley *et al.*, 2005) that model postmenopausal female patients, the clinical trial should incorporate patients from both sexes, but should be powered sufficiently such that statistical power remains high enough after stratification into three groups: males, premenopausal females and postmenopausal females. The potential effect of hormonal contraception in females on the outcome should also be considered. *Post hoc* analysis may subsequently evaluate the efficacy of PARP inhibitor therapy, based, for instance, on disease subtype, disease severity, co-morbidities and the presence or absence of concomitant therapies.

This analysis has, for the most part, focused on repurposing for acute indications. However, we should keep in mind that for the therapy of any patients with serious diseases, there is the consideration of the quality of life and the residual life expectancy and what balance the patient wishes. As discussed above, these choices are made on a daily basis in oncology, and considering many diseases have no disease modifying or preventative treatments (e.g. devastating neurodegenerative or neuroinflammatory diseases, or chronic metabolic diseases where the prognosis is poor and no therapeutic alternatives are known), we believe that these options should not be discarded for fear of a bad outcome, but investigated, monitored and addressed. Industry-sponsored trials versus investigator-initiated trials may be more feasible with different endpoints and may also present with different sets of challenges (Table 5). Although the complexities of testing PARP inhibitors for non-oncological indications are quite substantial, it is hoped that this review – which incorporates

Table 5

Potential clinical development pathways for repurposing PARP inhibitors for non-oncological diseases

	Industry path	Academic path
Mechanism	Company-sponsored trials	Investigator-initiated trials
Scale, Approach	Small, mid-size, large trials. Large trials, targeting the largest commercial indication are feasible. Complex trial designs can be implemented.	Small, mid-size trials; less complex design, more straightforward endpoints. (But NIH, VA is well suited to run even large-scale trials.)
Results	Registration endpoints completed, Data published, Regulatory approval obtained; on-label use of the PARP inhibitor.	Meaningful clinical endpoints completed, Data published; the results change of clinical practice, off-label use of the PARP inhibitor.
Pharmaceutical marketing after successful trials	Permitted for on-label use	Not permitted for off-label use
Challenges	Attract industry interest to consider non-oncological indications; consider trials in historically challenging indications	Obtain support (e.g. grants) for the trial; clinical trial infrastructure can be a challenge in an academic environment
Commercial considerations	The indication must be commercially attractive for the company	Minimal
Potential downsides	Trial may not show efficacy; many SAEs are expected in the trials because of the underlying disease; relatedness can be difficult to ascertain; SAEs noted in the trial endanger the already existing (approved) oncology indications	Trial may not show efficacy; unexpected adverse events in patients
Most suitable indications	Initial smaller-scale trials, followed by large, multi-centre trials are feasible. Indications which require large patient numbers and complex trial designs and survival endpoints (e.g. septic shock and, stroke studies). Chronic studies (e.g. Parkinson's disease). All other indications (ALI, TBI, pancreatitis) are also feasible.	Smaller, single-centre trials are more feasible. Indications where signs of efficacy may be obtained in smaller trials (e.g. pancreatitis, ALI); focused trials of TBI, exploratory trials in septic shock focusing on biomarker endpoints.

the collective knowledge and scientific and medical judgement of many investigators active in the field of PARP – will stimulate renewed interest and further preclinical and clinical research and development activity in this area.

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Author contributions

All authors contributed to writing and editing the paper and are listed in alphabetical order. C.S. prepared the first draft of the manuscript, coordinated the completion of the work with all co-authors and finalized the paper.

Conflict of interest

The authors declare no conflicts of interest.

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