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Molecular Case Studies

RESEARCH REPORT

Treatment-emergent neuroendocrine prostate cancer with a germline *BRCA2* mutation: identification of a candidate reversion mutation associated with platinum/PARP-inhibitor resistance

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Abstract Neuroendocrine prostate cancer (NEPC) is a highly aggressive histologic subtype of prostate cancer associated with a poor prognosis. Its incidence is expected to increase as castration-resistant disease emerges from the widespread use of potent androgen receptor-targeting therapies, such as abiraterone and enzalutamide. Defects in homologous recombination repair genes, such as *BRCA1/2*, are also being increasingly detected in individuals with advanced prostate cancer. We present the case of a 65-yr-old man with a germline *BRCA2* mutation who developed explosive treatment-emergent, small-cell neuroendocrine prostate cancer. He achieved a complete response to platinum-containing chemotherapy, but a limited remission duration with the use of olaparib, a poly(ADP-ribose) polymerase (PARP) inhibitor, as maintenance therapy. Upon relapse, tumor genomic profiling revealed a novel 228-bp deletion in exon 11 of the *BRCA2* gene. The addition of the anti-PD1 drug pembrolizumab to olaparib was ineffective. This case highlights the ongoing challenges in treating neuroendocrine prostate cancer, even in the setting of homologous recombination repair deficiency.

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INTRODUCTION

Metastatic castration-sensitive prostate cancer is typically treated in the first-line setting with androgen deprivation therapy in combination with either the chemotherapeutic drug docetaxel or a newer, orally active hormonal agent, such as abiraterone or enzalutamide (Swami et al. 2020). Treatment resistance, however, is universal and associated with transdifferentiation to a small-cell neuroendocrine histology in ~17% of cases (Aggarwal et al. 2018). Treatment-emergent small-cell neuroendocrine prostate cancer (t-SCNC) has few effective therapeutic options. Platinum-based chemotherapy is typically used as first-line therapy, although median survivals are only 7 mo (Wang et al. 2014). Improvements in the treatment of t-SCNC are, therefore, urgently needed.

[6]These authors contributed equally to this work.

Germline (*g*) and somatic mutations in homologous recombination repair (HRR) genes—such as *BRCA1/2*, *ATM*, *PALB2*, *RAD51D*, and *CHEK2*—are estimated to occur in 12% and 25% of individuals with castrate-resistant prostate cancer (CRPC), respectively (Sartor and de Bono 2018). Although the superiority of platinum-based chemotherapy has not been established in this setting (Mateo et al. 2018), as it has for other types of HRR-deficient adenocarcinomas (Robson et al. 2017; Moore et al. 2018; Golan et al. 2019), the effectiveness of poly (ADP-ribose) polymerase (PARP) inhibitors has been proven (de Bono et al. 2020). In contrast, neuroendocrine prostate cancer has demonstrated sensitivity to platinum agents (Apostolidis et al. 2019; Corn et al. 2019), but there are no clinical trial reports on the use of PARP inhibitors in this setting. Given the expanding linkage between platinum sensitivity and responsiveness to PARP inhibiton in malignancies characterized by HRR deficiency, it is reasonable to study this treatment paradigm in patients with HRR-deficient t-SCNC.

Here, we report the case of a man with an aggressive t-SCNC carrying a *gBRCA2* mutation who was treated with the PARP inhibitor (PARPi) olaparib following a major response to first-line platinum-based chemotherapy.

RESULTS

Clinical Presentation and Family History

Informed written consent was obtained from the patient for publication of this case report. A 65-yr-old Indian male, with a history of type 1 diabetes, presented to the Norwalk Hospital emergency department after 1 mo of progressive bilateral leg and scrotal edema as well as low back pain. Ultrasound showed an occlusive deep vein thrombosis (DVT) in the left common femoral vein with possible extension into the left external iliac vein. He underwent thrombectomy followed by treatment with enoxaparin. Computed tomography (CT) of the chest, abdomen, and pelvis showed thrombus in the left iliac veins, multiple enlarged retroperitoneal and left pelvic lymph nodes, and prostatic enlargement, measuring 5.1 × 5.3 × 5.3 cm. A bone scan showed several widely distributed osseous metastases involving the hips, spine, ribs, and sternum. The prostate-specific antigen (PSA) was 95 ng/mL. He underwent 12-quadrant prostate biopsies, which showed 50%–100% involvement in all cores with Gleason 8 (4 + 4) adenocarcinoma. The tumor was strongly positive for prostate-specific acid phosphatase (Fig. 1).

The patient began leuprolide androgen deprivation therapy (ADT) and abiraterone plus prednisone, choosing to avoid chemotherapy because of the potential impact of side effects on his employment. His initial symptoms gradually improved and he was soon able to return to work and carry out all activities of daily living (ADLs). The PSA declined to 0.2 ng/mL during the first 3 mo and remained low, as shown in Figure 2. After 12 mo, a bone scan showed decreased intensity and extent of all lesions, and CT showed no visceral disease (Fig. 2). Germline genetic testing revealed a pathogenic nonsense mutation in the *BRCA2* gene, predicting a truncated protein (Table 1 and next section). Family history was notable for a paternal uncle with prostate cancer and a maternal uncle with colon cancer, both in their 60s.

After 16 mo, the patient became acutely ill requiring hospitalization. Physical examination revealed scleral icterus, painful hepatomegaly, and profound global weakness. The aspartate transaminase was 424 U/L, alanine transaminase 241 U/L, total bilirubin 5.4 mg/dL, and lactate 5.0 mmol/L. The PSA was 0.5 ng/mL, serum chromogranin-A 7160 ng/mL (normal < 95), and neuron-specific enolase (NSE) 824 ng/mL (normal < 15). Abdominopelvic CT showed the liver was 27 cm in transverse dimension with innumerable new 2–3 cm hypodense lesions (Fig. 2). CT-guided liver biopsy showed poorly differentiated neuroendocrine carcinoma most consistent with small-cell carcinoma (Fig. 1). Immunohistochemical stains showed the tumor positive for CK20 (dot-like accentuation), synaptophysin, and

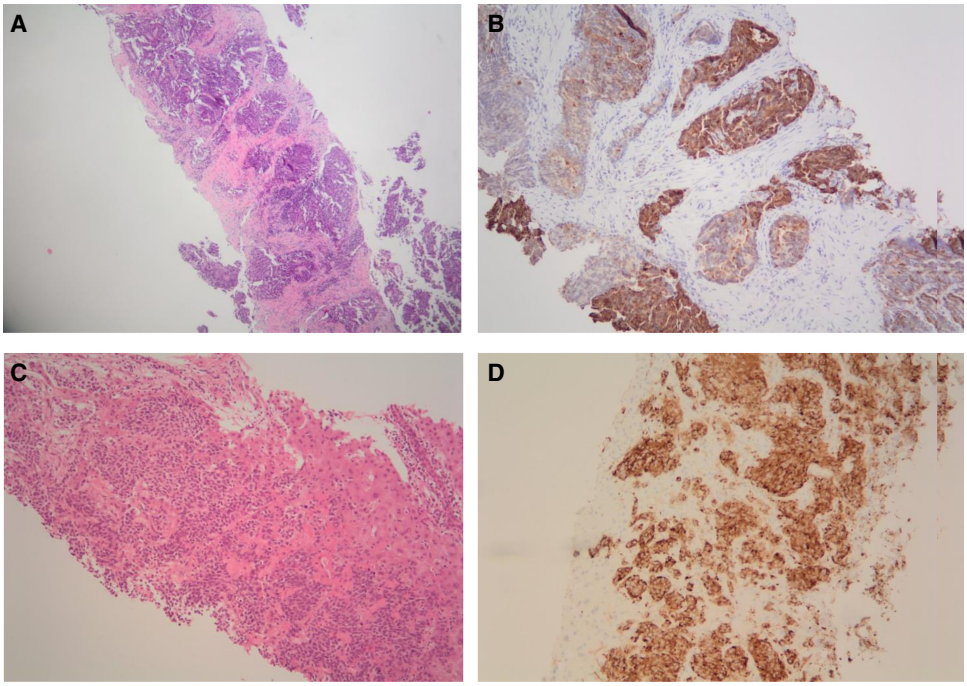

Figure 1. (*A*) Prostate adenocarcinoma: low-power view from left lateral mid prostate biopsy showing Gleason 4 + 4 = 8 pattern (4×). (*B*) Prostatic specific acid phosphatase (PSAP): low-power view from left lateral mid prostate biopsy showing positive cytoplasmic staining for PSAP by immunohistochemistry (IHC) (10×). (*C*) Liver small-cell carcinoma: low-power view of tumor in the liver with nests and sheets of uniform tumor cells with high nuclear to cytoplasmic ratio (10×). (*D*) Liver synaptophysin: IHC for synaptophysin showing membranous and cytoplasmic positive staining (20×).

chromogranin and negative for CK7, PSA, TTF-1, GATA-3, CDX-2, NKX3.1, and Merkel cell polyoma virus. There was loss of RB and the Ki-67 labeling index was >50%. There was insufficient tumor sample for genomic profiling prechemotherapy but this was performed subsequently (see below).

The patient began chemotherapy with etoposide and carboplatin every 3 wk and gradually improved with normalization of liver enzymes. After two cycles of treatment, the NSE declined to 18 ng/mL, chromogranin-A declined to 408 ng/mL, and CT scan showed markedly diminished hepatic metastases. After six cycles of chemotherapy, the chromogranin-A was 115 ng/mL, and CT showed near-complete resolution of hepatic metastases (Fig. 2). The patient was then placed on maintenance therapy with the PARPi olaparib (300 mg po bid). Olaparib was well-tolerated without significant side effects, but within 6 mo of treatment, CT scan showed recurrent hepatic metastases (Fig. 2). Upon the development of PARP inhibitor resistance, cell-free (cf) DNA analysis revealed only the pathogenic germline *BRCA2* mutant. Genomic profiling from a growing hepatic metastasis, however, revealed a 228-bp deletion in *BRCA2* that restored the open reading frame. This mutation results in near full-length BRCA2 protein and would be considered a reversion mutation (see the next section).

Because of the lack of known effective therapies for refractory t-SCNC, the patient was next treated with pembolizumab (200 mg every 3 wk) while olaparib was continued, given the preliminary efficacy of anti-PD1 immunotherapy drugs in patients with CRPC and an *HRR* gene mutation (Antonarakis et al. 2020). After three cycles of therapy, continued progression of disease was found by imaging and tumor markers (Fig. 2), and the patient began to lose weight and experience hepatic pain. He was again treated with platinum-based

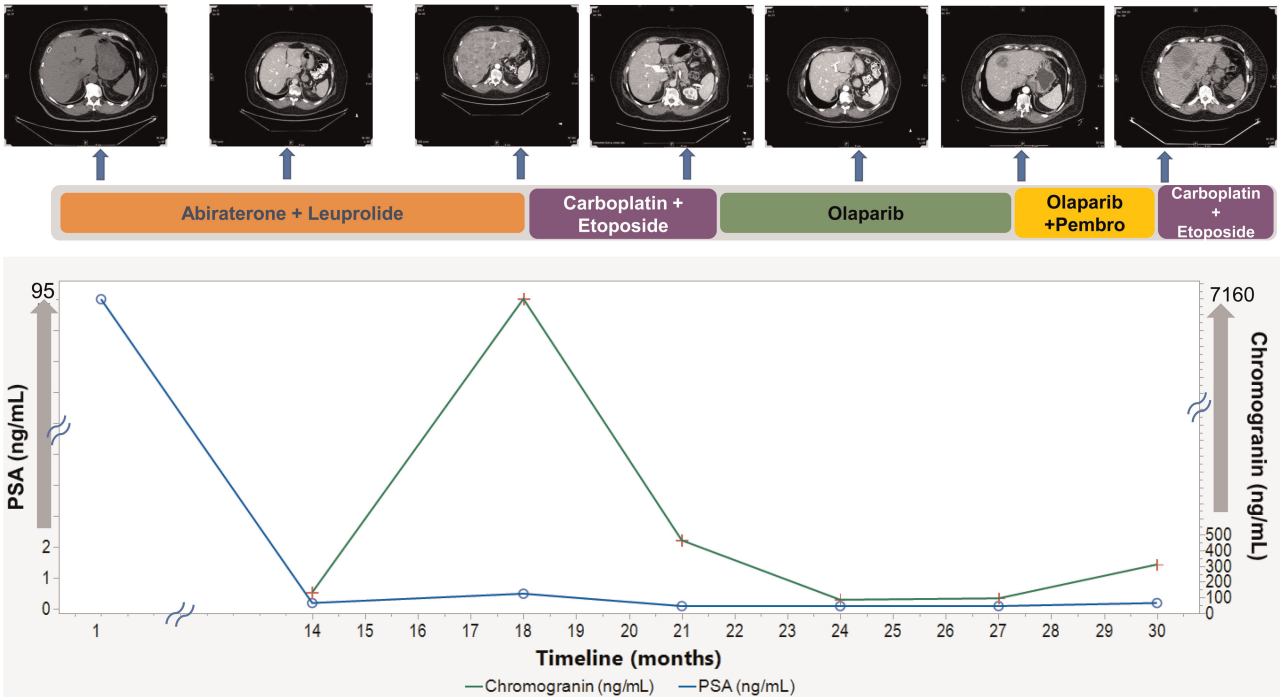

Figure 2. Clinical pattern of response to treatment. PSA and Chromogranin A measurement levels corresponding to the timeline showing therapy (*middle* panel). *Top* panels show corresponding axial CT images. N.B. Leuprolide continued but abiraterone was discontinued upon diagnosis of neuroendocrine carcinoma. (Pembro) Pembrolizumab.

chemotherapy, but after three cycles developed global progression of disease including innumerable brain metastases. Hospice care was chosen and he died 18 mo after the diagnosis of t-SCNC and 34 mo from diagnosis of metastatic prostate cancer.

Germline and Somatic Genomic Alterations

Germline genetic testing was performed prior to initiation of chemotherapy by the 30-gene Color Hereditary Cancer Risk Test (Color Genomics). This revealed the presence of a pathogenic *BRCA2* nonsense mutation (p.Ser1882*) at a mutation allele frequency (MAF) of 49.3%, which predicts truncation and loss of full-length functional protein. A variant of uncertain significance (VUS) in the *ATM* gene (p.I2185T) was also found (Table 1).

Later during the course of treatment and in the setting of resistance to PARP inhibitor therapy, blood for cfDNA was analyzed on the Guardant360 platform consisting of 74 cancer-associated genes (Guardant Health), revealing the same germline *BRCA2* mutation with a MAF of 46.9% and a somatic *BRCA1* (p.E255D) VUS with a MAF of 6.9% (Table 1). Liver biopsy confirmed SCNC and genomic profiling was performed on the MSK-IMPACT platform consisting of 468 genes (Memorial Hospital For Cancer & Allied Diseases) (Zehir et al. 2017). This revealed the following alterations; a somatic *BRCA2* mutation (p.V1810_C1885del) with a MAF of 56%, a VUS in *BRCA1*, benign *ALK, HGF*, and likely pathogenic *SETD2* mutations (Table 1). Copy-number alterations (CNAa): *PTEN, TNRFSF14, HNF1A, PIK3CD, PDCD1*, and *SETD8.* CNA from these genes are suggestive of multiple chromosomal level losses and deletion of the entire gene *PTEN* (Table 2). Structural variants: *MAP2K1, KMT2C2*, and *TMPRSS2-ERG* fusion (Table 2), supporting the transdifferentiation of the small-cell neuroendocrine cancer from the original prostate adenocarcinoma, as this

Table 1. Germline (g) and somatic (s) mutations

						Allele frequency (%)				
						Pre-platin/ PARPi	Relapse during PARPi			
						Color panel (g)	Guardant360 (g,s)	MSK-IMPACT (s)		
Gene symbol	Chromosome_position_change	Clinical significance	Variation type	Gene region	Protein variant	Saliva	Blood (cfDNA)	Tumor	Impact on translation	dbSNP ID
BRCA2	Chr 13:32340000_C > A/C > T	Pathogenic	SNV	Exonic	p.S1882*	49.3	46.9	-	Stop-gained	80358785
ATM	Chr 11: 108192129_T > C	VUS	SNV	Exonic	p.I2185T	51.9	44.3	-	Missense	779611511
BRCA1	Chr 17:43094766_C > T	VUS	SNV	Exonic	p.E255D	ND	6.9	39.6	Missense	62625299
BRCA2	Chr 13:32339783_G>227del	Reversion	Indel	Exonic	p.V1810_C1885del	ND	ND	56	In-frame	
ALK	Chr 2_29275099_C > G	Benign	SNV	Exonic	p.V681L	-	NR	42	Missense	
SETD2	Chr 3_47057420_G > C	Likely pathogenic	SNV	Exonic	p.R2122G	-	NR	39.9	Missense	1279854337
HGF	Chr 7_81757271_G > A	Benign	SNV	Exonic	p.R134C	-	NR	60.7	Missense	1032300573

Germline origin of *BRCA2* p.S1882* was determined by color hereditary cancer risk test. Guardant360 cannot distinguish germline from somatic origin.
MSK-IMPACT calls mutations against paired germline samples and does not report germline variants.
(ND) Not detected, (-) not determined, (NR) not reported (although sequenced, Guarant360 final report does not include variants of these genes), (SNV) single-nucleotide variant, (Indel) insertion deletion.

Table 2. Somatic alterations—copy-number alterations (CNAs) and structural variants (SVs)

Gene symbol	Type	Alteration	Location	Fold change	Panel
PTEN	Whole gene	Deletion	10q23.31	−3.2	MSK-IMPACT
TNFRSF14	Whole gene	Loss	1p36.32	−1.8	
HNF1A	Whole gene	Loss	12q24.31	−1.7	
PIK3CD	Whole gene	Loss	1p36.22	−1.8	
PDCD1	Whole gene	Loss	2q37.3	−1.7	
SETD8	Whole gene	Loss	12q23.31	−1.7	
MAP2K1	Translocation	t(14;15)(q31.2;q22.31) (Chr 14:g.84540589::Chr 15: g.66679738)	exon 1		
TMPRSS2-ERG	Deletion	c.56-537:TMPRSS2_c.18 + 8757:ERGdel	TMPRSS2 exon 1 to ERG exon 2		
KMT2C	Deletion	c.9453 + 378_c.11536del	Exons 41–44		

Amplification is considered when there is a greater than twofold change detected and deletion when <−2 of fold change is detected.

translocation is present in approximately half of prostate cancers (Mosquera et al. 2009), Additional findings included a tumor mutation burden (TMB) of 4.4 mutations per megabase and microsatellite instability (MSI) status stable.

The patient's somatic *BRCA2* (V1810_C1885del) mutation is a 228-bp deletion that includes the germline deleterious point mutation (S1882*) at the third to the last position, restoring the open reading frame, albeit a truncated predicted protein (Fig. 3A). This novel mutation could be considered a reversion mutation, if occurring in *cis* with the germline mutation. It has not been reported in the IRC Database of Reversion Mutations (https ://reversions.icr.ac.uk/), one of the largest of such databases (Pettitt et al. 2020). Because *BRCA2* allelic loss was not reported by MSK-IMPACT, we attempted to determine this by performing qualitative polymerase chain reaction (qPCR) and Sanger sequencing on microdissected tumor tissue (Fig. 3B,C). Gel electrophoresis of the PCR products from *BRCA2* mutation–specific primers revealed the presence of two distinct bands migrating at 230 and 450 bp. Sanger sequencing of each gel-isolated and -purified band confirmed the secondary deletion mutation but also revealed two electropherogram peaks for the wild-type and germline mutant alleles. TaqMan qPCR genotyping assay was performed to target the germline point mutation but showed the same heterogeneity of wild-type and mutant alleles; this was in part due to the inability to eliminate all normal cells from the biopsy samples. Although we could not confirm that the somatic *BRCA2* deletion was *cis* to the germline mutation, the concurrent emergence of resistance to platinum and PARPi therapies as well as several lines of evidence support this as a reversion mutation: (1) The affected region is frequently deleted in reversions; (2) the deletion includes the germline mutation and restores the open reading frame (ORF), an event unlikely to occur at the same location on the normal allele; and (3) the deletion is flanked by microhomology (CAA), a characteristic of many reversion mutations (Pettitt et al. 2020).

Although the Guardant360 assay did not detect our patient's deletion (cfDNA fragments in the assay are in general ~150 bp in length), by using deletion targeted primers on ctDNA taken at a later time, we were able to detect the deletion mutation from a blood sample (see Supplemental Fig. 1).

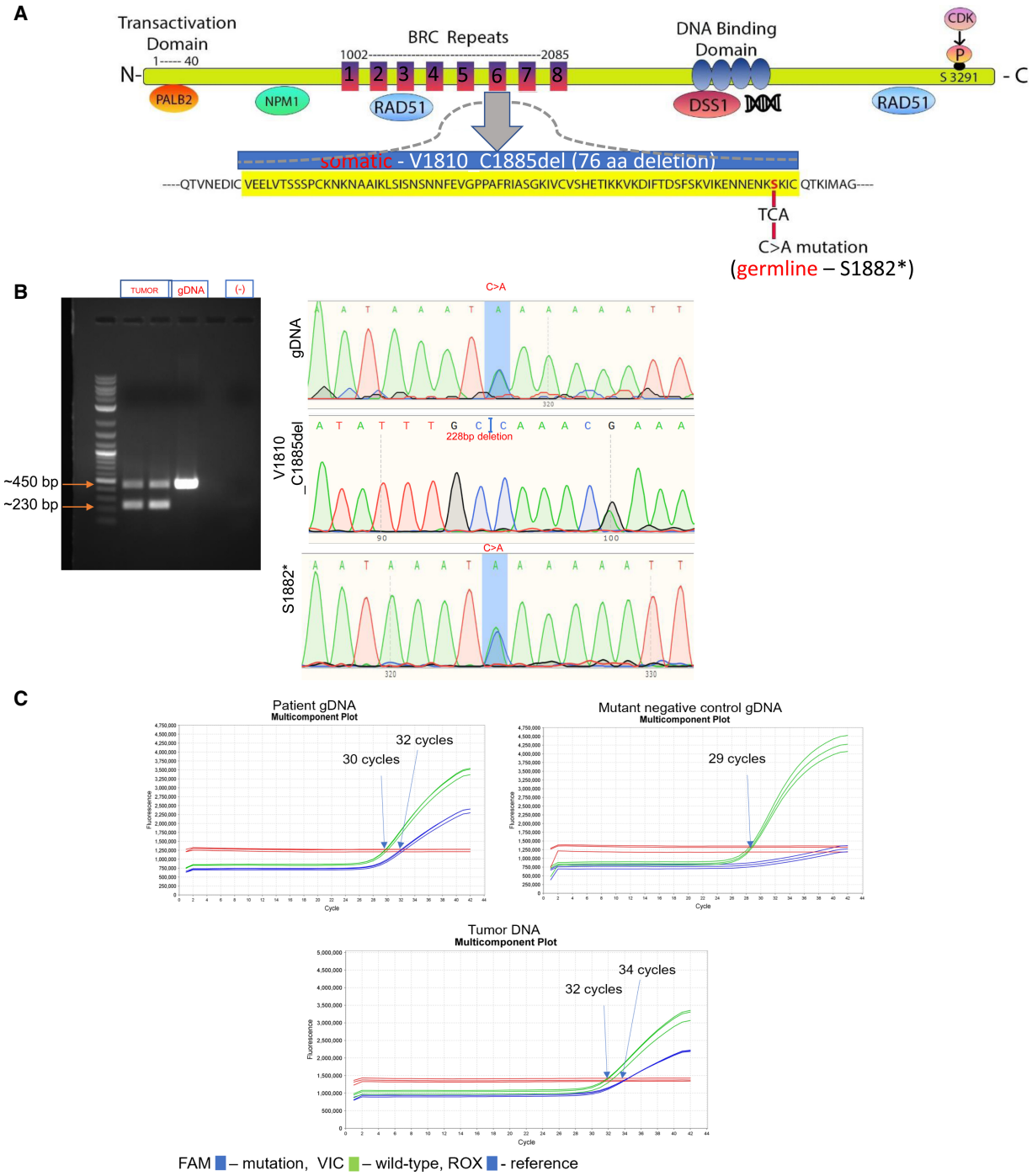

Figure 3. Detection of case patient *BRCA2* germline and somatic mutations in different tissue compartments. (*A*) BRCA2 domains showing location of patient's germline and somatic mutations. The patient has a germline mutation S1882*, which is a pathogenic nonsense mutation. During PARPi (olaparib) treatment, a somatic deletion mutation (V1810_C1885del) of 76 aa was detected. To confirm reversion status of the deletion mutant, Sanger and qPCR (*B*) were performed. As shown in *B*, gel electrophoresis (*left*) from the PCR performed on the primers designed outside the deleted region showed two clear bands of 230 bp and 458 bp and Sanger sequencing (*right*) on each of these bands confirmed the somatic deletion mutation but also showed two peaks, for the wild-type and germline point mutation. To further confirm these findings, we designed a TaqMan genotyping assay for the point mutation and performed qPCR (*C*). qPCR on gDNA showed the wild-type and point mutation alleles at 30 and 32 cycles, respectively. qPCR performed on PARPi resistant tumor tissue also detected both alleles.

DISCUSSION

The optimal management of individuals with incurable malignancies characterized by HRR deficiency is still evolving. Although germline mutations in *BRCA1/2* and *PALB2* predict response to both platinum-based chemotherapy and PARP inhibition, because of the requirement to repair double-strand DNA breaks through the error-prone, nonhomologous end joining pathway, the application of these therapies appears to be tumor-specific (Tutt et al. 2005). For example, platinum induction followed by PARP inhibitor maintenance has become a standard of care in the treatment of ovarian and pancreatic cancers with *gBRCA1/2* or *PALB2* mutations (Robson et al. 2017; Golan et al. 2019). In *gBRCA1/2*-mutated metastatic breast cancer, however, whereas platinum chemotherapy is being utilized in the first-line setting, PARP inhibitors are reserved for relapsed disease (Moore et al. 2018). The use of these genome-directed therapies is inherently more complicated in prostate cancer, which encompasses both castration-sensitive and castration-resistant adenocarcinomas as well as treatment-emergent and de novo neuroendocrine carcinomas.

Prostate adenocarcinoma with a *gBRCA1/2* mutation is associated with an aggressive clinical course and poor prognosis compared to nonmutated disease (Castro et al. 2013). Platinum-based chemotherapy has not been prospectively studied in HRR-deficient prostate cancer and there is no general consensus on whether it is more useful than standard therapies (Pomerantz et al. 2017; Mateo et al. 2018). On the other hand, the PARP inhibitors olaparib and rucaparib have both recently been approved by the U.S. Food and Drug Administration for use in recurrent CRPC with germline or somatic HRR gene mutations.

Neuroendocrine prostate cancer (NEPC) has traditionally been considered a rare, lethal subtype of prostate cancer (Akamatsu et al. 2018). Yet, the widespread use of and subsequent resistance to newer, potent anti-androgen drugs is increasing the incidence of NEPC, specifically t-SCNC (Aggarwal et al. 2018). This observation, coupled with an expansion in germline genetic testing of individuals with prostate cancer, will likely result in more oncologists encountering t-SCNC with HRR gene mutations. Although platinum-based chemotherapy has demonstrated efficacy as first-line therapy, there are no clinical trial results on the use of PARP inhibitors for relapsed disease or as maintenance therapy. Therefore, case reports and series are important in informing practice decisions until mature clinical trial data are reported.

To our knowledge, this is the first case report of germline *BRCA2*-mutated t-SCNC of the prostate. Our patient developed explosive t-SCNC after 16 mo of ADT plus abiraterone for Gleason 8 metastatic prostate cancer. He achieved a complete response with first-line platinum-based chemotherapy but a <6-mo remission duration, despite the use of a PARP inhibitor as maintenance therapy. Given this brief remission, which includes a recognized continued benefit of chemotherapy for ~2 mo after its completion, the benefit of olaparib was considered brief at best. The mechanism of PARP inhibitor resistance in our patient was revealed by tumor genomic profiling, which found a somatic *BRCA2* mutation. This *BRCA2* (V1810_C1885del) deletion mutant encodes for nearly full-length BRCA2 protein except for a deletion of the BRC repeat 6. This would most likely restore at least partial wild-type function as the other intact BRC repeats would allow for the necessary stabilization of RAD51–ssDNA interactions (Chatterjee et al. 2016; A. Monteiro pers. commun.). In support of this, the IRC Database of Reversion Mutations documents a case of platinum-resistant ovarian cancer with a *BRCA2* germline 5946delT reverting to a functional allele by deleting c.5954–6090 (aa 1977–2002), which is part of the BRC repeat 7 (Edwards et al. 2008).

The case presented also has clinical relevance regarding the uses of cfDNA analysis vs tumor profiling to detect *BRCA2* reversion mutations in patients with prostate cancer. The lack of detection of our reversion mutation by Guardant360 may relate to its large size (228 bp) relative to the typical reversion mutation, which is <100 bp (Pettitt et al. 2020).

Although some groups recommend cfDNA analysis over tumor biopsy for detection of the full spectrum of tumor heterogeneity (Carneiro et al. 2018), others have found that the assay has limitations in detecting clinically relevant alterations (including large somatic deletions) in a significant percentage of patients (Taavitsainen et al. 2019).

Both epigenetic and genetic changes have been linked to the lineage plasticity of prostate cancer (Ge et al. 2020). Altered transcriptional regulation by DNA methylation, histone modification, and chromatin remodeling plays a prominent role in the clonal evolution from hormone-sensitive adenocarcinoma to castration-resistant neuroendocrine cancer (Beltran et al. 2016). In our patient, we observed somatically acquired alterations in three histone lysine N-methyltransferase genes, including a missense mutation of *SETD2*, whole-gene loss of *SETD8* (*KMT5A*), and deletion in *KMT2C*. It has recently been shown that SETD2-mediated H3K36me3 down-regulates EZH2-catalyzed H3K27me3 chromatin repression and that SETD2 deficiency promotes the development of metastatic prostate cancer (Yuan et al. 2020). Similarly, SETD8 reduces EZH2-mediated cell proliferation, and reduction in SETD8 activity promotes oncogenesis (LeFave et al. 2015).

Our patient's genomic profile revealed a mutation in the *PTEN* gene and a *TMPRSS2-ERG* fusion, which are present in ~50% of prostate cancers (Akamatsu et al. 2018). Yet, we did not detect loss of *RB1* and *TP53* nor gene amplification of *MYCN* or *AURKA*, common alterations in NEPC (Beltran et al. 2011; Ge et al. 2020). We did not test for MYCN and AURKA overexpression by immunohistochemistry, however, and therefore cannot rule out their involvement in this case of t-SCNC.

Another case of *BRCA2*-alterered t-SCNC was recently reported by Turina et al. (2019). In contrast to our patient, their patient did not have a *gBRCA2* mutation but rather somatic complete copy-number loss of *BRCA2* found on tumor genomic profiling. The patient developed t-SCNC after 4 mo of ADT plus enzalutamide, had a complete response to etoposide/carboplatinum, and was in continued remission after 9 mo of olaparib; no further clinical detail was provided. Considering both cases, inactivation of *BRCA2*, whether through germline or somatic alteration, appears to render t-SCNC highly sensitive to platinum-based chemotherapy, although resistance to platinum/PARPi occurred within 6 mo in our patient as a result of the emergence of a *BRCA2* reversion mutation. Clearly, more reports of HRR-deficient t-SCNC are needed for a clearer understanding of the role of PARP inhibitors and the emergence of BRCA2 reversion mutations in response to platinum and/or PARPi therapy in this histologically and genetically defined subtype of prostate cancer.

In summary, the case presented provides evidence for initial platinum sensitivity of *gBRCA2*-mutated t-SCNC and emergence of a *BRCA2* somatic reversion mutation during PARPi maintenance therapy. The candidate reversion mutation was detectable through tumor genomic profiling but not routine cfDNA analysis. PARP inhibition following effective platinum-based chemotherapy may be a less promising strategy in *BRCA2*-mutated t-SCNC than in other HRR-deficient malignancies (Robson et al. 2017; Moore et al. 2018; Golan et al. 2019). Clinical trials testing different sequencing of platinum-chemotherapy and PARPi along with both serial cfDNA and tumor sampling next-generation sequencing (NGS) analyses are needed for a more informed treatment paradigm to emerge for the treatment of t-SCNC characterized by mutations in HRR genes.

METHODS

DNA Isolation and PCR Assay Design

Total genomic DNA isolation was performed from formalin-fixed paraffin-embedded (FFPE) tumor tissue sample (collected after PARPi treatment) by GeneRead DNA FFPE Kit (QIAGEN 180134) and from peripheral blood monocytes by Gentra Puregene Blood Kit (QIAGEN

158422). Double-strand DNA concentrations were determined by Qubit 2.0 Fluorometer (Invitrogen by Life Technologies). A primer pair (forward: CTTGATTCTGGTATTGA GCCAGT, reverse: ACCTTATGTGAATGCGTGCT) was designed to the *BRCA2* gene exon 11 to sequence somatic mutation as well as point mutation. PCR product DNAs were isolated from the agarose gel by QIAquick Gel Extraction Kit (QIAGEN 28704) and sent for Sanger sequencing (Genewiz). For the mutation analysis by qPCR, TaqMan SNP Genotyping Assay (ThermoFisher Scientific) was designed for point mutation (S1882*) and qPCR was performed on QuantStudio 7 Flex (ThermoFisher Scientific).

ADDITIONAL INFORMATION

Data Deposition and Access

Two *BRCA2* variants (S1882*, V1810_C1885del) focused on in this report have been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV001451935 and SCV001451938, for S1882* and V1810_C1 885del, respectively.

Ethics Statement

Informed written consent was obtained for the patient for the study, Genomic Profiling in Cancer Patients (Memorial Sloan Kettering Cancer Center IRB, ClinicalTrials.gov ID: NCT01775072).

Competing Interest Statement

The authors have declared no competing interest.

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