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European Association of Urology

Review – Prostate Cancer

DNA Repair in Prostate Cancer: Biology and Clinical Implications

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Article info

Article history:
Accepted August 12, 2016

Associate Editor:
James Catto

Keywords:
Prostate cancer
DNA repair
PARP
BRCA
Personalized medicine
DNA damage

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Abstract

Context: For more precise, personalized care in prostate cancer (PC), a new classification based on molecular features relevant for prognostication and treatment stratification is needed. Genomic aberrations in the DNA damage repair pathway are common in PC, particularly in late-stage disease, and may be relevant for treatment stratification.
Objective: To review current knowledge on the prevalence and clinical significance of aberrations in DNA repair genes in PC, particularly in metastatic disease.
Evidence acquisition: A literature search up to July 2016 was conducted, including clinical trials and preclinical basic research studies. Keywords included *DNA repair, BRCA, ATM, CRPC, prostate cancer, PARP, platinum, predictive biomarkers*, and *hereditary cancer*.
Evidence synthesis: We review how the DNA repair pathway is relevant to prostate carcinogenesis and progression. Data on how this may be relevant to hereditary cancer and genetic counseling are included, as well as data from clinical trials of PARP inhibitors and platinum therapeutics in PC.
Conclusions: Relevant studies have identified genomic defects in DNA repair in PCs in 20–30% of advanced castration-resistant PC cases, a proportion of which are germline aberrations and heritable. Phase 1/2 clinical trial data, and other supporting clinical data, support the development of PARP inhibitors and DNA-damaging agents in this molecularly defined subgroup of PC following success in other cancer types. These studies may be an opportunity to improve patient care with personalized therapeutic strategies.
Patient summary: Key literature on how genomic defects in the DNA damage repair pathway are relevant for prostate cancer biology and clinical management is reviewed. Potential implications for future changes in patient care are discussed.

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http://dx.doi.org/10.1016/j.eururo.2016.08.037

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1. Introduction

While therapeutic options for patients with advanced prostate cancer (PC) have improved over the last decade, castration-resistant PC (CRPC) remains a lethal disease [1]. Recently, relevant studies have identified genomic defects in DNA repair in advanced and primary PC. This has led to clinical studies that provide a strong rationale for developing PARP inhibitors and DNA-damaging agents in this molecularly defined PC subgroup. Following the successful development of targeted agents for molecularly defined subpopulations in other cancer types [2,3], there may be an opportunity to potentially improve patient care in PC via personalized therapeutic strategies. In this article, we review the biology and clinical implications of deleterious inherited or acquired DNA repair pathway aberrations in PC.

2. Evidence acquisition

A literature search for clinical trials and preclinical basic research studies up to July 2016 was conducted. Keywords for the included "DNA repair", "BRCA", "ATM", "CRPC", "prostate cancer", "PARP", "platinum", "predictive biomarkers", and "hereditary cancer".

3. Evidence synthesis

3.1. The molecular landscape of primary and advanced PC

Advances in genomics have permitted the identification of putative drivers of carcinogenesis and cancer progression. These genomic data provide for precise molecular tumor subclassification that extends beyond traditional histologic descriptions. For optimal utility, molecular clusters should provide prognostic or predictive information relevant for patient care [4].

Several studies have depicted the genomic landscape of primary prostate tumors [5–7]. Recently, The Cancer Genome Atlas Research Network (TCGA) reported on whole-exome sequencing of a series of 333 localized PCs [8]. Seven subgroups were defined on the basis of certain gene fusions involving the ERG/ETS transcription factor family (ERG, ETV1/4, and FLI1) or recurrent mutations in specific genes (*SPOP*, *FOXA1*, and *IDH1*); these subgroups differ with regard to androgen receptor (AR) signaling activity, DNA methylation, and microRNA expression.

In the TCGA study, in which Gleason ≥8 tumors represented 26% of the cohort, 62/333 (19%) tumors had deleterious germline or somatic aberrations in genes key to the DNA damage repair pathway (*BRCA2*, *BRCA1*, *CDK12*, *ATM*, *FANCD2*, *RAD51C*). Six of these aberrations involved a *BRCA2* K3326* nonsense germline variant, which arguably does not greatly impact protein function despite a modest association with risk of cancer [9], and 23 cases had heterozygous deletions of *FANCD2* or *RAD51* without evidence of biallelic inactivation; consequently, the proportion of localized PCs with impaired DNA repair function is probably less than 19%.

Next-generation sequencing studies of metastatic tumors identified enrichment of mutations in DNA repair genes among patients with lethal disease [10,11]. To provide a systematic analysis of the genomic landscape of CRPC and its potential relevance for patient care, the Stand Up To Cancer (SU2C)-Prostate Cancer Foundation (PCF) International Dream Team pursued whole-exome and transcriptome sequencing of 150 biopsies from metastatic CRPC (mCRPC) [12]. Higher prevalence of aberrations in key DNA repair genes (23%), *TP53* (53%), *RB1* (21%), the PTEN-PI3K pathway (49%), and *AR* (63%) in mCRPC than in localized disease was confirmed. It is not yet clear if this enrichment is secondary to a tumor evolution process in response to therapy exposure, or purely suggests markers of more aggressive PCs (Table 1).

Table 1 – Prevalence of DNA repair gene mutations and deletions described in studies on localized and metastatic prostate cancer

Study	Disease status	Samples	Gene frequency							
		(*n*)	Homologous recombination				MMR		NER	
SU2C-PCF CRPC	CRPC metastasis	150	*BRCA1*	0.7%	*CDK12*	4.7%	*MLH1*	1.3%	*ERCC2*	1.3%
genomic landscape			*BRCA2*	13.3%	*CHEK2*	3.0%	*MSH2*	3.0%	*ERCC5*	1.3%
[12]			*ATM*	7.3%	*PALB2*	2.0%	*MSH6*	2.0%		
UM PC genomics	CRPC metastasis	50	*BRCA1*	0%	*CDK12*	6.0%	*MLH1*	2.0%	*ERCC2*	2.0%
[11]			*BRCA2*	12.0%	*CHEK2*		*MSH2*	2.0%	*ERCC5*	12.0%
			ATM	6.0%	*PALB2*	0%	*MSH6*	2.0%		
UM PC genomics	Treatment-naïve tumors	11	*BRCA1*	0%	*CDK12*	0	*MLH1*	0	*ERCC2*	0
[11]			*BRCA2*	1/11	*CHEK2*	0	*MSH2*	1/11	*ERCC5*	0
			ATM	1/11	*PALB2*	0	*MSH6*	1/11		
Weill Cornell/Broad	Prostatectomy for localized	109	*BRCA1*	1.8%	*CDK12*	0	*MLH1*	0	*ERCC2*	0
[6]	or locally advanced PC		*BRCA2*	0%	*CHEK2*	0	*MSH2*	0	*ERCC5*	0
	(somatic only)		*ATM*	2.8%	*PALB2*	1.8%	*MSH6*	0.9%		
TCGA localized PC	Localized PC	333	*BRCA1*	1.0%	*CDK12*	2.0%	*MLH1*	0.3%	*ERCC2*	0.6%
[8]			*BRCA2*	3.0%*	*CHEK2*	0%	*MSH2*	0.3%	*ERCC5*	0.3%
			ATM	4.0%	*PALB2*	0%	*MSH6*	1.5%		

MMR = mismatch repair; NER = nucleotide-excision repair; PC = prostate cancer; CRPC = castration-resistant PC; SU2C-PCF = Stand Up To Cancer-Prostate Cancer Foundation; UM = University of Michigan; TCGA = The Cancer Genome Atlas.

With regard to DNA repair genes, the SU2C-PCF study identified inactivation of key DNA repair genes in at least 23% of cases, including homologous recombination (HR)–mediated repair genes (most commonly *BRCA2* and *ATM*) and mismatch repair (MMR) genes (*MLH1*, *MSH2*). Other DNA repair mechanisms are also likely to be impacted because of known influences of the AR in nonhomologous end-joining (NHEJ), and possibly aberrations in nucleotide excision repair (NER) and base excision repair (BER).

Intrapatient tumor heterogeneity represents a challenge for genomic stratification of PC in the clinic. Several studies have comprehensively observed an overall higher degree of heterogeneity within primary prostate tumors than in advanced disease [13–15]. This is likely to be related to: (1) bottlenecks in the metastatic process that limit metastatic spread and growth; (2) the capacity of metastatic tumor cells to seed other metastasis and even reseed the primary tumor; and (3) the selection of resistant clones driven by treatment exposures. Alterations in DNA repair genes have been related to increased mutational burden and may generate increased intrapatient heterogeneity; specific studies addressing the impact of genomic instability on treating the diverse subtypes of this common disease are now needed.

3.2. The DNA damage response pathway: a general overview

At any time, the DNA in human cells is constantly being damaged. If there is a deficient repair of this damage, genome stability is compromised, which can contribute to tumorigenesis. Damage can occur endogenously (due to spontaneous hydrolysis of bases or reaction of DNA with naturally occurring reactive oxygen species or alkylating agents) or can be induced by exogenous agents (eg, radiation and toxins). To protect their genome integrity, cells have evolved a complex signaling machinery for recognizing and repairing damage that includes several pathways with complementary and partially overlapping functions. Different forms of DNA damage trigger a response from different branches of this complex system. The main workflow is as follows; when genomic insults are detected, cell-cycle checkpoints are activated to halt the cell cycle and allow the cellular machinery to repair the DNA damage. If the repair is successful, the cell can continue its normal cycle; otherwise, programmed cell death or senescence programs are triggered. If the DNA repair mechanisms are dysfunctional, genomic instability, which is one of the hallmarks of carcinogenesis, ensues.

When damage is limited to one of the DNA strands (single-strand breaks or base modifications), different repair mechanisms can be deployed. These include BER, single-strand break repair (SSBR), NER, and MMR. Each of these pathways uses the complementary undamaged strand as a template to ensure fidelity of repair. BER is mainly activated to repair endogenous oxidative or alkylated base damage [16]. PARP1 and PARP2 are involved in detecting single-strand breaks, which are formed either directly or as intermediates in BER, and help to coordinate the SSBR response. The NER machinery is responsible for repairing bulky adducts such as those induced by UV light, for which the ERCC family of proteins are key mediators. The MMR pathway corrects mutations formed during DNA replication and recombination. The MSH and MLH family of genes are, among others, critical for MMR. The primary mechanisms involved in DNA double-strand break (DSB) repair comprise the HR system and NHEJ. HR requires a sister chromatid as template and is therefore restricted to the S/G2 phases of the cell cycle. It restores the original DNA code error-free. Key mediators of this pathway include BRCA1, BRCA2, PALB2, ATM, ATR, RAD51, MRE11, CHEK2, and XRCC2/3. In contrast, NHEJ functions by ligating broken DNA ends without the use of a template and is therefore functional throughout the cell cycle. The error-prone mode of NHEJ action leads to errors that are permanent and can drive genomic instability (Fig. 1). SSBs that are not repaired before DNA replication takes place will collapse replication forks, leading to formation of DSBs, which then require HR for repair and continued replication [17].

3.3. DNA repair defects play a relevant role in carcinogenesis and PC progression

Prostate carcinogenesis is mediated, as in other cancers, by the accumulation of genetic and epigenetic aberrations; these molecular changes can be inherited or be the result of altered AR transcriptional activity, changes in chromatin architecture, oncogenic replication, error-prone DNA repair, or defective cell division. The sum of these processes confers survival and growth advantage to the transformed cell. Many of these alterations are induced by factors of the microenvironment, particularly the immune system. Chronic inflammation with continued oxidative stress contributes to carcinogenesis of the prostate epithelium by inducing genomic damage. Deficient DNA repair response and defective apoptotic checkpoint control can then lead to permanent incorporation of these genome abnormalities.

AR signaling is critical not only for normal development of the prostate gland but also for prostate carcinogenesis. Genomic instability is related to AR transcriptional activity, and the cross-regulation between AR signaling and DNA damage response pathways appears to be relevant for PC progression [18]. Nevertheless, the role of AR in genome instability is only partly understood [19,20].

Rearrangements between the androgen-regulated *TMPRSS2* gene and the ETS genes *ERG*, *ETV1*, and *ETV4* are common in PC; these appear to be early events contributing to, but not sufficient on their own, prostate carcinogenesis, and are at least partly lineage-specific [5]. AR-driven transcription can result in increased DNA DSB generation at transcriptional hubs, probably as a result of topoisomerase-IIβ enzyme activity, leading to complex structural rearrangements across the genome [21,22]. Mechanistically, this is supported by AR binding to specific chromosomal sites creating a proximity to otherwise distant chromatin loci [20]. *TMPRSS2-ERG* translocation is probably the commonest example of such processes [23,24].

Interestingly, some PCs are characterized by high numbers of rearrangements. Many of these tumors have

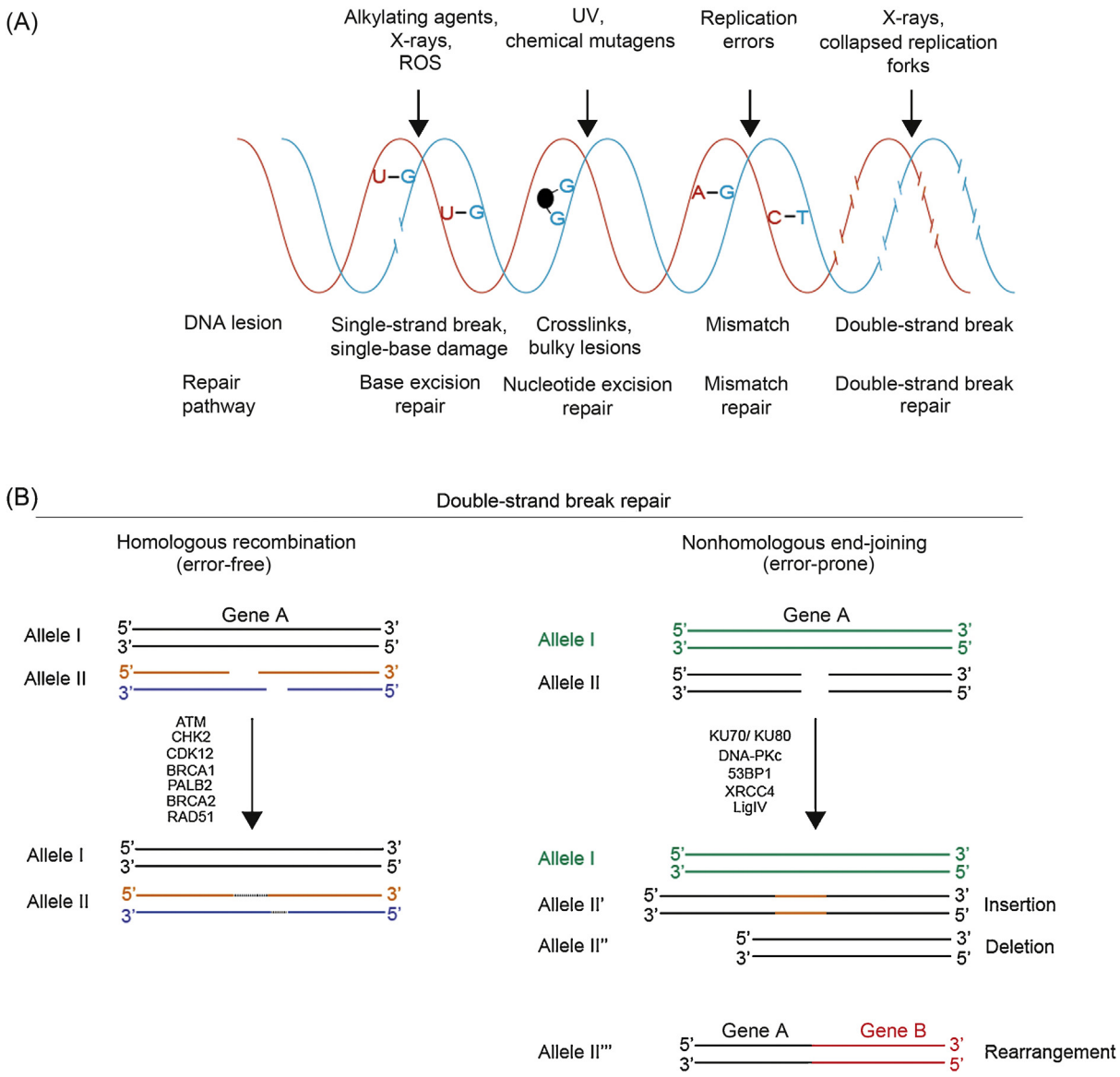

Fig. 1 – An overview of the most important DNA damage–inducing stresses and the corresponding molecular pathways that eukaryotic cells established to repair these. Diverse environmental and endogenous stresses can damage DNA, causing either single-strand (lilac) or double-strand (red box) DNA breaks. Eukaryotic cells developed various molecular mechanisms that repair such damage. DNA double-strand breaks are the most toxic DNA damage, and can be lethal for a cell if not properly repaired. The two most common and best-studied DNA double-strand repair pathways are homologous recombination (HR; error-free) and nonhomologous end-joining (NHEJ; error-prone). HR is limited to the S/G2 phases of the cell cycle and requires a sister chromatid as repair template. Key pathway components are highlighted. NHEJ is active mostly during the G1 phase of the cell cycle and can lead to structural genomic alterations (rearrangements), loss of genomic material (deletion), or insertion of additional nucleotides as a consequence of its imprecise nature. Key pathway components are highlighted.

oncogenic mutations in the *SPOP* gene that stabilize proteins including AR and its transcriptional regulators. Mechanistically, *SPOP* mutant tumors rely predominantly on NHEJ-based DSB repair (while reducing error-free HR-mediated DSB repair activity) [25].

The pattern of genomic aberrations may partly depend on deficiencies in specific DNA repair pathway branches. It has been shown that loss of MMR function induces a hypermutated microsatellite unstable genotype [12]. Somatic complex rearrangements in *MSH2* and *MSH6*, as well as somatic and germline truncating mutations in these two genes, have been described as the most common mechanism for MMR-deficient prostate tumors [26,27]. BRCA2-deficient PCs also present specific mutation signatures enriched in deletions and with higher mutational burden than wild-type–BRCA2 tumors [28,29].

3.4. *Inherited mutations in DNA repair genes and PC risk*

Hereditary germline mutations in DNA repair genes are associated with a higher risk of PC. This results in one gene allele being dysfunctional in every cell, with the second

allele commonly lost by a second hit (mutation, deletion, epigenetic silencing) [30]. Germline mutations in *BRCA2* increase the risk of developing PC (relative risk 8.6 in men <65 yr) [31,32]; their role in the development and progression of breast, ovarian, and pancreatic cancers is also well established. Moreover, inherited mutations in other DNA repair genes such as *PALB2*, *MLH1*, *MSH2*, and *PMS2* also appear to be associated with PC risk [33]. While the proportion of patients carrying a germline *BRCA1/2* mutation is low (1–2%) among the general population of primary PC patients, a multicenter study lead by the SU2C-PCF consortium in metastatic PC patients estimated the prevalence of germline *BRCA2* mutations as 5.3% in the setting of advanced disease; when a panel of 20 DNA repair genes was considered, 82/692 (11.8%) of patients with metastatic disease carried an underlying germline mutation [34]. Interestingly, age at diagnosis and family history of PC did not identify the mutation carriers, although there was enrichment among patients with a family history of cancer. It is therefore now critical to reconsider current guidelines for germline DNA testing; this could be relevant not only for treatment stratification but also in triggering cascade genetic testing for relatives who may be candidates for targeted cancer screening programs.

At present there is no consensus on how to manage this high-risk population with regard to screening for PC. To address this issue, the IMPACT study is evaluating targeted PC screening in men with germline *BRCA1/2* (gBRCA1/2) mutations. Annual prostate-specific antigen (PSA) tests are performed, and a biopsy is triggered if PSA >3ng/ml. A total of 1522 gBRCA1/2 mutation carriers and 959 controls had been recruited at last reporting. Preliminary results have revealed a higher incidence of PC in g*BRCA2* mutation carriers (3.3% vs 2.6% in g*BRCA1* mutation carriers, <2% for controls), who also have a higher likelihood of intermediate/high risk. Final results from these studies are awaited to ascertain the optimal screening strategies for this population [35].

Inherited mutations impairing the MMR function (Lynch syndrome) have been associated with an almost fivefold higher risk of PC, although additional work is needed to determine precise risks [36].

3.5. Impact of DNA repair defects on clinical outcome and response to treatment in PC

The relevance of somatic loss of function of DNA repair genes in the treatment of CRPC is still not clear, as neither the TCGA (primary tumors) nor the SU2C/PCF (metastatic disease) landscape studies reported follow-up clinical outcome data. Prospective studies looking at whether this molecular classification results in clinically relevant stratification for prognosis and treatment response are needed [8,12]. There are data on clinical outcome according to gBRCA1/2 in localized disease. In a series of more than 2000 patients with localized PC, including 61 *BRCA2* and 18 *BRCA1* mutation carriers, 23% of gBRCA1/2 mutation carriers developed metastasis after 5 yr of radical treatment, compared to 7% of noncarriers ($p = 0.001$). Cause-specific survival was significantly shorter among carriers (8.6 yr) compared to noncarriers (15.7 yr; $p = 9 \times 10^{-8}$). Subgroup analysis confirmed g*BRCA2* mutations as independent factor for poor prognosis [37]. The poorer outcome for g*BRCA2* mutation carriers seems to be particularly relevant for patients treated with radical radiotherapy in comparison to surgery, although the patient numbers evaluated were too small to support a robust claim [38]. The exact biological reasons underlying this poorer outcome remain to be fully elucidated; data from small series suggest that these tumors remain sensitive to taxanes [39,40].

3.6. Using DNA repair defects as a therapeutic target: PARP inhibitors

Over the last decade, exploitation of the vulnerabilities of tumor cells with DNA repair gene defects has been pursued in different tumor types, most successfully in ovarian and breast cancers. The identification of a subgroup of mCRPC with DNA repair defects with a similar genomic profile provides a strong rationale for developing the same therapeutic strategies for this molecular subtype of PC [10].

Poly (ADP-ribose) polymerases (PARP) are a family of enzymes involved, primarily, in transcriptional regulation and in detecting and localizing other DNA repair proteins to DNA single strand breaks. Activation of PARP1 and PARP2 triggers the damage response and recruits of key effectors of repair.

The fundamental basis for inhibiting PARP as anticancer therapy is the established biological concept called synthetic lethality: two genomic events that are each relatively innocuous individually become lethal when occurring together [41]. When PARP1/2 are pharmacologically inhibited, SSBs cannot be repaired and eventually progress to toxic DSBs. If a cell is competent in repairing damage, it will be able to fix the DSB. However, if a cell is lacking HR repair capacity (eg, *BRCA1*, *BRCA2*, *PALB2* or *ATM* is dysfunctional or lost), then PARP inhibiton would become lethal.

Two landmark studies demonstrated in 2005 specific killing of cell lines in which BRCA1/2 had been silenced or lost by the PARP inhibitor (PARPi) KU-0059436 (later named AZD2281, olaparib) [42,43]. In these studies, PARP inhibition led to γH2AX accumulation and the absence of RAD51 foci formation in BRCA-deficient models. Subsequent studies have revealed similar effects for other PARP inhibitors now in clinical development, and demonstrated that sensitivity to PARP inhibition also appears when other HR proteins besides BRCA1/2 are nonfunctional or lost [44,45].

This mechanistic interpretation of PARPi-associated synthetic lethality may, however, be a simplification of the underlying biological effect. It is now clear that PARP1 is involved in other DNA damage responses as well as SSBR, with reported functions in DNA replication and repair of stalled replication forks [46,47]. Moreover, certain PARP inhibitors may also have a direct cytotoxic effect by trapping PARP at DNA SSBs. These trapped PARP enzymes eventually induce replication fork stalling, which results in cell cycle arrest and apoptosis [48].

Lastly, of particular relevance to PC, PARP1 is involved in transcriptional regulation and has been implicated in AR

signaling and ERG function [49,50]. This direct interaction between PARP1 and ERG, as well as an interaction between PI3K/PTEN pathway aberrations and HR DNA repair [51,52], also raised hopes for a wider target population. However, these mechanisms have not been confirmed in human clinical trials to date [53,54].

3.7. Clinical development of PARP inhibitors in PC

A first-in-man clinical trial of olaparib among a cohort of patients with advanced solid tumors enriched in gBRCA1/2 mutation carriers provided critical proof of concept and clinical data on the exquisite antitumor activity of this drug in BRCA-deficient tumors [55]. Since then, olaparib has been evaluated in several phase II/III studies, mainly in ovarian cancer as a single agent, until granted US Food and Drug Administration and European Medicines Agency approval in 2014 for advanced ovarian cancer associated with *BRCA1/2* mutations [56–59].

With regard to mCRPC patients, a few carriers of deleterious gBRCA1/2 mutations were enrolled in the initial trials of olaparib, and showed promising tumor responses. In a phase 2 basket trial including 298 g*BRCA1/2* mutation carriers with different tumor types, eight mCRPC patients were enrolled (1 *BRCA1* mutant carrier, 7 *BRCA2* cases) [60]. Half (4/8) of the mCRPC patients experienced a radiologic partial response; the median progression-free survival for all eight patients was 7.2 mo, with two patients responding for over 1 yr. Of note, 4/8 patients had prior treatment with platinum-based chemotherapy before receiving olaparib. In line with data suggesting some degree of secondary cross-resistance [61], only 1/4 patients who were exposed to platinum responded to olaparib, compared to 3/4 of those who were platinum-naïve.

Other PARP inhibitors are in clinical development; data for PC patients are primarily from g*BRCA1/2* mutation carriers with PC who participated in early clinical trials of these compounds. Preclinical studies of BMN673 (Biomarin/Medivation) demonstrated high potency in inhibiting PARP [62], and tumor responses were seen in *BRCA1/2* mutation carriers across tumor types in a phase 1 clinical trial [63]. Rucaparib (AG-014699/CO-338, Pfizer/Clovis Oncology) and veliparib (ABT-888, Abbott Laboratories) have mainly been developed so far in combination with chemotherapies or other targeted agents [64,65].

The antitumor activity of PARP inhibitors as single agents in patients besides g*BRCA1/2* mutation carriers has been investigated in two studies. During the first-in-man trial of niraparib (MK-4827, Merck/Tesaro), an expansion cohort for "sporadic" CRPC patients was pursued. Eighteen patients received niraparib at the recommended phase 2 dose (300 mg QD). One patient achieved a >50% decrease in PSA, remaining on treatment for 10 mo [54]. Three more patients had significant declines in circulating tumor cell (CTC) counts for >6 mo. The trial was unable to associate responses with either PTEN or ERG expression.

More recently, results from the first stage of a phase 2 investigator-initiated adaptive study of olaparib in mCRPC have been reported, raising interest in developing PARP inhibitors for this disease. The TOPARP study conducted in the UK included a first stage (TOPARP-A) aimed at testing the antitumor activity of olaparib in a "sporadic" mCRPC population (not known to be g*BRCA1/2* mutation carriers and not selected based on any prior knowledge of the genomic background) [66]. The primary endpoint of the study was the response rate, using a composite definition of response: radiologic response according to RECIST 1.1 and/or PSA declines >50% and/or conversion in CTC count from poor (>5 CTC/7.5 ml of blood) to positive prognostic profile (≤5 CTC/7.5 ml of blood), confirmed in at least two readings 4 wk apart. Progression-free and overall survival were explored as secondary endpoints. Response to olaparib was evaluated in 49/50 patients who received at least one dose of olaparib. These were all mCRPC patients progressing on docetaxel and, for all but one, on abiraterone and/or enzalutamide. Some 58% of patients also progressed on cabazitaxel before participating in the study. Of the 49 patients, 16 fulfilled at least one of the response criteria, including 11 cases with a PSA decline >50% and 6/32 with radiologic partial responses among the patients with measurable disease. The antitumor activity observed was strongly associated with the presence of mutations or homozygous deletions in DNA repair genes, evaluated by next-generation sequencing for metastatic biopsies collected at trial entry. Seven patients were found to have biallelic loss of *BRCA2*, either by germline or somatic mutations and deletions, with all seven responding to therapy. In five cases, mutations impacting *ATM* function were found; 4/5 responded to olaparib, including patients with germline and somatic mutations, and two patients with a single-allele mutation in the ATM kinase domain and no evidence of biallelic loss. Moreover, four cases with biallelic events in other genes involved in DNA damage response, including *PALB2*, *FANCA*, and *BRCA1*, showed benefit, primarily involving prolonged CTC conversions. Only two patients responding to olaparib did not have a clear DNA repair defect according to genomic analysis. Several long response durations were observed, including four patients benefiting for >1 yr. Patients with defects in DNA repair genes exhibited improved progression-free and overall survival from treatment initiation, although the preliminary survival data reported will need to be re-evaluated after longer follow-up.

The promising results in this first stage of the TOPARP study led to initiation of a second trial (TOPARP-B) with prospective selection of patients with aberrations in DNA repair genes; the objectives are to validate the antitumor activity seen in patients with the most common mutations (*BRCA2*, *ATM*) and to acquire critical data on sensitivity to olaparib for patients with mutations or deletions in less commonly affected genes.

The tolerability profile of PARP inhibitors is manageable, with anemia, thrombocytopenia, fatigue, and gastrointestinal toxicities (primarily nausea) the most frequent. In the TOPARP-A trial, anemia (20%) and fatigue (12%) were the most common grade ≥3 adverse events; gastrointestinal toxicities were less relevant than reported for ovarian cancer [67]. Hematologic toxicities and fatigue were also

the dose-limiting events determining the recommended dose for other PARP inhibitors such as BMN673 and niraparib [54,63].

PARP inhibitors are also being evaluated in combination trials in mCRPC. An obvious strategy is to combine PARPi with DNA-damaging agents, mostly chemotherapy agents, to achieve a synergistic effect by blocking the response to chemotherapy-induced DNA damage. In a trial of veliparib and the alkylating agent temozolamide [65], 2/26 treated patients experienced PSA declines of >30%; the rate of grade 3–4 anemia and thrombocytopenia was 15% and 23% respectively. Overlapping hematologic toxicities also represent a major hurdle for combining platinum chemotherapies and PARPi.

An alternative approach would be to aim for a synthetic lethal interaction rather than a synergistic effect. Preclinical data demonstrating enhanced death of prostate tumor cells when combining HDAC and PARPi exemplify an opportunity for clinical development [68].

Lastly, trials combining PARPi with AR-targeting agents may be of interest on the basis of the crossregulation of both pathways and the central role of hormonal therapy in PC. Preliminary results from a randomized trial combining veliparib and abiraterone determined that 27% of patients had aberrations in DNA repair genes; this subgroup experienced high response rates to the combination and, remarkably, to abiraterone alone [53]. Data from a randomized trial combining abiraterone and olaparib are also expected. However, interpretation of putative predictive biomarkers of response in combination trials may be challenging.

3.8. DNA damaging agents: should they be reconsidered for PC?

Platinum salts are part of standard management for other tumor types, but their use in PC has been limited since phase 3 trials of the orally available platinum derivative satraplatin failed to meet the primary endpoint of overall survival (OS) improvement [69]. However, some antitumor activity has been described for carboplatin, cisplatin, and satraplatin in mCRPC. This, together with the possibility now of identifying DNA repair–defective tumors and data on DNA repair mutations and response to platinum from ovarian cancer studies, has raised interest in re-evaluating the role of platinum agents in this disease.

Recently, Kumar et al reported longer benefit from carboplatin for cases with HR defects in a retrospective series of patients ($p = 0.002$ for duration of treatment, $n = 21$). Small case series have reported tumor responses to carboplatin in mCRPC patients with biallelic *BRCA2* loss [70]. Nonetheless, the mechanisms involved in sensitivity to platinum and PARPi may be similar but not identical, and further investigation of cross-sensitivity and cross-resistance between agents is now needed following data from ovarian cancer studies. For example, the predominance of NER in repairing platinum-generated adducts warrants specific clinical trials [71,72].

A few clinical trials have explored combinations of carboplatin and taxanes for PC. One of the most relevant was a phase 2 study of carboplatin and docetaxel, followed by cisplatin and etoposide on progression. The study recruited 120 patients with mCRPC with prespecified clinicopathologic characteristics suggestive of more aggressive, arguably less AR-dependent disease [73]. With median OS of 16 mo, the radiological response rate was ~30% for both first- and second-line combinations. The tolerability was relatively acceptable, with only three cases of febrile neutropenia.

Use of the topoisomerase inhibitor mitoxantrone in PC has declined as several other therapies became available over the last decade. However, the main mechanism in the cytotoxicity of mitoxantrone is disruption of DNA synthesis and repair, so re-evaluation of its activity in molecularly defined populations may be of interest.

4. Conclusions

The identification of a subgroup of PCs with lethal disease with genomic deleterious aberrations of DNA repair genes supports further evaluation of this biomarker-driven treatment stratification of advanced PC in registration studies. If the efficacy of this strategy is, it might also be possible to apply it to earlier disease stages, including high-risk locally advanced disease.

Further studies are now needed to clinically qualify multiplex predictive biomarkers of DNA repair–defective PCs, particularly for the less common genomic aberrations that cause this phenotype. On the basis of recent studies indicating that these aberrations are common in the germline DNA of patients with metastatic PC, somatic and germline DNA testing for patients with advanced PC should be considered in view not only of the therapeutic consequences for the patient but also the possibility of pursuing targeted screening in this population. A major limitation at present for adoption of this strategy is the implementation and standardization of genomic testing in the community setting, but the decreasing costs of next-generation sequencing and lessons learned from stratified therapies in other diseases will help us to pursue more precise care for PC patients.

Author contributions: Johann S. de Bono had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Mateo, Boysen, de Bono.
Acquisition of data: Mateo, Boysen, de Bono.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: Mateo, Boysen.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: None.
Obtaining funding: None.
Administrative, technical, or material support: None.
Supervision: Barbieri, Bryant, Castro, Nelson, Olmos, Pritchard, Rubin, de Bono.
Other: None.

Financial disclosures: Johann S. de Bono certifies that all conflicts of interest, including specific financial interests and relationships and

affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: None.

Acknowledgments: J. Mateo and J.S. de Bono acknowledge funding from an Experimental Cancer Medical Centre grant, Cancer Research UK, Prostate Cancer Foundation, Prostate Cancer UK, a Biomedical Research Centre grant to the ICR/Royal Marsden, and a Medical Research Council - Prostate Cancer UK - Movember Fellowship to J. Mateo. G. Boysen was supported by a Marie Sklodowska-Curie International Individual Postdoctoral Fellowship (CDELP) and Prostate Cancer UK (PG13-036). C.E. Barbieri is a Damon Runyon Clinical Investigator supported (in part) by the Damon Runyon Cancer Research Foundation, and supported by the US National Cancer Institute (K08CA187417-02), the Prostate Cancer Foundation, and the Urology Care Foundation (Rising Star in Urology Research Award). H. Bryant is supported by Yorkshire Cancer Research (SS012PHD). C.C. Pritchard is supported by a CDMRP award (PC131820). The Prostate Cancer Unit at CNIO is supported by CRIS Fundación contra el cancer. E. Castro is the recipient of a Juan de la Cierva Grant from Ministerio de Economía y Competitividad (Spain). D. Olmos is also supported by Fundación Científica de la Asociación Española Contra el Cáncer. The Prostate Cancer Foundation provided support to the following authors through Young Investigator Awards: J. Mateo, C.E. Barbieri, D. Olmos, and C.C. Pritchard.

References

[1] Lorente D, Mateo J, Perez-Lopez R, de Bono JS, Attard G. Sequencing of agents in castration-resistant prostate cancer. Lancet Oncol 2015;16:e279–92.
[2] Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
[3] Flaherty KT, Robert C, Hersey P, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 2012;367:107–14.
[4] Rubin MA, Girelli G, Demichelis F. Genomic correlates to the newly proposed grading prognostic groups for prostate cancer. Eur Urol 2015;69:2–5.
[5] Baca SC, Prandi D, Lawrence MS, et al. Punctuated evolution of prostate cancer genomes. Cell 2013;153:666–77.
[6] Barbieri CE, Baca SC, Lawrence MS, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–9.
[7] Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214–20.
[8] Abeshouse A, Ahn J, Akbani R, et al. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25.
[9] Meeks HD, Song H, Michailidou K, et al. BRCA2 polymorphic stop codon K3326X and the risk of breast, prostate, and ovarian cancers. J Natl Cancer Inst 2016;108, djv315.
[10] Beltran H, Yelensky R, Frampton GM, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol 2013;63:920–6.
[11] Grasso CS, Wu Y-M, Robinson DR, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–43.
[12] Robinson D, Van Allen EM, Wu Y-M, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161:1215–28.
[13] Kumar A, Coleman I, Morrissey C, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med 2016;22:369–78.
[14] Beltran H, Prandi D, Mosquera JM, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298–305.
[15] Gundem G, Van Loo P, Kremeyer B, et al. The evolutionary history of lethal metastatic prostate cancer. Nature 2015;520:353–7.
[16] Wood RD, Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. Science 2011;1284:241–83.
[17] Saleh-Gohari N, Bryant HE, Schultz N, Parker KM, Cassel TN, Helleday T. Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks. Mol Cell Biol 2005;25:7158–69.
[18] Goodwin JF, Schiewer MJ, Dean JL, et al. A hormone-DNA repair circuit governs the response to genotoxic insult. Cancer Discov 2013;3:1254–71.
[19] Polkinghorn WR, Parker JS, Lee MX, et al. Androgen receptor signaling regulates DNA repair in prostate cancers. Cancer Discov 2013;3:1245–53.
[20] Lin C, Yang L, Tanasa B, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell 2009;139:1069–83.
[21] Ju B-G, Lunyak VV, Perissi V, et al. A topoisomerase IIβ-mediated dsDNA break required for regulated transcription. Science 2006;312:1798–802.
[22] Wu C, Wyatt AW, McPherson A, et al. Poly-gene fusion transcripts and chromothripsis in prostate cancer. Genes Chromosomes Cancer 2012;51:1144–53.
[23] Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
[24] Mani R, Tomlins SA, Callahan K, et al. Induced chromosomal proximity and the genesis of gene fusions in prostate cancer. Science 2010;326:2009–11.
[25] Boysen G, Barbieri CE, Prandi D, et al. SPOP mutation leads to genomic instability in prostate cancer. Elife 2015;4:e09207.
[26] Pritchard CC, Morrissey C, Kumar A, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014;5:4988.
[27] Chen Y, Wang J, Fraig MM, et al. Defects of DNA mismatch repair in human prostate cancer. Cancer Res 2001;61:4112–21.
[28] Decker B, Karyadi DM, Davis BW, et al. Biallelic BRCA2 mutations shape the somatic mutational landscape of aggressive prostate tumors. Am J Hum Genet 2016;98:1–12.
[29] Castro E, Jugurnauth-Little S, Karlsson Q, et al. High burden of copy number alterations and c-MYC amplification in prostate cancer from BRCA2 germline mutation carriers. Ann Oncol 2015;26:2293–300.
[30] Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC. Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci U S A 1992;89:3367–71.
[31] Kote-Jarai Z, Leongamornlert D, Saunders E, et al. BRCA2 is a moderate penetrance gene contributing to young-onset prostate cancer: implications for genetic testing in prostate cancer patients. Br J Cancer 2011;105:1230–4.
[32] Leongamornlert D, Mahmud N, Tymrakiewicz M, et al. Germline BRCA1 mutations increase prostate cancer risk. Br J Cancer 2012;106:1697–701.
[33] Eeles R, Goh C, Castro E, et al. The genetic epidemiology of prostate cancer and its clinical implications. Nat Rev Urol 2014;11:18–31.
[34] Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA repair gene mutations in men with metastatic prostate cancer. N Engl J Med 2016;375:443–5. http://dx.doi.org/10.1056/NEJMoa1603144.
[35] Bancroft EK, Page EC, Castro E, et al. Targeted prostate cancer screening in BRCA1 and BRCA2 mutation carriers: results from the initial screening round of the IMPACT study. Eur Urol 2014;66:489–99.

[36] Haraldsdottir S, Hampel H, Wei L, et al. Prostate cancer incidence in males with Lynch syndrome. Genet Med 2014;16:553–7.
[37] Castro E, Goh C, Olmos D, et al. Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. J Clin Oncol 2013;31:1748–57.
[38] Castro E, Goh C, Leongamornlert D, et al. Effect of BRCA mutations on metastatic relapse and cause-specific survival after radical treatment for localised prostate cancer. Eur Urol 2015;68:186–93.
[39] Gallagher DJ, Cronin AM, Milowsky MI, et al. Germline BRCA mutation does not prevent response to taxane-based therapy for the treatment of castration-resistant prostate cancer. BJU Int 2012;109:713–9.
[40] Gallagher DJ, Gaudet MM, Pal P, et al. Germline BRCA mutations denote a clinicopathologic subset of prostate cancer. Clin Cancer Res 2010;16:2115–21.
[41] Dobzhansky T. Genetics of natural populations. Xiii. Recombination and variability in populations of Drosophila pseudoobscura. Genetics 1946;31:269–90.
[42] Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21.
[43] Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005;434:913–7.
[44] McCabe N, Turner NC, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(-ADP-ribose) polymerase inhibition. Cancer Res 2006;66:8109–15.
[45] Murai J, Huang S-YN, Renaud A, et al. Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. Mol Cancer Ther 2014;13:433–43.
[46] Patel AG, Sarkaria JN, Kaufmann SH. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc Natl Acad Sci U S A 2011;108:3406–11.
[47] Bryant HE, Petermann E, Schultz N, et al. PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. EMBO J 2009;28:2601–15.
[48] Murai J, Huang SN, Das BB. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res 2012;72:5588–99.
[49] Schiewer MJ, Goodwin JF, Han S, et al. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov 2012;2:1134–49.
[50] Brenner JC, Ateeq B, Li Y, et al. Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. Cancer Cell 2011;19:664–78.
[51] Kumar A, Fernandez-Capetillo O, Carrera AC. Nuclear phosphoinositide 3-kinase controls double-strand break DNA repair. Proc Natl Acad Sci U S A 2010;107:7491–6.
[52] McCabe N, Hanna C, Walker SM, et al. Mechanistic rationale to target PTEN-deficient tumor cells with inhibitors of the DNA damage response kinase ATM. Cancer Res 2015;75:2159–65.
[53] Hussain M, Daignault S, Twardowski P, et al. Co-targeting androgen receptor (AR) and DNA repair: a randomized ETS gene fusion-stratified trial of abiraterone + prednisone (Abi) +/− the PARP1 inhibitor veliparib for metastatic castration-resistant prostate cancer (mCRPC) patients (pts) (NCI9012). J Clin Oncol 2016;34(Suppl):5010.
[54] Sandhu SK, Schelman WR, Wilding G, et al. The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. Lancet Oncol 2013;2045:1–11.
[55] Fong PCP, Boss DDS, Yap T, et al. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009;361:123–34.
[56] Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med 2012;366:1382–92.
[57] Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 2010;376:235–44.
[58] Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014;15:852–61.
[59] Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 2010;376:245–51.
[60] Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol 2015;33:244–50.
[61] Fong PC, Yap T, Boss DS, et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 2010;28:2512–9.
[62] Shen Y, Rehman FL, Feng Y, et al. BMN 673, a novel and highly potent PARP1/2 inhibitor for the treatment of human cancers with DNA repair deficiency. Clin Cancer Res 2013;19:5003–15.
[63] de Bono JS, Mina L, Gonzalez M, et al. First-in-human trial of novel oral PARP inhibitor BMN 673 in patients with solid tumors. J Clin Oncol 2013;31(Suppl):2580.
[64] Ihnen M, zu Eulenburg C, Kolarova T, et al. Therapeutic potential of the poly(ADP-ribose) polymerase inhibitor rucaparib for the treatment of sporadic human ovarian cancer. Mol Cancer Ther 2013;12:1002–15.
[65] Hussain M, Carducci MA, Slovin S, et al. Targeting DNA repair with combination veliparib (ABT-888) and temozolomide in patients with metastatic castration-resistant prostate cancer. Invest New Drugs 2014;32:904–12.
[66] Mateo J, Carreira S, Sandhu S, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015;373:1697–708.
[67] Mateo J, Moreno V, Gupta A, et al. An adaptive study to determine the optimal dose of the tablet formulation of the PARP inhibitor olaparib. Target Oncol 2016;11:401–15.
[68] Chao OS, Goodman OB. Synergistic loss of prostate cancer cell viability by coinhibition of HDAC and PARP. Mol Cancer Res 2014;12:1755–66.
[69] Sternberg CN, Petrylak DP, Sartor O, et al. Multinational, double-blind, phase III study of prednisone and either satraplatin or placebo in patients with castrate-refractory prostate cancer progressing after prior chemotherapy: the SPARC trial. J Clin Oncol 2009;27:5431–8.
[70] Cheng HH, Pritchard CC, Boyd T, Nelson PS, Montgomery B. Biallelic inactivation of BRCA2 in platinum-sensitive metastatic castration-resistant prostate cancer. Eur Urol 2016;69:992–5.
[71] Ang JE, Gourley C, Powell B. Efficacy of chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of poly(ADP-ribose) polymerase inhibitor resistance: a multi-institutional study. Clin Cancer Res 2013;44:1–31.
[72] Ceccaldi R, O'Connor KW, Mouw KW, et al. A unique subset of epithelial ovarian cancers with platinum sensitivity and PARP inhibitor resistance. Cancer Res 2015;75:1–8.
[73] Aparicio AM, Harzstark AL, Corn PG, et al. Platinum-based chemotherapy for variant castrate-resistant prostate cancer. Clin Cancer Res 2013;19:3621–30.