RESEARCH BRIEF

BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma

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ABSTRACT A key resistance mechanism to platinum-based chemotherapies and PARP inhibitors in *BRCA*-mutant cancers is the acquisition of *BRCA* reversion mutations that restore protein function. To estimate the prevalence of *BRCA* reversion mutations in high-grade ovarian carcinoma (HGOC), we performed targeted next-generation sequencing of circulating cell-free DNA (cfDNA) extracted from pretreatment and postprogression plasma in patients with deleterious germline or somatic *BRCA* mutations treated with the PARP inhibitor rucaparib. *BRCA* reversion mutations were identified in pretreatment cfDNA from 18% (2/11) of platinum-refractory and 13% (5/38) of platinum-resistant cancers, compared with 2% (1/48) of platinum-sensitive cancers ($P = 0.049$). Patients without *BRCA* reversion mutations detected in pretreatment cfDNA had significantly longer rucaparib progression-free survival than those with reversion mutations (median, 9.0 vs. 1.8 months; HR, 0.12; $P < 0.0001$). To study acquired resistance, we sequenced 78 postprogression cfDNA, identifying eight additional patients with *BRCA* reversion mutations not found in pretreatment cfDNA.

SIGNIFICANCE: *BRCA* reversion mutations are detected in cfDNA from platinum-resistant or platinum-refractory HGOC and are associated with decreased clinical benefit from rucaparib treatment. Sequencing of cfDNA can detect multiple *BRCA* reversion mutations, highlighting the ability to capture multiclonal heterogeneity.

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INTRODUCTION

Cancers with defective homologous recombination repair (HRR) have been shown to be exquisitely sensitive to platinum-based chemotherapies and PARP inhibitors (1, 2). The best characterized mechanism for HRR deficiency is the presence of deleterious mutations in *BRCA1* or *BRCA2* (*BRCA*), which are predominantly frameshift or nonsense mutations that result in truncated BRCA proteins. PARP inhibitors demonstrate synthetic lethality in cancer cells with HRR deficiency and clinical efficacy for cancers harboring deleterious germline or somatic *BRCA* mutations, including ovarian, breast, prostate, and pancreatic carcinomas (3–8).

Multiple potential mechanisms of acquired resistance to platinum-based chemotherapies and PARP inhibitors have been described, although few have been identified in clinical samples (9). A key mechanism of clinical resistance is the acquisition of reversion mutations, which are somatic base substitutions or insertions/deletions (indels) that are typically close to the primary protein-truncating mutation and restore the open reading frame (ORF) of the gene and functional protein, switching the neoplastic cell from HRR deficient to proficient (10–12). The restoration of HRR function within the cancer promotes drug resistance by enabling DNA-damage repair induced by PARP inhibitor and/or platinum-based chemotherapy, undermining the basis of synthetic lethality and ultimately promoting cell survival. Reversion mutations in multiple HRR pathway genes, including *BRCA1, BRCA2, RAD51C, RAD51D*, and *PALB2*, have been reported in ovarian, prostate, and breast carcinomas as a mechanism of acquired resistance to platinum-based chemotherapies and PARP inhibitors (13–18).

Recent studies have identified somatic *BRCA* reversion mutations in circulating cell-free DNA (cfDNA) in germline *BRCA* mutation carriers with ovarian, fallopian tube or primary peritoneal carcinoma [collectively termed high-grade ovarian carcinoma (HGOC); refs. 19, 20]. However, to date there is no reported study in a large prospective cohort of patients with HGOC to determine the prevalence of *BRCA* reversion mutations after prior platinum-based chemotherapies and assess their relationship with clinical efficacy of PARP inhibitor treatment.

Therefore, to study primary and acquired resistance to the PARP inhibitor rucaparib, we sequenced cfDNA from blood plasma samples collected before and after rucaparib treatment in patients (n = 112) with *BRCA*-mutant carcinomas enrolled in the phase II study of rucaparib in relapsed HGOC, ARIEL2 (NCT01891344).

RESULTS

Detection of *BRCA* Reversion Mutations in Pretreatment Samples

To study primary resistance to rucaparib, we first performed targeted next-generation sequencing (NGS) of cfDNA extracted from plasma samples collected just prior to rucaparib treatment (pretreatment) in 112 patients with germline or somatic *BRCA*-mutant HGOC in ARIEL2 (Supplementary Fig. S1; refs. 21, 22). The median cfDNA extracted from the 2–3 mL plasma samples was 19.1 ng (range, 4.8–1428 ng), and the median unique molecule coverage was 1,971 × (range, 586 × –6,029 ×).

Because *TP53* is ubiquitously mutated in HGOC, the presence of *TP53* mutations in cfDNA can be used as an indicator of the presence of neoplastic DNA in the bloodstream (23). Somatic *TP53* mutations were detected in 96% (107/112) of the sequenced pretreatment cfDNA samples, indicating that shedding of neoplastic DNA was frequent in patients with relapsed HGOC. Of these cases, primary deleterious *BRCA* mutations (germline or somatic) were detected in 97 cfDNA samples, which was required to determine whether a secondary mutation restores the ORF (i.e., reversion). A significantly lower level of the serum marker CA-125 was found at study enrollment in patients with no *TP53* or *BRCA* mutations detected in the cfDNA (P = 0.025; Supplementary Fig. S2). The median somatic *TP53* mutation allele frequency (MAF) detected in the cfDNA was 3.4% (range, 0.13%–80.4%; Supplementary Table S1). Primary somatic *BRCA* mutations accounted for 31% (30/97) of patients, and germline *BRCA* mutations accounted for the remainder (Table 1); the median somatic *BRCA* MAF detected in the cfDNA was 5.7% (range, 0.18%–77.4%). The observed MAFs of the primary somatic *BRCA* and *TP53* mutations were significantly correlated (R = 0.93; P < 0.0001), supporting the notion that these are clonal driver mutations of HGOC (Supplementary Fig. S3).

The 97 patients with sequenced cfDNA samples containing the primary deleterious *BRCA* mutations had a median of three prior chemotherapy regimens (range, 1–4 regimens; Table 1). Of these 97 patients, 48 were classified as platinum-sensitive, 38 as platinum-resistant, and 11 as platinum-refractory at study entry based on time to relapse following the most recent platinum-based chemotherapy. Interestingly, we found *BRCA* reversion mutations in cfDNA from 18% (2/11) of platinum-refractory and 13% (5/38) of platinum-resistant cancers, compared with 2% (1/48) of platinum-sensitive cancers (P = 0.049; Fig. 1). Apart from platinum sensitivity, no other baseline characteristics were significantly associated with the presence of *BRCA* reversion mutations (Supplementary Table S2).

Sequencing of cfDNA identified *BRCA* reversion mutations in both *BRCA1* (n = 4) and *BRCA2* (n = 4; Fig. 1). All four cases with *BRCA1* reversion mutations in pretreatment cfDNA were found within the large exon 11 of *BRCA1*, whereas the *BRCA2* reversion mutations were found within and outside of exon 11 of *BRCA2* (Supplementary Fig. S4). Although the *BRCA* reversion (secondary) mutations were somatic events, the primary deleterious *BRCA* mutations preceding those events were either germline (n = 5) or somatic (n = 3) in origin. The eight patients with *BRCA* reversion mutations detected in cfDNA samples had received a median of four prior chemotherapy regimens (range, 3–4 regimens) and had a median platinum-free interval of 0.7 months (range, 0–10.9 months). Three of the five platinum-resistant cases had a platinum-free interval of <1 month, suggesting that their disease was at the borderline of platinum-resistant and platinum-refractory.

To confirm the mutations detected in cfDNA, pretreatment plasma samples were available for six of the eight patients with *BRCA* reversion mutations for sequencing

Table 1. Baseline characteristics of patients with primary *BRCA* mutations detected in pretreatment cfDNA samples from ARIEL2

	Patients with cfDNA sequenced ($n = 112$)	Patients with primary *BRCA* mutations detected in cfDNA ($n = 97$)
Median age (range), y	60.5 (33–82)	60 (33–82)
Histologic classification, *n* (%)		
Serous	108 (96.4)	93 (95.9)
Endometrioid	2 (1.8)	2 (2.1)
Mixed	2 (1.8)	2 (2.1)
BRCA mutation type, *n* (%)		
Germline	71 (63.4)	67 (69.1)
Somatic	41 (36.6)	30 (30.9)
BRCA gene with deleterious mutation, *n* (%)		
BRCA1	69 (61.6)	60 (61.9)
BRCA2	43 (38.4)	37 (38.1)
Number of prior chemotherapy regimens, *n* (%)		
1	12 (10.7)	8 (8.2)
2	11 (9.8)	10 (10.3)
3	52 (46.4)	47 (48.5)
4	37 (33)	32 (33)
Platinum response to last therapy, *n* (%)		
Sensitive	56 (50)	48 (49.5)
Resistant	44 (39.3)	38 (39.2)
Refractory	12 (10.7)	11 (11.3)
Median time since cancer diagnosis (range), mo	49.4 (12–186.8)	49.6 (12–186.8)

by an alternative targeted NGS-based cfDNA assay (see Methods). This independent NGS-based cfDNA assay also identified *BRCA* reversion mutations in all six patients (Supplementary Table S1). Overall, a highly significant correlation of MAF for the primary deleterious *BRCA* mutations was found between the two independent NGS-based cfDNA assays (Supplementary Fig. S5).

In addition to cfDNA analysis, tumor tissues (archival and/or pretreatment biopsies) for NGS were available from 95 of 97 patients. The same primary deleterious *BRCA* mutations were detected by NGS of cfDNA and tumor tissues in all cases (Supplementary Table S1). The zygosity of the primary *BRCA* mutations was evaluable for 85% (82/97) of tumors. In all evaluable cases (82/82), the primary *BRCA* mutations were homozygous, suggesting loss of heterozygosity of the wild-type allele and biallelic inactivation (Fig. 1). This finding is consistent with the previous report of ubiquitous biallelic inactivation of *BRCA* in HGOC (24).

Of the 97 patients with sequenced cfDNA samples containing primary *BRCA* mutations, 48 had matched pretreatment tumor biopsies (collected within 28 days prior to the first dose of rucaparib) for NGS, including four of the eight patients with *BRCA* reversion mutations detected in cfDNA (Fig. 1). A *BRCA* reversion mutation was also identified by NGS in each of the four available pretreatment tumor biopsies. In addition, sequencing of pretreatment tumor biopsies identified a large *BRCA1* deletion event [366 base pairs (bp)] that restored the ORF from a patient with platinum-refractory cancer (Fig. 1). Due to the size of cfDNA fragments (approximately 170 bp) and short paired-end sequence reads (150–175 bp), this large deletion was not initially detected by the NGS-based cfDNA assays, but a *post hoc* bioinformatics analysis of the cfDNA sequencing data did identify the 366-bp deletion of *BRCA1*.

Overall, a significant association was found between detection of *BRCA* reversion mutations in matched pretreatment tumor biopsies and cfDNA ($P < 0.0001$). In contrast, *BRCA* reversion mutations were not detected in sequenced archival tumor specimens ($n = 97$) collected at the time of primary diagnosis prior to any platinum-based treatment for ovarian cancer [10% (5/48) vs. 0% (0/97); $P = 0.0034$].

Compared with NGS of pretreatment tumor biopsies, we found sequencing of cfDNA detected additional *BRCA* reversion mutations at baseline that also restored the ORF, highlighting the ability of the NGS-based cfDNA assay to capture multiclonal heterogeneity (Fig. 1; Supplementary Table S1). For instance, a patient with platinum-resistant cancer and a primary somatic *BRCA1* mutation (c.1045G>T; p.E349*) had four unique *BRCA1* reversion mutations detected in cfDNA, including base substitutions that resulted in a single amino acid change and deletions that restored the ORF (Fig. 2). In comparison, in the pretreatment tumor biopsy, only one of the four *BRCA1* reversion mutations was identified.

Another patient with platinum-resistant cancer and a primary somatic *BRCA1* mutation (c.2679delG; p.K894fs) had eight unique *BRCA1* reversion mutations detected in cfDNA, consisting of different lengths of deletions (ranging from

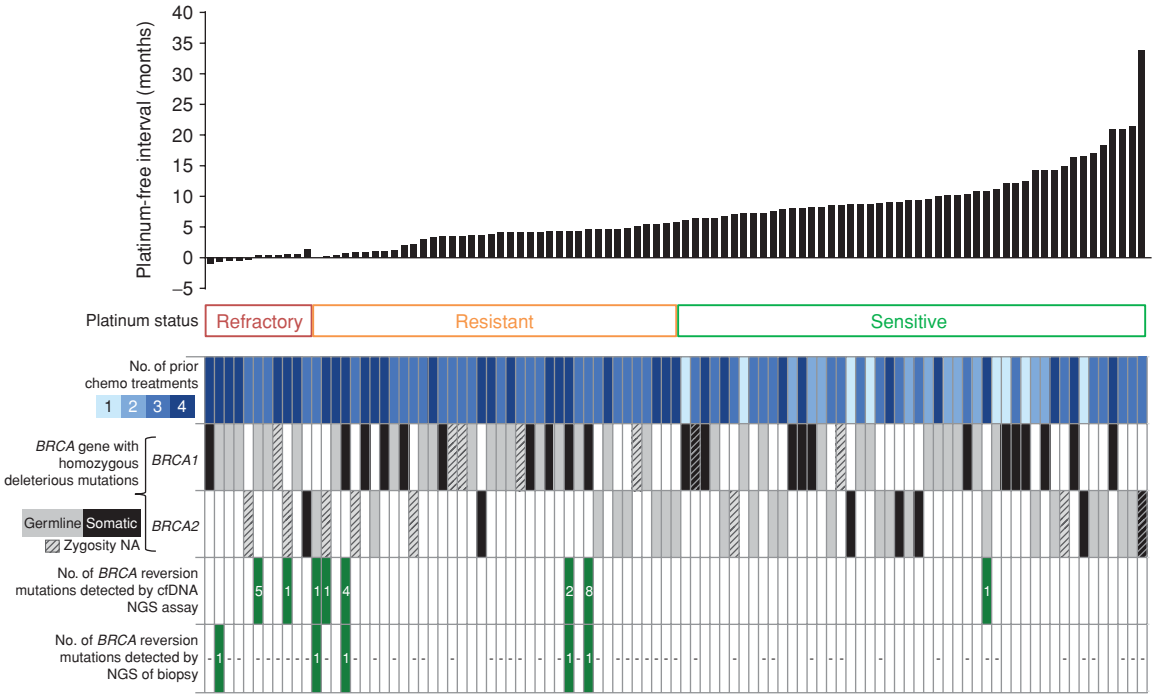

Figure 1. Detection of *BRCA* reversion mutations in pretreatment cfDNA and tumor biopsy. The platinum-free interval and platinum status is based on the patient's most recent platinum-based treatment. The primary deleterious *BRCA* mutations are categorized based on their germline or somatic origin. All tumors with evaluable zygosity of the *BRCA* mutation were found to be homozygous; tumors with indeterminate zygosity are indicated with hatching. The number of *BRCA* reversion mutations detected by each assay is indicated; an empty cell denotes that no reversion mutation was detected. A dash within a cell denotes a pretreatment tumor biopsy sample was not available for sequencing.

deletions of 2 to 29 bp; Supplementary Table S1). Of the eight *BRCA1* reversion mutations in cfDNA, only one was detected in the contemporaneous tumor biopsy. Similarly, a germline *BRCA1* mutation carrier (c.3770_3771delAG; p.E1257fs) with platinum-refractory cancer had five unique *BRCA1* reversion mutations in cfDNA that were deletions ranging from 1 to 31 bp. A paired tumor biopsy was not available for this patient.

Sufficient tumor biopsy specimens were available from 8 of the 41 platinum-resistant or platinum-refractory cases without secondary *BRCA* reversion mutations for laser-captured microdissection of neoplastic cells to determine whether reversion to the wild-type *BRCA* sequence was present. However, we did not observe reversion to the wild-type BRCA sequence in any of the cases tested (data not shown).

BRCA Reversion Mutations in Pretreatment cfDNA Predict Primary Resistance to Rucaparib

We hypothesized that patients without *BRCA* reversion mutations would be more sensitive to rucaparib therapy than those with reversion mutations. Consistent with our hypothesis, patients with *BRCA*-mutant cancers but no *BRCA* reversion mutations detected in pretreatment cfDNA had significantly longer progression-free survival (PFS) after rucaparib treatment than those with reversion mutations, with a median PFS of 9.0 and 1.8 months, respectively (HR, 0.12; 95% CI, 0.05–0.26; $P < 0.0001$; Fig. 3A). Furthermore, within the platinum-resistant or platinum-refractory *BRCA*-mutant subgroup, patients without *BRCA* reversion mutations had significantly longer PFS than those with reversion mutations, with a median PFS of 7.3 and 1.7 months, respectively (HR, 0.16; 95% CI, 0.07–0.42; $P < 0.0001$; Fig. 3B). In fact, five of the seven platinum-resistant or platinum-refractory cases with pretreatment *BRCA* reversion mutations had progressive disease as defined by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST) at the first scheduled response assessment (8 weeks following rucaparib treatment; Supplementary Fig. S6). In addition, all seven of these platinum-resistant or platinum-refractory cases with *BRCA* reversion mutations had an increase from baseline in sum of target lesions as defined by RECIST (Fig. 3C). On the other hand, most patients without *BRCA* reversion mutations derived clinical benefit from rucaparib, even though they had been resistant to their last platinum-based chemotherapy.

We next explored whether the MAF of the *BRCA* reversion mutations detected in pretreatment plasma was associated with rucaparib PFS. Linear regression analysis showed a nonlinear association between *BRCA* reversion MAF and PFS ($P = 0.69$; Supplementary Fig. S7).

Detection of *BRCA* Reversion Mutations in Postprogression Samples

To study acquired resistance to rucaparib, we performed NGS of cfDNA extracted from plasma samples collected immediately following progression while on rucaparib treatment in

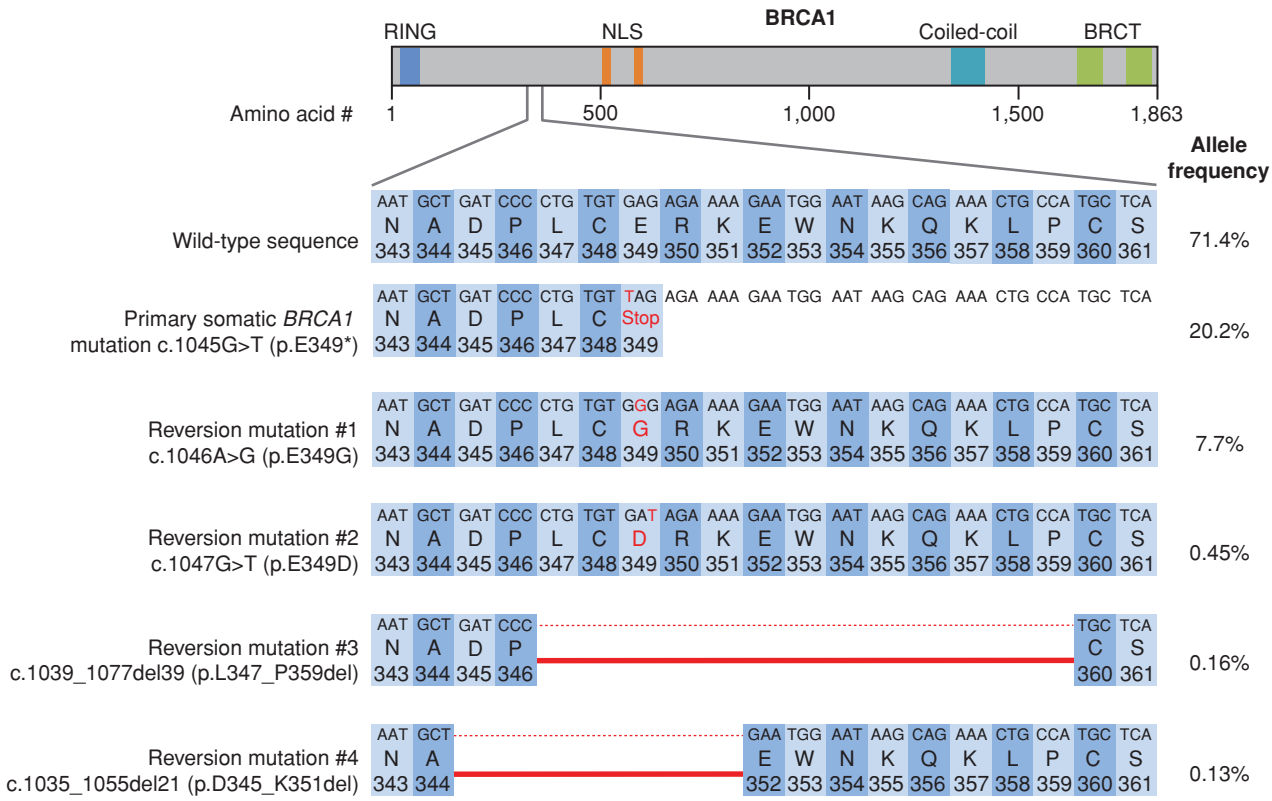

Figure 2. Multiple *BRCA1* reversion mutations detected in pretreatment cfDNA from one patient. Detected allele frequencies of the primary somatic *BRCA1* mutation (c.1045G>T) and the four unique reversion mutations by NGS of cfDNA extracted from a pretreatment plasma sample. The nucleotide and corresponding protein sequence changes of the primary and reversion mutations are shown in red.

78 patients who had *BRCA*-mutant carcinomas (Supplementary Fig. S1). Similar to pretreatment plasma samples, somatic *TP53* and *BRCA* mutations were detected in 81% (63/78) of postprogression plasma samples (Supplementary Table S1). The median *TP53* MAF detected in postprogression cfDNA was 5.7% (range, 0.17%–68.8%; Supplementary Table S1).

Postprogression plasma samples were available from seven of the eight patients with *BRCA* reversion mutations detected in pretreatment plasma samples. As expected, *BRCA* reversion mutations detected before treatment were also detected after progression (Supplementary Table S3). For patients with multiple *BRCA* reversion mutations, changes were observed in the relative MAFs of the different reversion mutations (normalized to *TP53* MAF to control for variation in the neoplastic component of cfDNA), suggesting potential expansion of certain clonal fractions. For example, in the patient with platinum-resistant cancer and a primary somatic *BRCA1* mutation (c.2679delG; p.K894fs), the reversion mutation c.2740_2750del11 increased from a relative MAF of 7.2% before treatment to 25.9% after progression, and seven additional *BRCA* reversion mutations were detected after progression (Supplementary Fig. S8).

Furthermore, sequencing postprogression plasma samples identified eight additional patients with *BRCA* reversion mutations not found in pretreatment plasma samples (Supplementary Table S3). Most of these patients derived clinical benefit on rucaparib treatment, with a median PFS of 9.0 months, before eventually progressing. These *BRCA* reversion mutations were found in all three platinum status groups: platinum-refractory ($n = 1$), platinum-resistant ($n = 5$), and platinum-sensitive ($n = 2$). The acquired *BRCA1* reversion mutations were found within and outside of exon 11 of *BRCA1*, whereas the *BRCA2* reversion mutations were all outside of exon 11 of *BRCA2* (Supplementary Fig. S4). The primary *BRCA* mutations for all eight of these cases were germline in origin (Supplementary Table S1).

To study the kinetics of acquisition of *BRCA* reversion mutations, we profiled cfDNA collected at multiple time points between pretreatment and postprogression. Interestingly, in four of the eight patients with acquired *BRCA* reversion mutations, the reversion mutations were detected in plasma samples collected prior to progression, at a median of 3.4 months (range, 0.7–8.3 months) before progression. By contrast, the remaining four patients had *BRCA* reversion mutations detected in plasma samples only at the time of radiologic/clinical progression.

DISCUSSION

In this study, we sequenced cfDNA from a large cohort of patients with recurrent, *BRCA*-mutant HGOC and found that *BRCA* reversion mutations were more commonly detected in pretreatment cfDNA from patients with platinum-resistant or platinum-refractory than platinum-sensitive

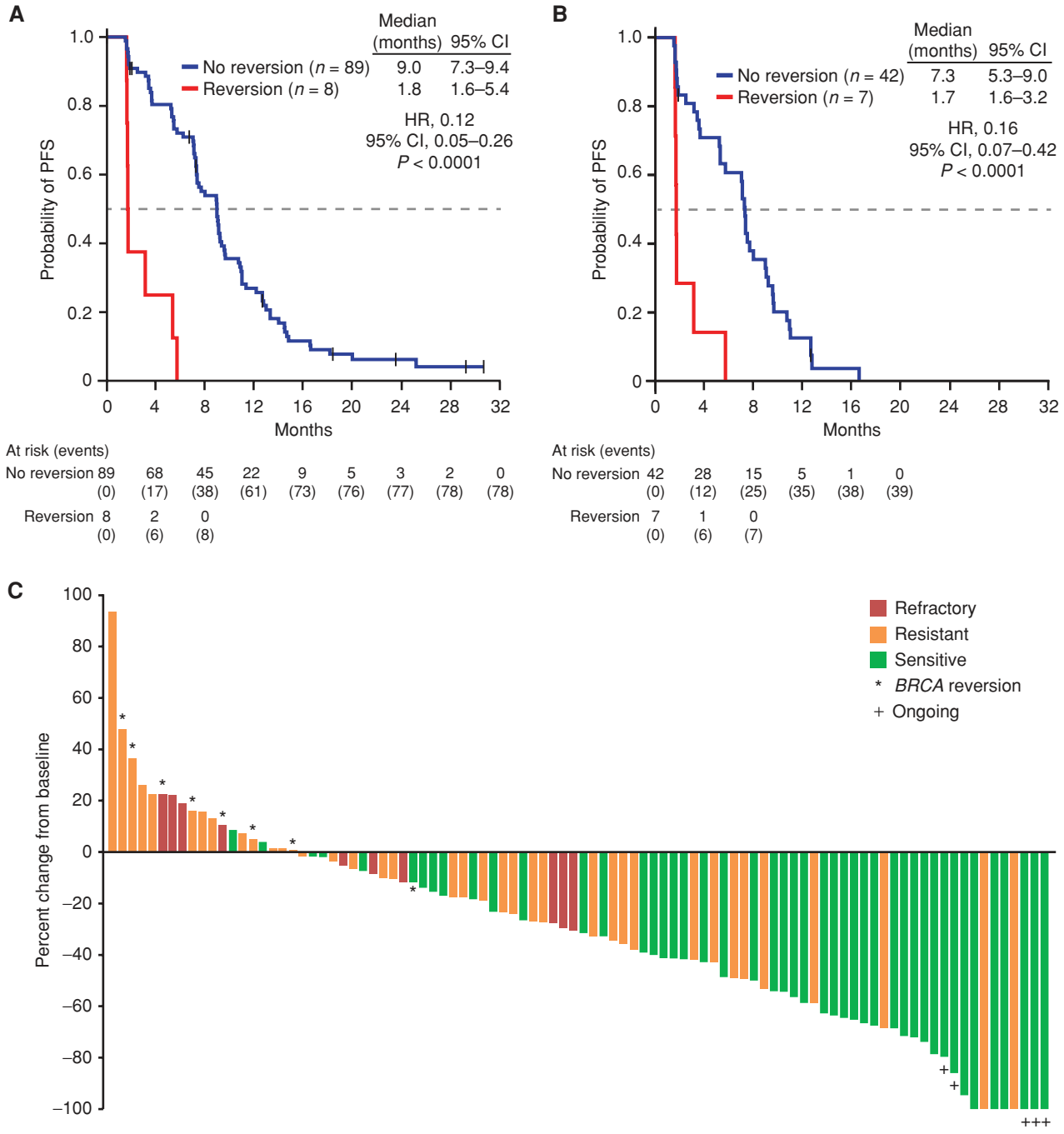

Figure 3. Patients without *BRCA* reversion mutations detected in pretreatment cfDNA have significantly longer rucaparib PFS in (**A**) all *BRCA*-mutant cases and (**B**) platinum-resistant or platinum-refractory *BRCA*-mutant cases. **C,** Best percentage change from baseline in sum of longest diameter of target lesions per RECIST as assessed by the investigators for patients with both baseline and postbaseline measurements.

disease at study entry. Previous studies examining *BRCA* reversion mutations in cfDNA were restricted to germline *BRCA* mutation carriers with HGOC (19, 20). We found that *BRCA* reversion mutations were also detected in cfDNA from patients with deleterious somatic *BRCA* mutations. Furthermore, we showed that patients without *BRCA* reversion mutations in cfDNA prior to starting therapy had significantly longer PFS when treated with the PARP inhibitor rucaparib than patients with reversion mutations. Patients without reversion mutations at baseline appeared to benefit from rucaparib treatment, suggesting that resistance to platinum does not always predict resistance to rucaparib.

A critical question is how the detection of *BRCA* reversion mutations should affect clinical decision-making in patients on PARP inhibitor therapy. The answer may depend on whether the reversion mutation is detected in a tumor

biopsy or in cfDNA. In the case of a tumor biopsy, we previously observed a *RAD51D* reversion mutation in one progressing lesion, whereas the rest of the metastatic sites were stable (17). Continuing PARP inhibitor therapy in this setting seems reasonable, potentially combined with local treatment of the progressing site (i.e., radiation or surgical resection). On the other hand, the finding of reversions in cfDNA reflects a more global tumor assessment. We had four patients in whom reversion mutations were detected in plasma samples prior to radiologic progression according to RECIST criteria, a median of 3.4 months prior to progression (range, 0.7–8.3 months), and four patients in whom reversion mutations were detected at the time of progression. In no cases were reversion mutations identified in cfDNA that did not associate with concurrent or imminent progression, suggesting that these findings warrant a change in therapy.

Reversion mutation in an HRR gene such as *BRCA* is an acquired resistance mechanism observed for the different clinically tested PARP inhibitors, including olaparib, talazoparib, and rucaparib (15–18). The utility of changing from one PARP inhibitor to another is uncertain. Given the impact of reversion mutations in restoring DNA homologous recombination–directed repair, a cancer with a *BRCA* reversion mutation that has just progressed on one PARP inhibitor should not be treated with another single-agent PARP inhibitor. The next generation of clinical trials is evaluating a variety of therapies after PARP inhibitor progression, and we suggest that these trials should stratify patients at trial entry for the presence of reversion mutations in tumor tissue and/or cfDNA.

The association between *BRCA* reversion mutations and platinum resistance found in this study is consistent with previous studies that sequenced ovarian carcinomas (13, 14, 20). In addition, the frequency of pretreatment *BRCA* reversion mutations in platinum-resistant or platinum-refractory cancers observed in this study (14%) was similar to the frequency found in a recent study of cfDNA from a smaller cohort of patients with platinum-resistant or platinum-refractory ovarian cancer (21%; 4/19; ref. 20).

Because equal volumes of plasma samples from the same blood draw were used to perform two independent NGS-based cfDNA assays in this study, the low starting volume of 2–3 mL of plasma and resulting cfDNA extraction yield may not be optimal for detecting low allele frequency indels in cfDNA. Therefore, to estimate the prevalence of *BRCA* reversion mutations, we required the sequenced cfDNA samples to harbor the primary somatic *BRCA* and *TP53* mutations, indicating shedding of neoplastic DNA with detectable key driver mutations. From a typical 10 mL whole blood draw, an average yield of approximately 5.5 mL of plasma would likely produce higher cfDNA extraction yield, potentially resulting in even higher probe coverage and sensitivity.

BRCA reversion mutations were identified in pretreatment and postprogression cfDNA from a minority of patients with platinum-resistant or platinum-refractory HGOC in this study, suggesting the existence of other primary and acquired resistance mechanisms. In addition to reversion mutations, other mechanisms of resistance to platinum agents and PARP inhibitors have been reported by others (9). A potential mechanism of resistance for cancers with *BRCA1* mutations in exon 11 is increased expression of a naturally occurring alternative splice isoform that lacks exon 11 but still has residual BRCA1 activity (25). If this resistance mechanism was commonly at play, we would expect a lower rate of *BRCA1* reversion events in carcinomas with exon 11 mutations than in those with mutations outside of exon 11, which was not the case in this series. Another potential mechanism of chemoresistance recently found in ovarian carcinomas is promoter fusion that results in overexpression of the drug efflux pump MDR1 (14). Unfortunately, the NGS-based cfDNA assays that were tested in this study do not sequence the regions around the *ABCB1* gene, which encodes MDR1, to detect such fusion events. Future studies may incorporate assays that can assess the frequency of *ABCB1* fusion events and other resistance mechanisms to PARP inhibitors.

In summary, this study found *BRCA* reversion mutations were detected in cfDNA of platinum-resistant or platinum-refractory HGOC and are associated with decreased clinical benefit from PARP inhibitor therapy. For patients who relapsed from prior chemotherapies and need to urgently find the next treatment options, this type of minimally invasive assay can efficiently detect *BRCA* reversion mutations that predict resistance to PARP inhibitors and may provide information on tumor heterogeneity.

METHODS

Patient Cohort and Sample Collection

Archival tumor and pretreatment and postprogression tumor biopsies were collected from patients who enrolled in ARIEL2, an international, multicenter, two-part, phase II open-label study (21, 22). Part 1 of ARIEL2 enrolled patients who received at least one prior platinum-based regimen and had platinum-sensitive disease. Part 2 enrolled patients who had received at least three, but no more than four, prior chemotherapy regimens.

Only patients in whom a germline or somatic *BRCA* mutation was identified were included in this analysis. Blood plasma samples were prospectively collected before treatment (during the 28-day screening period and just prior to day 1 of the first cycle of rucaparib treatment), during treatment (day 1 of subsequent treatment cycles), and after progression.

PFS was defined as the time from the first dose of rucaparib to investigator-assessed disease progression or death from any cause. Tumor response was assessed by the investigators using RECIST, with computed tomography scans at screening and every 8 weeks during treatment. Serum CA-125 measurements were taken at screening (before rucaparib), day 1 of each cycle, the end of treatment, and when clinically indicated. The cutoff date for the clinical efficacy data was December 31, 2017.

Patients whose disease progressed during their most recent platinum-based treatment were classified as platinum-refractory. Patients whose disease relapsed within 6 months after the last platinum agent was administered were classified as platinum-resistant; those whose disease relapsed after more than 6 months were classified as platinum-sensitive.

ARIEL2 was approved by the institutional review board at each study site and was done in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Conference on Harmonisation. Patients provided written informed consent before participation.

Plasma Sample Preparation and NGS of cfDNA

Whole blood was collected in the S-Monovette 9 mL hematology EDTA tubes. Within 30 minutes of blood collection, the EDTA tubes were centrifuged at 800 × *g* for 10 minutes. After transferring plasma into 1 mL aliquots, the plasma samples were centrifuged a second time at 14,000 rpm for 10 minutes to remove any remaining cellular debris and stored at −70°C or below prior to cfDNA extraction.

Blinded plasma samples of equal volume (2–3 mL) were sent to Guardant Health and Foundation Medicine for targeted NGS using the Guardant360 (cfDNA assay 1; ref. 26) and FoundationACT (cfDNA assay 2; ref. 27), respectively. Details of the bioinformatics methods for read processing and variant calling are described in the analytic validation studies of the two NGS-based cfDNA assays (26, 27). Both cfDNA assays are based on the Illumina HiSeq sequencing platform and sequence a panel of cancer-related genes, including all exons of *BRCA1, BRCA2*, and *TP53*. The Guardant360 assay was used to sequence 112 pretreatment and 78 matched postprogression plasma samples. For confirmation of mutations detected by the Guardant360 assay, the FoundationACT assay was utilized to sequence 28 pretreatment plasma samples.

BRCA reversion mutations included the following: (i) a base substitution that changed a nonsense mutation to a missense mutation and (ii) an insertion/deletion that restored the ORF. For an insertion/deletion to restore the ORF and be classified as a reversion mutation, the combined effect of the primary deleterious mutation and secondary mutation should produce a nucleotide change that is divisible by three. For instance, if a primary deleterious mutation is a deletion of 2 bp and the secondary mutation is an upstream or downstream deletion of 4 bp, the two mutations result in a net deletion of 6 bp (divisible by three). A larger intragenic deletion that deletes the primary deleterious mutation can also result in restoration of the ORF. Large intragenic deletions that can restore the ORF were visually confirmed using the Integrated Genomics Viewer (28).

NGS of Tumor Specimens

Sequencing of formalin-fixed paraffin-embedded tumor specimens was performed using the Foundation Medicine T5 NGS-based assay (29), which sequences the exons of 287 cancer-related genes, including *BRCA1, BRCA2*, and *TP53*. Of the 112 patients with cfDNA sequenced, 97 had archival tumors and 59 had pretreatment tumor biopsies for NGS.

Classification of deleterious *BRCA* mutations was previously described (21). We defined primary *BRCA* mutation as a deleterious *BRCA* mutation that was detected in the tumor tissue. Due to differences in variant annotation between the different NGS assays, we verified that the cfDNA change of the primary *BRCA* mutations detected in the plasma sample was the same as that of the mutations detected in the tumor tissue.

To identify reversion to the wild-type *BRCA* sequence, DNA was extracted from tumor cells that were laser-captured, microdissected, and sequenced for specific *BRCA* mutations by Sanger sequencing (University of Washington, Seattle, WA) as previously described (13, 30). The BROCA-HR assay was used to sequence DNA extracted from whole blood to identify germline *BRCA* mutations (30).

Statistical Methods

Comparisons of CA-125 levels in patients with and those without *BRCA* or *TP53* mutations detected in pretreatment cfDNA were analyzed using the Mann–Whitney test. Pearson correlation coefficient was calculated for the MAF of primary somatic *BRCA* and *TP53* mutations.

Comparisons of *BRCA* reversion mutation frequencies in different patient subgroups (e.g., platinum status) were analyzed using the Fisher exact test. Assessment of cfDNA as a molecular marker of efficacy was a prospectively planned, exploratory objective of ARIEL2. PFS was analyzed using the Kaplan–Meier method and a Cox proportional hazard model, and the log-rank test was used to compare the PFS of patients with and those without *BRCA* reversion mutations.

Disclosure of Potential Conflicts of Interest

K.K. Lin has ownership interest (including stock, patents, etc.) in Clovis Oncology. A. Oaknin is a consultant/advisory board member for Roche, AstraZeneca, PharmaMar, Clovis Oncology, Tesaro, and Immunogen. A.V. Tinker reports receiving a commercial research grant from AstraZeneca and has received honoraria from the speakers bureau of AstraZeneca. C. Say has ownership interest (including stock, patents, etc.) in Clovis Oncology. L.-T. Vo has ownership interest (including stock, patents, etc.) in Clovis Oncology. J.D. Isaacson is VP, Biostatistics and Data Management at Clovis Oncology and has ownership interest (including stock, patents, etc.) in Clovis Oncology. L. Maloney is Senior Director, Clinical Science, at Clovis Oncology and has ownership interest (including stock, patents, etc.) in Clovis Oncology. D.M. O'Malley reports receiving commercial research grants from Agenus, Ajinomoto Co., Inc., Iovance Biotherapeutics, Inc., Janssen Research and Development, LLC, Ludwig Institute for Cancer Research Ltd., New Mexico Cancer Care Alliance, NRG Oncology, PRA Intl, Regeneron Pharmaceuticals, Inc., Serono Inc., Stemcentrx, Inc., Tesaro, Array BioPharma Inc., TRACON Pharmaceuticals, AstraZeneca Lp, Bristol-Myers Squibb Co., Clovis Oncology, Gynecologic Oncology Group, ImmunoGen, Inc., INC Research, Inc., and inVentiv Health Clinical, and is a consultant/advisory board member for TapImmune, Agenus, AbbVie, Regeneron, Myriad, Novocure, GOG, Roche, AstraZeneca, Immunogen, OncoQuest, Tesaro, Ambry, Clovis, and Janssen. S.K. Chambers is a consultant/advisory board member for Heron. C.L. Scott reports receiving a commercial research grant from Sierra Oncology, reports receiving other commercial research support from AstraZeneca and Clovis Oncology, is a consultant/advisory board member for AstraZeneca, and has given uncompensated expert testimony for AstraZeneca. G.E. Konecny has received honoraria from the speakers bureau of Clovis and is a consultant/advisory board member for Tesaro. R.L. Coleman reports receiving commercial research grants from Clovis, AstraZeneca, Merck, and Roche and is a consultant/advisory board member for Clovis, AstraZeneca, Genmab, Tesaro, Roche, Agenus, and Immunogen. H. Giordano has ownership interest (including stock, patents, etc.) in Clovis Oncology, Inc. T.C. Harding has ownership interest (including stock, patents, etc.) in Clovis Oncology, Inc. I.A. McNeish is a consultant/advisory board member for Clovis Oncology, AstraZeneca, and Tesaro. No potential conflicts of interest were disclosed by the other authors.

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