Germline and somatic mutations in prostate cancer: focus on defective DNA repair, PARP inhibitors and immunotherapy

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“The understanding that PCa harbors mutations in the DNA repair genes has emerged together with the development of PARP inhibitors, that is, drugs that are designed to target DNA repair deficient and *BRCA*-mutated cancer”

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It is paramount important for pathologists and clinicians, who are involved in the management of prostate cancer (PCa) patients, to understand the role of germline (heritable) mutations that affect PCa biology and risk, somatic (acquired) mutations that occur in PCa and the effect on potential therapies in light of these mutations. Germline mutations are inherited pathogenic variants and are present in all nucleated cells of the body [1]. Germline genetic testing can identify the presence of germline mutations in genes associated with cancer risk, including mutations in components of DNA repair pathways, such as *BRCA1* and *BRCA2* genes. Such genetic alterations increase an individual's predisposition and/or susceptibility to a certain disease, including PCa and breast cancer, due to the impairment of cells' ability to recover from other mutations (Supplementary Figure 1). When a germline mutation in a tumor suppressor gene is inherited, development of cancer is more likely, but not certain. Testing can be performed on blood lymphocyte DNA or on a combination of blood lymphocyte and buccal cells (from saliva) [2].

Acquired mutations to the germline DNA refer to tumor-associated genetic changes that are not heritable. Sequencing technologies for somatic mutations (also referred to as tumor-acquired genetic mutations) require PCa material: cancer-containing biopsies, surgical material, and circulating tumor cells or circulating tumor DNA in the blood. Somatic mutations observed in tumor tissue may change over time due to selective pressure from therapy and genetic instability. These mechanisms are at the base of acquired resistance to a given therapy. Repeat testing of tumor DNA may be needed during the course of the treatment. Testing from primary or metastatic sites or blood may help guide treatment options [3]. Multiple independent clones of tumor cells have been identified at different stages of disease progression by sequencing serial plasma and tumor samples. A distinct mechanism of resistance at different sites that emerged and regressed depending on treatment selection pressure have been suggested [4].

Tumor-based testing has the potential to identify germline mutations that have implications for inherited cancer predisposition. If somatic testing identifies a mutation in a gene associated with PCa predisposition (e.g., *BRCA*), referral to a genetic counselor for confirmatory germline testing is indicated. Tumor testing should never be used to substitute for germline testing.

Mutations in DNA repair genes in PCa

Functional loss of *BRCA1* or *BRCA2* & PCa risk

BRCA1 and *BRCA2* are tumor suppressor genes responsible for DNA repair. The loss of *BRCA1* or *BRCA2* leads to a defect in double-strand break (DSB) repair by means of the so-called homologous recombination repair (HRR) process. This influences the ability of the cell to maintain genome stability [5] with the development of genomic alterations, such as selected rearrangements, copy number alterations, base pair substitutions, deletions and insertions. Inability to control somatic mutations in genes known as oncogenes or tumor suppressor genes may lead to tumorigenesis. Failure in the BRCA pathway favors the activation of alternative repair pathways less effective (small deletions or insertions) and has been related to the enhancement of tumor development (Supplementary Figure 2) [6,7]. For germline *BRCA2* mutation carriers, the relative risk of developing PCa by age 65 years is estimated to be 2.5- to 8.6-fold compared with noncarriers [8]. Lifetime risk of PCa by the age of 80 years is, respectively, between 19 and 61%, and between 7 and 26% for carriers of *BRCA2* and *BRCA1* mutations [9]. Men with *BRCA2* mutations present at a younger age with higher Gleason score tumors, higher frequency of lymph node and distant metastases, and higher PCa-specific mortality [10].

In a study analyzing men undergoing active surveillance (AS), carriers of cancers with *BRCA2* mutation often have a tumor grade reclassification. This requires the adoption of definitive treatment, compared with noncarriers. Upstage at 5 and 10 years is as high as 50%, and 78% in carriers of *BRCA2* mutations, when compared with 22 and 40% in noncarriers [11,12]. The poor outcome associated with *BRCA*-related PCa discourages clinicians to recommend AS to eligible carriers; however, AS may be feasible for *BRCA* carriers with low-risk PCa if they are monitored very closely and patients are compliant [13].

BRCA mutation carrier & breast cancer in men

It is worth mentioning in this setting that the risk for breast cancer in men is increased to approximately 8% with mutation of the *BRCA2* gene, being only 1% with a *BRCA1* mutation. Breast cancer in men is quite rare in the population, the lifetime risk being of 0.1% [14]. There is scant knowledge on the clinical and pathological characteristics and features of male *BRCA1/2* mutation carriers and breast cancer. In general, prognosis in male patients with breast cancer with *BRCA1* and *BRCA2* mutations is worse in comparison to those without such mutations.

Knowledge of the status of *BRCA* mutations can have implications for therapy and can affect survival. Breast cancers with *BRCA* mutations have responded better to chemotherapy based on platinum [15]. PARP inhibitors are used in women with advanced breast cancer. In the Phase III OlympiAD patients treated with olaparib had a progression-free survival benefit but no overall survival benefit versus standard therapy in patients with HER2-negative metastatic breast cancer with a germline *BRCA* mutation. In this trial 2% of patients enrolled were male [16].

Germline mutations other than *BRCA1* & *BRCA2*

ATM [17], CHEK2 [18] and PALB2 [19] also are involved in homologous recombination DNA repair. They have been associated with increased PCa risk, although such germline mutations have less clinical information available and suggest lower risk of developing PCa compared with *BRCA2*.

Germline mutations in the mismatch repair (*MMR*) genes (*MLH1*, *PMS2*, *MSH2* and *MSH6*) are seen in the Lynch syndrome, an inherited condition that predisposes individuals to an increased risk of developing many different types of cancers, even at a young age. Several studies suggest a modest increased risk for PCa in men with this syndrome [20]. *HOXB13* G84E is a germline variant associated with increased risk of developing PCa [21]. However, this variant is neither clearly associated with increased disease aggressiveness, nor should it influence treatment decision making.

Emerging data suggest that *NBS1*, *FANCA* and other DNA repair genes are associated with increased PCa risk and choice of treatment. However, further investigations are needed before clinical action is warranted.

Prostate cancer-specific multigene panels

Advances in the technology of DNA sequencing have clarified the genomic landscape of metastatic castration-resistant prostate cancer (mCRPCa). Sequencing of somatic mutations in tumor biopsies (primary prostate tissue or metastatic lesion) can use multigene panels and molecular profiling. There are several next-generation sequencing

panels used to compare mutations for different quantitative assessments of prognosis and resistance to therapy. A critical review on the efficacy and accuracy of these panels has been recently published by Cucchiara *et al.* [22].

As an example, the panel from Foundation Medicine tests 315 genes related to cancer. Tumors possessing DNA repair pathway mutations are quite common in advanced stages of the neoplasia, thus showing a poor prognosis related to these mutations. Between 20 and 25% of persons with mCRPCa can present mutation in the DNA repair pathway (such as *BRCA* genes) [23].

There are at least ten clinical germline multigene panels specifically designed for PCa patients that can be currently ordered in the USA. All panels include *BRCA1* and *BRCA2*. In a cohort of 3607 PCa patients who underwent clinical testing, about 10% had informative results for PCa risk management and 3.4% had pathogenic variants in *PALB2*, *CHECK2*, *RAD51D* or *RAD51C*, which have a prognostic and/or predictive utility [24].

The aim of such panels is that potential ‘driver’ mutations are identified and that allows a personalized treatment of cancer. The understanding that men with mCRPCa can harbor a mutation in the DNA repair pathway has been the basis to develop trials that evaluate the clinical response of various clinical therapies.

Treatment strategies

PARP inhibitors

The understanding that PCa harbors mutations in the DNA repair genes has emerged together with the development of PARP inhibitors, that is, drugs that are designed to target DNA repair deficient and *BRCA*-mutated cancer.

PARPs are enzymes responsible for the repair of single-strand breaks of the DNA through a mechanism called base-excision repair. Such single-strand breaks, when PARP is inhibited, lead to double-strand breaks at replications forks. This is usually repaired via the HRR pathway involving genes such as *BRCA2*, *BRCA1* and *RAD51*. In *BRCA1/2*-mutated tumors, these double-strand breaks cannot be efficiently repaired, leading to the death of the cells.

A very recent study on Lynparza (olaparib; a PARP inhibitor) in men with mCRPC has shown a statistically significant and clinically meaningful improvement in the primary end point of radiographic progression-free survival with Lynparza versus enzalutamide or abiraterone in men with mCRPC selected for *BRCA1/2* or *ATM* gene mutation (NCT02987543). Median overall survival in *BRCA1/2* or *ATM*-mutated patients at the interim analysis was 18.5 months with olaparib compared with 15.11 months with physician’s choice hormonal therapy (HR: 0.64; 95% CI: 0.43–0.97; $p = 0.0173$) [25,26]. Of notice, previous trial testing PARP inhibitors in unselected mCRPC patients did not show significant efficacy [27].

With the advancement in the genetic sequencing, mutations in the DNA repair pathway can be suspected and detected earlier in the course of the disease, even in the diagnostic phase. Testing therapies based on PARP inhibitors into the metastatic castrate-sensitive phase or even as adjuvant therapy seem reasonable considering the small and distinct target population that might benefit from this class of drugs.

Not all patients with DNA repair deficiency respond to PARP inhibitors. Multiple mechanisms for resistance have been postulated. At present, which mechanisms contribute to PARP inhibition resistance in PCa, is still unclear. One potential mechanism is through the so-called secondary ‘reversion’ mutation. Ashworth *et al.* [28] have shown that additional mutations can restore the *BRCA2* protein treating *BRCA2*-deficient ovarian or pancreatic cancer cell lines to PARP inhibitors or cisplatin. *BRCA2* reversion mutations can detect cell-free DNA in men with PCa that previously responded to talazoparib and olaparib, and then became resistant [29].

Additional therapeutic strategies

PARP inhibitors are being used alone or in combination with other treatments such as:

- Radiotherapy (RT);
- Various chemotherapies;
- Abiraterone (second-generation antiandrogen);
- Enzalutamide (second-generation antiandrogen);
- PDL1 immunotherapies (pembrolizumab and durvalumab).

In the localized disease setting there are no prospective studies comparing outcomes for patients with homologous recombination-deficient PCa for primary treatment with surgery versus RT. Prostate cancer with defects in DNA repair genes may be particularly susceptible to RT as demonstrated in preclinical models [30]. The administration of

RT causes multiple forms of DNA damage, including damage to the bases, DNA single-strand breaks and DSB. The impaired ability for DSB repair due to *BRCA1* mutations contributes to radiation sensitivity. Interestingly, *BRCA1* expression restores radiation resistance in *BRCA1*-defective cancer cells through enhancement of transcription-coupled DNA repair [31]. Future trials may combine PARP inhibitors with RT to synergize with PARP inhibitors or to overcome resistance.

Platinum-based chemotherapy may represent a treatment option in HRD-selected PCa patients. So far, few cases of dramatic and sustained response to carboplatin/cisplatin therapy have been recently reported. All cases harbored a biallelic inactivation of the *BRCA2* gene [32,33]. Multiple trials are now testing platinum-based chemotherapy in PCa patients with HRR mutations. Moreover, a new trial (EA8152) will test the combination between carboplatin–paclitaxel chemotherapy with or without the addition of the PARP inhibitor veliparib in an unselected mCRPC cohort. The HRR mutational status of each patient at baseline will be considered as a stratification element to evaluate treatment responses in both subgroups.

Concerning antiandrogen therapy and PARP inhibitors, the prognostic impact of HRR mutations on sensitivity or resistance to hormonal therapy remains unclear at this time [34]. In a randomized Phase II trial testing abiraterone with or without veliparib [35] in an unselected mCRPC population, patients with HRR mutations had a longer progression free-survival (13.8 months) compared with patients without HRR mutations (7.8 months), regardless the treatment received. In the HRR-mutated patients, the addition of veliparib did not significantly improve outcomes. This means that HRR-mutated population may be more responsive to abiraterone compared with the nonmutated PCa patients. However, Annala *et al.* found that patients with DNA repair defects show an inferior response to hormonal therapies [36].

Immunotherapy

Immunotherapy enhances the antitumor effect of the host immune system. Efficacy against a variety of cancers has been shown. A fundamental key in the efficiency of immunotherapy is the development of tumor-specific antigens (also called neoantigens). These neoantigens distinguish normal cells from tumor cells. They can induce a tumor-specific immune response.

A defective DNA repair can increase the frequency of mutations. This is considered an important element in the development of the antitumor immune response. In particular, *BRCA1* mutations are associated with a higher mutation burden in breast cancers that are triple negative [37]. Mutations in the *BRCA2* gene have been observed in melanoma patients with a better response to anti-PD-1 therapy [38]. Such findings support the potential use of the somatic and germline mutation status of DNA repair genes, to predict the response to immunotherapy.

Tumors harboring mutations in the MMR pathway with consequent microsatellite instability (MSI) have been reported to be more responsive to immunotherapy, based upon a hypermutated phenotype [39]. Pritchard *et al.* have found complex *MSH2* and *MSH6* mutations in 10% of mCRPC from a rapid autopsy series [40]. The same group reported that *MMR* mutations might be enriched in patients with ductal adenocarcinoma histology [41]. In the Phase II trial combining pembrolizumab to enzalutamide for patients with mCRPC progressing on enzalutamide (NCT02312557) only one of the three responders' patients had MSI and DNA repair defects [42].

Further investigations are needed to determine the relationship between MSI and response to immunotherapy in PCa.

Conclusion

Information about germline and tumor somatic mutations has increasing importance in the management of men with PCa. The knowledge that approximately 25% of men with mCRPCa may harbor a mutation in the DNA repair gene pathway has led to trials to evaluate the response of various therapies. PARP inhibitors are compounds that target *BRCA*-mutated or DNA repair-deficient cancers. Tumors harboring mutations in *BRCA1* and *BRCA2* genes and in the MMR pathway have been reported to be more responsive to immunotherapy because of their defective DNA repair system and the consequent higher tumor neoantigens. Ongoing and future trials are expected to address these hypotheses and to test a new potential treatment strategy for this selected PCa patient population.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2019-0745

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