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ORIGINAL REPORT

Reversion of *BRCA1/2* Germline Mutations Detected in Circulating Tumor DNA From Patients With High-Grade Serous Ovarian Cancer

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ABSTRACT

Purpose

Germline *BRCA1* or *BRCA2* mutations in patients with high-grade serous ovarian cancer (HGSC) are associated with favorable responses to chemotherapy. However, secondary intragenic (reversion) mutations that restore protein function lead to clinically significant rates of acquired resistance. The goal of this study was to determine whether reversion mutations could be found in an unbiased manner in circulating cell-free DNA (cfDNA) to predict treatment response in HGSC.

Patients and Methods

Plasma and tumor samples were obtained from 30 patients with HGSC with either *BRCA1* or *BRCA2* germline mutation. Two cohorts were ascertained: patients with a malignancy before undergoing primary HGSC debulking surgery (n = 14) or patients at disease recurrence (n = 16). Paired tumor and plasma samples were available for most patients (24 of 30). Targeted amplicon, next-generation sequencing was performed using primers that flanked germline mutations, whose design did not rely on prior knowledge of reversion sequences.

Results

Five patients were identified with intragenic mutations predicted to restore *BRCA1/2* open reading frames, including two patients with multiple independent reversion alleles. Reversion mutations were only detected in tumor samples from patients with recurrent disease (five of 16) and only in cfDNA from patients with a tumor-detected reversion (three of five). Findings from a rapid autopsy of a patient with multiple independent reversions indicated that reversion-allele frequency in metastatic sites is an important determinant of assay sensitivity. Abundance of tumor-derived DNA in total cell-free DNA, as measured by *TP53* mutant allele frequency, also affected assay sensitivity. All patients with reversions detected in tumor-derived DNA were resistant to platin- or poly ADP ribose polymerase inhibitor-based chemotherapy.

Conclusion

Reversion mutations can be detected in an unbiased analysis of cfDNA, suggesting clinical utility for predicting chemotherapy response in recurrent HGSC.

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INTRODUCTION

Patients with high-grade serous ovarian cancer (HGSC) with a germline *BRCA1* or *BRCA2* (*BRCA1/2*) mutation have higher response rates and longer progression-free intervals than mutation-negative patients when treated with platin-based therapies or poly ADP ribose polymerase (PARP) inhibitors.[1-3] However, durable responses in women with advanced disease at diagnosis are uncommon and the development of acquired chemoresistance often occurs during disease recurrence. Several acquired chemoresistance mechanisms have been described in HGSC, including reversion mutations involving intragenic alterations at or adjacent to a germline mutant *BRCA1/2* allele.[4-7] Prior exposure to chemotherapy during an earlier malignancy, such as breast cancer, has also been associated with the presence of reversion mutations and primary chemoresistance in patients with a subsequent diagnosis of ovarian cancer.[7]

Because knowledge of tumor reversion status, and, therefore, likely chemoresistance, may influence treatment planning, we sought to determine if *BRCA1/2* reversion mutations could be identified in cell-free DNA (cfDNA) as a noninvasive test in patients with primary or recurrent HGSC.

ASSOCIATED CONTENT

Data Supplement
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Advantages of using cfDNA rather than tissue biopsy specimens or examination of ascitic fluid in patients with recurrent disease include the opportunity for serial sampling to examine mutation allele frequencies over time, avoidance of invasive peritoneal biopsy, and the reduced availability of significant ascites because of bevacizumab-mediated control.[8]

In general, secondary reversion mutations must occur close to the germline allele to restore the reading frame before a stop codon is reached. Therefore, most reversions should be able to be detected in 170 base-pair (bp) cfDNA fragments without prior knowledge of the sequence of any reversion event. Although some reversion mutations can fall outside this size range (eg, whole exon deletions),[6] these will be difficult to detect in an unbiased manner in cfDNA due to the small size range of circulating DNA.[9]

Here, we assessed cfDNA from patients with primary or recurrent HGSC for *BRCA1/2* reversion mutations. We demonstrate that targeted amplicon sequencing of tumor and cfDNA samples can detect reversions. Furthermore, we investigated detection of subclonal reversion mutations in cfDNA in a patient with multiple reversion events who underwent a rapid autopsy.

PATIENTS AND METHODS

Patient Cohort and Sample Collection

The patient cohort comprised 30 women diagnosed with epithelial ovarian, peritoneal, or fallopian tube cancer between 1992 and 2012 who were recruited to the Australian Ovarian Cancer Study (AOCS).[10] Ethics board approval was obtained at all institutions for patient recruitment, sample collection, and research studies. Written informed consent was obtained from all participants in this study. All patients were diagnosed with serous carcinoma of high-grade (grade 2 or grade 3) and any stage (International Federation of Gynecology and Obstetrics stage I to stage IV), and carried either a germline *BRCA1* or *BRCA2* mutation. All 30 patients (100%) received platin-based chemotherapy during primary treatment, and 28 (93%) additionally received a taxane at first-line treatment (Data Supplement).

Prior malignancy cohort. The group of patients who had a prior malignancy consisted of 14 patients with *BRCA1* or *BRCA2* germline mutation who had a cancer diagnosis before their diagnosis of epithelial ovarian, peritoneal, or fallopian tube cancer (Data Supplement). Thirteen patients had a prior breast cancer, and two of those patients had contralateral metachronous breast cancer before their ovarian cancer diagnosis. Treatment of the prior malignancy comprised chemotherapy (two of 14 patients), radiotherapy (five of 14 patients), or both chemotherapy and radiotherapy (two of 14 patients). A blood sample was collected on the same day or up to 3 days before the primary surgery for ovarian cancer. An ovarian tumor sample collected at surgery was available for 12 patients (86%).

Recurrent cohort. The recurrent cohort comprised 16 patients with *BRCA1* or *BRCA2* germline mutation from whom a blood sample was collected during recurrence of their ovarian cancer. For 12 of these patients, a recurrent tumor or ascites sample was also available. Recurrent, end-stage tumor or ascites samples typically were collected 5 days before the blood sample collection, but, in some cases, collection was considerably earlier or later (Data Supplement). A primary tumor and/or blood sample was also available for four patients. This cohort included seven patients with *BRCA1/2* germline mutation described in Patch et al,[6] including four in whom a reversion had been identified by whole-genome sequencing (WGS) of the relapse tumor. The tumor samples for these cases were sequenced at greater read depth across *BRCA1/2*, except for the patient on whom a rapid autopsy was performed (AOCS-167, patient 5), where targeted deep sequencing had previously been achieved (mean read depth, $> 8{,}000\times$).[6]

Sample Collection and Nucleic Acid Isolation

Blood samples were collected in EDTA or acid citrate dextrose Vacutainer tubes (BD, Franklin Lakes, NJ), and plasma was isolated by centrifugation at $1{,}500 \times g$ for 15 minutes, followed by a second centrifugation at $4{,}000 \times g$ for 10 minutes within 5 days of blood-sample collection (range, 0 to 5 days; mean, 2 days). For patients 1 through 5, in whom reversions were identified, plasma was isolated within 1 day of blood collection except for patient 4, in whom plasma was isolated 4 days after blood collection. Plasma was stored at −80°C until required. Circulating cfDNA was isolated from 1 mL of plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen, Hilden, Germany), per manufacturer's instructions. For solid tumor samples, frozen tissue was cryosectioned and whole-tissue sections were used for DNA extraction. Tumor cells were isolated from ascites using Dynabeads Epithelial Enrich (Thermo Fisher Scientific Life Sciences, Waltham, MA), followed by DNA extraction. DNA extraction of tumor and ascites samples was performed with the DNeasy blood and tissue kit (Qiagen), per manufacturer's instructions. DNA from tumor and ascites was quantitated using the Qubit dsDNA BR assay (Thermo Fisher Scientific Life Sciences); DNA was stored at −20°C.

Reversion Detection in Tumor and Circulating Tumor DNA

Screening for *BRCA1/2* reversions was performed using a targeted amplicon-sequencing approach similar to TAm-Seq.[11] Briefly, primers flanking the known germline mutations were designed for each mutation (amplicon size, 113 to 170 bp) and a common sequence (CS) was added to the 5′ end (Data Supplement). For insertion-and-deletion (INDEL) germline mutations causing a frameshift and downstream premature stop codon, where possible, amplicons were designed to cover the entire sequence altered by the frameshift. This was possible for eight of the 18 cases (13 unique germline mutations) with frameshift mutations. Screening for *TP53* mutations in cfDNA was performed using the targeted amplicon approach, using CS primers flanking the known somatic *TP53* mutations identified in Patch et al.[6] *TP53* mutations were not screened for in patient 3 because of low cellularity and subsequent limiting amounts of DNA from the ascites sample.

Library generation involved two polymerase chain reaction (PCR) amplifications, the first using the CS gene-specific primers and the second using PE-CS primers (Paired End, PE1 & PE2 sequences for Illumina sequencing).[6] Libraries were purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) and bioanalyzed using a high-sensitivity DNA chip (Agilent, Glostrup, Denmark). Libraries were sequenced on a MiSeq using paired end 150-bp sequencing (v2 reagents; Illumina, San Diego, CA). For several samples, multiple libraries were prepared and sequenced to examine reproducibility of variant detection.

Variant Calling in Tumor and Circulating Tumor DNA

Sequence alignment was performed as described previously.[6] Briefly, sequence data were adaptively trimmed and aligned to the amplicon sequences (GRCh37/hg19 assembly), mpileup files generated by SAMtools 0.1.18[12] were used to calculate read depth using VarScan version 2.3 (http://varscan.sourceforge.net/).[13] The mean read depth for each sample was calculated, and ranged from 15,627 to 729,787 per sample (Data Supplement). Variant calling was performed on the amplicon-aligned bam file or the mpileup file using VarScan (version 2.3), MuTect[14] (GATK version 3.1; https://www.google.com/#q=GATK+version+3.1), and IndelGenotyper[15] (GATK version 1.0.4905; gatkforums.broadinstitute.org). The variant calls were merged to generate a single vcf file for each sample and the variants were normalized using Mutalyzer (https://www.mutalyzer.nl/),[16] annotating each variant in the HGVS (Human Genome Variation Society) notation. The allele frequency of each mutation was subsequently calculated using the read-depth information

from VarScan and manual interrogation in Integrative Genomics Viewer (IGV; http://software.broadinstitute.org/software/igv/).[17] Variants with fewer than five supporting reads or in regions with less than 500× coverage were discounted from further analysis.

Variant Classification

Variants in *BRCA1/2* were classified either as the known germline mutation, a reversion, or a nonreversion variant, depending on the location and type of variant, and based on the following rules:

1. For cases with germline mutations that were single nucleotide variants, reversion mutations were additional single nucleotide variants in the same codon as the germline mutation that would cause nonsense mutations to become missense or silent variants, or cause missense mutations to become silent.
2. Reversions for INDEL germline mutations were additional INDELs that would restore the open reading frame (ORF).
3. Nonreversion variants were all other sequence variants that failed to restore the ORF or revert a missense mutation.

These rules do not allow identification of back mutations, large-scale deletions, or splicing alterations. Back mutations, which would appear as a reduced allele frequency of the germline mutation, are difficult to identify because of the rarity of circulating tumor (ctDNA) in cfDNA, and such minimal changes in allele frequency would be unidentifiable. Germline and reversion mutations were manually examined in IGV; reversions that were not seen in the same reads as the germline mutation (ie, on the same allele) were discounted.

Variants in *TP53* were classified as the known somatic mutation or another variant; all mutations were manually reviewed in IGV.

Sensitivity and Specificity

A case was considered a true positive if at least one reversion identified in the matched tumor sample was called in more than one sequencing run of the cfDNA. A case was considered a false negative if a reversion was observed in the tumor sample but not in the matched cfDNA. True-negative cases were those in which no reversion was identified in cfDNA or the matched tumor.

RESULTS

Patient Cohorts and Personalized Assays

To examine *BRCA1/2* reversion mutations, we ascertained two cohorts of patients with *BRCA1/2* germline mutations: patients with HGSC who had had a prior malignancy (Prior Malignancy, n = 14), and patients whose samples were collected during HGSC recurrence (Recurrent, n = 16; Data Supplement). Matching tumor and plasma samples were available for most patients (24 of 30; Data Supplement), allowing us to relate reversion mutation status of the tumor to detection in the plasma. The tumor material available for the Prior Malignancy cohort was collected at HGSC primary surgery, whereas the tumor samples for the Recurrent cohort were ascites samples collected during disease recurrence or tumor deposits collected at rapid autopsy. A recent analysis found that ascitic cells from patients with HGSC represent > 90% of the overall somatic mutation burden,[18] suggesting that prevalent, and, therefore, clinically relevant, reversion mutations should be identifiable in ascites. Personalized PCR assays were designed for each germline *BRCA1/2* mutation by generating primers flanking each germline allele, and with the resulting PCR product spanning 100 to 170 bp (Data Supplement) to allow amplification of cfDNA. PCR amplification incorporated sequencing adaptors and sample-specific barcodes, and the subsequent amplicons underwent deep next-generation sequencing.

Reversion Status of Tumor Samples

We first characterized tumor samples to determine their ongoing germline mutation or reversion status. PCR amplification of sequences flanking each allele and deep sequencing showed that known germline *BRCA1/2* mutations were correctly called in an unbiased manner in all tumor and ascites samples. Additional variants were identified in tumor samples from seven patients in the Prior Malignancy group (none to four per patient; mean, 1; n = 12) and nine tumor samples in the Recurrent group (none to six per patient; mean, 1.87; n = 12; Data Supplement). Any variant that restored the ORF or wild-type protein sequence of a germline allele was regarded as a putative reversion event. The remaining variants, both single-base substitutions and INDELs, that failed to restore the reading frame and, therefore, predicted *BRCA1/2* protein function, were discounted.

Reversion mutations were detected in the relapse tumor samples of four patients in the Recurrent cohort (designated patients 1 through 4; Fig 1A; Data Supplement), including two independent reversion events in patient 1. The four tumors with reversions included three in which we had previously identified the reversion alleles by WGS,[6] providing validation of the PCR-based amplicon sequencing. Adding to the group of samples with reversions for subsequent analysis of cfDNA was a patient on whom rapid autopsy was performed and who had 12 independent, high-confidence reversion events, also previously characterized by WGS and deep amplicon sequencing (patient 5).[6] In contrast with the findings of Norquist et al,[7] no reversions were identified in tumor material from the Prior Malignancy cohort. However, we only had record of prior chemotherapy in four of 14 patients and this may have accounted for our findings. The Prior Malignancy cohort, therefore, added to control blood samples collected for cfDNA analysis at initial debulking surgery in the Recurrent cohort.

Reversion Detection in cfDNA

With knowledge of the reversion status of the tumor samples, we then analyzed cfDNA. As with the tumor samples, known *BRCA1/2* germline mutations were called in all cfDNA material. Additional variants were called in 22 of the 30 patients (Data Supplement); however, reading-frame–restoring reversion mutations were only identified in the Recurrent cohort relapse cfDNA samples, and, in each case, they corresponded to those seen in the tumor DNA (Fig 1A; Data Supplement). As a further measure of specificity, matching cfDNA collected before chemotherapy was available for patient 1, but, in contrast to the cfDNA sample collected during recurrence, no reversion was found. We were unable to detect any evidence of the reversions seen in the tumors of patients 3 and 4 in their matched cfDNA. We repeated the library generation and sequencing for patients 1 through 5, and noted variation in the detection of the reversions in cfDNA between sequencing runs (Fig 1A; Data Supplement).

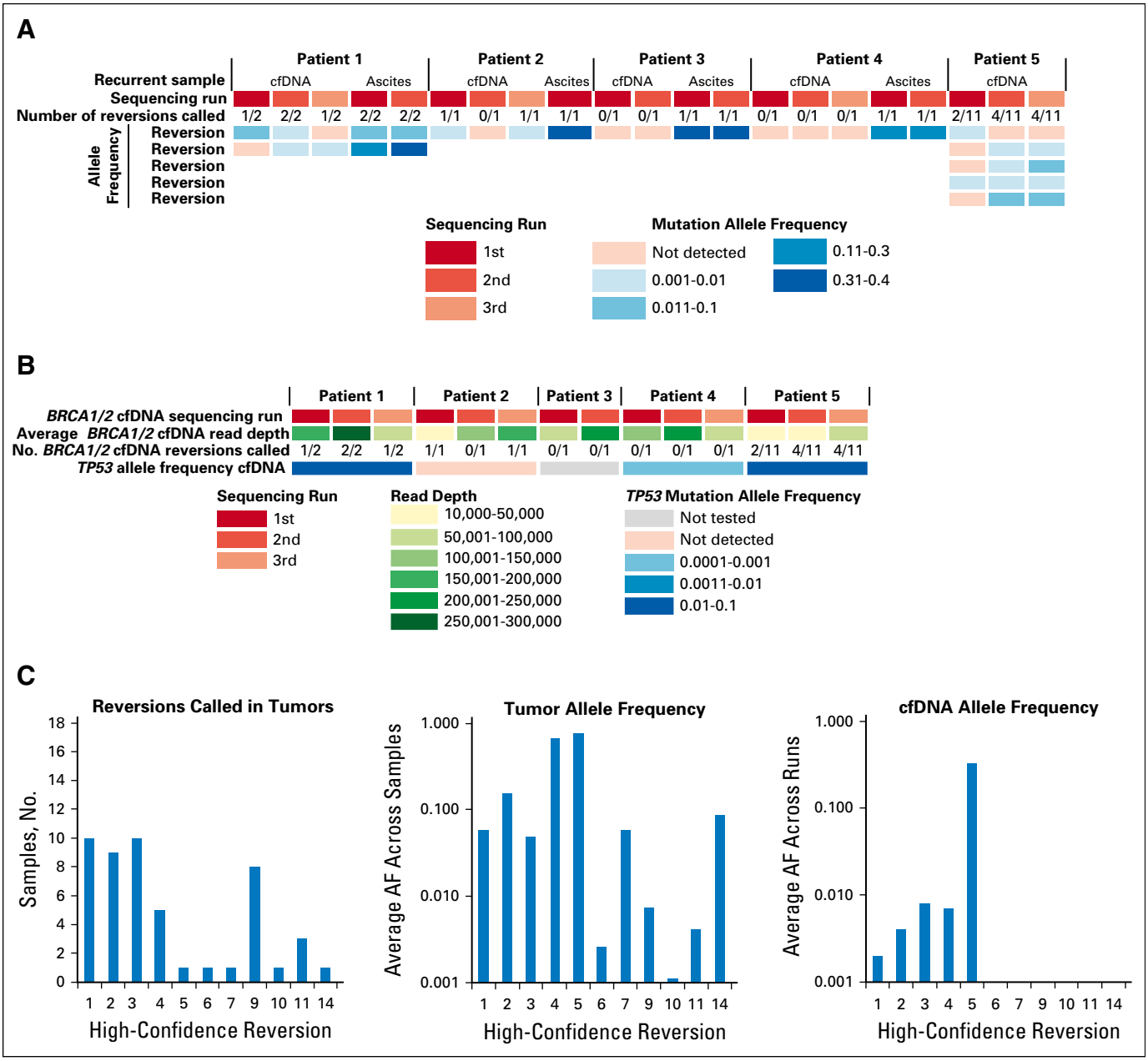

Fig 1. *BRCA1/2* reversion mutations are present in tumor and cfDNA samples of patients with recurrent high-grade serous ovarian cancer. (A) *BRCA1/2* reversion mutations were detected in recurrent tumor and some cfDNA samples from patients 1 through 5. (B) Investigation of factors affecting assay sensitivity, including sequencing read depth and abundance of circulating tumor DNA, as indicated by *TP53* mutation allele frequency. (C) In patient 5, the abundant reversions, as indicated by identification in multiple tumor samples and/or at high tumor allele frequency, were those detected in cfDNA. cfDNA, cell-free DNA.

cfDNA Assay Sensitivity

Using the status of matching tumor tissue as a reference and cfDNA results, each case was categorized as a true or false positive or true or false negative. The assay showed a specificity of 1.00 (no false positives) and sensitivity of 0.60, with a positive predictive value of 1.00 and a negative predictive value of 0.90.

To better understand factors that may affect assay sensitivity, we reviewed the nature of each germline *BRCA1/2* mutation (Data Supplement), patient clinical history, and sequencing metrics. Not surprisingly, we noted that reversions were detected more readily in sequencing runs with higher read depth (Fig 1B).

TP53 mutations are almost invariant in HGSC[19] and are typically clonal,[20,21] thus providing a useful reference for the proportion of ctDNA sequences in cfDNA.[11] Therefore, we determined the *TP53* mutation status for the four tumor samples from our previous WGS study[6] and performed targeted amplicon sequencing of the corresponding site in the recurrent cfDNA samples. The *TP53* mutant allele frequency was $> 1\%$ in patients 1 and 5, in whom reversion alleles were detected. By contrast, the *TP53* mutant allele frequency was $< 0.1\%$ in patient 4, in whom we were unable to detect the reversion allele that had been seen in the corresponding tumor (Data Supplement). We note that 4 days

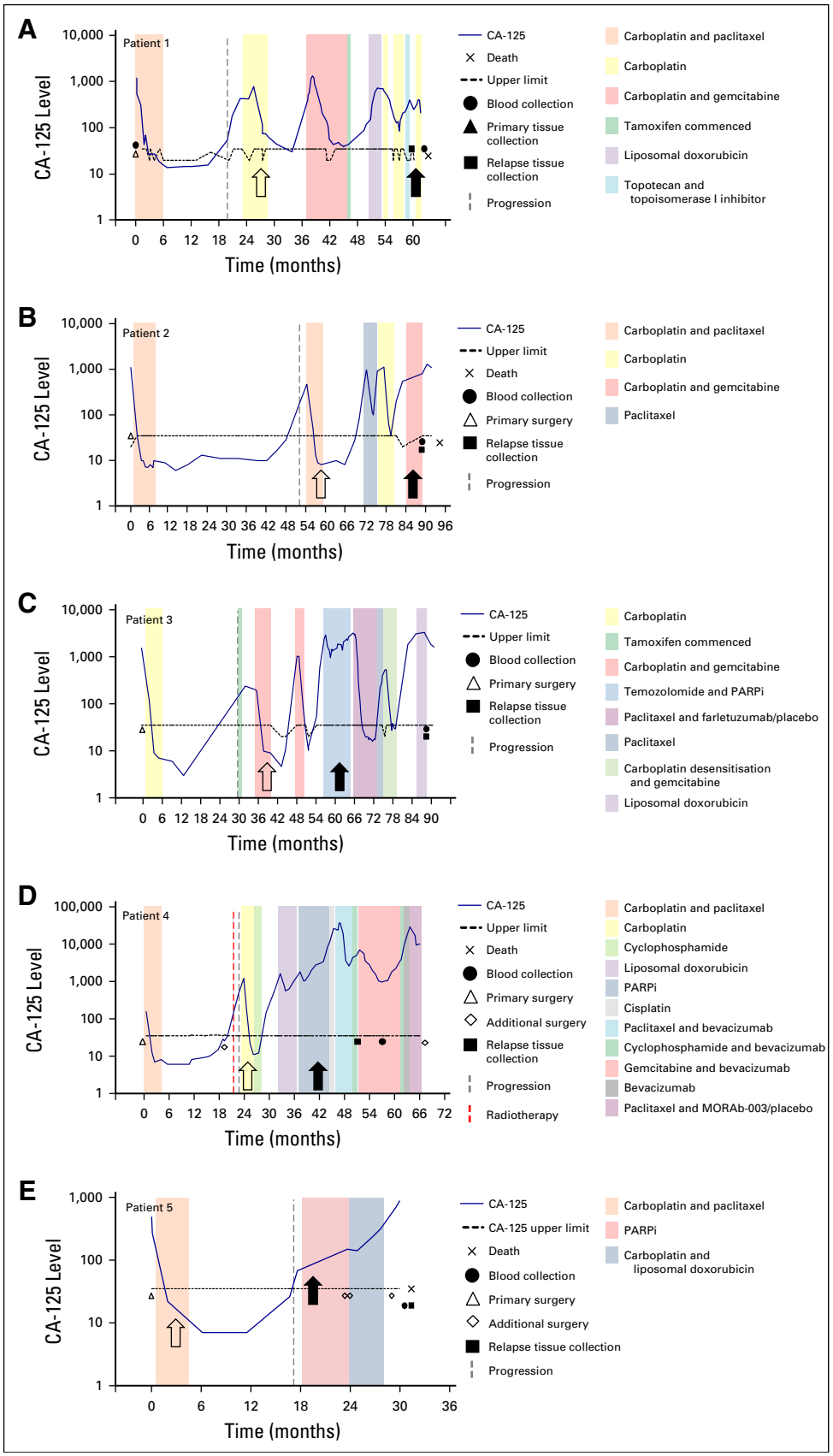

Fig 2. Clinical synopsis of patients with *BRCA1/2* reversions. (A-E) All patients showed a response to first-line treatment, which comprised debulking surgery and platin-based chemotherapy, and development of chemoresistance after progression of disease (gray dashed line) as indicated by elevated CA-125 (blue line) above the upper limit (dotted line). Patient responses to chemotherapy treatment are indicated by arrows. (A) Clinical synopsis of patient 1. After progression, the patient showed a partial response to two lines of platin-based chemotherapy (open arrow) and then displayed no response to later lines of chemotherapy, including carboplatin at line 6 (black arrow) around the time recurrent ascites fluid and blood were collected when reversions were present. (B) Clinical synopsis of patient 2, who had a complete response to platin-based chemotherapy at line 2 (open arrow) and no response to platin-based chemotherapy at line 5 (black arrow) at the time reversions were observed in the recurrent ascites fluid and blood samples. (C) Clinical synopsis of patient 3. After progression, the patient had a complete response to two lines of platin-based chemotherapy (open arrow) before showing no response to a PARPi (black arrow). The recurrent samples were collected at the last line of treatment when the patient showed no response to chemotherapy. (D) Clinical synopsis of patient 4. The patient had a complete response to carboplatin treatment after progression (open arrow) but developed resistance and showed no response to PARPi or cisplatin (black arrow). (E) Clinical synopsis of patient 5. After progression, the patient did not respond to PARPi treatment (black arrow) and recurrent samples harboring reversions were collected at rapid autopsy. PARPi, poly ADP ribose polymerase inhibitor.

elapsed between blood collection and isolation of plasma in patient 4, during which time nucleated white blood cell rupture may have diluted the ctDNA. Surprisingly, the *TP53* mutation was not detected in the cfDNA of patient 2, yet the tumor reversion mutation was detectable in the cfDNA of this patient.

Patient 5, with multiple independent reversion mutations and systematic tumor sampling at autopsy, provided a valuable opportunity to investigate the impact on assay sensitivity of reversion allele frequency in tumor deposits. The reversions identified in the cfDNA of patient 5 (reversions 1 through 5) were those present in multiple tumor samples and/or reversions at an average allele frequency of at least 10% (detection of reversion *v* average tumor allele frequency across sites; Pearson correlation, 0.766; $P = .00598$; Fig 1C).

Clinical Utility of Reversion Detection

Finally, we considered whether the detection of reversion alleles in cfDNA could be of clinical value. Consistent with the known responsiveness of patients with *BRCA1/2* germline mutation to platinum-based treatment, patients 1 through 5 showed durable responses to debulking surgery and first-line adjuvant carboplatin treatment, and four of the five patients had complete responses to a subsequent line of platinum-based treatment during initial disease relapse (Figs 2A-2E; Data Supplement). However, in all cases where reversions were subsequently found in cfDNA, they had become resistant to platin- or PARP-inhibitor–based chemotherapy at the time of blood collection. As another example of potential utility, patient 4 was treated with a PARP inhibitor and cisplatin but failed to respond. However, this patient subsequently had a partial response to gemcitabine and bevacizumab treatment (Fig 2D), demonstrating clinical circumstances where detection of a reversion allele may influence alternative drug selection in the recurrent setting.

DISCUSSION

Other than the length of remission after earlier treatment with platinum-based agents (the platinum-free interval), regrettably, there are no predictive markers of response currently used to guide drug selection in recurrent HGSC. Patients with germline *BRCA1/2* mutations generally show favorable responses to platin-based therapies and PARP inhibitors compared with patients who are mutation negative. However, these patients may develop chemotherapy resistance associated with reversion mutations at recurrence. Therefore, a biomarker to assist in directing their chemotherapy treatment would be valuable. Patch et al[6] described multiple mechanisms of acquired chemoresistance in HGSC; in this study, we focused on the detection of reversion mutations in cfDNA.

We examined cfDNA isolated from patients with germline *BRCA1/2* mutations and HGSC, comparing control samples collected at primary surgery with those obtained after disease progression. Our findings show that detection of *BRCA1/2* reversion mutations in cfDNA by targeted amplicon sequencing is feasible and indicates poor response to platin-based therapy or PARP inhibition. We noted variation in the detection of the reversions in cfDNA between sequencing runs (Fig 1A; Data Supplement), indicating limits to the sensitivity of the current assay. Through access to samples from a patient with multiple reversions on whom a rapid autopsy was performed, we found that detection of the reverted allele was influenced by the abundance of reverted clone(s) in metastatic sites. Other factors affecting assay sensitivity included the proportion of ctDNA in the cfDNA, and/or the read depth of the sequencing of cfDNA (Figs 1B and 1C). Inability to detect reversions to wild-type sequence (back mutations[7]), due to abundant wild-type *BRCA1/2* gene sequence contributed to the cfDNA from normal cells, represents an additional current limitation.

Our findings encourage the development of assays with improved sensitivity and consistency, perhaps through use of molecular barcoding and methods to reduce PCR and sequencing error, followed by the evaluation of larger numbers of samples collected globally from patients with primary HGSC after treatment of a prior malignancy, recurrent HGSC, and other malignancies associated with germline *BRCA1/2* mutations. These studies require a focus on demonstrating an association between detection of reversions and poor response to PARP inhibitors and platin-based therapies compared with non-platin–based chemotherapies. The use of a noninvasive biomarker to direct treatment in a clinical trial of patients with recurrent disease is attractive to demonstrate clinical benefits, such as improvement in survival and through detection of reversions early in recurrence, and thereby avoiding use of ineffective therapy.

In summary, this study demonstrates that analysis of cfDNA can detect reversion mutations in an unbiased manner and has the potential after further evaluation to be used to direct treatment in recurrent HGSC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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REFERENCES

1. Alsop K, Fereday S, Meldrum C, et al: BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: A report from the Australian Ovarian Cancer Study Group. J Clin Oncol 30:2654-2663, 2012
2. Fong PC, Boss DS, Yap TA, et al: Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361: 123-134, 2009
3. Fong PC, Yap TA, Boss DS, et al: Poly(ADP)-ribose polymerase inhibition: Frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 28:2512-2519, 2010
4. Stronach EA, Chen M, Maginn EN, et al: DNA-PK mediates AKT activation and apoptosis inhibition in clinically acquired platinum resistance. Neoplasia 13:1069-1080, 2011
5. Cowin PA, George J, Fereday S, et al: LRP1B deletion in high-grade serous ovarian cancers is associated with acquired chemotherapy resistance to liposomal doxorubicin. Cancer Res 72:4060-4073, 2012
6. Patch AM, Christie EL, Etemadmoghadam D, et al: Whole-genome characterization of chemoresistant ovarian cancer. Nature 521:489-494, 2015
7. Norquist B, Wurz KA, Pennil CC, et al: Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. J Clin Oncol 29:3008-3015, 2011
8. Pujade-Lauraine E, Hilpert F, Weber B, et al: Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol 32:1302-1308, 2014
9. Newman AM, Bratman SV, To J, et al: An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 20: 548-554, 2014
10. Tothill RW, Tinker AV, George J, et al: Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res 14:5198-5208, 2008
11. Forshew T, Murtaza M, Parkinson C, et al: Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med 4:136ra68, 2012
12. Li H, Handsaker B, Wysoker A, et al: The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078-2079, 2009
13. Koboldt DC, Zhang Q, Larson DE, et al: VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 22:568-576, 2012
14. Cibulskis K, Lawrence MS, Carter SL, et al: Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31:213-219, 2013
15. McKenna A, Hanna M, Banks E, et al: The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297-1303, 2010
16. Wildeman M, van Ophuizen E, den Dunnen JT, et al: Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. Hum Mutat 29:6-13, 2008
17. Robinson JT, Thorvaldsdóttir H, Winckler W, et al: Integrative genomics viewer. Nat Biotechnol 29:24-26, 2011
18. Choi YJ, Rhee JK, Hur SY, et al: Intraindividual genomic heterogeneity of high-grade serous carcinoma of the ovary and clinical utility of ascitic cancer cells for mutation profiling. J Pathol 241:57-66, 2017
19. Ahmed AA, Etemadmoghadam D, Temple J, et al: Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. J Pathol 221: 49-56, 2010
20. Bashashati A, Ha G, Tone A, et al: Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol 231:21-34, 2013
21. McPherson A, Roth A, Laks E, et al: Divergent modes of clonal spread and intraperitoneal mixing in high-grade serous ovarian cancer. Nat Genet 48: 758-767, 2016

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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