case report

Acquired Resistance to Poly (ADP-ribose) Polymerase Inhibitor Olaparib in *BRCA2*-Associated Prostate Cancer Resulting From Biallelic *BRCA2* Reversion Mutations Restores Both Germline and Somatic Loss-of-Function Mutations

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INTRODUCTION

Mutations of the *BRCA1* or *BRCA2* gene (*BRCA1/2*) confer an increased lifetime risk of developing breast, ovarian, pancreatic, and prostate cancers, among others.[1,2] BRCA1/2-deficient cancer cells from germline *BRCA1/2* mutation carriers often lose the second *BRCA1/2* allele through deletion of all or part of chromosome 17q or 13q, respectively, or inactivating point mutations or small insertions or deletions.[3-6] Loss of both alleles leads to impaired homologous recombination of double-strand DNA breaks and increased sensitivity to radiation, platinum-based chemotherapy, and poly (ADP-ribose) polymerase (PARP) inhibitors.[7,8]

PARP inhibitors target PARP1 and PARP2 enzymes that bind single-strand DNA breaks and catalyze post-translational modification of DNA repair proteins.[9] In the absence of functional BRCA1 or BRCA2 protein, PARP1/2 inhibition compromises DNA repair and leads to cell-cycle arrest and apoptosis.[10] PARP inhibitors are US Food and Drug Administration approved for the treatment of ovarian and breast cancers with germline *BRCA1* and *BRCA2* mutations, but they also have antitumor activity in castration-resistant prostate cancer (CRPC) carrying germline or somatic mutations in genes involved with DNA repair, such as *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *FANCA*, *CHEK2*, and *CDK12*.[11] In a cohort of 16 patients with metastatic CRPC (mCRPC) carrying mutations in DNA repair genes, PARP inhibitor olaparib achieved response rates as high as 88%.[11] These results fostered ongoing clinical trials of PARP inhibitors in mCRPC and supported breakthrough therapy designation of olaparib by the US Food and Drug Administration for the treatment of *BRCA1/2*- and *ATM*-mutated mCRPC in January 2016.

In germline *BRCA1/2* mutation carriers treated with platinum-based chemotherapy or PARP inhibitors, resistance eventually develops through several mechanisms, including acquisition of somatic *BRCA1/2* mutations that restore the open reading frame (ie, *BRCA* reversion mutations) of the germline allele, which in turn restores production of functional BRCA1/2 protein.[12-18] *BRCA* reversion mutations have been reported in *BRCA*-mutated ovarian, breast, and pancreatic cancer cell lines with acquired resistance to platinum compounds or PARP inhibitors.[13,15-18]

Here we report a case of acquired resistance to PARP inhibitor olaparib in *BRCA2*-mutant mCRPC resulting from multiple acquired reversion mutations detected by circulating tumor DNA (ctDNA) analysis that restored both the *BRCA2* germline mutation and the somatic second-hit loss-of-function mutation on the second allele. We also report the prevalence of *BRCA2* reversion mutations among a large cohort of 1,534 patients with mCRPC who underwent ctDNA testing.

METHODS

Blood for cell-free DNA (cfDNA) analysis was drawn during the patient's regularly schedule clinic visit. The cfDNA next-generation sequencing (NGS) analysis was performed at Guardant Health (Guardant360; Redwood City, CA), a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State Department of Health–approved laboratory. Barcoded sequencing libraries were generated from 5 to 30 ng of plasma cfDNA. The exons of 73 cancer genes were captured using biotinylated custom bait oligonucleotides (Agilent, Santa Clara, CA), resulting in a capture footprint of 148,000 base pairs (78 kb). The mean cfDNA loaded into each sequencing reaction was 22 ng (range, 5 to 30 ng). Samples were paired-end sequenced on an Illumina HiSeq 2500 (San Diego, CA), followed by algorithmic reconstruction of the digitized sequencing signals. The coverage depth across all coding sequences in all samples averaged approximately 15,000×. Illumina sequencing reads were mapped to the hg19/GRCh37 human reference sequence, and genomic alterations in cfDNA were identified from Illumina sequencing data by proprietary bioinformatic algorithms. These algorithms quantify the absolute number of unique DNA fragments at a given nucleotide position, thereby enabling ctDNA to be quantitatively measured as a fraction of total cfDNA. The Guardant360 assay detects single-nucleotide variants, indels, fusions, and copy-number alterations in cfDNA with a reportable range of ≥ 0.04%, ≥ 0.02%, ≥ 0.04%, and ≥ 2.12 copies, respectively.[19,20] This research was approved by the Quorum institutional review board for the generation of deidentified data sets for research purposes (Guardant protocol) and the Northwestern University institutional review board (protocol STU00205723).

CASE REPORT

The patient was a 63-year-old white male of Ashkenazi Jewish ancestry who underwent a radical prostatectomy revealing Gleason 5 + 4 = 9 adenocarcinoma,[21] with involvement of seminal vesicles, perineural invasion, and negative margins. He received adjuvant androgen-deprivation therapy and radiation therapy (70 Gy in 35 fractions) and developed biochemical recurrence 1 year later, when he was treated with bicalutamide. Two years later, prostate-specific antigen (PSA) rose to 218 ng/mL. Computed tomography scan showed retroperitoneal and pelvic lymphadenopathy and a vertebral body metastasis. He received seven cycles of docetaxel followed by prolonged control of disease with 13 cycles of cabazitaxel before a new liver metastasis was identified on scans. Liver biopsy confirmed prostate adenocarcinoma, and NGS (Foundation Medicine, Cambridge, MA) of the liver biopsy identified two mutations in *BRCA2*: c.5946delT (p.Ser1982fs*, also known as 6174delT) and c.5754_5755delTA (p.His1918fs*5). *BRCA2* allelic loss was not reported. Tissue NGS also revealed *CDKN2a* (p16INK4a H83Y; p14ARF A97V), as well as losses of *PTEN* and *FAS* and 12 variants of unknown significance. Germline testing confirmed a heterozygous *BRCA2* c.5946delT mutation in the patient, which was inherited from his father, who had died as a result of colon cancer at age 81 years.

After liver biopsy, the patient was treated with olaparib (400 mg twice per day), resulting in rapid reduction of PSA from 821 to 300 ng/mL and improvement of lymphadenopathy and liver lesions. One year after starting olaparib, PSA rose to 779 ng/mL. Computed tomography scans showed stable adenopathy and liver lesions, but bone scan demonstrated marked progression of disease. ctDNA analysis was performed at the time of disease progression during olaparib treatment. The patient died 3 months later.

RESULTS

Analysis of ctDNA identified the *BRCA2* c.5946delT (p.Ser1982fs*) mutation at a mutant allele fraction (MAF) of 42.4%, consistent with germline origin, and the c.5982_5983delTA (p.His1918fs) mutation at 23.6%, consistent with secondary somatic mutation. ctDNA also detected 11 additional somatic *BRCA2* mutations not identified in the pre-PARP liver biopsy specimen (Table 1), all of which occurred at low MAFs (range, 0.1% to 1.0%), consistent with subclonal somatic origin. Nine of these 11 somatic *BRCA2* mutations occurred in cis with the germline mutation, and three overlapped with the original germline mutation (Fig 1). All occurred within zero to 52 nucleotides of the c.5946delT germline mutation and restored the *BRCA2* open reading frame. Interestingly, the two remaining somatic *BRCA2* mutations

Table 1. Summary and Description of *BRCA2* Mutations Identified by ctDNA Analysis

Mutation (HGVS designation)	Protein (HGVS designation)	Indel Type	Functional Consequence	Indel Length (nt)	Net Loss Resulting From Reversion (nt)	MAF in ctDNA (%)	Location Regarding Germline Mutation
Allele 1							
c.5946delT*	p.Ser1982fs	Deletion	Germline loss of function	−1	NA	42.4	NA
c.5946_5990delTGGAAAATCTGTCCAGGTATCAGATGCTTCATTACAAAACGCAAG	p.Ser1982_Ala1996del	Deletion	Somatic reversion	−45	−46	1.0	Cis
c.5949_5952dupAAAA	p.Ser1985fs	Duplication	Somatic reversion	+4	−3	0.5	Cis
c.5964_5998delATCAGATGCTTCATTACAAAACGCAAGACAAGTGT	p.Ser1989fs	Deletion	Somatic reversion	−35	−36	0.4	Cis
c.5959_5966delCAGGTATC	p.Gln1987fs	Deletion	Somatic reversion	−8	−9	0.3	Cis
c.5992_6005delCAAGTGTTTTCTGA	p.Gln1998fs	Deletion	Somatic reversion	−14	−15	0.3	Cis
c.5941_5956delGCAAGTGGAAAATCTGinsA	p.Ala1981_Val1986delinsIle	Insertion-deletion	Somatic reversion	−15	−15	0.3	Cis
c.5994_5999delAGTGTTinsTATC	p.Gln1998fs	Insertion-deletion	Somatic reversion	−3	−3	0.2	Cis
c.5998_6008delTTTTCTGAAATinsCAA	p.Phe2000fs	Insertion-deletion	Somatic reversion	−8	−9	0.2	Cis
c.5944_5952delAGTGGAAAA	p.Ser1982_Lys1984del	Deletion	Somatic reversion	−9	−9	0.1	Cis
Allele 2							
c.5754_5755delAT	p.His1918fs	Deletion	Somatic secondary mutation	−2	NA	23.6	Trans
c.5736delA	p.Glu1912fs	Deletion	Somatic reversion	−1	−3	0.2	Trans*
c.5748_5754delTTCACATinsC	p.Ser1917_His1918del	Insertion-deletion	Somatic reversion	−6	−6	0.1	Trans*

NOTE. The c.5946delT mutation corresponds to the 6174delT mutation in *BRCA2*; c.5946delT uses the HGVS nomenclature, and 6174delT uses the Breast Cancer International Consortium nomenclature.

Abbreviations: ctDNA, circulating tumor DNA; HGVS, Human Genomic Variation Society; indel, insertion or deletion or compound insertion/deletion; MAF, mutant allele fraction; NA, not applicable; nt, nucleotide.

*Somatic *BRCA2* mutations causing reversion of the secondary loss of function mutation.

(c.5736delA and c.5749_5754delTCACAT) occurred in close proximity to the putative somatic second hit: c.5754_5755delTA. Both were in cis to the c.5754_5755delTA mutation, and both were predicted to restore the open reading frame, suggesting that these acquired somatic variants occur on the alternate allele relative to the germline *BRCA2* c.5946delT mutation. In addition to multiple *BRCA2* mutations, ctDNA analyses revealed the following alterations: *TP53* F113fs; *GATA3* D336D; *ARID1A* S1755T; *MYC* P72A; and amplification of *MYC*, *KRAS*, *CCND2*, and *BRAF*.

Because the biallelic reversion of both germline and truncal somatic *BRCA1/2* alterations contrasted the generally accepted model of monoallelic reversion of germline *BRCA1/2* mutations, we attempted to estimate the relative prevalence of germline versus somatic *BRCA1/2* reversion events in patients with mCRPC using a large genomic database including comprehensive ctDNA results from more than 40,000 patients with a variety of solid tumors. Between October 5, 2015, and April 25, 2017, 1,765 samples from 1,534 unique patients with mCRPC underwent ctDNA testing (Guardant Health), which included

complete sequencing of all *BRCA1* and *BRCA2* exons and exon-intron borders. Of these, 24 patients (1.6%) had a deleterious *BRCA2* mutation falling within the germline MAF (40% to 80%). There were no putative germline mutations in the *BRCA1* gene in this mCRPC cohort. Five of these 24 patients were receiving either a PARP inhibitor or platinum-based chemotherapy at the time of the blood draw. Two of the five patients, one receiving olaparib and one carboplatin, had *BRCA2* reversion mutations detected by the ctDNA analysis. Therefore, in this germline mutation–positive, platinum- or PARP-exposed cohort, the frequency of *BRCA2* reversion was 40% (n = 2 of 5). A third case of reversion was identified, but the patient had no previous exposure to platinum-based chemotherapy or PARP inhibitors.

DISCUSSION

We report a case of acquired resistance to olaparib in *BRCA2* germline–positive mCRPC resulting from multiple acquired *BRCA2* reversion mutations of both the germline mutation and a second-hit somatic mutation on the opposite allele. This case is similar to one recently reported by Goodall et al,[22] in which acquired reversion mutations restored the open reading frame of not only the primary germline mutation but also the secondary loss-of-function mutation. Although previous studies in ovarian cancer have established that reversion of the germline allele is necessary and sufficient to restore normal BRCA protein function, this case suggests functional comparability of the variants despite their origin (ie, somatic or germline). This observation challenges the established model of *BRCA1/2* reversion as restricted to germline mutations and suggests that the germline or somatic origin of the allele may not play a critical biologic role in this mechanism of resistance.

Furthermore, this case is a powerful illustration of convergent evolution of multiple *BRCA2* reversion mutations arising in different clones of the metastatic lesion or within multiple metastases (Fig 2), as has been described.[23] Other studies of acquired resistance have compared ctDNA with tissue-based testing on biopsies from multiple metastatic lesions in the same patient. These studies have shown that a single tissue biopsy often does not capture the full spectrum of acquired resistance mutations, whereas ctDNA may provide a more global summary of tumor heterogeneity, as seen in this case.[24,25] ctDNA analyses also enable monitoring and early detection of mutations driving treatment resistance to PARP inhibitors, with meaningful clinical implications.

Once a *BRCA1/2* mutation is detected, longitudinal monitoring with ctDNA can be relevant for early detection of reversion *BRCA1/2* mutations to predict resistance to PARP inhibitors, as illustrated by the case presented here. In women with platinum-resistant ovarian cancer, presence of *BRCA* reversion mutations was a more accurate predictor of response to subsequent platinum or PARP inhibitor therapy than duration of response to previous lines of platinum therapy.[26] Another study identified reversion of germline *BRCA1/2* mutations in high-grade serous ovarian carcinoma using ctDNA and was able to predict treatment responses.[27] There are limited data on the prevalence of *BRCA* reversion mutations and rates of resistance to platinum or PARP inhibitors in mCRPC. Estimates of *BRCA1/2* reversion rates in women with

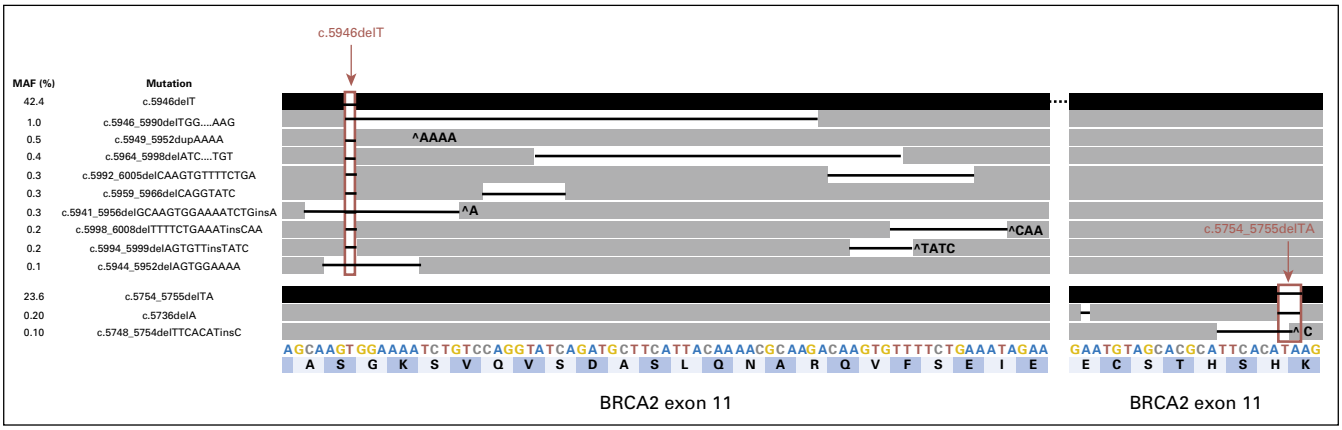

Fig 1. Schematic representation of the germline c.5946delT and secondary c.5754_5755delTA loss-of-function mutations (black bars; top row and third row from bottom) in relation to the acquired somatic reversion mutations (dark gray bars). Black lines between bars represent the nucleotides deleted. ^ indicates an insertion. Letters across the bottom two rows represent the nucleotide (A, C, G, T) and amino acid (dark and light blue bars) sequences of wild-type *BRCA2* exon 11.

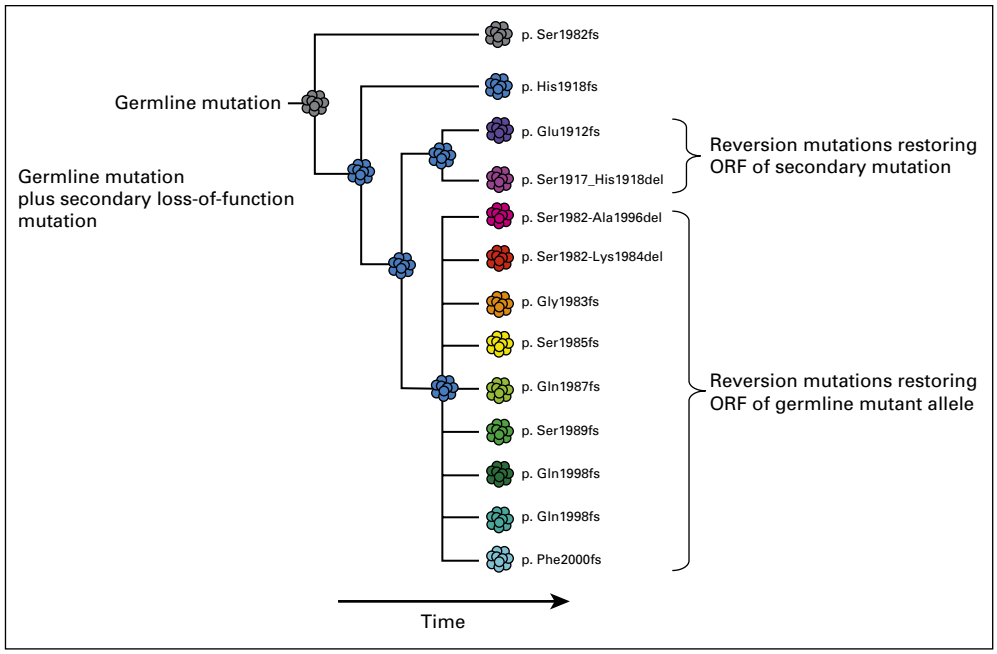

Fig 2. Circulating tumor DNA profiling of a patient experiencing disease progression during treatment with a poly (ADP-ribose) polymerase inhibitor, showing a known germline frameshift mutation and somatic second-hit frameshift mutation, as well as 11 additional frameshift mutations. Phasing the mutation using a Dollo parsimony model allows a presumptive evolutionary history of the tumor population to be inferred. Both somatic and germline lineages contain multiple independent revertant subclones. ORF, open reading frame.

platinum-resistant ovarian cancer range from 25% to 70%, but these are based on small series.[18,26] Analysis of genomic data from large databases may be one way to overcome this limitation. Our ctDNA NGS study estimates a frequency of 40% among patients with mCRPC carrying *BRCA2* germline mutations exposed to platinum or PARP therapy. However, caution should be used when interpreting the reversion frequency reported here, because it is based on a small series of platinum- or PARP inhibitor–exposed patients. Larger prospective studies are needed to determine the true frequency of reversion mutations in a platinum- or PARP-exposed cohort.

There are several limitations to our study. Although genomic testing on tissue before PARP inhibitor therapy was performed for the index case, this information was not available for the additional patient cases showing evidence of reversion mutation. All patients underwent cfDNA analysis at the time of clinical progression, suggesting that they had developed platinum or PARP inhibitor resistance, but the duration of their response during therapy or presence of reversion mutations before exposure is unknown. With regard to the retrospective cohort analysis, our *BRCA2* germline mutation rate was lower than that previously described in the literature.[28] Possible explanations for this include exclusion of putative germline missense and nonsense mutations in the analysis and overly restrictive germline MAF thresholds resulting in exclusion of putative germline mutations in patients with more severe allele imbalance. Lastly, one patient case with evidence of a reversion mutation had no prior exposure to PARP inhibitors or platinum. Review of the patient's treatment history revealed treatment with taxane-based chemotherapy, radium-223, and mitoxantrone. The latter is a DNA intercalating agent used in the treatment of breast cancer, prostate cancer, and acute myeloid leukemia. Interestingly, Ikeda et al[29] reported a patient with Fanconi anemia with biallelic *BRCA2* mutations and previous exposure to mitoxantrone for acute myeloid leukemia. At the time of relapse, a bone marrow biopsy was performed, and a patient-derived cell line showed loss of the Fanconi anemia phenotype because of monoallelic reversion of the *BRCA2* mutation and restoration of wild-type *BRCA2* function. The authors suggest that DNA intercalating agents such as mitoxantrone may have the ability to induce reversion mutations and lead to resistance.

Compared with biopsy, cfDNA analyses allow easier monitoring and potentially earlier detection of mutations that result in treatment resistance. cfDNA analysis, which allows detection of both somatic and germline mutations in a single test, is well suited to distinguish whether a somatic *BRCA* mutation represents a second-hit loss of function or a reversion of the germline *BRCA* mutation. To make the distinction, the exact location of the mutations must be known,

because to restore the reading frame, a revert must be located near the inactivating mutation (ie, before the end of the same exon).[27] Furthermore, cfDNA may provide a more global summary of tumor heterogeneity and the full spectrum of acquired resistance mutations than a single tissue biopsy.[24,25] The case presented here illustrates convergent evolution of multiple *BRCA2* reversion mutations arising in different clones of the metastatic lesion or within multiple metastases (Fig 2), as has been described elsewhere.[23] Incorporation of routine cfDNA analyses into standard of care of *BRCA1/2*-mutated cancers treated with PARP inhibitors or platinum-based chemotherapy requires validation of the germline calls from cfDNA but may allow early detection of treatment resistance and subsequent change in therapy before significant disease progression.

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