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Circulating tumor DNA genomic profiling reveals the complicated olaparib-resistance mechanism in prostate cancer salvage therapy: A case report

YuanF *et al.* ctDNA profiling reveals olaparib-resistance mechanism

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Abstract

BACKGROUND

The poly (ADP-ribose) polymerase (PARP) inhibitor olaparib has displayed superior clinical effect in metastatic castration-resistant prostate cancer (mCRPC) patients with the homologous recombination repair (*HRR*) genes mutations. However, when a patient's tumor tissue volume is insufficient for genomic profiling of *HRR* gene mutations, circulating tumor DNA (ctDNA) may be useful in helping to determine and monitor the efficacy of olaparib, as well as in abiraterone-combination treatment, and for understanding any resistance mechanism related to such mutations.

CASE SUMMARY

A 61-year-old man who was diagnosed with metastatic prostate adenocarcinoma was initially hormone sensitivity, showing high Gleason score ($5 + 5 = 10$) and absolute positive rate (14/14 biopsied specimens). Following failure of several standard therapies, the patient progressed to mCRPC. Surprisingly, the patient showed good response to olaparib-abiraterone-prednisone combination treatment (an androgen-deprivation therapy, provided as the 'final choice' in China). Serum total prostate-specific antigen (TPSA) level reduced and symptoms remitted for 4 months. However, thereafter, serum TPSA levels began slowly increasing, indicating development of olaparib resistance. Subsequent comprehensive genomic profiling of ctDNA, screening 508 cancer-related genes by next-generation sequencing, identified 10 somatic variants as well as 3 copy number alterations. Two identified reverse missense mutations in partner and localizer of BRCA2 (*PALB2*) may have recovered the reading frame, restoring function of the primary germline *PALB2* mutation and causing resistance to the PARP inhibitor olaparib.

CONCLUSION

Reverse mutations in *PALB2*, discovered *via* genomic profiling of ctDNA, may represent a potential resistance mechanism against olaparib in mCRPC.

Key Words: mCRPC; olaparib; ctDNA; PALB2; resistance mechanism; reverse missense mutations

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Core Tip: This case report describes the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib treatment response in a patient with metastatic castration-resistant prostate cancer (mCRPC). The patient's course of response to 'final choice' therapy (olaparib-abiraterone-prednisone combination treatment), consisting of serum total prostate-specific antigen (TPSA) level reduction and symptom remission for 4 months followed by TPSA rise, prompted comprehensive genomic profiling of circulating tumor (ct)DNA, which revealed reverse missense mutations in the partner and localizer of BRCA2 (*PALB2*) gene. Timely multigene testing by ctDNA, especially in mCRPC, can determine the most appropriate and accurate therapeutic approach and explore the resistance mechanism.

INTRODUCTION

The International Agency for Research on Cancer produced ¹an update on global cancer burden using the GLOBOCAN 2020 estimates of cancer incidence and mortality^[1]. ¹With an estimated almost 1.4 million new cases and 375000 deaths worldwide, prostate cancer was ranked as the second most frequent cancer and the fifth leading cause of cancer death among men. Asia is traditionally considered a low-incidence area for this type of cancer, but the incidence and mortality rates are rapidly increasing across the continent^[2]. In mainland China^[3], prostate cancer is now the sixth most commonly occurring malignant tumor. According to the 2020 estimates of global burden, prostate cancer accounted for 120000 of new cases, ranking as the ninth most frequent cancer in China^[1]. Patients who progress to metastatic castration-resistant prostate cancer (mCRPC) considered the most serious stage usually suffer from unfavorable clinical prognosis combined with poor quality of life^[4].

Treatment options during the disease progression to mCRPC are limited and mainly consist of continued androgen-deprivation therapy (ADT) combined with one of the new endocrine drugs such as abiraterone acetate plus prednisone or with docetaxel chemotherapy plus prednisone. Poly (ADP-ribose) polymerase (PARP) inhibitors, such as olaparib, rucaparib, niraparib or talazoparib, have displayed promising clinical results, with prolonged survival duration and improved life quality in patients with mutations in the homologous recombination repair (*HRR*) gene who are suffering from various cancers, including ovarian^[5], breast^[6], pancreatic^[7], and prostate^[8, 9]. Recent studies have also shown the potential therapeutic effect of the PARP inhibitor olaparib in mCRPC patients with deleterious mutations in genes belonging to the DNA damage repair (*DDR*). Thereinto, harmful mutation of *BRCA2* may act as a particularly useful marker for therapeutic response to PARP inhibitor. Indeed, the published results from the study named PROfound^[10] indicate a helpful clinical effect with olaparib treatment in patients with *BRCA1/2* or *ATM* deleterious mutations. For patients without *DDR* deleterious mutations, it is reported that olaparib combining with abiraterone plus prednisone could be the effective treatment options^[11].

The subpopulation of mCRPC patients with *HRR* gene partner and localizer of BRCA2 (*PALB2*) mutation has been reported to have the good objective response and prostate-specific antigen (PSA) response^[8], but due to the relatively low mutation frequency of *PALB2*, there are limited reports of clinical benefit. Moreover, the Food and Drug Administration has approved olaparib for adult patients with germline or somatic *HRR* gene-mutated mCRPC, who have progressed following prior treatment with enzalutamide, abiraterone or docetaxel chemotherapy. The evaluation of patients' *HRR* gene mutation was based on tumor tissue, but in routine clinical practice, especially for mCRPC patients, it is a challenge to acquire metastatic tumor tissue biopsies and of sufficient volume for evaluative processing.

Circulating tumor (ct)DNA can be obtained as a 'liquid biopsy' in a minimally invasive manner and as such can serve as a surrogate tumor specimen, providing a real-time snapshot of a patient's overall tumor burden. To date, however, the impact of ctDNA on clinical decision-making for prostate cancer remains unclear and there is a lack of prospective studies in the literature to advance this important topic.

CASE PRESENTATION

Chief complaints

A 61-year-old man presented to Chongqing University Cancer Hospital with complaint of continuous lumbosacral pain that had lasted for 3 months.

History of present illness

The patient reported a 3-month history of lumbosacral pain.

3

History of past illness

The patient had no past illness.

Personal and family history

The patient had no personal and family history.

Physical examination

None

Laboratory examinations

The patient showed a markedly increased level of serum total (T)PSA (787 ng/mL; normal: < 4 ng/mL) but normal neuron-specific enolase (4.32 ng/mL).

Imaging examinations

Multiple bone lesions were firstly found by the lumbar magnetic resonance imaging (MRI) (Figure 1A). Then, these lesions and other bones were revealed to have high metabolic activity by bone scanning (Figure 1B). The prostate MRI showed that the seminal vesicle and bladder wall were invaded and pelvic lymph nodes were metastasized (Figure 1C). Computed tomography (CT) showed no metastasis in lung.

HISTOLOGICAL EXAMINATIONS

Ultrasound-guided transperineal prostate needle biopsies were obtained (14 specimens in total), and histology confirmed the diagnosis of prostate adenocarcinoma. The specimens showed the Gleason score was highest (5 + 5 = 10) together with an absolute positive rate (14/14). The biopsied tissues tested through immunohistochemistry staining of serial sections excluded a neuroendocrine component [PSA (+), CK-L (+), P504S (+), AR (++)>95%, NKX3.1 (+), Ki-67 30% (+), Syn (-), CK34βE12 (-), CgA (-), CD56 (-), P63 (-)] (Figure 1D).

MULTIDISCIPLINARY EXPERT CONSULTATION

none

FINAL DIAGNOSIS

Metastatic hormone-sensitive prostate cancer (mHSPC) of pT4N1M1b type and stage IV.

TREATMENT

First-line treatment and outcome

ADT therapy (triptorelin, an LHRH agonist) in combination with docetaxel (three weekly doses of 75 mg/m²) without prednisolone, was applied as the initial treatment^[12-14] fitting with the patient's mHSPC diagnosis and high tumor burden^[15]. Two cycles of this therapeutic intervention led to significant relief in the patient's self-reported pain as well as a substantial drop in TPSA level to 0.45 ng/mL (Figure 2A). However, at that point, the docetaxel had to be stopped due to severe bone marrow inhibition and liver toxicity. Therefore, ADT was continued as monotherapy.

Second-line treatment and outcome

After 5 months, the patient developed lumbosacral pain and showed a rebound of increased TPSA. Testosterone levels remained suppressed throughout entire treatment period, indicating disease progression to mCRPC. To determine the new endocrine therapy resistance or sensitivity, he was investigated the classification and respective number of circulating tumor cells (CTCs) along with the expression of AR-V7 mRNA^[16]. Based on the negative findings, we had confidence in the decision for abiraterone-prednisone administration as maintenance therapy (Figure 2B and C). After 6 months of this treatment, the patient showed a decreased TPSA level (3.02 ng/mL; Figure 2A). Fortunately, the disease remained effectively controlled by this therapy for nearly 1 year.

Third-line treatment and outcome

The re-emergence of an elevated TPSA level to 69.57 ng/mL manifested disease progression again (Figure 2A). At the time, the patient's CTCs number was increased together with AR-V7 mRNA overexpressions, which confirmed that he had developed abiraterone resistance (Figure 2B and C). Then, he was deemed eligible for a phase III double-blind, randomized, placebo-controlled, multicenter clinical trial to assess the

safety and efficacy of proxalutamide (a new androgen receptor [AR] antagonist) in patients with mCRPC who failed abiraterone acetate and docetaxel therapy. Of note, as of the writing of this report, the trial is ongoing. Unfortunately, the patient's disease progressed rapidly during this treatment, as reflected by TPSA level increased to 601 ng/mL (Figure 2A) with somnolence and severe bilateral lower limb edema.

Fourth-line treatment and outcome

The first genetic testing, performed with the patient's peripheral blood leukocytes, had detected no germline *BRCA1/2* deleterious mutations (Figure 2A). Therefore, we chose to re-challenge the tumor by administration of abiraterone together with the PARP inhibitor olaparib. After only 1 mo of the combined treatment, the patient's TPSA level began to decrease rapidly (from 601.61 ng/mL to 119.65 ng/mL), and in the months thereafter he maintained a low level (8.02 ng/mL) (Figure 2A). Electrocardiography (commonly known as ECG) findings and the patient's mental state were significantly improved. The patient also reported decreased pain and required a reduced amount of morphine dosage (30 mg, q12h, p.o.). After 5 mo, however, the TPSA level began to rise again and the patient reported increasing carcinomatous pain.

OUTCOME AND FOLLOW-UP

When the disease progressed the third time and we sought to reperform a second comprehensive genomic profile assessment, we chose to use peripheral ctDNA samples due to the difficulty of obtaining a tumor tissue biopsy (Figure 2A). A total of 508 cancer-related genes were sequenced by next-generation sequencing. The *HRR* gene *PALB2* germline pathogenic mutation and two somatic mutations were discovered (Figure 3, Table 1). *PALB2*, as a tumor suppressor belonging to the *HRR* gene, physically interacts with *BRCA2* leading to the subsequent recruitment of proteins to DNA breaks and plays a crucial role in repairing double-strand breaks through homologous recombination [17]. Some studies have associated *PALB2* harmful mutations with therapeutic benefit attained from PARP inhibitors [8, 18]. For example, the TOPARP-B

study has shown that patients with mutations of *PALB2* or somatic cell dysfunction can benefit from olaparib therapy [8].

Meanwhile, 10 somatic variants genes were found: *AR*, *PTEN*, *TP53*, *CHD1*, *NOTCH2*, *FGFR1*, *LHCGR*, *PIK3C2G*, *FLT4* and *CDC25C* (Table 1). In particular, two reverse missense mutations in *PALB2* were suspected as functioning to recover a truncated polypeptide chain translated from the germline *PALB2* mutation (Table 1, Figure 3). Two somatic missense mutations of *PALB2* which were c.751_752delCAinsTT and c.751_753delCAGinsTAC (Figure 3) shared the same loci with the germline deleterious variant c.751C>T. Bringing about the in-frame deletion and insertion variants, the *PALB2* mutations would contribute to restoring the entire reading frame and to supporting the correct functioning to repair DNA double-stranded breaks *via* the HRR pathway. Therefore, we reasoned that these two reversing mutations of *PALB2* may represent the mechanism of resistance to olaparib and abiraterone in this patient. In the future, functional assays are needed to validate this potential drug-resistance mechanism as indicated by these ctDNA profile findings.

Finally, the patient's ECOG scale increased and he attained a score of 4. The patient showed clouding of consciousness and developed pain throughout the body, and he died without further rescue efforts.

DISCUSSION

In the case described herein, a mCRPC patient who had undergone treatment by many differing therapy achieved surprisingly clinical response to combination therapy of olaparib and abiraterone-prednisone. Next-generation sequencing of the patient's ctDNA revealed 13 relevant gene mutations, including in the *HRR* gene *PALB2*. Intriguingly, a subgroup of prostate cancer patients who benefit from the treatment of PARP inhibitors have been identified, and these individuals show a trend towards loss-of-function mutations in the *HRR* gene^[8, 9, 18, 19]. In the TOPARP-A, TOPARP-B and PROfound studies, olaparib monotherapy was shown to exert antitumor activity against mCRPC with *HRR* or *DDR* gene alterations. The PROfound study^[20] indicated

that radiologic progression-free survival (rPFS) was significantly longer in the olaparib group than in the group treated with the physician's choice of enzalutamide or abiraterone (median: 7.4 mo vs 3.6 mo in patients with *BRCA1/2* and *ATM* mutations; 5.8 mo vs 3.5 mo in patients with all 15 *HRR* genes' mutations). Similarly, the TOPARP-A study showed mCRPC patients carrying the *HRR* gene mutation had a median rPFS of 9.8 mo, being 6.1 mo longer than that of mCRPC patients with wild-type *HRR* gene. The TOPARP-B study showed that the mCRPC patients with *PALB2* mutation had a median rPFS of 5.3 mo in response to treatment with olaparib. The gene scope of our first genetic testing was limited to the *BRCA1* and *BRCA2* genes, and indicated that the patient did not carry the germline *BRCA1* or *BRCA2* mutation. Based on the conclusion from the Study 08 (NCT01972217)^[11] that olaparib in combination with abiraterone provided efficacious clinical benefit for patients with mCRPC compared to abiraterone alone and regardless of *HRR* mutation status, we chose the olaparib and abiraterone-prednisone combination therapy for our patient. In detail, the Study 08 revealed that olaparib and abiraterone provided median rPFS of 13.8 mo in and intention-to-treat population and 17.8 mo in an *HRR* mutation-positive subgroup.

Our patient achieved rPFS of 5 mo with the PARP inhibitor olaparib and abiraterone-prednisone combination therapy. This response time was overall consistent with olaparib single-drug use previously reported in the literature but was relatively worse than that with the olaparib and abiraterone-prednisone combination therapy. The reason for this may be that our patient had received the abiraterone treatment and developed resistance to such before the olaparib. It is important to note here that Study 08 had recruited patients who had not received prior abiraterone treatment, only having received docetaxel. In addition, there is a dual model of synergy between PARP inhibitor and ADT^[21, 22]. PARP is involved in AR-dependent transcription and PARP inhibitor impairs this process, at the same time, the AR regulates transcription of DNA repair genes and androgen depletion impairs *HRR*, which might produce a so-called "BRCA-ness" phenotype that renders susceptibility to PARP inhibitor. Amplification of the *AR* gene, as observed *viactDNA* profiling of our patient, would lead to continuous

activation of downstream signaling pathway(s), overcoming the extrinsic androgen inhibition^[23, 24] and precluding triggering of the “BRCA-ness” phenotype. The synergy between PARP inhibitor and ADT would not be able to be established in such a patient, which would explain why our patient’s clinical response was worse.

The *PALB2* reverse mutations are another important feature of our patient. There was a research about mCRPC patient who achieved 9-months clinical effect by olaparib with germline *PALB2* p.L253Ifs*2 mutation. During that patient’s disease progression, two reverse somatic mutations in *PALB2* were found by ctDNA^[25]. Similarly, we detected two somatic reverse mutations and a germline p.Q251* nonsense mutation in *PALB2* at our patient, which may have restored the reading frame and the homologous recombinational function. Once the repair function of homologous recombination is restored, the synthetic lethality on which PARP inhibitors work will be broken, and patient will inevitably be resistant to PARP inhibitor therapy.

Our case also emphasizes the importance of blood-based liquid biopsies and genomic profiling by means of ctDNA. Tumor tissue can be much more difficult to obtain for this evaluative purpose, particularly from advanced cancer patients who are at higher risk in or counter-indicated for surgery or patients who cannot tolerate biopsy for other reasons; another complication is that tumor tissues, in general, may show negative pathological results if the patient has already started or undergone treatment. ctDNA is thus an attractive, minimally invasive alternative, which can used as a practical tool to profile tumor dynamics over time, elucidating features with tumor progression and overcoming spatial heterogeneity of tumors. For our patient, the genetic testing of ctDNA indicated a complicated mechanism of disease response and progression. First, the discovered *HRR* gene *PALB2* germline mutation could explain the rapid response of to the PARP inhibitor olaparib. Second, the two *PALB2* somatic reverse mutations and *AR* gene amplification could underlie the relative shorter response time. Gogolaet al^[26] had reported on activation of the PI3K-AKT-mTOR signal transduction pathway as a mechanism of resistance. These activated oncogenic pathways may cause the expression of homologous recombination genes, which were compensating for DNA double-

breaks. By the way, the activated signaling pathways can accelerate the progression of cell cycle or allow cells to evade apoptosis^[27]. Because our patient carried mutations in the *PTEN* and *FGFR1* genes, it is intriguing to consider that *FGFR1* amplification with a loss-of-function mutation in *PTEN* can directly or indirectly activate the RAS-RAF-MAPK/ERK signaling pathway as well as that of PI3K-AKT-mTOR^[28]. Further validation at the functional level is warranted. According to the patient's ctDNA sequencing results, we were able to evaluate the patient's treatment and response course thoroughly.

Several limits to this clinical evaluative approach still need to be addressed. First, with full respect to the patient's willingness, comprehensive genomic profiling is typically performed only when resistance to olaparib presents. Owing to our patient's rapid disease progression, we were unable to adjust the treatment strategy based on the ctDNA sequencing result. Second, we were unable to distinguish whether the two somatic *PALB2* mutations discovered had been present originally or resulted from the abiraterone combination treatment. If the ctDNA comprehensive sequencing (of 508 genes) had been performed earlier, in lieu of sequencing only the *BRCA1* and *BRCA2* genes, then the abiraterone combination treatment may have been started earlier. In that situation, the detection of germline *PALB2* aberration at a relatively early stage in the disease course may have allowed for the patient to receive olaparib monotherapy, while not being enrolled into the proxalutamide clinical trial, or the abiraterone combination treatment. Third, *PALB2* as a cancer susceptibility gene increases the hazard of breast cancer (absolute risk: 41%-61%). The ⁸ National Comprehensive Cancer Network (commonly known as ¹³ NCCN) Genetic/Familial High-Risk assessment: Breast, Ovarian and Pancreatic (Version 1.2022) recommends that the *PALB2* germline mutation carrier should start annual mammograms, with consideration of tomosynthesis and breast MRI with contrast, at age of 30 years and that the healthcare team open discussions into the option of risk-reducing mastectomy. *PALB2* gene mutations are also associated with susceptibility to cancers of the ovary (absolute risk: 3%-5%), pancreas (absolute risk: 5%-10%), and breast in males. The NCCN: Prostate cancer (Version 1.2022) also

recommends germline multigene testing that includes (at least) *BRCA1/2* and *PALB2* in its testing panel. Our patient carried a *PALB2* germline pathogenic variation, so that his offspring would carry a 50% likelihood of harboring the same variation. As such, we would suggest that first- and second-degree relatives visit a genetic counselor to further evaluate whether they carry the proband's same *PALB2* mutation; if so, the relative should receive genetic counseling to gain a sufficient understanding of the correlative cancer risk, further screening options and risk-reduction strategies. This will allow the positive carrier to better protect against the onset of related cancers or at least promote their ability to suspect and seek timely assessment to detect a cancer much earlier. Although we made such suggestions to our patient's relatives, none have accepted our suggestion as of the writing of this case report; nonetheless, this is part of our routine strategy of care and we will continue such efforts in the future.

CONCLUSION

Herein, we have described a comprehensive genomic profiling of ctDNA in a mCRPC patient which revealed *HRR* gene germline *PALB2* mutation, including reverse mutations and others affecting known cancer-related signaling pathways. Using the ctDNA sequencing results, we were able to analyze the intrinsic mechanism underlying the patient's rapid response and resistance to the PARP inhibitor and abiraterone combination therapy. On one hand, our case demonstrated that a patient with *HRR* gene *PALB2* mutation can benefit from PARP inhibitor treatment. On the other hand, the case showed the feasibility of ctDNA sequencing to guide treatment, indicate prognosis and analyze resistance and its underlying mechanisms, with ctDNA serving as a surrogate for limited or unavailable tumor tissue. Overall, though, the case provides a real-world example of how timely multigene testing can be of great importance for selecting the most precise therapeutic approach for cancer patients, especially for those with mCRPC.

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