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Case report

The dynamic landscape of *BRCA1* reversion mutations from indel to SNV in a patient with ovarian cancer treated with PARP-inhibitors and immunotherapy

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ABSTRACT

Background: Reversion mutations of somatic BRCA mutations are an important source of resistance within ovarian cancer. Furthermore, these reversion mutations are known to change over the course of treatment. Better understanding of the mechanisms leading to reversion mutations and the role of serial ctDNA collection in detecting changes to overall landscape of resistance mutations over time is needed to guide treatment in the metastatic setting.

Methods: Here we study a case of metastatic ovarian cancer undergoing multiple lines of treatment with collection of three serial ctDNA samples. These samples were analyzed by Guardant Health next generation sequencing to detect somatic alterations and their associated mutant allele frequency (MAF) as % cfDNA.

Results: Analysis of our initial ctDNA collection, taken during PARP-inhibitor therapy, revealed a nonsense BRCA-1 mutation (c. 2563C > T p. Q855*), consistent with the BRCA 1 somatic mutation detected on tumor tissue analysis. Initial analysis also revealed a reversion mutation (c.2535_2576del) resulting in an in-frame deletion of the somatic BRCA-1 alteration. The second collection, taken while still on PARP-inhibitor therapy, re-demonstrated this indel reversion mutation along with a second indel reversion mutation (c.2546_2587del), again resulting in an in-frame deletion of the somatic BRCA-1 mutation. The final ctDNA, collected upon initiation of immunotherapy, revealed 4 novel SNV reversion mutations (c.2564A > C, c.2564A > T, c.2565G > T, and c.2565G > C). These SNV reversion mutations result in missense amino acid changes rather than insertions or deletions within the BRCA-1 somatic mutation. The previous indel reversion mutations were no longer detected.

Conclusions: This study illustrates the role of serial ctDNA analyses in the detection of resistance mutations and the dynamic nature of reversion mutations with multiple lines of treatment. While other studies have described both indels and SNVs that occur in tandem, a change in the types of reversion mutations detected across changing therapies has never before been described. Further studies regarding the unique selective pressures arising from use of multiple types of therapy is needed to fully explain this phenomenon.

1. Introduction

Within ovarian cancer, *BRCA1* & *BRCA2* are two of the most common and best studied tumor suppressor genes [1, 2]. Mutations in these genes can affect DNA repair by homologous recombination (HR), allowing mutations that drive carcinogenesis [3]. Ovarian cancers with *BRCA1 & BRCA2* mutations are sensitive to PARP-inhibitors and platinum-based therapies. These therapies harness the defective DNA repair by creating double strand DNA breaks and "staling" of the PARP protein at the replication fork, preventing progression of mitosis [4, 5, 6, 7, 8, 9, 10].

There are multiple mechanisms of PARP-inhibitor resistance including the development of drug efflux pumps, loss or mutation of PARP1 target protein binding site, and changes to enzymes involved in down-stream metabolites of PARP resulting in unrestrained replication [11, 12, 13, 14, 15]. Changes in overall replication fork biology with mutations in PARP-independent protein pathways also protect stalled replication forks and allow their progression [16, 17, 18, 19, 20, 21].

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Another important mechanism is reversion mutation in the *BRCA* gene which restore previously absent BRCA function and ability of cells for homologous recombination [22, 23, 24, 25]. Reversion mutations have been described in several studies of ovarian cancer after exposure to platinum-based therapies and PARP-inhibitors [23, 24, 25, 26, 27, 28]. They are the most common cause of PARP-inhibitor resistance, and are generally single nucleotide variations (SNVs) or point deletions/insertions (indels) causing frameshifts near the original mutations [21]. They restore the open reading frame of the *BRCA* gene, leading to functioning proteins that cause PARP-inhibitor and chemotherapy resistance by reinstating down-stream DNA repair [22, 24]. They are usually late events that reflect changes to sub-clonal populations and can occur in anywhere between 25-70% of ovarian cancers following platinum-based chemotherapy and PARP-inhibitors [24, 26, 27, 28]. Previous studies have reported the existence of reversion mutations in circulating-tumor DNA (ctDNA) in patients with advanced ovarian, prostate and breast cancer [29, 30]. Our group has previously reported detection of *BRCA2* reversion mutations, using serial ctDNA in a patient with prostate cancer [31].

The detection of reversion mutations allows identification of those patients who will later progress on therapy. In fact, rates of progression can reach up to 33.3% in 36 months on olaparib and 50% in 12.8 months on rucaparib [32, 33]. In a study of patients with *BRCA*-mutated ovarian cancer, patients with no ctDNA reversion mutation detected prior to initiation of rucaparib had significantly longer median PFS on rucaparib compared to those with ctDNA reversion mutations identified (9.0 vs 1.8 months, respectively) [34]. For patients that have developed PARP-inhibitor resistance, evidence exists for the use of immunotherapy as next line treatment [35]. The use of serial ctDNA has allowed detection of acquired resistance mutations over time, especially given the clinical infeasibility of performing serial tumor tissue sampling to detect resistance [30, 36]. In some cases, ctDNA analysis allowed detection of reversion mutations that were not present on tumor pathologic samples [27]. Current models of reversion mutations focus on individual mutations [21] however, with the use of ctDNA analysis to capture heterogeneity, multi-clonal reversion mutations for PARPi resistance within the same individual have been described [30, 34, 37].

Here we present a case illustrating the dynamic landscape of reversion mutations within one patient with ovarian cancer who underwent several treatments, including PARP-inhibitors and immunotherapies. This patient also underwent serial ctDNA analyses that demonstrated a dynamic landscape of reversion mutations.

2. Methods

During routine clinic visits, the patient underwent ctDNA analysis with blood draws on three separate occasions. 2 samples of 10 mL of peripheral blood were collected in Streck tubes and submitted for clinical plasma ctDNA analysis using Guardant360 (Guardant Health, Redwood City, CA) as previously described [38, 39]. The next-generation sequencing (NGS) testing was performed in a CLIA-certified and College of American Pathologists accredited laboratory. Guardant360 plasma ctDNA NGS testing detects SNVs, indels, copy number amplifications, and fusions in up to 74 genes, including full exonic sequencing of *BRCA1*. The average sequencing depth of the platform is approximately 15,000x and it uses oligonucleotide barcodes with up to 30ng for library preparation. These barcodes and sequencing libraries are used to reconstruct molecules while minimizing error. Previous validation studies of the platform revealed clinical sensitivity of $\geq$85% and ability to detect SNVs and indels down to a mutation allele frequency (MAF) of 0.01% [39]. Clinical testing reports somatic alterations and their associated MAF as % ctDNA. Patient written and verbal consent was obtained prior to analyses of these results. Given that this was a study of a single case and consent was obtained, further IRB approval was waived.

3. Description of case and results

The patient is a 71-year-old with diagnosis of recurrent stage IIIC papillary serous adenocarcinoma of the ovary. *BRCA1* positive somatic mutation was detected using Foundation One sequencing of metastatic tumor tissue. The patient was negative for germline *BRCA1* mutation. The patient initially underwent resection and 6 cycles of adjuvant paclitaxel/carboplatin. About one year later, she was shown to have progressive disease on CT scans and biopsy-confirmed liver and cecal metastases. Over the next seven years she underwent resection of abdominopelvic disease and chemotherapy including abraxane/bevacizumab, gemcitabine/carboplatin, topotecan, navelbine, pemetrexed, altretamine, capecitabine.

Seven years after diagnosis, the patient was treated with the PARP-inhibitor olaparib 400mg BID (Figure 1). At this time, she had known metastases to the right pleura, two abdominal masses and CT scan with new liver and splenic metastases. She was subsequently treated with 3 cycles of carboplatin AUC5 q 21 days. Repeat scan showed progressive disease (PD) with increasing size of hepatic and splenic lesions and patient was started on a second PARP-inhibitor, rucaparib 600mg BID. At this point, blood was sent for plasma ctDNA analysis (Guardant360, Guardant Health) which identified multiple somatic genomic alterations (Table 1), including two in *BRCA1*: the patient's known nonsense mutation, c.2563C > T (p.Q855*) at 33.3% MAF, as well as a reversion mutation comprising a 42-nucleotide deletion, c.2535_2576del, at 0.3% MAF. The original non-sense mutation in *BRCA1* (c. 2563C > T p. Q855*; Figure 2) was an SNV in codon 855 resulting in premature truncation, loss of function and resultant malignant phenotype. This mutation is consistent with the *BRCA*1 somatic mutation detected on tumor tissue analysis. The MAF of 33.3% is of clinical significance as several studies have shown it to be a surrogate marker of tumor burden [40, 41, 42]. The reversion mutation detected (c.2535_2576del) resulted in an in-frame deletion within the *BRCA1* transcription domain (Figure 2), resulting in a loss of the original mutated codon 855 and restoring transcription.

The patient was continued on rucaparib and 6 months later another ctDNA analysis was obtained. This was due to increasing CA125 from 145.6 to 271.7 and progression of hepatic metastasis on imaging, again indicating progressive disease (PD). In this sample, three *BRCA1* mutations were detected: the previous c.2563C > T (p.Q855*) nonsense mutation at a MAF of 50.7% (increased from 33.3% as depicted in Figure 3A), the previous c.2535_2576del at 0.1% (decreased from 0.3% as depicted in Figure 3B), and a new reversion, another 42 nucleotide in-frame deletion encompassing the original non-sense *BRCA1* mutation, c.2546_2587del at 0.2% MAF.

After 1 year, patient was transitioned from rucaparib to nivolumab/ipilumumab and after a few months of therapy, a third ctDNA was drawn. At this point, CT scan showed stable disease (SD) in hepatic, splenic and abdominal metastases (1.17% change from prior scan per RECIST criteria). Results from this third ctDNA analysis, after the initiation of immunotherapy, are very distinct. The MAF of the original *BRCA1* nonsense mutation decreased from 50.7% to 27.2% (Figure 3A). Notably, the previously detected in-frame deletions were no longer detected and instead, 4 previously undetected single nucleotide variants (SNVs) emerged (Figure 3B): c.2564A > C, c.2564A > T, c.2565G > T, and c.2565G > C at 2.2%, 1.8%, 1.8%, and 0.8% MAF, respectively. All of the subclonal *BRCA1* SNVs (c.2564A > C, c.2564A > T, c.2565G > T, and c.2565G > C) occur within the same codon as the original nonsense mutation (c.2563C > T). Given this configuration, the original p.Q855* nonsense mutation, in conjunction with the sub-clonal SNVs c.2564A > C, c.2564A > T, c.2565G > T, and c.2565G > C, are predicted to result in missense amino acid changes p.Q855S, p.Q855L, p.Q855Y, and p.Q855Y, respectively, and continuation of *BRCA1* transcription (Figure 2).

In addition to the described mutations in *BRCA1*, several other alterations and amplifications were detected on ctDNA over the three time points (supplemental Tables 1-2, Supplemental Figures 1-3). The alteration with the next highest MAF was an alteration in *TP53* (V216M). This

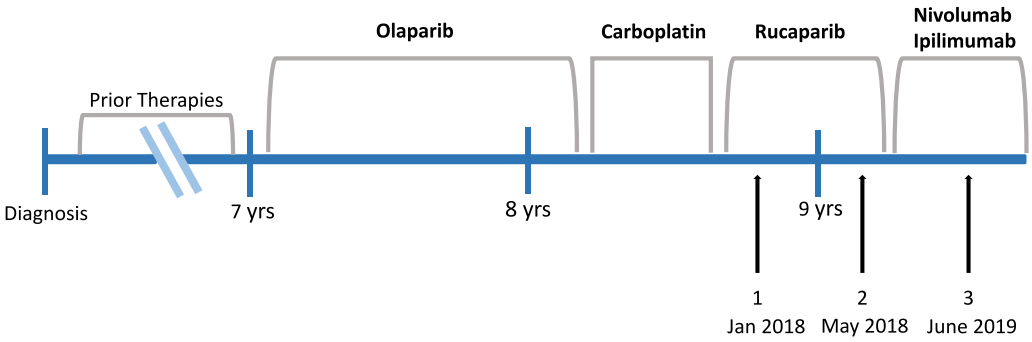

Figure 1. Lines of therapy with corresponding ctDNA collections.

Table 1. Mutant Allele Frequencies of Detected BRCA1 ctDNA by Collection.

ctDNA	1	2	3
c.2563C > T (original BRCA1 mutation)	33.3%	50.7%	27.3%
c.2535_2576del	0.3%	0.1%	0
c.2546_2587del	0	0.2%	0
c.2564A > C	0	0	2.2%
c.2564A > T	0	0	1.8%
c.2565G > T	0	0	1.8%
c.2565G > C	0	0	0.8%

There are the two reversion mutations prior to initiation of immunotherapy.

mutation followed a similar pattern to the *BRCA1* mutation with an increase from 23% to 46.8% between the first and second collection. It subsequently decreased to 27.2% on the third collection after initiation of immunotherapy. All other alterations had significantly smaller MAF but showed similar dynamic changes between types of therapy (Supplementary Figures 1-2).

4. Discussion

This novel case report demonstrates a dynamic landscape of *BRCA1* reversion mutations from indels to SNV with multiple lines of treatment. While other studies have described both indels and SNVs that occur in tandem, this difference in types of reversion mutations detected across changing therapies has never before been described. While we focused on *BRCA1* which had the highest MAF, these dynamic changes were seen with the other alterations detected on ctDNA. The cause of these changes is unclear, however they may be related to differing selective pressures introduced by the unique mechanisms of PARP-inhibitors, which rely on defective DNA repair mechanisms, in contrast to immunotherapy, which rely on activation of T lymphocytes against tumor antigens [43, 44]. For example, the subclones carrying the initial in-frame deletions observed within the initial two ctDNA analyses may have disappeared because while they were particularly successful against evading PARP-inhibition, the lack of continued PARP-inhibitor therapy no longer selected for them.

The mechanism for emergence of SNV reversion mutations, rather than the previous indel reversion mutations observed in this patient, is not clear. One possible explanation is that these mutations were present previously at undetectable levels and the changing treatments allowed for proliferation of these sub-clones over others. However, notably, these new mutations arose in the setting of decreasing MAF of the original *BRCA1* mutation, presumably indicating decreasing overall tumor burden. Another possible explanation is varied shedding of ctDNA from metastatic sites, which is known to be affected by tumor location, size and vascularity [45, 46]. It is possible that as metastatic disease sites grew, and presumably experienced changes in vascularity, the passive release of ctDNA changed to favor certain sub-clones over others. Further investigation regarding the selective pressures induced by immunotherapy is required to better understand this unexpected change to ctDNA milieu observed in this patient. This is of particular importance as immunotherapy is being used more widely across tumor types in clinical trial settings and in conjunction with PARP inhibition to potentially increase efficacy of checkpoint inhibition.

Increase in the original *BRCA1* mutation between first and second ctDNA collections points to overall increasing disease burden, as confirmed by progression of metastatic sites on imaging. This

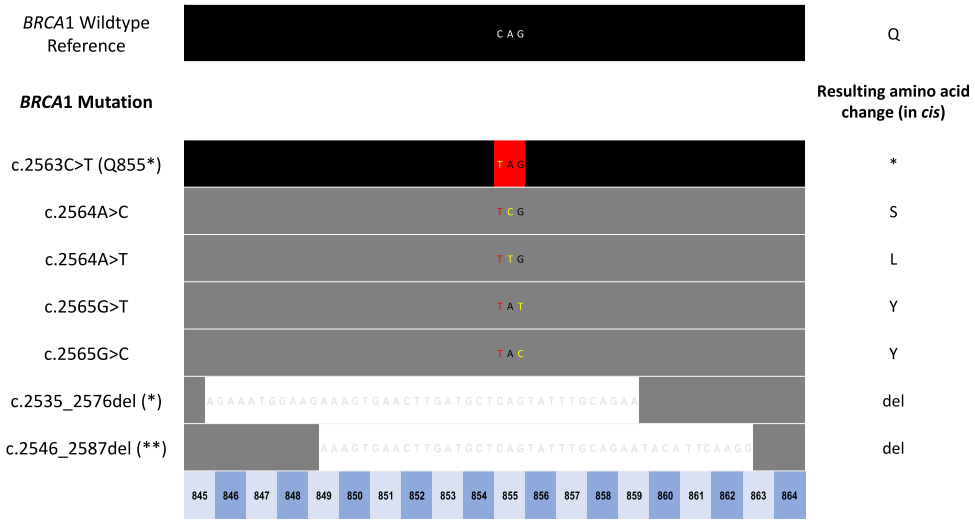

Figure 2. Genomic representation of detected ctDNA *BRCA1* mutations

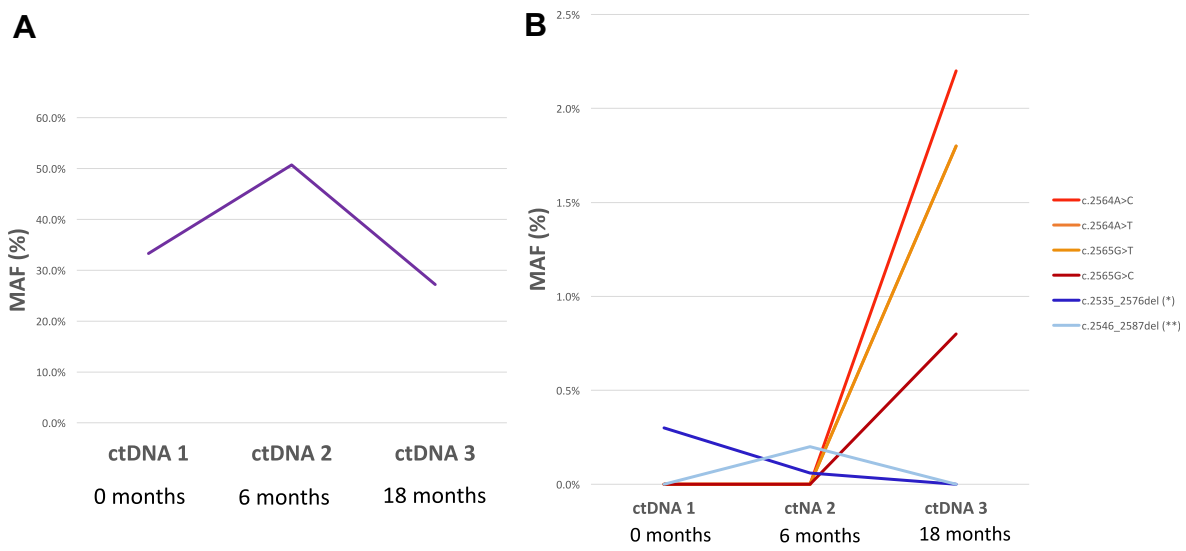

Figure 3. A. Original somatic *BRCA1* mutation (Q855*) over three serial ctDNA analyses. B. Dynamic landscape of reversion mutations over three serial ctDNA analyses

proliferation of the original nonsense mutation points to resistance mechanisms apart from the reversion mutations described above. These resistance mechanisms may be due to development of efflux pumps, changes to the PARP1 protein binding site, changes in enzymes processing down-stream metabolites or changes to overall replication fork biology as previously mentioned. Our study of ctDNA is limited in detection of these alternative resistance mechanisms.

This case study illustrates the role of serial ctDNA analyses in the detection of resistance mutations, the dynamic nature of reversion mutations and the development of multiple mutations within the same individual. The novel shift to SNV reversion mutation clones detected in the reversion mutation landscape are previously undescribed and could possibly be related to differing selective pressures between treatment types. Further studies regarding the unique selective pressures and resistance mechanisms arising from use of multiple types of therapy is needed to fully explain this observed phenomenon.

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The authors declare no conflict of interest.

Additional information

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