Inhibition of polyADP-ribosylation in cancer: Old and new paradigms revisited.

Inhibition of poly(ADP-ribosyl)ation in cancer: Old and new paradigms revisited

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Abstract

Inhibitors of poly(ADP-ribose) polymerases actualized the biological concept of synthetic lethality in the clinical practice, yielding a paradigmatic example of translational medicine. The profound sensitivity of tumors with germline BRCA mutations to PARP1/2 blockade owes to inherent defects of the BRCA-dependent homologous recombination machinery, which are unleashed by interruption of PARP DNA repair activity and lead to DNA damage overload and cell death. Conversely, aspirant BRCA-like tumors harboring somatic DNA repair dysfunctions (a vast entity of genetic and epigenetic defects known as “BRCAness”) not always align with the familial counterpart and appear not to be equally sensitive to PARP inhibition. The acquisition of secondary resistance in initially responsive patients and the lack of standardized biomarkers to identify “BRCAness” pose serious threats to the clinical advance of PARP inhibitors; a feeling is also emerging that a BRCA-centered perspective might have missed the influence of additional, not negligible and DNA repair-independent PARP contributions onto therapy outcome. While regulatory approval for PARP1/2 inhibitors is still pending, novel therapeutic opportunities are sprouting from different branches of the PARP family, although they remain immature for clinical extrapolation. This review is an endeavor to provide a comprehensive appraisal of the multifaceted biology of PARPs and their evolving impact on cancer therapeutics.

Keywords

Poly(ADP-ribose) polymerase; BRCAness; Tankyrase; DNA repair; Synthetic lethality; Targeted cancer therapies

1. Introduction

When the poly(ADP-ribose) polymerase (PARP) superfamily was discovered, back in the early 1960s [1], it was hardly predictable that abundant and rather elementary molecules, namely poly(ADP)ribose (PAR) units, would drastically impact upcoming scientific research.

The PARP superfamily encompasses at least 17 enzymes involved in several biological processes, including transcriptional regulation, DNA repair, cell cycle regulation, inflammation, hypoxic response, spindle pole function, oncogene-related signaling, and cell death [2], [3] and [4]. As some PARP family members catalyze mono- rather than poly(ADP)ribosylation, a more accurate nomenclature has been recently proposed that renames PARPs as ADP-ribosyltransferases diphtheria toxin-like (ARTD) [5]. PARP1 (ARTD1) is the founding member as well as the most abundant and best-characterized protein within this large family. Although historically reviewed as a DNA repair enzyme [6], PARP1 is being increasingly implicated in cell homeostasis at several levels, including transcriptional and epigenetic mechanisms [3]. PARP1's closest relative, PARP2 (ARTD2), displays almost overlapping functions [7]. Outside the archetypal PARP1 and PARP2, latest reports have implicated PARP3 (ARTD3) in DNA repair, mitotic spindle integrity, and telomerase regulation [8] and [9]. PARP4 (ARTD4) is part of a cytoplasmic ribo-nucleoprotein structure, known as the vault complex, whose function is uncertain, although it has been related to multidrug resistance in some cell lines [2]. PARP5A (ARTD5) and PARP5B (ARTD6), also referred to as tankyrase 1 (TNKS) and tankyrase 2 (TNKS2), stand out as founders of an alternative branch of the PARP *summa*, involving telomere and mitotic spindle-related functions, as well as regulation of the Wnt signaling. Consequently, this latter line of investigation is now being

inflated with high clinical expectations [10]. Other PARPs have been less extensively studied, with initial information attesting to their potential role as effectors in cell division, cell viability, and transduction of cytoskeletal cues [10] and [11].

Historically, PARP couples with BRCA. Germline inactivation of BRCA1/2 tumor suppressor genes strongly predisposes to breast and ovarian cancer as well as other malignancies, including pancreatic and prostate cancer [12] and [13]. Tumor suppressors are hardly accessible to pharmacologic interception; however, the concept of synthetic lethality (broadly referred to as non-oncogene addiction) has outlined an intriguing avenue for targeting tumor suppressor defects [14]. Two genes are synthetically lethal if depletion of either alone is viable whereas concurrent loss results in cell death [15]. This notion has been epitomized and successfully extrapolated to the clinical practice by PARP inhibitors, which are effective in BRCA-related tumors due to sabotage of functionally interdependent DNA repair routes that ultimately leads to cellular toxicity [16].

It is expected that a broader spectrum of tumors, displaying inherent or acquired DNA repair dysfunction regardless of the leading genetic lesion, exhibit a BRCA-like behavior and benefit from PARP-targeted strategies [17]. After almost one decade of eager investigation and high expectations, enthusiasm has been tempered by disappointing trial outcomes, wherein “BRCAness” could not obviously predict favorable responses [18]. Therefore, upgrading PARP inhibitors beyond experimental use seems to be facing many roadblocks. Lagging behind, new drug development efforts are chasing additional members of the PARP superfamily, namely tankyrases, which may hold a clinical value as integral components of several cancer-related pathways [10]. In this review, we focus on fortune and downfalls of PARP-directed approaches and underscore emerging lines of investigation, including and beyond the archetypal PARP-DNA repair duo.Table 1 summarizes recently completed and ongoing trials with first- and last-generation PARP inhibitors, on which we will touch in the following sections.

2. Rationale for PARP-targeted therapeutics

2.1. DNA repair mechanisms

A complex network of molecular players is allocated for preserving genomic integrity from a myriad of endogenous and environmental insults. DNA single-strand breaks (SSBs) are repaired by the base-excision repair (BER), the nucleotide-excision repair (NER) or the mismatch repair (MMR) pathways, whereas double-strand breaks (DSBs) engage either homologous recombination (HR) or non-homologous end joining (NJEJ) [19]. The BER machinery recognizes single-base lesions resulting from oxidation, alkylation and deamination; it involves a large number of enzymes, including DNA glycosylases, APE1, ERCC1, DNA polymerase beta, DNA ligase 3, and PARP1 itself [20]. NER is activated by helix-distorting lesions and is the main pathway for the repair of UV-induced DNA alterations [19]. MMR removes replication errors, *i.e.*mismatched nucleotides and little insertion/deletion loops. Defects in this pathway result in high mutation rate and cancer, including hereditary non-polyposis colorectal cancer [19].

DSBs are more threatening, because the complementary strand is not always available for faithful repair. The HR pathway is operative exclusively in the S and G2 phases of the cell cycle, where the accessibility of a homologue template, usually a sister chromatid, ensures accurate DNA repair. It starts with binding of the MNR complex (MRE11/RAD50/NBS1) to DSBs, followed by recruitment of CtIP and activation of MRE11-mediated DSB resection [21]. The resultant ssDNA is then bound by RAD51, a very critical step which ultimately leads to template-dependent DNA synthesis. BRCA1 and BRCA2 play a role in RAD51 loading, together with other HR components, such as ATM, H2AX, PALB2, RPA, RAD52, and proteins of the Fanconi anemia pathway [22]. Unlike HR, NHEJ is operative throughout the cell cycle and mediates direct ligation of broken DNA ends without requiring a template DNA [23]; therefore, it is highly mutagenic

2.2. PARP1 in DNA damage repair

Huge efforts have been devoted to elucidate the role of PARP1 and PARP2 in DNA repair [24], with higher emphasis on PARP1 as it accounts for the vast majority of PAR synthesis [25]. It is well-established that, following DNA damage, PARP1 senses and binds to DNA strand interruptions, whereby it becomes catalytically activated. Using NAD^+ as a substrate, PARP1 catalyzes the attachment of PAR polymers onto acceptor nuclear targets, including transcription factors, histones, and PARP1 itself [3]. Besides relaxing chromatin structure, this post-translational modification results in reduced affinity and consequent PARP1 dissociation from DNA, allowing for access to an orchestrated network of repair enzymes [26] and [27]. Although PARP1 has long been annotated as a BER effector by assisting the assembly of a core-complex comprising XRCC1, Polβ, and DNA ligase III [27] and [28], recent data propose an indirect mechanism, according to which PARP1 activity would be dispensable for BER sheer execution, and would be rather engaged to seize potentially detrimental SSB intermediates and to promote their resolution [29]. Recently, PARP1 contribution to SSB repair has also been extended to MMR and NER [30] and [31]. Besides its canonical role in sensing SSBs, PARP1 was reported to bind to and assist the repair of additional damaged DNA structures, including stalled replication forks and DSBs, influencing both HR and NHEJ [32],[33] and [34].

Overall, these findings emphasize the complexity of PARP-related DNA repair routes and rationalize the relevance of this enzyme in the safeguard of genome stability.

2.3. BRCA1/2 in DNA repair

BRCA1 is involved in multiple steps of HR-directed DNA repair. It binds to and enters phosphorylated CtIP into the damaged site, resulting in MRE11-mediated resection [35]. Moreover, it interacts with PALB2 to engage the BRCA1–PALB2–BRCA2 complex [36]. Then, BRCA2 directly binds and recruits RAD51 onto the ssDNA resected end, which is propaedeutic for the ensuing recombination events [37]. Besides coordinating HR execution, BRCA1 plays a role in both the G1/S and G2/M cell cycle checkpoint regulation in response to DNA damage, again preserving genomic integrity [38]. In keeping with these notions, germline mutations of BRCA1/2 aggravate genetic instability, thereby increasing the lifetime risk of developing breast, ovarian, prostate, and pancreas cancers [13].

2.4. Supporting notions for targeting PARPs as anticancer strategy

Two conceptually unrelated anticancer strategies vindicate the use of PARP inhibitors as either single agents or in combination with chemotherapy or radiotherapy. The rationale for combination therapies is obviously linked to PARP1 as an integral element of the DNA damage response (DDR), which cancer cells rewire to resist DNA-damaging drugs [39]. Unfortunately, DDR is poorly compliant to pharmacologic obstruction; several DDR inhibitors are currently tested in the clinic, but they generally display unfavorable toxicity profiles [40]. Since PARP1 impacts many of DDR circuits, PARP inhibitors are expected to overwhelm DDR and kill cancer cells [41].

The most intriguing application of PARP inhibitors is monotherapy in HR-defective cancers, a molecular setting in which synthetic lethality is exploited. This notion has unlocked the therapeutic potential to selectively kill cancer cells by capitalizing on their inherent vulnerabilities including genomic instability, a well-known hallmark of cancer [42] and [43]. Faulty genome surveillance delivers a double-edged outcome: while facilitating the acquisition of tumor-promoting lesions, it needs to be buffered by compensatory routes to escape DNA damage overload and cell death. The ensuing reliance on these offsetting pathways can be therapeutically harnessed to drive toxicity [40] and [44]. This is particularly pertinent to hereditary BRCA1/2 mutated cancers, in which ineffective HR-driven DNA repair offers a permissive milieu for overriding cell tolerance to DNA damage [45] and [46]. In the BRCA-mutation setting, as well as in any genetic background resulting in HR dysfunction, PARP blockade causes replication-associated lesions that cannot be repaired by inherently defective HR, thereby encouraging the activation of compensatory, error-prone DNA repair pathways. This leads to genomic instability, non-viable genetic errors and, eventually, cell death[17] and [47].

2.5. Proposed mechanisms for PARP-BRCA synthetic lethality

The exact molecular mechanism underlying the selective killing of HR-deficient cells by PARP inhibition is yet incompletely understood and is a matter of intense debate. An accepted explanation is based on the assumption that PARP1-inhibited cells accumulate unrepaired SSBs, which are eventually converted to DSBs when encountered by the replication machinery; in the absence of BRCA1/2, HR repair is disabled and cells reroute to alternative low-fidelity DNA repair pathways, thus hastening genomic instability and cell death [47] (Fig. 1). PARP1 *per se* has a controlling role in NHEJ [34], supporting a model whereby contextual deficiency of PARP1 and BRCA would lead to a shift toward NHEJ, resulting in synthetic lethality [48]. Another proposed theory is that PARP1 and HR-related proteins mediate distinct pathways for the restart of stalled replication forks; therefore, PARP inhibition would be selectively toxic in HR-defective cells [49].

A conceptually different interpretation recalls the notion that auto-ADP ribosylation is necessary for PARP1 dissociation from DNA. Hence, the so called "PARP1-trapping model" has been theorized suggesting that PARP inhibitors capture PARP1 onto DNA repair intermediates [29]; these PARP-DNA aggregates are likely to impede DNA replication and require functional HR for efficient restart [49]. This analysis has been recently integrated by Murai et al., who demonstrated that trapped PARP-DNA is *per se* a more toxic lesion than unrepaired SSBs. These PARP-DNA structures necessitate efficient HR for resolution, thus explaining the synthetic lethal effect [50]. Consistent with PARP inhibitors being cellular poisons rather than catalytic inhibitors, the extent of PARP trapping by different inhibitors positively correlates with cytotoxicity [51].

Overall, despite the fragmented nature of these data, there is no evidence of mutual exclusion. A coherent picture would posit that PARP inhibition may generate as many lesions as the range of individual DNA repair nodes affected, including stalled replication forks, DSBs and trapped intermediates, all of which are substrates for HR. In the absence of functional HR, unwarranted activation of low-fidelity NHEJ drives accumulation of non-viable errors and selective toxicity.

2.6. BRCAness and BRCA-like tumors: broadening therapeutic routes

The concept of "BRCAness" was first established by Ashworth in 2004 to include clinical and biological features that some sporadic tumors share with those harboring germline BRCA mutations [52]. The finding that depletion of HR repair components other than BRCA1/2 (*i.e.* the MRN complex, PALB2, RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, and FANCC) is synthetic lethal with PARP inhibition provides proof of concept that HR deficiency, regardless of the underlying genetic lesion, can be therapeutically exploited to expand the pool of patients entitled to PARP-targeted therapy [17] and [53]. For example, preclinical data indicate that PARP inhibitors are very toxic in ATM-deficient cancer cells [54] and prove also effective in a variety of other BRCA-unrelated HRR deficient tumors, including microsatellite unstable colorectal cancers with MRE11 mutations [55] and RAD51C-deficient cancer cells [56].

ATM, MRE11 and RAD51, as well as other HR repair genes, are actually disrupted by genetic or epigenetic mechanisms in a significant proportion of sporadic tumors [53] (Fig. 2). This outlines the rationale for molecular profiling of non-BRCA genes to guide patient enrollment into clinical trials. PARP inhibitors are already used in phase I/II studies involving/enriched in ATM-deficient tumors (NCT01618136; NCT01063517), and many other synthetic lethal interactions are likely to enter inclusion criteria in prospective clinical trials.

Functional dependencies might also involve HR-unrelated proteins that yet provide ancillary paths to "BRCAness". The mitotic serine/threonine kinase Aurora A is frequently amplified in cancer. In preclinical models, overexpression of Aurora A impairs RAD51 recruitment, thus disabling DSB repair and sensitizing cells to PARP inhibition [57]. Recently, much interest has been focused on PTEN loss as a major determinant of BRCA-like features and sensitivity to PARP inhibitors and DNA damaging agents, including a case report in a patient [58], [59], [60] and [61] (Fig. 2). This issue remains in fact controversial: Shen et al. reported that PTEN depletion impairs HR-driven repair by decreasing the expression of RAD51 [62], but more recent data indicate that this is unlikely to be a general mechanism [63], [64] and [65]. It is also confounding that, although PTEN-null status is reported to positively identify PARP inhibitor responsiveness, yet

experimental PTEN down-regulation does not score positive in unbiased RNA interference drug sensitization screens to PARP inhibitors [66].

More compelling evidence associates BRCAness to non-germline BRCA status (Fig. 2). Somatic mutations of BRCA1/2 are rare in sporadic cancers, but inactivation of these genes may occur *via* disparate mechanisms. This is particularly relevant to breast and ovarian cancer. Triple negative breast cancer (TNBC), mostly classified as basal-like (BL) by gene expression analysis, shares common clinical and pathologic traits with familial BRCA1-mutated breast cancers, including high frequency of P53 mutation, aneuploidy, high pathologic grade, and relative sensitivity to DNA-damaging chemotherapeutics[67] and [68]. Reportedly, the molecular mechanisms underlying this similarity include transcriptional down-regulation or (more frequently) epigenetic silencing of BRCA1 [69], [70], [71] and [72]. Unlike BRCA1, BRCA2 functional inactivation is less clear. BRCA2 promoter is rarely hyper-methylated in breast cancer, but BRCA2 transcriptional down-regulation is frequently associated to amplification of the EMSY gene, occurring in up to 13% of sporadic, mostly hormone-positive breast cancers [73] (Fig. 2). EMSY binds to and disables a transcriptional activation domain on BRCA2 exon 3 [74]; however, whether EMSY amplification contributes to BRCAness is still debated [53].

Ovarian cancer is a promising BRCA-like tumor as well. Molecular profiling analysis reported HR dysfunction in up to 50% of high-grade-serous ovarian cancers (HGSOC) [75]. A number of mechanisms are thought to account for this phenotype, including BRCA1/2 somatic mutations, hypermethylation of the BRCA1 promoter, EMSY amplification, inactivation of the Fanconi anemia pathway, epigenetic silencing of RAD51C, and mutations of several other HR repair components and related proteins, including ATM, ATR, BARD1, BRIP1, MRE11A, PALB2, RAD50, RAD51D [76] and [77].

3. PARP blockade in cancer therapy

3.1. PARP inhibitors as chemo-/radio-potentiating agents

As early as in 1980, Durkacz and colleagues used the still immature, low-potency PARP inhibitor 3-aminobenzamide (3-AB) to derail DNA damage repair and enhance the cytotoxicity of dimethyl sulfate, a DNA alkylating agent [78]. Since then, a huge number of preclinical studies legitimated PARP inhibitors as sensitizing agents to DNA-damaging drugs and radiotherapy [79], [80], [81] and [82]. The first clinical trial in patients was initiated in 2003 and allowed safety, pharmacokinetic and pharmacodynamic evaluation of the PARP inhibitor AG014699 (rucaparib, displaying a half maximal inhibitory concentration [IC50] of 1.4 nM by*in vitro* assays with purified PARP1 [83]) in combination with temozolomide in advanced solid tumors [84]. However, the subsequent phase II study in melanoma [85], as well as additional independent clinical trials, featured a common (albeit not universal) shortcoming of combinatorial strategies with PARP inhibitors, namely, enhanced toxicity. Myelotoxicity was the main dose-limiting concern, in the face of variable response rates [86], [87], [88] and [89]. The need to reduce the dosage of either chemotherapy or PARP inhibitor (or both) to overcome excessive toxicity raises obvious questions about the real contribution of PARP inactivation to combinatorial regimens. Therefore, in spite of relentless efforts, still no definitive conclusions can be drawn as to which combination regimen would enhance the therapeutic index as compared with chemotherapy alone.

3.2. PARP inhibitors as single agents in tumors with germline HR deficiency

In 2005, two landmark studies fueled momentum to PARP-targeted strategies by providing evidence that PARP1 inactivation is synthetic lethal with BRCA1/2 deficiency, both *in vitro* and *in vivo* [90] and [91]. Further preclinical studies followed to endorse the use of PARP inhibitors in the “BRCAness” context of HR dysfunction driven by genetic alterations other than BRCA1/2 (see 3.3), envisaging an expanded therapeutic window for prospective clinical applications [17].

The very first evidence of clinical benefit with a PARP inhibitor as single agent was provided in 2009 by Fong and colleagues [92], who conducted a dose-escalation study of olaparib (a potent PARP inhibitor with *in vitro*IC50 values of 5 nM and 1 nM against PARP1 and PARP2, respectively, and higher than 1 μM against TNKS [93]) in patients with

advanced breast, lung, prostate, and ovarian cancer, registering striking objective responses exclusively in the germline BRCA1/2 mutation setting, with manageable side effects[92]. These results were confirmed in an expansion cohort of BRCA-mutated ovarian cancer patients [94]and in paired phase II clinical trials, which outlined encouraging dose-dependent response rates of 33% and 41% in BRCA-mutant ovarian and breast cancer, respectively [95] and [96].

The following studies with olaparib and other PARP inhibitors as monotherapy have been largely (although not uniformly) promising, especially in the ovarian cancer setting [18] and [97]. These findings have spurred the progression into phase III development, whose results are eagerly awaited.

3.3. Is a synthetic lethal approach possible in cancers with sporadic HR deficiency?

The use of monotherapy with PARP inhibitors was extended to sporadic tumors with suspected HR dysfunction, including HGSOC and TNBC. Gelmon et al. documented for the first time the clinical efficacy of olaparib in sporadic HGSOC patients. However, the same study reported an unpredicted failure in the breast cancer setting, casting a shadow that would subsequently put into question the actual effectiveness of PARP-targeted therapeutics in sporadic TNBC [18]. The road toward an effective use of PARP inhibitors in TNBC has been complicated by misleading trials with iniparib, a proposed non-competitive agent that raised high optimism in a phase II clinical trial [98]. The startling failure of the subsequent phase III study [99]and the retraction of iniparib due to lack of PARP inhibitory activity [100] depressed further advances and underscored the need for more accurate trial designs. It is conceivable that deeper mechanistic insights into TNBC may put forward novel therapeutic strategies to maximize the efficacy of PARP inhibitors.

More encouraging data have emerged in the ovarian cancer setting, where niraparib (previously MK-4287, with *in vitro* IC50 of 3.8 nM and 2.1 nM for PARP1 and PARP2, respectively, and less than 330-fold potency against PARP3, PARP4 and TNKS [93]) showed significant antitumor activity [97], and maintenance therapy with olaparib considerably improved progression free survival in patients with platinum-sensitive HGSOC [101]. The less compelling efficacy of PARP inhibitors in sporadic breast cancer as compared with the ovarian setting could reflect either inherent setbacks imposed by the high heterogeneity of TNBC or inadequate trial design, suffering from small sample size and lack of biomarker-driven inclusion criteria. Another explanation could be the higher prevalence of HR defects in ovarian cancer compared to breast cancer [75]. Outside the breast and ovarian cancer settings, antitumor activity of PARP inhibition with MK-4287 is also documented in non-small-cell lung cancer and prostate cancer, holding the promise for further expansion of PARP-targeted strategies [97].

3.4. Combination of PARP inhibitors with targeted agents

A step forward in the landscape of PARP inhibitors involves the rational combination with targeted agents known to derange HR, which is expected to induce BRCAness. A striking example has been provided by inhibition of cyclin-dependent kinase 1 (CDK1), a core-component of the cell cycle apparatus that is also involved in BRCA activity [102]. CDK1-mediated phosphorylation of BRCA is required for the formation of repair foci at the sites of DNA damage. Consistently, neutralization of CDK1 impairs BRCA function both *in vitro* and *in vivo*, thus mirroring BRCA-deficiency and imparting susceptibility to PARP inhibition [102]. These preclinical data have encouraged the initiation of a phase I clinical trial testing the combination of the PARP inhibitor veliparib (formerly ABT-888, a very selective inhibitor with *in vitro* IC50 of 8.3 nM and 11 nM toward PARP1 and PARP2, and 1000-fold less activity against TNKS [93]) with the CDK inhibitor dinaciclib in patients with advanced solid tumors (NCT01434316). This trial is ongoing, and results are not yet available.

Another opportunity to bring about BRCAness stems from the PI3K pathway, which is commonly activated in a wide array of tumors. PI3K inhibition has been reported to decrease the expression of BRCA1 and BRCA2, thereby disabling HR-mediated repair and sensitizing BRCA wild-type TNBC cells and xenografts to PARP inhibition [103]. Mechanistically, PI3K inhibitors stimulate ERK-mediated activation of ETS transcription factors, which in turn negatively control BRCA expression [103]. Based on these findings, a phase I clinical trial is currently underway to evaluate concurrent PARP/PI3K inhibition in TNBC and HGSOC patients (NCT01623349).

ETS transcription factors are more generally hyperactivated in many tumor types, including prostate cancer, Ewing's sarcoma, breast cancer, and melanoma [104]. In a substantial percentage of prostate tumors (50%), ETS overexpression due to genomic rearrangements increases the formation of DNA DSBs through reduced BRCA expression; therefore, PARP blockade has been successfully applied to exacerbate the DNA damage phenotype and kill ETS fusion-positive cells in preclinical models [104]. At present, a phase II study with olaparib has been approved in castration-resistant prostate cancers, also including the assessment of ETS fusion frequency and association with response rates (NCT01972217).

Combinatorial strategies with PARP and other targeted agents also include inhibitors of HSP90 or histone deacetylases, which are expected to reduce the expression of HR-related proteins by mechanisms involving proteasomal degradation and transcriptional regulation, respectively [57].

Tyrosine kinases have been reported to interfere with DNA damage repair to support tumor development. One example is c-ABL, known to increase RAD51 levels and promote drug resistance. In preclinical models, targeting c-ABL by imatinib mesylate enhances radiosensitivity and chemosensitivity of cancer cells [105], suggesting that combining clinically applicable c-ABL inhibitors with PARP inhibitors may prove beneficial. EGFR is positively implicated in both HR (by regulating BRCA1 function) and NHEJ dynamics (through binding with DNA-PK) [106] and [107]; accordingly, in a variety of preclinical models, inhibition of constitutively active EGFR hampers DNA repair and enhances susceptibility to PARP obstruction [108],[109] and [110]. In the clinic, combination therapy with olaparib and EGFR inhibitors is being currently evaluated in phase I and phase II studies in patients with head and neck cancer (NCT01758731) and EGFR-mutant non-small cell lung cancer (NCT01513174), respectively.

A striking synthetic lethality relationship was recently evidenced between PARP and HER2 inhibition in HER2-overexpressing breast cancer cells. In this setting, the efficacy of PARP inhibitors was not ascribed to disturbance of DNA repair but rather to interception of the NF-κB pathway, which critically contributes to the growth and survival of HER2-overexpressing mammary tumors [111].

Aberrant activation of c-MYC is associated with resistance to DNA damaging agents. Very recently, a key role for this master oncogene in promoting PARP-dependent DNA repair has been uncovered, possibly explaining the chemoresistant phenotype of MYC-overexpressing cells. Basically, c-MYC liberates PARP1 from the inhibitory interaction with the adaptor protein BIN, which enhances DNA repair and resistance. Therefore, PARP inhibitors could be employed to sensitize MYC-driven tumors to DNA damaging agents[112].

Finally, hypoxia is known to impinge on DNA repair pathways by decreasing the expression and activity of HR-related proteins. Therefore, hypoxic cells acquire defects in HR and are susceptible to PARP inhibition as a consequence of the so called “contextual synthetic lethality” [113]. As hypoxic subregions due to uneven blood supply are present in solid tumors, PARP inhibitors could be more widely used in combination with anti-angiogenesis strategies to enhance selective cell killing. A phase I study has been conducted in which PARP blockade by olaparib was combined with VEGFR inhibition by cediranib in ovarian and breast cancer patients, documenting encouraging activity in the ovarian setting, despite significant hematologic toxicity [114]. Instead, the combination of olaparib with the anti-VEGF monoclonal antibody bevacizumab is well-tolerated and awaits phase II studies to assess the clinical efficacy of this contextual synthetic lethal approach [115]. In parallel, other PARP inhibitors are under phase I clinical evaluation with bevacizumab and chemotherapy in various tumor types (NCT01459380; NCT00989651).

4. Mechanisms of resistance to PARP blockade

4.1. Resistance to PARP inhibitors: a common roadblock

The success of cancer targeted therapies is counteracted by the emergence of multiple resistance mechanisms, involving integrated cell autonomous and non-autonomous routes that compensate for the drug-induced neutralization of individual signaling pathways. This issue has been traditionally investigated with regard to targeted agents against

catalytically hyperactive oncoproteins [116], but instances of drug tolerance have also been documented in the context of non-oncogene addiction. There is now compelling evidence that the presence of BRCA1/2 mutations *per se* does not vouch for durable antitumor response in patients treated with PARP inhibitors [117]. Several mechanisms leading to both intrinsic and acquired resistance to PARP inhibitors have been discovered at the preclinical level and some of them have been eventually validated in cancer patients [118] and [119].

4.2. Recovery of BRCA function by secondary mutations

The first experimental evidence that tumors may escape PARP inhibitor-mediated toxicity was provided by two independent groups in 2008, dampening the enthusiasm swelled only three years earlier with the discovery of the PARP-BRCA synthetic lethal paradigm [120] and [121]. In both studies, drug resistance was linked to secondary mutations that restore the open reading frame and the original function of BRCA2, thereby reinstating HR competence. Different from common oncogene-related resistance mechanisms, whereby missense genetic lesions occur within the drug target that preclude compound binding, refractoriness to PARP inhibitors arises from genetic deletions in the synthetic lethal partner (BRCA2) that disarm BRCA/PARP functional dependency. Selective drug pressure is likely to foster the emergence of pre-existing resistant clones in a Darwinian fashion. In keeping with this notion and akin to accredited escape routes to tailored therapies, data from a single unresponsive patient unveiled the presence of secondary BRCA1 mutation in rare cells of pre-treatment material from the matched tumor [122]. It has been proposed that resistance to PARP inhibitors may be paradoxically promoted by the very same DNA repair defects (and the ensuing genome instability) that drive initial PARP inhibitor sensitivity [123].

In the future, it will be crucial to identify novel therapeutic avenues to overcome restoration of BRCA1/2 proficiency. Some hints in this direction have been advanced by Helleday and collaborators, who identified 6-thioguanine, a purine analog able to induce DSBs, as an effective agent in eradicating genetically-reverted BRCA2 mutant tumors resistant to PARP inhibition [124]. However, additional strategies that selectively halt tumors exhibiting acquired resistance to PARP inhibitors are urgently warranted, as are further studies to definitely assess the clinical incidence of HR-restoration through genetic reversion of BRCA1/2.

4.3. Loss of 53BP1 expression

New mechanistic insights into resistance to PARP inhibitors were provided by two landmark studies which disclosed BRCA1/2-independent routes to retrieved HR competency. Herein, loss of P53-binding protein 1 (53BP1), a protein implicated in DNA repair response, was reported to rescue HR proficiency in BRCA1-mutated cells, thus installing cell tolerance to both DNA-damaging agents and PARP inhibition[125] and [126]. Mechanistically, 53BP1 prevents resection of ssDNA, which is preparatory for the ensuing recombination events, as specified in 2.1; therefore, loss of 53BP1 unleashes DNA end-resection and HR competency, proving “synthetically viable” with BRCA1 deficiency [127].

53BP1 expression is reduced in a significant fraction of triple-negative and BRCA1/2-associated breast cancers, possibly accounting for therapy unresponsiveness [126]. Decreased 53BP1 levels have also been detected in BRCA1-mutant ovarian carcinoma patients that developed secondary resistance to PARP inhibitors and platinum compounds [128]. However, it should be noted that there is no objective consensus as to how 53BP1 loss impacts tumor response in the clinical setting: 53BP1 expression was shown neither to unequivocally correlate with BRCA1 status nor to segregate with resistance to platinum therapy, as it would have been expected from previous findings [129]. Hence, larger studies are needed to determine whether 53BP1 expression may predict DNA repair status in biomarker-driven clinical trials.

4.4. Residual activity of mutant BRCA

The notion that secondary mutation of BRCA1 may elicit tolerance to genotoxic agents or PARP inhibitors was complemented by the finding that discrete BRCA1 mutations can differently impinge on drug response. In particular, the C61G mutation within the N-terminal RING domain of BRCA1 yields a mutant BRCA1 protein still retaining partial biological activity, which suffices for reduced drug sensitivity [130]. Residual BRCA1 activity may also result from HSP90-mediated stabilization of a BRCA1 variant with a mutation in the BRCT domain that impairs protein folding but

does not completely prevent protein activity. In this case, HSP90 is engaged under PARP inhibitor selective pressure to prevent protease-mediated degradation of the misfolded hypomorphic BRCA1, resulting in functional recovery of HR [128]. Overall, this evidence advises the opportunity to incorporate testing of individual BRCA1 mutations into biomarker-driven clinical trials.

4.5. Upregulation of efflux transporters

Overexpression of ATP-binding cassette transporters (ABC), such as P-glycoprotein (P-gp) and ABCG2, is universally recognized to mediate drug exclusion and therapy failure [131]. Several lines of evidence indicate that drug efflux-based resistance occurs also for PARP inhibitors; notably, this mechanism can be reversed by administration of P-gp inhibitors [132] and [133].

Mechanistically, a number of possible explanations as to how PARP obstruction enhances efflux pump expression have been proposed. P53 is known to negatively regulate P-gp expression and is frequently defective in PARP-deficient cells; hence, lack of functional P53 response may account for resistance [132]. Another hypothesis calls attention to the well-established role of PARP1 in transcriptional regulation and chromatin remodeling. Although there is no direct experimental evidence that PARP1 is able to bind the P-gp promoter to repress transcription, such a regulatory complex might actually be operative in cells and therefore liable to PARP inhibitor-mediated disengagement; this would ultimately unleash P-gp expression and convey drug resistance [134].

Given the relatively recent development of clinical studies, there is little knowledge as to whether and how frequently P-gp overexpression occurs in patients; nevertheless, data gathered so far surmise a likely therapeutic applicability. Hints for circumventing this resistance machinery have also been proposed. For example, either administration of PARP inhibitors with low affinity for P-gp or concomitant P-gp obstruction have already been productively implemented at the preclinical level and hold promise for prospective extrapolation to the clinical setting [133] and [135].

4.6. Aberrant PARP expression and activity

Inactivation of PARP by reduced protein expression appears to exert different (if not opposite) effects compared with catalytic inactivation by pharmacologic inhibition [119]. Indeed, initial evidence suggests that low PARP levels (and activity) attenuate responsiveness to PARP inhibitors [90]. In hindsight, this apparent discrepancy is sound: decreased PARP protein might be selectively advantageous to withstand the “poisoning” activity of drug-induced DNA-PARP aggregates [50]. This hypothesis is in agreement with results from insertional mutagenesis screens that identify PARP1-depleted cells as up to 100-fold more resistant to olaparib as compared with wild type cells [136]. Therefore, PARP1-depleted cells may experience loss of cell viability (through SSB repair inhibition) but also resistance to PARP inhibition (through preclusion of PARP-trapping cytotoxic effect).

5. Predictive biomarkers of sensitivity to PARP inhibitors

5.1. Array-based procedures: “BRCAness” signatures

Validation of predictive biomarkers for PARP-directed therapeutics is a major task and a difficult endeavor. Genetic testing for BRCA1/2 mutations is being increasingly integrated in the clinical management of patients diagnosed with breast and ovarian cancer. However, this criterion may underestimate the real fraction of patient with BRCA-like tumors likely to benefit from PARP inhibition. For instance, epigenetic silencing of BRCA1/2 would not be detected, thereby excluding a consistent population of responding patients.

The need to tackle overall dysfunctional HR for broadening the therapeutic span of PARP inhibitors – independently of BRCA1/2 – brings with it the inherent hurdle to identify predictive biomarkers for patient selection through readily applicable procedures. Targeted genotyping assays, even when using custom panels, may reveal inadequate due to the high number of genes involved in DNA repair response and the heterogeneous nature of their genetic/epigenetic inactivation. Therefore, array-based strategies, including gene expression profiling, comparative genomic hybridization,

and quantitative copy number analysis by multiplex ligation-dependent probe amplification, have been implemented to build BRCA1/2-like classifiers for the generation of “BRCAness” signatures [137], [138] and [139].

In a complementary perspective, BRCAness profiling by proteomic approaches pinpointed molecular patterns of HR deficiency and proved to positively associate with response rate and disease recurrence, thus showing good predictive value [140] and [141]. An alternative avenue was recently proposed by Abkevich and colleagues, who successfully developed an “HRD score” (homologous recombination deficiency score) for detection and quantification of homologous recombination defects by a DNA-based assay [142]. Overall, these procedures may expedite a more accurate appraisal of BRCAness and could be integrated in clinical trials. However, a major liability is the lack of univocal correlation between a given genetic footprint and response to therapy [130]. Indeed, individual genetic lesions have been shown to differently impact drug response phenotypes despite their converging onto identical genetic profiles. This hints that functional rather than molecular assessment of HR proficiency might be more valuable for patient stratification [130].

5.2. Functional assays to classify HRR-proficient and -defective tumors

The evaluation of HR function through surrogate biomarkers directly in patient biopsies is now deployed in clinical trials. The focal accumulation of RAD51 at the DNA lesion is a widely recognized indicator of HR integrity; consequently, absence of RAD51 nuclear foci following DNA damage is a functional biomarker of HR dysfunction [57]. Immunofluorescence-based detection of RAD51 foci, coupled with quantification of additional DNA repair-related proteins, has been successfully applied to classify tumors as either HR-competent (RAD51 foci-positive) or HR-defective (RAD51 foci-negative), with a strong predictive value for chemotherapy response [143], [144] and [145]. A possible drawback of this method is that RAD51 expression is cell cycle-dependent, which increases the likelihood of incurring in false-negative outcomes. It should also be noted that a retained ability to form RAD51-containing foci does not universally segregate with HR competence. Hence, this criterion could erroneously exclude tumors still amenable to PARP inhibition [146]. Another key protein involved in DNA repair is histone H2AX, which also assembles as foci at DNA DSBs in the setting of HR-competence, where it becomes phosphorylated (gamma-H2AX) to attract additional effectors. Therefore, the degree of gamma-H2AX foci is widely employed to assess the extent of DNA damage in patients [147].

An inherent limitation of these procedures is the need for fresh tissues, whose availability is not always predictable. Circulating tumor cells (CTCs) in the peripheral blood of patients are permanently accessible and, in principle, would allow for non-invasive response monitoring and biomarker discovery research in longitudinal studies [148]. Therefore, studies are underway to score gamma-H2AX and RAD51 foci through CTC isolation and processing [149].

Finally, it is increasingly evident that high PARP activity identifies HR-defective cells [150], possibly as a compensatory feedback. A surrogate for PARP activity, which is leveraged for dose selection during the development of PARP inhibitors, is detection of PAR polymers. Therefore high PAR levels, as assessed by PAR polymers, may classify BRCA-like tumors [151].

6. Beyond Dna repair

6.1. Targeting angiogenesis and metastasis with PARP inhibitors

The extreme versatility of the PARP family highlights the existence of DNA repair-independent functions that may be targeted for cancer treatment. Although long underrated, these mechanisms include transcriptional regulation, mitotic functions, and interference with oncogenic signaling pathways [4].

PARP1 regulates transcription through mechanisms as diverse as relaxation of chromatin structure, direct regulation of transcription factors, association to enhancer- and/or promoter-binding complexes, and modulation of DNA methylation [152]. Furthermore, other PARPs (including PARP2, PARP9/ARTD9, and PARP14/ARTD8) have been reported to modulate transcription-factor complexes [153]. Therefore, blocking PARPs offers a unique opportunity to tackle the aberrant activity of transcription factors in cancer [154]. The clinical relevance of this notion is obvious, as

transcription factors promote cancer progression, including proliferation, angiogenesis and metastasis, but have proven as yet hostile to pharmacologic targeting.

Numerous studies suggest that PARP1 inhibition impairs angiogenesis both *in vitro* and in mouse models[155], [156] and [157]. Specifically, PARP inhibition is reported to abolish vessel formation and endothelial cell migration in response to VEGF. This activity has not received full mechanistic elucidation, but at least partially it can be ascribed to the impairment of PARP-mediated transcription of pro-angiogenic cues such as syndecan-4 and Id-1 [158]. Preclinically, PARP1 down-regulation reduces angiogenic sprouts and decreases aggressiveness in xenografts of melanoma cells [159].

PARP1 also controls (at both transcriptional and translational levels) the expression of molecules involved in the epithelial–mesenchymal transition, such as vimentin and Snail1 [160] and [161]; interception of this activity, coupled with the anti-angiogenic effects described above, likely accounts for the anti-metastatic outcome of PARP inhibition in a mouse model of melanoma [161]. Similarly, PARP1 hampers ETS fusion-positive prostate cancer cell invasion, intravasation and metastasis by disrupting ETS-mediated transcription of metastasis-related genes, such as EZH2 [104].

Recent findings indicate that PARP1 positively regulates the activity of key components of hypoxic adaptation and of the inflammatory response, namely HIF1-α and NF-κB, respectively [162]; as a consequence, PARP inhibition reduces HIF-1α- and/or NF-κB-driven tumor proliferation, angiogenesis and metastatic ability in several preclinical models [4] and [111]. Beyond PARP1, other members of the PARP superfamily have been reported to affect NF-κB signaling. As an example, the mono(ADP)-ribose polymerase PARP10 (ARTD10) inhibits NF-κB nuclear translocation and transcriptional outcome in response to interleukin-1β and tumor necrosis factor-α [163]. These findings unveil a novel scenario that entails the possibility to interfere with microenvironmental capabilities, pointing to non-obvious aspects of PARP inhibition that are very far from HR-based vulnerabilities.

6.2. Additional roles of PARPs

As briefly outlined above (see Section 6.1), one major non-repair function of PARP1 is transcriptional regulation. In particular, PARP1-mediated modification of histones in response to environmental stimuli unties the chromatin architecture, thereby allowing transcription [3]. Not mutually exclusive, PARP1 contribution to transcriptional events also entails functional interaction with the RNA polymerase II-related apparatus as well as with a large number of transcription factors (including NF-κB, Elk1, Oct-1, Sox2, nuclear receptors, and others), triggering either stimulatory or inhibitory effects [152]. PARP1 is also reported to act as an exchange factor by assisting the release of inhibitory molecules and the subsequent engagement of stimulatory elements to transcriptional complexes. Neuronal differentiation provides a paradigmatic example of this mode of action, whereby PARP1-modified corepressors dissociate from the promoter region while specific coactivators are recruited to elicit a neurogenic program [164]. Moreover, PARP is documented to enhance muscle-specific gene transcription, further strengthening the emerging notion of a context-specific (or lineage-dependent) role [165]. In this context, PARP1 may also exert functions irrespective of its catalytic activity. For instance, it individually acts as a scaffold to allow functional cooperation among several regulatory molecules at the promoter sites, including the p300/CBP acetyltransferase [166]. Increasing attention is being placed onto additional epigenetic functions of PARP1, including inhibition of the DNA methyltransferase Dnmt1, cooperation with the methylcytosine dioxygenase Tet2, and interaction with the transcriptional insulator protein CTCF [152].

Beyond PARP nuclear activities, interphase cytosolic functions are starting to emerge, which encompass relieve of microRNA-mediated translational repression and assembly of the so-called stress granules — *i.e.*, macromolecular structures embedding RNA-binding proteins that control mRNA translation and stability upon stress conditions. This attests to PARP involvement (in particular PARP12/ARTD12, PARP13/ARTD13, TNKS, and PARP15/ARTD7) in cytosolic post-transcriptional gene regulation [167].

It is important to emphasize that PARP activity is strictly NAD^+-dependent. NAD^+ is a key cofactor for redox reactions (central to cellular energetics and metabolism) as well as for non-redox events, including signaling pathways and gene regulation [168]. NAD^+ cellular availability is warranted by both *de novo* synthesis (from tryptophan) and three individual

salvage pathways (involving nicotinic acid, nicotinamide, and nicotinamide riboside), which collectively titer the cellular levels of NAD^+ and therefore influence the reciprocal activity of PARPs and that of other NAD^+-dependent enzymes [168] and [169]. Unwarranted PARP1 activation due to extensive DNA damage dramatically depletes NAD^+ cellular supplies and triggers NAD^+ resynthesis through the salvage pathway. This process results in exhaustive ATP consumption and diverts cells from apoptosis to necrotic cell death, supporting the use of PARP inhibitors to mitigate oxidative damage during pathologic conditions such as inflammatory diseases and reperfusion injury [169]. In a context-dependent manner, another mode of cell death can be triggered, which is referred to as "parthanatos" (PARP1-dependent cell death): in this case, PARP1 hyper-activation and the subsequent depletion of NAD^+ induce the nuclear translocation of AIP (apoptosis-inducing factor) from mithocondria, ultimately leading to caspase-independent cell death [170].

An additional non-repair function of PARPs relates to safeguarding of the mitotic fidelity checkpoint by localizing to centrosomes, where they catalyze the poly(ADP)-ribosylation of centrosomal proteins[171] and [172], and by interacting with the tumor suppressor protein CHFR [173]. The mitotic checkpoint enables accurate chromosome segregation during cell division by arresting the cell cycle in case of faulty chromosome attachment to the microtubule-based mitotic spindle. Disabling this machinery leads to chromosome instability and aneuploidy, a gross form of genetic instability that (similar to DNA repair deficiencies) sustains tumorigenesis while representing an intrinsic weakness of the cancer phenotype[174]. In this perspective, PARP inhibitors have been proposed in combinatorial regimens to selectively kill cancer cells resistant to microtubule-stabilizing agents [173].

Novel lines of investigations may help maximize the efficacy of PARP-related approaches. Recent preclinical data have emphasized the link between PARP inactivation and the induction of a senescent phenotype [175]. This could be particularly relevant in the advanced tumor setting, where key effectors of therapy-induced senescence are likely to be inactivated, resulting in drug resistance [176]. Hence, PARP targeting holds promise as a pro-senescence therapy against cancer. Novel prospects may also stem from the recent finding that PARP inhibition-driven senescence is associated with the secretion of immunoregulatory molecules by senescent cells, resulting in a strong anti-tumor immune response [177]. The notion of a "senescence-associated secretory phenotype" is now emerging as a response to sustained DNA damage, which involves communication with the microenvironment and could be therapeutically exploited [178].

6.3. Tankyrases 1 and 2: promising targets in anti-cancer drug development

Recent studies on other members of the PARP superfamily, namely tankyrase 1 (TNKS) and its closest related homologue, tankyrase 2 (TNKS2), have disclosed unanticipated paths for therapeutic intervention. Tankyrases 1 & 2 were originally implicated in telomere maintenance through their ability to poly(ADP)-ribosylate the telomeric repeat binding factor 1 (TRF1), a negative regulator of telomere length. This post-translational modification enables TRF1 disengagement from telomeres and allows telomerase access for chromosome end elongation (Fig. 3) [179] and [180]. Therefore, TNKS was proposed as a potential target for telomere-targeted anticancer therapy, with potential synergistic or additive efficacy with existing telomerase inhibitors [181]. The essential drawback of telomerase-directed approaches is the lag period required for cell senescence to occur, which could foster the emergence of secondary resistance. Combinatorial approaches with TNKS inhibitors are expected to integrate selective killing of cancer cells – by definition of tumor contexts that are more reliant on telomerase activity for their unrestrained growth – with shorter intervals needed to attain cell crisis and senescence [181] and [182]. Supporting this assumption, genetic TNKS downregulation combined with telomerase inhibition was reported to synergistically induce telomere shortening in both gastric and lung cancer cell lines [183] and [184]. Consistently, overexpression of TNKS confers resistance to telomerase-targeted agents [181]. As mentioned in the Introduction, these events are also modulated by PARP3, which suppresses telomerase activity [9]. In this perspective, broad-range PARP inhibitors may elicit non-obvious outcomes depending on the relative affinities for either TNKS or PARP3.

Multiple lines of evidence delineate TNKS as a mitotic regulator. First, it forms a complex with a number of spindle-associated proteins, including NuMA, ATM, and BRCA1, to allow proper spindle pole focusing and

polarity [185], [186] and [187]. Of note, PARP3 also enters this network by associating to and positively regulating both NuMA and TNKS, thereby favoring mitotic spindle integrity, telomere stability and efficient mitotic progression (Fig. 3) [8]. TNKS promotes sister telomere resolution before anaphase: indeed, in the absence of TNKS, sister chromatids remain associated at their telomeres due to protein–protein interactions, which results in end-to-end fusions through the NHEJ machinery (Fig. 3) [188], [189] and [190]. It is worth noting that the TNKS sequence lacks a nuclear localization signal, which complicates our understanding of how TNKS can localize at telomeres both in interphase and in mitosis [191]. A cell cycle- and TRF1-dependent distribution of TNKS has been proposed, whereby in interphase TNKS localizes not only to telomeres (in association with TRF1) but also at nuclear pore complexes; at mitosis, when the nuclear pore complexes are disassembled, TNKS shuttles to the pericentriolar matrix of mitotic chromosomes through a nuclear import mechanism that is likely regulated by TRF1 [191]. This issue is currently the object of active debate. TNKS also resides at the centrosomes and promotes centrosome maturation by interacting with several proteins, including the mitotic kinetics regulator, Miki, and the Centrosomal P4.1-associated protein CPAP (Fig. 3) [192] and [193]. Notably, as discussed in Section 6.2, PARPs other than TNKS are known to affect centrosome events [171] and [172]. Hence, it seems that poly(ADP)-ribosylation activity, mediated by different PARPs and affecting a diverse array of substrates, operates as a general mechanism to assure centrosome function.

The mitotic role of TNKS implies appealing therapeutic implications. RNA interference-mediated depletion of TNKS leads to either mitotic arrest or senescence in several cell lines, depending on the status of a number of negative cell-cycle regulators/tumor suppressors [10]. Nevertheless, it remains unclear whether this aspect may be clinically pursued. There is a perplexing dichotomy between TNKS genetic depletion and pharmacologic blockade, in that spindle-related defects are much feebler upon chemical inhibition as compared with TNKS silencing [187]. Coherently, we also have observed that patent growth arrest, as achieved by shRNA-mediated TNKS knockdown, is not phenocopied by TNKS pharmacologic blockade (our unpublished results). This could imply that residual activity in drug-treated cells may suffice for rescuing mitotic defects; alternatively, TNKS scaffolding function (disrupted by TNKS-shRNA but spared by the drug) and catalytic activity (lost anyhow) differently impinge on the cell mitotic apparatus. Strategies involving TNKS inhibition in BRCA-associated cancer have been advanced to combine spindle dysfunction with DNA repair defects. Indeed, a pioneering paper documented a synthetic lethal relationship between TNKS down-regulation and BRCA dysfunction, owing to exacerbation of BRCA1-related mitotic defects [194].

A breakthrough in the landscape of TNKS-targeted strategy is the discovery that TNKS1/2 positively modulates the canonical Wnt/β-catenin pathway. Specifically, tankyrase-mediated poly-ADP-ribosylation of axin, the concentration-limiting component of the β-catenin destruction complex, triggers axin degradation by the proteasome machinery, thus enabling β-catenin to translocate into the nucleus and switch on the Wnt program (Fig. 3) [195]. The Wnt signaling network is deeply involved in embryonic development, stemness, cell fate determination, neuronal migration, and cancer [196]. Genetic inactivation of APC or oncogenic mutational activation of β-catenin yields aberrant Wnt activation in more than 80% of colorectal cancers[197]. While this pro-tumorigenic apparatus provides very few actionable targets for rational drug design, TNKS may offer one such opportunity. This assumption fueled cumulative drug development efforts to pharmacologically inhibit these enzymes, leading to the identification of several compounds characterized by increasingly higher specificity and bioavailability. Indeed, converging lines of evidence indicate that pharmacologic TNKS blockade restrains Wnt signaling and tumor growth in APC-mutant colorectal tumors both *in vitro* and *in vivo* [195], [198] and [199]. One conceivable caveat to this approach could be a limiting intestinal toxicity [198].

Drug combination strategies may be useful to unleash the full therapeutic potential of TNKS inhibition. To this aim, positive hints have been provided by studies reporting enhanced efficacy of MEK- and EGFR-directed agents in combination with TNKS inhibitors in KRAS-mutant colorectal cancer and non-small cell lung cancer cell lines, respectively [198] and [200].

TNKS1/2 play an additional role in cellular response to insulin by regulating the plasma membrane targeting of storage vesicles containing the glucose transporter GLUT4 and the insulin-responsive aminopeptidase (IRAP) to facilitate glucose uptake (Fig. 3) [201].

Finally, TNKS are also involved in DNA repair by stabilizing the NHEJ protein DNA-PK (Fig. 3) [202]. Although still in their infancy, all these studies provide proof of concept that TNKS inhibitors could deliver therapeutic benefit as telomere-targeted agents and/or in the management of Wnt-driven malignancies.

7. Concluding remarks and challenges

More than three decades have elapsed since Durkacz and colleagues forecasted the therapeutic potential of PARP inhibitors. After years of fortune and downfalls in the clinical arena, it is fair to say that the impact of precision medicine with PARP inhibitors has been incremental rather than transformative. Despite a sustained trend toward better responses in BRCA-mutant patients, it is increasingly evident that BRCA mutations as such prove neither necessary nor sufficient to convey drug sensitivity [18]. Therefore, the identification of molecular determinants of drug response other than BRCA still remains a major challenge.

The implementation of high-throughput functional genomics, including large-scale siRNA sensitization screens, has led to the identification of potentially actionable synthetic lethal candidates [203]. However, the clinical relevance of such an approach is not obvious. One major hurdle is that artificial down-regulation of individual genes in immortalized cell lines does not recapitulate the genetic framework and molecular rewiring that may develop in germline null genotypes. Moreover, genetic downregulation does not always result in the same biological outcome as pharmacologic inhibition, thus raising the possibility that siRNA-based investigation may neglect (or overestimate) pursuable targets.

Integrative molecular and functional characterization of tumors may expedite the identification of BRCA-like behaviors. In this respect, BRCAness signatures and classifiers are intriguing, although they will require prospective large-scale clinical validation before entering conventional clinical practice. As a methodological caveat, the lack of gold standards to detect BRCAness still remains an unmet challenge, which currently precludes cross-study evaluation.

The inconclusive clinical experience with non-hereditary TNBC suggests that the most favorable application of PARP inhibitors should be expected in less heterogeneous (or better profiled) tumors and underlines the need to refine biomarker-driven clinical trials so as to enrich for potential responders and avoid the dilution of favorable outcomes. Efforts will be also needed to identify therapeutic restraints to secondary resistance. Assessment of multiple biopsies or circulating cancer cells at different time points, including at the emergence of resistance, may help advance this field. Besides, a key contingency for the optimization of PARP inhibitors will be to analyze the consequences of single-agent *versus* combination strategies, as well as intermittent *versus* continuous treatment schedules.

A step forward in our knowledge about the manifold biology of PARP and relative clinical extrapolation might derive from understanding whether (and how productively) more selective compounds can overtake the broad-spectrum PARP neutralization currently achieved with the majority of available competitive inhibitors. Such information would allow a more accurate interpretation of both basic research studies and clinical trial outcomes. Indeed, olaparib targets several members of the PARP family and also displays off-target effects (including Akt and ERK kinases, likely contributing to antitumor activity to some extent), whereas the highly selective inhibitor veliparib does not display any recognized PARP-unrelated effects [204]. At present, however, studies are as yet immature to draw a solid comparison of the consequences of pan-PARP *versus* selective PARP targeting. Conversely, the road to selective tankyrase inhibition appears more practicable due to the lower homology between tankyrases and other PARPs (with the exception of the shared catalytic domain), although considerable skepticisms exists as to whether TNKS1 *versus* TNKS2 selectivity may be attainable [93].

Another issue is to ascertain whether the DNA-damaging insults produced by prior chemotherapy-based regimens may impact prospective treatments by aggravating tumor heterogeneity, which would boost the appearance of resistant clones. PARP inhibitors and chemotherapeutics share a number of overlapping resistance mechanisms [94] and [121], but this notion does not apply universally [97] and [205]. The idiosyncratic nature of DNA repair mechanisms (and of their faulty counterparts) adds further complexity to an already intricate matter and will likely disorient both the interpretation of trial outcomes and the definition of eligibility criteria. Additional burdens may be imposed by the notion

that biomarker expression and prognostic value are context-dependent and may have different relevance in different tumor types (as epitomized by the different extent of response in sporadic BRCA-like breast and ovarian cancers).

Despite intense research efforts, progress toward regulatory approval of PARP inhibitors is stalling. New functional settings are emerging in which PARP inhibitors might be therapeutically relevant — including tumor-promoting inflammation, cell senescence, angiogenesis, and metastasis. New inhibitors are being developed against more recent members of the PARP family, such as tankyrases, which have provided fresh knowledge on additional facets of PARP-mediated tumor biology. We are eager to see whether and how further advances in this field will impact treatment decisions.

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Table 1
Overview of completed and ongoing clinical trials with PARP inhibitors.www.clinicaltrials.gov.

Drug a	Phase	Intervention	Cancer type	Patient stratification
Olaparib	Phase I[a]	Combination	Ovarian, breast, esophagus, lung, brain, uterin, head and neck Ewing's sarcoma, fallopian, endometrial, cervical, peritoneal, colorectal, melanoma, pancreatic, other unspecified solid tumors	No
		Combination	Breast, ovarian, peritoneal, fallopian, other unspecified solid tumors	BRCA mut
		Monotherapy	Unspecified solid tumors	No
		Monotherapy	Ovarian	BRCA mut
	Phase II	Combination	Breast, ovarian, fallopian, peritoneal, prostate	No
		Combination	Gastric, pancreatic	ATM loss, BRCA/FA pathway mut
			Lung	EGFR mut
		Monotherapy	Prostate, Ewing's sarcoma, ovarian	No
		Monotherapy	Ovarian, breast, prostate, pancreatic, peritoneal, fallopian, other unspecified solid	BRCA mut
	Phase III	Combination	Gastric	No
		Monotherapy	Colorectal cancer	MSI status
			Breast, ovarian	BRCA mut
Veliparib	Phase I	Combination	Peritoneal, fallopian, ovarian, pancreatic, lymphoma, leukemia, lung, bladder, liver, rectal, breast, melanoma, colorectal, gastric prostate, brain, other unspecified solid tumors	No
		Combination	Breast, ovarian, other unspecified solid tumors	BRCA/FA pathway mut
		Monotherapy	Gastric, ovarian, breast, fallopian, peritoneal, leukemia, lymphoma other unspecified solid tumors	No
		Monotherapy	Breast, ovarian, other unspecified solid tumors	BRCA/FA pathway mut, BRCA1 promoter methylation
	Phase II	Combination	Lung, ovarian, fallopian, peritoneal, breast, head and neck, cervical, prostate, pancreatic, liver, colorectal, brain, melanoma other unspecified solid tumors	No
		Combination	Pancreatic, breast, ovarian	BRCA/PALB2 mut
			Ovarian	non-BRCA
		Monotherapy	Pancreatic, breast, ovarian	No
		Monotherapy	Breast, fallopian, ovarian, peritoneal	BRCA mut
	Phase III	Combination	Breast	BRCA mut
Rucaparib	Phase I	Combination	Unspecified solid tumors	No
		Monotherapy	Ovarian, fallopian, peritoneal	No
	Phase II	Combination	Breast	BRCA mut
		Monotherapy	Ovarian, fallopian, peritoneal	No
		Monotherapy	Breast, ovarian, pancreatic	BRCA mut
	Phase III	Monotherapy	Ovarian, fallopian, peritoneal	BRCA mut, other HR gene alteration
BMN-673	Phase I	Combination	Unspecified solid tumors	No
		Monotherapy	Leukemia, lymphoma, myelodisplastic syndrome	No
		Monotherapy	Breast, ovarian, lung, prostate, pancreatic, Ewing's sarcoma	BRCA mut
	Phase II	Monotherapy	Ovarian, breast, peritoneal, other unspecified solid tumors	BRCA mut
	Phase III	Monotherapy	Breast	BRCA mut
Niraparib	Phase I	Combination	Ovarian, brain, melanoma, other unspecified solid tumors	No
		Monotherapy	Leukemia, unspecified solid tumors	No
	Phase III	Monotherapy	Breast, ovarian	BRCA mut
			Breast	BRCA mut, non-HER2
CEP9722	Phase I	Combination	Lymphoma, unspecified solid tumors	No
		Monotherapy	Unspecified solid tumors	No
	Phase II	Monotherapy	Unspecified solid tumors	BRCA mut, other HR gene alteration
E7016	Phase I	Combination	Unspecified solid tumors	No
	Phase II	Combination	Melanoma	wt BRAF
AZD-2461	Phase I	Monotherapy	Unspecified solid tumors	No
INO-1001	Phase I	Combination	Melanoma	No
E7449	Phase I/II	Combination	Melanoma, breast	No
		Monotherapy	Ovarian, B-cell malignancies	ATM mut

[a] Iniparib is not included in the table due to its poor selectivity toward PARPs.

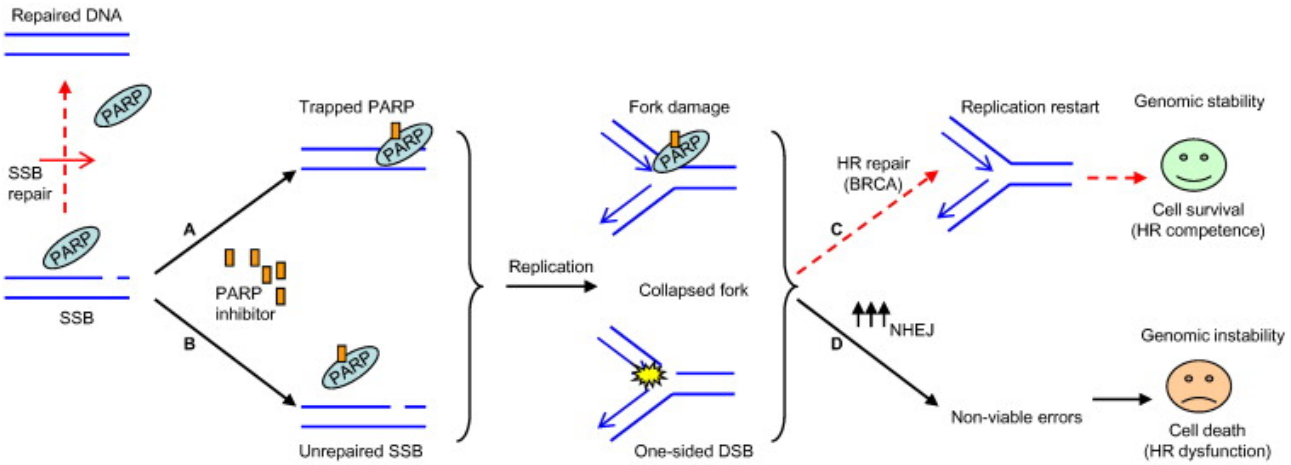

Fig. 1.

Molecular pathways underlying PARP/BRCA synthetic lethality. Red dotted lines indicate processes impaired by PARP blockade in HR-defective cells. In the presence of PARP inhibitors, SSB repair is precluded and either PARP is trapped onto DNA (A) or unrepaired SSBs are converted to DSBs by collision with the replication machinery (B). In both cases, resultant replication fork damage requires operational HR for efficient restart (C). HR-deficient BRCA mutant cells redirect to alternative, error-prone DNA repair pathways (D), undergoing genomic instability and cell death.

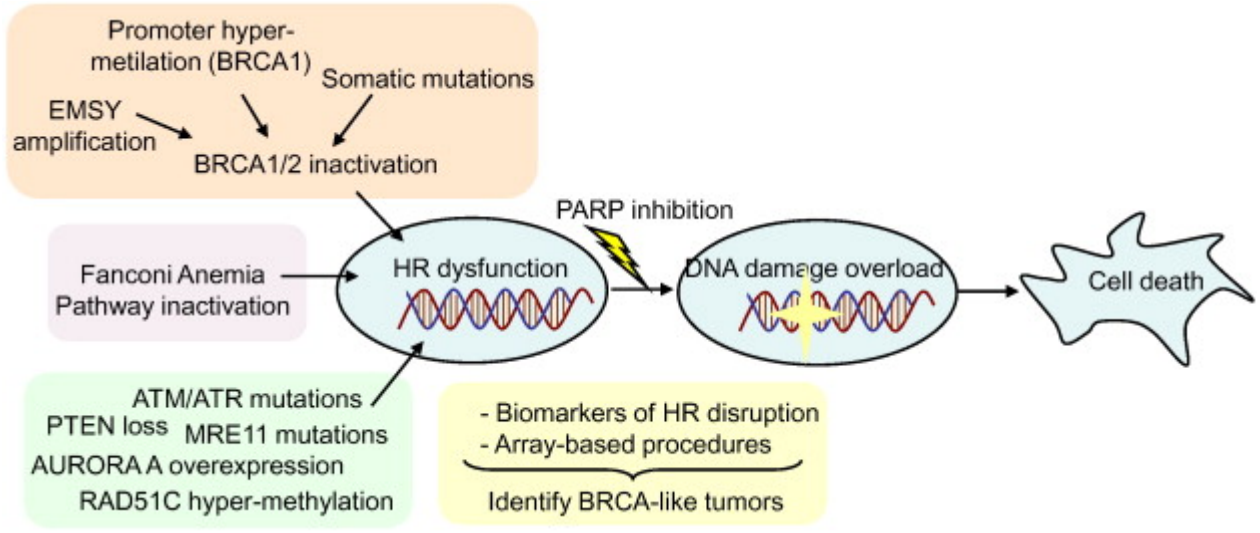

Fig. 2.

BRCA-like behavior in sporadic tumors. BRCAness may arise from genetic and epigenetic lesions (left panels) that phenocopy germline BRCA$^{-/-}$ background and disable HR-directed DNA repair in sporadic cancer. PARP blockade in this setting is expected to drive selective toxicity. Identifying BRCAness (yellow panel) through genetic testing, functional assays or array-based procedures can potentially widen the therapeutic span of PARP-targeted strategies.

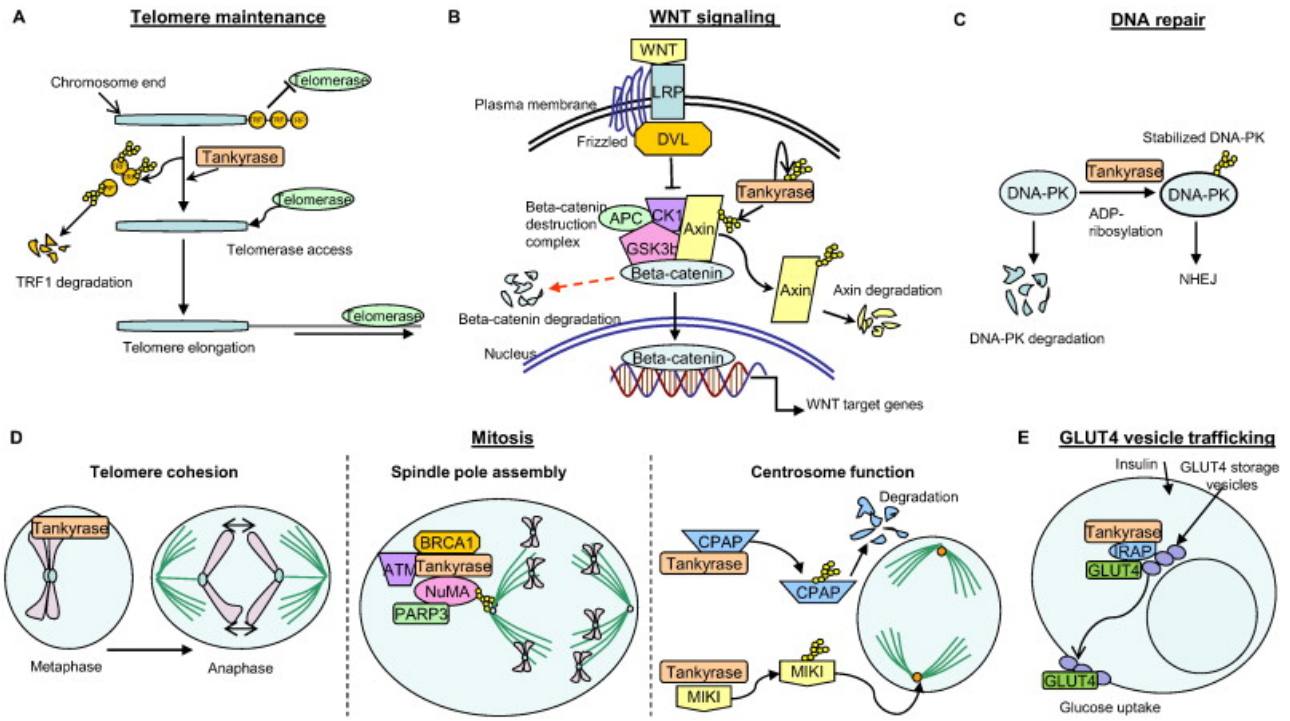

Fig. 3.

Overview of Tankyrase functions.(A) Tankyrase promotes telomere elongation by PARsylating and displacing TRF1 from telomeres, thereby enabling telomerase loading onto chromosome ends; (B) Tankyrase-mediated PARsylation of axin and ensuing axin degradation lead to disruption of the betacatenin destruction complex. Released beta-catenin translocates into the nucleus and drives WNT-dependent transcription; (C) Tankyrase PARsylates DNA-PK, a key player in NHEJ-mediated DNA repair, and unleashes it from proteasome-mediated degradation; (D) Tankyrase displays a multifaceted role in mitosis. First, it promotes sister chromatid resolution before anaphase (left panel); second, it forms a complex with ATM, BRCA1 and NuMA, thereby PARsylating NuMA and ensuring spindle pole bipolarity (middle panel); finally, TNKS regulates centrosome function by interacting with centrosome-associated proteins, such as CPAP and MIKI; (E) TNKS1/2 regulates exocytosis of GLUT4-storage vesicles upon insulin stimulation through interaction with IRAP.