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PARP Inhibition and Beyond in BRCA-Associated Breast Cancer in Women: A State-Of-The-Art Summary of Preclinical Research on Risk Reduction and Clinical Benefits

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Highlights of the Study

- Female breast cancer patients experiencing BRCA1/2 mutation may benefit from PARP inhibition.
- PARP inhibitors combined with platinum-based chemotherapy may offer better clinical results than monotherapy.
- Mechanisms of clinical resistance to PARP inhibitors require future research.

Keywords
Female breast cancer · BRCA1/2 mutation · PARP inhibitors · Synthetic lethality · PARP inhibitor resistance

Abstract
In mammalian cells, DNA damage response initiates repair by error-free homologous recombination (HRR) or by error-prone non-homologous end joining (NHEJ). DNA damage is detected by PARP proteins that facilitate this repair, both in normal cells and in cancer cells. Cells containing BRCA1/2 mutations have an HRR-deficient repair mechanism which may result in unrepaired one-ended double-strand breaks and stalled replication forks, considered as the most lethal cell damage. Here, we review the state of the art of the role of Poly (ADP-ribose) polymerase (PARP) inhibitors as a precision-targeted anticancer drug in BRCA1/2-mutated female breast cancer. Although knowledge is incomplete, it is assumed that the main role of the archetype PARP1 in the cell nucleus is to detect and adhere to single-strand breaks. This mediates possible damage repair, after which cells may continue replication; this process is called synthetic lethality. As for PARP clinical monotherapy, progression-free survival has been observed using the FDA- and EMA-approved drugs olaparib and talazoparib. In the case of combined drug therapy, a synergy has been demonstrated between veliparib and platinum drugs. Information regarding adverse effects is limited, but hematological effects have been described. However, there is need for multicenter trials, preferably conducted without commercial guidance and funding. Some of the available trials reported resistance to PARP inhibitors. In this review, we also describe the various causes of resistance to PARP inhibitors and research indicating how resistance can be overcome.

Introduction

Breast cancer is the most prevalent cancer in women, with a global incidence of 2.3 million cases in 2020. Overall mortality in the same year recorded 685,000 deaths [1], although prevention, based on mammographic X-ray

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screening, has been a long-standing effort [2–6]. Patients harboring a germline BRCA1 or BRCA2 mutation suffer from an autosomal dominant genetic disorder in these tumor suppressor genes [7–11]. This special category of patients is predisposed to develop breast cancer. The prevalence among patients with HER2-negative breast cancer and without traditional risk factors for harboring germline BRCA mutation was reported to be 5.8% [12]. Mutations in BRCA genes may become clinically evident for BRCA 1 (location 17q21) before the age of 40 years and for BRCA2 (location 13q12.3) before the age of 50 years [13]. A European prospective cohort study of women with a BRCA mutation has estimated the cumulative breast cancer risk for BRCA1/2 mutation carriers as 67–72% by the age of 80 years and 55–60% by the age of 70 years [14]. These numbers are in fair concordance with the absolute risk of breast cancer through 80 years of life, associated with protein-truncating variants in 8 genes, being of the order of 55% for BRCA1 and 45% for BRCA2. This is based on a recent population-based international study involving a total of 113,000 women consisting of more than 60,000 women with an invasive breast tumor and 53,000 controls [15]. The age at which first cancer in BRCA1/2-mutated patients is discovered is an indicative risk factor for bilateral breast cancer. Diagnosis before age 41 years carries a 23.9% risk and is reduced to 12.6% in those aged 41–49 years [16, 17]. Notably, in a subgroup analysis in Icelandic patients with a BRCA2 mutation, an adverse effect regarding prognosis was seen in women with estrogen-positive breast cancer [17, 18]. Apart from the aggravating bilateral prophylactic mastectomy [19, 20], regular mammography, ultrasound examination, or MRI represent important risk-reducing imaging modalities for BRCA1/2 carriers [21]. Lifestyle changes, such as eating healthy food, overall outcomes in BRCA1/2-mutant carriers have not been promising or are not decisive [22–24].

As for treatment, precision-targeting molecules such as PAR (Poly ADP-ribose) polymerases or PARPs are a welcome novel addition to the therapeutic arsenal [25, 26]. The goal of this review is to provide a comprehensive overview of the biomechanism of PARPs and the clinical results obtained so far with these agents, focusing on PARP inhibitors.

Data Collection

For this review, we searched biomedical and life science journals in the PubMed archive. We identified 129 relevant articles (regular peer-reviewed articles, early publications, reviews, and clinical trials) using the terms BRCA, BRCA-related breast cancer, PARP, and PARP inhibitor during the period January 1, 2007, to July 1, 2022. We reviewed 103 of these articles for this paper, having determined that the remaining 26 were beyond the scope of this review.

BRCA 1/2 Pathogenic Mutations

BRCA1 and BRCA2 proteins are essential to homologous recombination repair (HRR) of DNA breaks. BRCA1 is pivotal in the recognition and response to DNA damage. BRCA2 controls proteins such as RAD51 which is a DNA-dependent ATPase that interacts with replication protein A (RPA) to repair double-stranded breaks. This mechanism is mediated by the HRR system and the no-homologous endjoining (NHEJ) system [27]. Whereas the first is a slow but precise mechanism using the sister chromatid as a template, the latter is a fast, low-fidelity process that can introduce alterations in the sequence by using direct ligation of DNA ends. Nevertheless, both repair mechanisms are complimentary and essential for chromosome stability. A recent article by Palleschi et al. [25] describes these repair processes in detail. In short, following the protein biosynthesis of ADP-ribose, post-translational modifications take place to form the "biopolymer" poly(ADP-ribose) which consists of up to 400 linked ADP-ribose residues that form the polymerase known as PARP. The PARP family has 17 members; the archetype PARP1 is exclusively present in the cell nucleus, where it has a critical role in multiple DNA repair pathways [27]. According to the most accepted model, PARP1 is activated by DNA damage via its N-terminal DNA-binding domain. Experimental evidence indicates that PARP1 selects its target site via zinc fingers within this domain. Subsequently, the PARP1 molecule undergoes structural remodeling necessary for DNA damage repair [28]. Preclinical experiments demonstrated that inhibition of PARP activity renders cells susceptible to carcinogenic agents [29], suggesting that inhibition of the PARP protein has a role in carcinogenesis. Indeed, inhibition of PARP impedes the maturation of nascent DNA strands during DNA replication [30]. This brings about the production of double-strand breaks and stalled replication forks. Throughout the cell cycle, the NHEJ error-prone pathway is the predominant double-strand break repair mechanism. It has a higher efficiency than the HRR, which is primarily used in the S-phase. The repair mechanisms differ in the DNA end resection. In order to

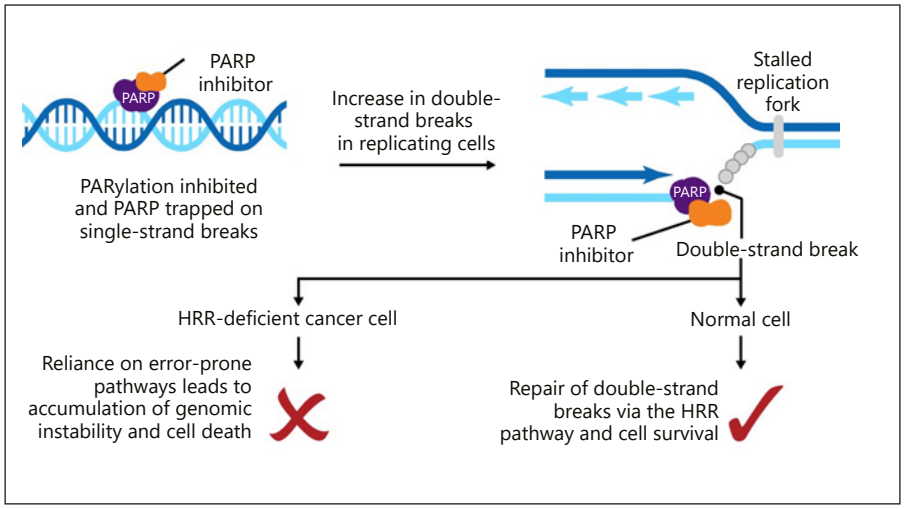

Fig. 1. Simplified representation of the DNA damage response (DDR) signaling pathways: synthetic lethality by PARP inhibitors in HRR-deficient cancer cells, whereas repair of double strand breaks occurs in normal, non cancerous cells according to the HRR pathway (reproduced from Cortesi et al. [36]). HRR, homologous recombination repair; PARP, poly(ADP-ribose) polymerase. Reproduced according to the licensing conditions of http://creativecommons.org/licenses/by/4.0/.

propagate, the cancer cell relies on the NHEJ system. This introduces DNA rearrangements, and, as a result, PARP inhibitor causes replication stress that results in killing of cancer cells, saving the normal cells due to their slower replication speed, and a more functional HRR system (see below, Fig. 1).

PARP Inhibitors for Treatment of Breast Cancer

Recent advances highlight the use of PARP inhibitors in the treatment of patients with BRCA-associated breast cancer [31–35]. Both the clinical results with PARPi's as mono agents and PARPi's as part of a combination therapy will be discussed below.

PARP Inhibitor Monotherapy

Two PARP inhibitors, olaparib and talazoparib, were approved by the FDA and EMA in 2018/2019. Olaparib was approved for the adjuvant treatment of adult patients with deleterious or suspected deleterious germline BRCA-mutated, HER2-negative, high-risk early breast cancer. This is specifically for patients who have been treated with neoadjuvant or adjuvant chemotherapy. Talazoparib was approved for the treatment of adults with deleterious or suspected deleterious germline BRCA mutation-positive, HER2-negative, locally advanced or metastatic breast cancer. This was based on the favorable results in the OlympiAD and EMBRACA Phase 3 studies [36]. A recent in-depth study focused on the efficacy, safety profile, and potential harms of PARP inhibitors in the treatment of patients with locally advanced or metastatic HER2-negative BRCA germline-mutated breast cancer patients [37]. This review of clinical data from 1,474 patients in 5 studies reported an improvement (HR 0.63; 95% CI: 0.56–0.71) and supports the use of PARP inhibitors as part of the therapeutic strategy in this population, the toxicity profile being no worse than chemotherapy. Two other meta-analyses on the efficacy and safety of PARP inhibitors came to similar conclusions. Chang et al. [38] reported a progression-free survival with outstanding overall efficacy (HR 0.56; 95% CI: 0.45–0.68) for PARP inhibitors versus chemotherapy. A subanalysis of their data confirmed these positive results for both BRCA1-mutant carriers (HR 0.65; 95% CI: 0.53–0.78) and BRCA2-mutant carriers (HR 0.63; 95% CI: 0.51–0.76) as well as for triple-negative cases (HR 0.62; 95% CI: 0.50–0.77). Further to this, Sun et al. [39] showed that progression-free survival significantly improved using the single-agent PARP inhibitor in BRCA1 patients (HR 0.64; 95% CI: 0.53–0.79) with similar results in triple-negative breast cancer patients (HR 0.65; 95% CI: 0.54–0.79). These researchers also reported adverse hematological events: neutropenia in 35% of the patients studied, anemia in 29%, and thrombocytopenia in 24%. A network meta-analysis compared talazoparib ($n = 241$) and olaparib ($n = 492$) for efficacy, safety, and acceptability and reported similar characteristics in patients with BRCA-mutated HER2-negative metastatic or advanced breast cancer [40]. They concluded that monotherapy with either agent can be regarded as an option for treating both categories of patients. FDA databases list monotherapy studies using veliparib and niraparib in heavily pretreated patients, but definite results are not available yet.

In this context, it needs to be mentioned that triple-negative breast cancer has an unfavorable prognosis as hormone or receptor treatments are not efficacious or are only minimally efficacious. This particularly aggressive subtype of breast cancer, often in women below 40 years, has been associated with reduced expression of repair genes. The average prevalence of this mutation has been estimated as 35% for BRCA1 and 8% for BRCA2 [41, 42].

PARP Inhibitors in Combination Therapies

As noted above, PARP inhibitor monotherapy works out favorably in patients, but some preliminary studies suggest that PARP inhibitors in combination therapy using (either prior or subsequent) cytostatic drugs may be more successful [43]. Indeed, treatment, with PARP inhibitors, of triple-negative cancer patients who received prior or subsequent platinum-based chemotherapy demonstrated beneficial results in the OlympiAD (NCT02000622) and EMBRACA (NCT01945775) open-label randomized, multicenter phase 3 trials with olaparib and talazoparib, respectively. This is an important finding as about two-thirds of the triple-negative patients are BRCA1 proficient and without homologous recombination deficiency.

These patients would not benefit from monotherapy with PARP inhibitors as they often experience resistance to platinum-based agents [44]. In this way, the synergy between both drugs is evident and has opened the way to evaluate PARP inhibitors in combination therapies. In the Brocade 3 trial (NCT02163694; randomized, double-blind, placebo-controlled, 36 countries, full results available after November 2021), the PARP inhibitor drug veliparib was combined with carboplatin and paclitaxel. These researchers concluded that "the addition of veliparib to a highly active platinum doublet, with continuation as monotherapy if the doublet were discontinued, resulted in significant and durable improvement in progression-free survival in patients with germline BRCA mutation-associated advanced breast cancer" [45].

The California Cancer Consortium Trial (NCT0114 9083, limited sample size, no randomization) investigated the efficacy of the PARP inhibitor veliparib with carboplatin or as a single agent in patients with germline BRCA1- or BRCA2-associated metastatic breast cancer. Out of 28 enrolled patients in phase 1, 27 patients experienced positive results from treatment with veliparib in combination with carboplatinum. In phase 2, the trial design was amended to assess the efficacy of single-agent velarib. In this phase, a total of 49 patients were enrolled, of which 22 BRCA1 carriers and 22 BRCA2 carriers could be evaluated. Leucopenia occurred in 43% of the patients in phase 1. In phase 2, dose adjustments were needed in 25% of the patients, and 6% of the patients could not be evaluated due to toxicities. The median best response duration for phase 1 patients was 28 weeks for BRCA1 patients and 26 weeks for the BRCA2 patients. In phase 2, these periods were 18 and 28 weeks, respectively. In spite of the limited number of eligible cases and the nonrandomized nature of the study, the authors concluded that the combination therapy was superior to single-agent veliparib treatment. Additionally, the results of an important phase 2 randomized trial (NCT 02595905) on cisplatin with or without veliparib in metastatic triple-negative breast cancer in 323 eligible patients were published by Sharma et al. [46]. Among the patients were 37 BRCA-mutant (BRCA+) carriers, 101 BRCA-like patients, as well as 110 non-BRCA-like patients. Progression-free survival was numerically improved for the BRCA+ and the BRCA-like group when combined treatment was applied, although for BRCA+ the difference with cisplatin alone was not significant. In the non-BRCA-like group, no benefit of veliparib addition was observed. Notably, when combined therapy was used, the authors observed a grade 3/4 neutropenia (46 vs. 19%) and anemia (23 vs. 7%). A tentative conclusion based on these available data is that the combined cisplatin and veliparib treatment is beneficial for patients with BRCA-like triple-negative breast cancer and merits further investigation.

In 2021, Turner et al. [47] reported on an open-label randomized phase 3 study of patients with mutated BRCA1/2 and HER2-negative advanced cancer and those who had relapsed within 12 months of adjuvant chemotherapy. The study compared the use of niraparib versus the chemotherapy chosen by the physician. However, after the preplanned interim analysis, recruitment was halted because of a high degree of discordance between local and central assessment of progression-free survival in the physician's choice of chemotherapy arm that resulted in informative censoring. Nevertheless, the authors state that there was clear evidence of the efficacy of niraparib in this patient population. The outcome of this study needs to be supported by further data.

To the best of our knowledge, no further important and/or final trial outcomes on the use of olaparib, niraparib, rucaparib, veliarib, talazoparib (for structural formulas see reference [48]) either as monotherapy or in combination with other agents have been published in the literature. It is unlikely that these results (of paramount importance for the treatment of triple-negative breast cancer patients) will be available soon; the list of ongoing

trials, published by the Educational Portal for Oncologists (see: oncologypro.esmo.org) reports that delays have occurred for various reasons.

Mechanisms of Resistance to PARP Inhibitors

In view of the invasive character of BRCA-driven breast cancer, PARPs are welcome candidates for precise targeting, but resistance to PARPs has been observed in patients. Below, we elaborate on the several mechanisms that play a role in this phenomenon.

The most common explanation for acquired resistance is the restoration of BRCA1/2 functionality by a process that restores the expression of functional BRCA, usually commonly called reversion mutation. This has been described in PARP inhibitor treatment of ovarian, prostate, and breast cancer [49, 50].

Decreased trapping of PARP inhibitor has been attributed to loss of PARG (an inhibitor of poly[ADP-ribose] glycohydrolase that may complement PARP inhibitors in the process of cancer cell death by synthetic lethality) [51]. Endogenous and exogenous DNA lesions may challenge the replication fork, and subsequent fork breakage can cause genome instability. To prevent tumorigenesis, the rescue of the fork is essential, and the action of RAD1 to stop cell cycle progression and other remodelers mediate fork recovery and its restart. This process allows the stalled replication forks to restart [52, 53]. However, this process of stabilization may also occur in cancer cells preventing their degradation due to PARP inhibitors. Thus, this restoration of stalled forks may result in uncontrolled duplication of cancer cells and resistance to PARP inhibitor [54].

Epigenetic changes in HRR may lead to PARP inhibitor resistance because of hypermethylation of BRCA1/2 genes without changing the DNA sequence. It is a frequent phenomenon during tumorigenesis that results in decreased expression of corresponding mRNA and generates a deficiency in the homologous recombination system thereby decreasing sensitivity to PARP inhibitors [55].

The upregulation of ABC drug-efflux transporter genes has often been found in chemotherapy-resistant breast cancers due to chromosomal translocation. The high expression of Abcb1b gene encodes for the ATP-dependent broad substrate specificity efflux transporter P-glycoprotein, which is responsible for similar increased efflux of PARP inhibitors. This weakens the efficacy of these drugs [53, 56–58].

Triple-negative breast cancer harbors germline BRCA 1/2 mutations and faulty 53BP1 in 10–20% of cases. This results in higher expression of RAD51, which compensates for functional loss of BRCA1/2 and reactivates HRR. The increase of the HRR mechanism curtails the action of synthetic lethality by PARP inhibitors, causing drug resistance (Fig. 1). Subsequently, cell survival occurs in a BRCA1/2-independent manner and explains the resistance to PARP inhibitor [59–64].

Strategies to Overcome PARP Inhibitor Resistance

Franchet et al. [65] and Gralewska et al. [66] have described mechanisms that lead to the activation of the cell cycle checkpoint proteins such as ATM (ataxia telangiectasia mutated), CHK2, ATR (ataxia telangiectasia and RAD3-related protein kinase) and its major downstream checkpoint serine/threonine checkpoint kinase CHK1 (Fig. 2). This activation is crucial for the proper coordination of multiple DNA repair processes to maintain genome stability. Replication fork stability is required to overcome the consequences of oncogene activation, dysfunctional checkpoints as well as radiation therapy and chemotherapy. Activation of ATR/CHK1 leads to the activation of another important nuclear kinase, called WEE1, which regulates the activity of various cyclin-dependent kinases (CDKs) through phosphorylation. WEE1 is primarily involved in the G2/M-phase checkpoints (Fig. 2) [67–69]. Research over the past 10 years has demonstrated that this enzyme, together with the extra-nuclear kinase PKMYT1, controls genetic stability in nonmalignant cells and can act as a tumor suppressor. Paradoxically, it can act as a pseudogene that recognizes DNA damage in malignant cells due to chemotherapy while also initiating their repair [70]. Understandably, the pharmacological suppression of this type of DNA damage repair process provides the rationale for targeting these kinases in cancer. Against this background, it has been postulated that inhibition of both PARP, WEE1, and the ATR/CHK1 pathway would help to overcome PARP inhibitor resistance in tumor cells and contribute to the restoration of HRR, fork or both. Thus, in cancers with prominent reproduction stress, such as breast cancer in patients with the *BRCA*1/2 mutation, targeting these different fork-stabilizing mechanisms by the combination of PARP inhibitor and inhibition of ATR would lead to increased DNA double-strand breaks and tumor cell death [71].

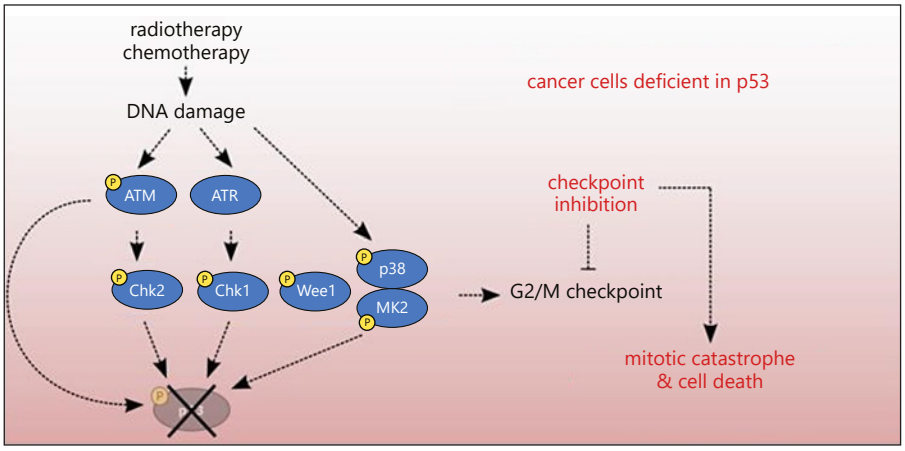

Fig. 2. Sensitizing cancer cells to DNA-damaging agent checkpoint inhibitors. Cancer cells deficient in tumor suppressor gene, like BRCA1/2, depend largely on checkpoint kinases to establish the G2/M checkpoint. Inhibition of checkpoint kinases in combination with DNA-damaging therapy leads to the G2/M checkpoint abrogation, mitotic catastrophe, and cell death. Notably, healthy cells are protected by 53BP1-dependent response (53BP1 is a p53 binding protein). (Reproduced and partially reproduced from reference, Benada and Macurek [67], according to the license conditions of http://creativecommons.org/licenses/by/4.0/.) p38, p38 MAPkinase (mitogen-activated protein kinase), responsive to cell damage to initiate apoptosis; MK2, (MAPKAPK2), a downstream substrate of p38MAPkinase, involved in transcript stability and cell proliferation. (For further abbreviations: see article text.)

Concluding Remarks and Future Directions

As discussed above, there is reliable evidence that stalled replication forks are a major source of double-strand breaks and drive the development of cancer cells. In order to protect the fork during replication, DNA replication proteins (polymerases) monitor and guide proper function, thereby preserving genome stability and cell health [72, 73]. However, during this process, a variety of obstacles may affect the function of these DNA polymerases and result in replication stress. An excellent article by Bainbridge et al. [74] discusses at length the function of these polymerases. Briefly, the stalling of polymerases is caused by unrepaired DNA lesions created by endogenous and exogenous sources, including impediments in DNA structures, repetitive sequences, and increased expression of oncogenes. Yet, through evolution, the jeopardized cell has developed an ingenious system to circumvent distorted templates by DNA damage tolerance. This process, taking place during the S-phase, enables DNA replication while circumventing the lesions and enabling cell repair. This process is called translesion DNA synthesis and occurs with the help of specialized DNA polymerases. These enzymes insert bases opposite the damaged bases. Basically, it is a strand exchange mechanism that anneals parent strands and generates newly synthesized strands to promote remodeling of the damaged structure of replication forks. It enables the cell to continue replication beyond the damaged template and maintain genome stability. When the replication fork is restructured, it is protected by the recombinase Rad51 that promotes the replication restart [75–77]. The polymerases that support the stabilization of the damaged fork belong to a large Y family. These DNA polymerases specialize in translesion DNA synthesis to complete replication beyond a damaged template using unique structural features that bind the damaged DNA [75].

Additionally, another mechanism that permits continuous replication beyond the damaged template is homologous recombination using a newly synthesized sister chromatid ("template switching") [76]. The abovementioned Y family polymerases apply a slow process that provides the time to repair the damage before the complete genome duplication takes place. Although this synthesis is not error-proof, each member of the Y family appears to assist in the high-fidelity repair of one specific kind of DNA damage [75, 76].

Notably, primase polymerase ("Primpol") is unique in the sense that the protein shares the same active site for the DNA polymerase and primase activities [78]. Also, it has been documented that Primpol may restart a stalled replication fork by acting as either a translesion DNA polymerase or by repriming DNA synthesis downstream of the lesion to reinitiate DNA synthesis [79, 80]. This is in line with a study by Pilzecker et al. [81] who demonstrated that Primpol has a critical antimutagenic activity

and may stimulate error-free homology directed repair. Indeed, a high number of Primpol-deficient tumors in breast cancer patients with invasive lobular and ductal carcinoma [81]. A study by Quinet et al. [82] suggests that Primpol is indispensable for cell survival in the absence of functioning BRCA1, implying that therapeutic targeting of this polymerase may be a useful strategy.

Thus, it is evident that the damaged cell may take recourse to several multi-connecting pathways to restore damaged DNA. As the replication fork has a central position in genome stability, there are ongoing studies to unravel the mechanisms that contribute to fork remodeling [83–85]. It is known that the tumor suppressor BRCA2 protects the stalled fork from degradation. Stress proteins such as ubiquitinated proliferating cell nuclear antigen (PCNA), Rad51 and Rad52 function as stabilizing factors. These proteins act alone or in combination with BRCA2 to harness the replication fork instability in BRCA-mutant breast cancer. Of note, with genotoxic stress, PCNA ubiquitination allows for replication of damaged DNA by recruiting lesion-bypass DNA polymerases [86, 87]. Intriguingly, this complex molecule protects both fork integrity and promotes resistance of BRCA-deficient cells to PARP inhibitors. Based on this knowledge, it has been suggested that ubiquitinated PCNA may even operate parallel to the BRCA-RAD51 pathway to protect the replication fork progression [88]. Further experimental evidence made clear that, in response to DNA replication stress, the fork may reverse its direction thus providing time to start the repair procedure and preventing double-strand breaks [89].

Detailed knowledge on various involved mechanisms is lacking and delays the translation from bench to bedside. For example, on the strength of the fact that the replication fork fulfils a central role in genome stability, several unsolved questions remain in relation to cancer treatment. Just to name a few: Is it possible to promote fork reversal and restart in a clinical setting [90]? What is the detailed biochemical relationship between fork stabilization and resistance to PARP inhibitors [91]? How does stalled fork stabilization work out in BRCA1/2-deficient cancer cells [92]?

On the clinical side, it is of immense importance to study the effectiveness of different PARP inhibitors for various subtypes of breast cancer [27, 93, 94]. As cancer is a disease caused by abnormalities in the genome, the mutational profile points to diagnosis and therapeutic options that include radiotherapy, chemotherapy, and immunotherapy along with precision agents such as PARP inhibitors as part of the treatment options. In this respect, the mutation profile also helps to understand why patients do not respond to treatment [95]. While for the most part, this paper deals with breast cancer, it appears that other cancers such as pancreatic, ovarian, and prostate malignancies may also be caused by BRCA deficiencies [96]. Understandably, the development of a broad biomedical perspective on the clinical use of PARP inhibitors for BRCA mutational female breast cancer is just one example. The progress in treatment of ovarian cancer may serve as an example of how PARP inhibitors may change the therapeutic landscape [97–103].

Dedication

The first author dedicates this article to Dr. Herman Koeter and Professor Kalevi Kairemo who made lifelong professional and personal efforts to implement reduction in animal testing and developments in patient care.

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Statement of Ethics

The authors have adhered to the principles of the article "Ethics in Medical Research and Publication" (*Int J Prev Med*. 2014; 5:1073–1082).

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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Author Contributions

Ernest K.J. Pauwels composed the first and later drafts of the manuscript and prepared the final text. Michel H. Bourguignon reviewed the drafts, made textual additions, selected references, and provided fresh data.

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