Routine Plasma-Based Genotyping to Comprehensively Detect Germline, Somatic, and Reversion BRCA Mutations among Patients with Advanced Solid Tumors

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Purpose: PARP inhibitors (PARPi) are efficacious in multiple cancers harboring germline (and possibly somatic) BRCA1/2 mutations. Acquired reversions can restore BRCA1/2 function, causing resistance to PARPi and/or platinum-based chemotherapy. The optimal method of identifying patients with germline, somatic, and/or reversion mutations in BRCA1/2 has not been established. Next-generation sequencing (NGS) of cell-free DNA (cfDNA) provides a platform to identify these three types of BRCA1/2 mutations.

Experimental Design: Patients with advanced breast, ovarian, prostate, or pancreatic cancer were tested using a clinically validated 73-gene cfDNA assay that evaluates single-nucleotide variants and insertion–deletion mutations (indels) in BRCA1/2, and distinguishes somatic/reversion mutations from germline mutations with high accuracy.

Results: Among 828 patients, one or more deleterious BRCA1/2 mutations were detected in 60 (7.2%) patients, including germline (n = 42) and somatic (n = 18) mutations. Common coexisting mutations included TP53 (61.6%), MYC (30%), PIK3CA (26.6%), BRAF (15%), and ESRI (11.5%). Polyclonal reversion mutations (median, 5) were detected in 9 of 42 (21.4%) germline BRCA1/2-mutant patients, the majority (77.7%) of whom had prior PARPi exposure (median duration, 10 months). Serial cfDNA demonstrated emergence of reversion BRCA mutations under therapeutic pressure from initial PARPi exposure, which contributed to subsequent resistance to PARPi and platinum therapy.

Conclusions: cfDNA NGS identified high rates of therapeutically relevant mutations without foreknowledge of germline or tissue-based testing results, including deleterious somatic BRCA1/2 mutations missed by germline testing and reversion mutations that can have important treatment implications. Further research is needed to confirm clinical utility of these findings to guide precision medicine approaches for patients with advanced malignancies.

Introduction

Deleterious BRCA1 or BRCA2 mutations represent the most common mechanism that can lead to homologous recombination deficiency (HRD), a feature that lends certain cancers increased sensitivity to PARP inhibitors (PARPi) and DNA-damaging agents, such as platinum-based chemotherapies (1–14). Germline testing for BRCA1 or BRCA2 is routinely recommended for patients with advanced malignancies based on evolving criteria proposed by various organizations such as the National Comprehensive Cancer Network (NCCN) (15). Currently, four PARPi (olaparib, talazoparib, rucaparib, and niraparib) have been approved as single-agent treatments by the FDA for advanced BRCA-mutated or certain HRD-positive breast and/or ovarian cancers (Table 1).

Data from The Cancer Genome Atlas (TCGA) suggest that 1%–6% of patients with breast, ovarian, prostate, or pancreatic cancer have a deleterious somatic BRCA1/2 mutation that could lead to HRD (16–19). While clinical criteria help identify patients who are more likely to harbor a germline BRCA mutation based on age of diagnosis, tumor histology or stage, and/or family history, there are no clinical criteria that accurately predict patients who have a somatic BRCA mutation. Tumors with somatic BRCA mutations may be responsive to PARPi and platinum chemotherapy (9, 20, 21).

Finally, recent case reports/series have highlighted the emergence of acquired reversion BRCA mutations under therapeutic pressure from PARPi and/or platinum-based therapies in breast, ovarian, pancreatic, and prostate cancers (22–35). BRCA reversions are somatic mutations that restore the open-reading frame of an existing germline and/or somatic loss-of-function BRCA mutation leading to reestablishment of BRCA protein function thus rendering the cell proficient in homologous recombination repair with resultant loss in sensitivity to PARPi and DNA-damaging agents.

Despite the potential clinical importance of identifying and distinguishing among germline, somatic, and reversion BRCA mutations,
Translational Relevance

PARP inhibitors (PARPi) are approved for treatment or maintenance therapy for several malignancies harboring deleterious germline or somatic BRCA1/2 mutations. Acquired reversions in BRCA1/2–positive cancers restore the BRCA reading frame, thereby conferring resistance to PARPi. The identification of patients with germline, somatic, and reversion mutations in BRCA1/2 could have important therapeutic implications, but the optimal method for identification is not yet established. In this study, we evaluated the utility of next-generation sequencing of cell-free DNA (cfDNA) to identify various types of BRCA1/2 mutations in individuals with advanced malignancies. We identified a high rate of BRCA1/2 mutations, including germline, somatic, and reversion mutations. Reversions were identified under therapeutic pressure after initial exposure to PARPi or DNA-damaging chemotherapy, and contributed to subsequent resistance to PARPi and platinum therapy. Plasma-based genotyping may help identify therapeutically relevant BRCA1/2 mutations and consequently influence clinical decision regarding PARPi for patients with advanced malignancies.

Table 1. Status of FDA reviews of PARPi for germline and somatic BRCA-mutated cancersa.

<table>
<thead>
<tr>
<th></th>
<th>Breast</th>
<th>Epithelial ovarianb</th>
<th>Pancreatic</th>
<th>Prostate</th>
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<td>Olaparib</td>
<td>Approved:</td>
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<td>Approved:</td>
<td>g/sBRCA*</td>
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<tr>
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<td>g/sBRCA*</td>
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<td>g/sBRCA*</td>
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<td>Approved:</td>
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<td>Approved:</td>
<td>g/BRCA*</td>
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<td>Talazoparib</td>
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<td>gBRCA*</td>
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</table>

Abbreviations: gBRCA, germline BRCA; sBRCA, somatic BRCA; HRD, homologous recombination deficiency.

*Please refer to FDA for most updated status of PARPi approvals.

†Including primary peritoneal and Fallopian tube cancer.

Materials and Methods

Study population

Eligible patients were identified from the Guardant Health deidentified database (Guardant Health) and included patients undergoing clinical Guardant360 testing between November 2016 and October 2017 with a diagnosis of stage III or IV breast, ovarian, pancreatic, and/or prostate cancer reported on the test requisition form. Only samples with evidence of tumor DNA present (e.g., at least one somatic mutation present) were included to determine prevalence estimates. Detailed clinical history and orthogonal germline testing status were not available for most cases and infeasible to collect due to the retrospective nature of this review of commercial laboratory results; however, all patients with BRCA reversions had their treatment history and germline BRCA status confirmed by the ordering clinician (only one reversion-positive patient did not receive dedicated germline testing to confirm the germline BRCA mutation detected in cfDNA). This research was approved by Quorum Institutional Review Board (IRB) for the generation of deidentified datasets for research purposes and all work was conducted in accordance with the Declaration of Helsinki.

cfDNA assay

Guardant360 is a 73-gene CLIA-certified, College of American Pathologists–accredited, New York State Department of Health–approved clinical cfDNA NGS test with analytic and clinical validation previously reported (43, 44). BRCA1/2 are evaluated for SNVs and as well as small (~50–70 bp) insertions/deletions (indels) using a proprietary bioinformatics pipeline that leverages unique molecular barcodes to reconstruct the original double-stranded cfDNA molecules. The coding regions and intron/exon boundaries of BRCA1/2 are fully sequenced. The reportable range for SNVs and indels is ≥0.04%, ≥0.02%, respectively, with a >99.9999% per-position analytic

BRCA Germline, Somatic, and Reversion Landscape in cfDNA

breast, ovarian, prostate, or pancreatic cancer tested with a CLIA-certified NGS cfDNA assay (Guardant360, Guardant Health). This rapid, minimally invasive assay can differentiate germline from somatic BRCA mutations, distinguish early/truncal from acquired subclonal variants, and identify reversions and coexisting tumor genomic mutations across 71 other cancer-associated genes all in a single blood test (42, 43).
specification (44). Copy-number gain and fusions are evaluated in a subset of genes. Sequencing reads are mapped to the hg19/GRCh37 human reference sequence.

Germline mutations are distinguished from somatic mutations using a proprietary bioinformatic algorithm previously described and validated (42, 45). Typically, only a small fraction of cfDNA is tumor-derived; the majority is germline-derived. The variant allele fraction (VAF) for a given mutation is the total number of cfDNA molecules harboring the mutation divided by the total number of unique cfDNA molecules at that position. Germline mutations are generally found at VAFs of approximately 50%, whereas the median VAF of somatic mutations using this assay is 0.46% (44). However, a gene with allele imbalance due to copy-number variation or loss of heterozygosity may have germline VAFs in both the 10%–30% and 70%–90% ranges. In addition, the VAF of somatic mutations in high shedding tumors can approach those more typical of germline VAFs. The bioinformatics algorithm considers the VAF of the mutation of interest relative to that of other somatic mutations in the sample and nearby germline single-nucleotide polymorphisms (SNP). Germline variants are expected to have VAFs similar to nearby germline SNPs, even with high allelic imbalance, whereas somatic mutations are expected to have different VAFs. This bioinformatics method has been shown to have high positive predictive value in calling the germline status of cfDNA-detected mutations (42).

Although clinical reporting of germline mutations using this assay differed over the timeframe of this study due to evolving technology and clinician preferences, all BRCA+ cases were reevaluated *in silico* using the same validated germline bioinformatics algorithm. Given recent advancements in the understanding of germline BRCA1 and response to PARP therapy, suspected germline alterations are now currently reported with a recommendation for confirmatory testing.

**Biomarker analysis**

Samples containing at least one loss-of-function BRCA1/2 mutation were further evaluated for the presence of additional mutations in the same BRCA gene as the deleterious mutation. Additional mutations were classified as reversions if they restored the open-reading frame of the original deleterious mutation and were confirmed to be in cis (i.e., allelic) with the original deleterious BRCA mutation. The Guardant360 assay utilizes molecular barcoding, which allows for tracking of individual molecules permitting accurate assessment of phase. Additional BRCA somatic mutations that were not reversions were classified as potential second hits if the mutation was predicted to be deleterious. Curation resources include COSMIC, cBioPortal for Cancer Genomics, UniProt, Integrative Genomics Viewer, and literature catalogued by PubMed.

**Statistical analysis**

Patients were considered BRCA positive in this study if at least one sample contained a germline and/or somatic loss-of-function BRCA1 or BRCA2 mutation, which most commonly included out-of-frame indels leading to premature protein truncation. Patients with only synonymous alterations, alterations that are known to have no functional impact (i.e., benign variants), or variants of uncertain significance (VUS) were deemed BRCA negative for the purpose of this study. Comparisons of BRCA mutation prevalence to that in The Cancer Genome Atlas (TCGA) literature were performed using a two-sided Fisher exact test (16–19). Co-occurrence of other mutations was determined on a per patient basis by summarizing all unique non-synonymous mutations present from all samples available for review.

Nonsynonymous benign variants and VUS were excluded from counts to focus on co-occurring mutations most likely to be contributing to cancer progression.

**Results**

**Prevalence of functionally relevant BRCA1/2 mutations in cfDNA of patients with advanced cancer**

From a total of 828 patients with advanced breast, ovarian, pancreatic, or prostate cancer, 60 (7.2%) patients had at least one BRCA1/2 loss-of-function mutation, either in germline (*n* = 42 patients) or somatic only (*n* = 18 patients; Fig. 1). The prevalence of germline and somatic BRCA mutations by cancer type was similar to that reported by TCGA (16–19), but 2.5× higher among men with prostate cancer (8.1% vs. 3.3%; *P* = 0.0184; Fig. 2). This difference was largely explained by the number of cfDNA-identified germline BRCA2 mutations and is an expected finding because the cfDNA-tested population was comprised of only patients with advanced cancers where they are known to be more prevalent compared with TCGA which includes patients with early-stage cancers. Our estimates are in line with what has been reported in genomic profiling studies in metastatic prostate cancer (46–48).

**Germline BRCA1/2 mutations**

The 42 patients with a cfDNA-identified deleterious germline mutation had mutations in BRCA1 (*n* = 9 patients) or BRCA2 (*n* = 33 patients) and had breast (*n* = 17), ovarian (*n* = 5), pancreatic (*n* = 4), or prostate (*n* = 16) cancer. Five of these patients had at least one sample positive for an additional loss-of-function somatic mutation in the same gene as the germline mutation, which may represent the second hit in the form of a mutational event.

**Somatic BRCA1/2 mutations**

Eighteen patients without evidence of a germline mutation had a deleterious somatic mutation in BRCA1 or BRCA2 (*n* = 9 patients each) and had breast (*n* = 14), ovarian (*n* = 2), or prostate (*n* = 2) cancer. One patient had two deleterious somatic BRCA1 mutations.

**Reversion BRCA1/2 mutations**

In 9 of the germline BRCA+ patients, at least one plasma sample contained additional somatic mutations consistent with a BRCA reversion. A total of 72 unique reversions were identified in 13 samples from the 9 patients (Table 3; Supplementary Table S1). In 10 of 13 (76.9%) samples containing a reversion, there were multiple reversions detected indicative of polyclonal origin. The median number of unique reversions per sample was 5 (range 1–16) and per patient cumulatively across all samples was 6 (range 1–24). In one patient, the reversion impacted both a germline BRCA2 mutation as well as a somatic deleterious BRCA2 mutation (the “second hit”), as has been previously described previously (26). While reversions were not found in patients with ovarian or pancreatic cancer in this cohort, it is likely due to the small number of patients assessed (7 BRCA-positive ovarian cancers and 4 BRCA-positive pancreatic cancers).

**Oncogenic mutations coexisting in BRCA+ cancers**

Additional somatic mutations commonly co-occurred in BRCA+ cancers, notably PIK3CA activating mutations (11 of 31 breast cancers; 35.5%), ESRI mutations (7 of 20 BRCA2+ breast cancers; 35%), and AR ligand–binding domain mutations and/or copy number gain (4 of 18 prostate cancers; 22.2%). Copy number gain across multiple genes was...
common in BRCA+ breast and prostate cancers, which may be reflective of patterns of genome instability resulting from defective DNA repair systems as is the hallmark of BRCA-deficient tumors (Fig. 3; refs. 49, 50).

**BRCA1/2 reversions and association with therapy resistance**

Among the 9 patients with at least one plasma sample positive for a reversion, all had prior exposure to therapies known or reported to lead to BRCA reversions (Table 2). Seven of the 9 (77.8%) had received a
PARPi (median duration: 10 months) prior to the time the reversion(s) were first detected. Six patients had initially responded to the PARPi, but had progressed at the time of sample collection while the remaining patient (patient F) did not have a clinical response. In addition to a PARPi, 6 patients also had prior exposure to a platinum-based chemotherapy ($n=5$) or investigational DNA-damaging agents ($n=1$).

The remaining two of the nine reversion–positive patients (patients A and B), did not have prior treatment with a PARPi or platinum agent, but both had received mitoxantrone, a DNA cross-linking agent, which has been reported to lead to development of BRCA reversions (51). Each had only one reversion detected, whereas all of the patients who had prior PARPi exposure had multiple reversions detected.

Two patients with a reversion had samples collected from multiple timepoints enabling study of the temporal evolution of the BRCA reversion. The first plasma sample for patient F was drawn following exposure to cisplatin, etoposide, paclitaxel, carboplatin, and capecitabine and identified a somatic PIK3CA-activating mutation at 3.8% VAF, but no somatic BRCA mutations were detected (Fig. 4A). Following PARPi treatment for 3 months without clinical benefit a second cfDNA sample revealed a rise in the VAF of the PIK3CA mutation as well as a new BRCA2 reversion. The rapid appearance of the reversion suggests it may have been present just below the limit of detection of the assay at baseline prior to the PARPi, and possibly contributed to her short time to progression on the PARPi. Patient H had plasma samples collected before, during and after progressing on a PARPi. At the time of progression, cfDNA testing revealed eight reversions in BRCA2 that were not present in the original tissue from the liver metastasis collected prior to PARPi therapy nor in three serial cfDNA analyses prior to PARPi therapy (Fig. 4B). Following the PARPi, the patient was then treated with a combination of carboplatin and docetaxel, but experienced rapid disease progression. Another cfDNA test interestingly revealed emergence of 13 new BRCA2 reversions, possibly explaining the intrinsic resistance to the platinum combination. While it is possible that taxotere may have been effective against the initial reversion clones, carboplatin may have resulted in the selection of other clones proficient in DNA repair (i.e., those with an acquired reversion) resulting in disease progression.

**Discussion**

This is the largest series to date evaluating three clinically relevant types of BRCA1/2 mutations among patients with advanced breast, ovarian, pancreatic, and prostate cancer using a plasma-based NGS assay, identifying a 5.1% and 2.3% prevalence of germline and somatic BRCA loss-of-function mutations, respectively. Restricting analysis to

| Table 2. Treatments received prior to first detection of a BRCA reversion mutation. |

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cancer type</th>
<th>Origin of deleterious BRCA mutation(s)</th>
<th>Duration of PARPi (mo)</th>
<th>Other systemic cancer therapies</th>
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<tr>
<td>A</td>
<td>Breast</td>
<td>gBRCA2</td>
<td>n/a</td>
<td>ET, capecitabine, everolimus, paclitaxel, erubulin, gemcitabine, mitoxantrone, vinorelbine</td>
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<td>B</td>
<td>Prostate</td>
<td>gBRCA2</td>
<td>n/a</td>
<td>ADT, taxotere, enzalutamide, abiraterone acetate, cabazitaxel, mitoxantrone</td>
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<td>C</td>
<td>Breast</td>
<td>gBRCA2</td>
<td>11</td>
<td>ADT, docetaxel, cabazitaxel,</td>
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<tr>
<td>D</td>
<td>Prostate</td>
<td>gBRCA2 + sBRCA2</td>
<td>12</td>
<td>ADT, docetaxel, cabazitaxel,</td>
</tr>
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<td>E</td>
<td>Breast</td>
<td>gBRCA2</td>
<td>8</td>
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<td>F</td>
<td>Breast</td>
<td>gBRCA2</td>
<td>3</td>
<td>Carboplatin, etoposide, paclitaxel, carboplatin, capetcitabine</td>
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<tr>
<td>G</td>
<td>Breast</td>
<td>gBRCA1</td>
<td>12</td>
<td>Gencitabine, carboplatin, PD-1 inhibitor</td>
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<td>H</td>
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<td>gBRCA2</td>
<td>10</td>
<td>ADT, radium-223, abiraterone acetate, PD-1 inhibitor</td>
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<td>I</td>
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<td>Carboplatin</td>
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Abbreviations: ADT, androgen deprivation therapy; ET, endocrine therapy; gBRCA, germline BRCA; sBRCA, somatic BRCA.

**Figure 3.**

Co-occurring mutations among germline or somatic BRCA-positive patients. Only genes with a functionally significant mutation found in at least 10% of patients within a given cancer type are shown.
# BRCA Germline, Somatic, and Reversion Landscape in cfDNA

Table 3. Reversions detected in the cfDNA of 9 germline BRCA1/2-positive patients.

<table>
<thead>
<tr>
<th>Pt</th>
<th>Sample</th>
<th>Gene</th>
<th>Mutation</th>
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<th>Exon</th>
<th>Reversions, n</th>
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<td>A</td>
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<td>BRCA2</td>
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<td>Germline</td>
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<td>c.5550_5577del28insC</td>
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<td>B</td>
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<td>BRCA2</td>
<td>c.4876_4877delAA</td>
<td>Germline</td>
<td>11</td>
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<td>C</td>
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<td>c.2385_2386insAA</td>
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<td>16</td>
<td>c.2401_2404del20, c.2404_2429del12, c.2415_2455del41, c.2570_2387del18, c.2362_2396del18, c.2397dupA, c.2354_2364del11, c.2392dupC, c.2366_2373del8, c.2395_2414del20, c.2352_2374del23, c.2379_2390del12, c.2380_2397del18, c.2385_2386insAAA, c.2414_2436del23, c.2401_2419dup19</td>
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<td>D</td>
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<td>BRCA2</td>
<td>c.5946delT</td>
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<td>BRCA2</td>
<td>c.3689delC</td>
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<td>7</td>
<td>c.3593dupA, c.3668_5700del33, c.3680_3681delTG, c.3699_3705del5, c.3676_3693del18, c.3656_3666del11, c.3687_3712del27</td>
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<td>F</td>
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(Continued on the following page)
only germline mutations would have missed 30% of potentially clinically targetable loss-of-function BRCA mutations, as these were detected in tumor only. Rucaparib is currently FDA-approved specifically for treating relapsed ovarian cancer with a somatic BRCA1/2 mutation and there are concerted efforts to study PARPi in tumors containing somatic BRCA mutations (20). Several PARPi have received FDA breakthrough therapy designation for somatic BRCA-mutated cancers and numerous PARPi clinical trials are ongoing with promising early results reported (9, 21). Thus, testing for somatic BRCA mutations increases the ability to identify potential therapeutic

<table>
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<th>Gene</th>
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*Reported previously in Carneiro and colleagues (26).

Table 3. Reversions detected in the cfDNA of 9 germline BRCA1/2-positive patients. (Cont’d)

Figure 4.
Evolution of BRCA reversions and other somatic mutations detectable by cfDNA NGS following various systemic therapies. Systemic therapies the patient received prior to each sample are listed. A, Patient F – a woman with germline BRCA2+ metastatic triple-negative breast cancer initially diagnosed at age 38. B, Patient H – a man with germline BRCA2+. Gleason score 9 (4+5) metastatic prostate adenocarcinoma initially diagnosed at age 46.
negative breast cancer. PI3K inhibitors are also being investigated in and tumor somatic second hit would restore (11.9%) had additional somatic loss-of-function mutations. Among germline-positive cases, we found 5 of 42 (11.9%) had additional somatic loss-of-function events, which cfDNA testing is well-suited to evaluate using a single blood draw. Importantly, individuals with BRCA reversion mutations are likely to have a poor response to PARPi and likely to DNA-damaging agents, such as platinum and anthracyclines, given the similar mechanisms of action. A post hoc analysis of data from the ARIEL2 trial showed a significantly shortened median progression-free survival with rucaparib in patients with BRCA-mutated recurrent ovarian cancer that had a reversion mutation detected in cfDNA prior to PARPi treatment compared with those that did not (1.8 vs. 9.0 months; ref. 33). Similar findings were observed in the subset of platinum resistant/refractory ovarian cancers suggesting that the presence of a reversion mutation may be a better predictor of PARPi nonresponse than platinum sensitivity status. Detection of reversion mutations may therefore be useful in informing strategies involving sequential use of PARPi with chemotheraphy or a different PARPi, combination therapy after single-agent PARPi failure, and PARPi rechallenge after a period of another therapy.

Our data also highlight the utility of cfDNA testing in identifying polyclonal resistance mechanisms as a median of six distinct reversions per patient was detected in this series. Tumor biopsy of a single lesion may not inform the full spectrum of reversion mutations. For example, among 48 patients with matched pre-rucaparib cfDNA samples and NGS-tested tissue biopsies collected in the ARIEL2 trial, 4 patients had reversions detected in both cfDNA and tissue (33). Among these four patients, cfDNA identified 15 distinct reversions, whereas tissue identified four distinct reversion mutations (i.e., one in each patient). However, a fifth patient had a large (366 base pair) deletion reversion mutation that was detected in tissue only (though in a post hoc analysis, evidence of the deletion was observed in cfDNA). Reversion mutations may go undetected in cfDNA in low shedding tumors or in cases of large deletion reversion events, which are limitations of cfDNA testing. As such, tissue biopsy of progressing lesions may be considered in patients with a suspected reversion and/or biopsying the growing tumor at the time of a mixed response. Future studies are needed to evaluate how to best stratify the use of cfDNA results and tissue biopsies for optimal management.

In addition to BRCA reversion mutations, comprehensive cfDNA profiling in patients experiencing disease progression may inform customized therapeutic approaches, including combination therapies, by identifying other established and emerging genomic targets as well as resistance mutations that may be driving tumor progression. We observed a high frequency of classic acquired somatic resistance mutations such as in ESR1 or AR in this series, which indicate lack of efficacy of estrogen- or androgen receptor-directed therapies, respectively (52, 53). We also frequently observed BRCA mutations co-occurring with PIK3CA mutations in patients with breast cancer. The FDA recently approved the PI3-kinase (PIK) inhibitor, alpelisib, for PIK3CA-mutant metastatic hormone receptor–positive, HER2-negative breast cancer. PI3K inhibitors are also being investigated in combination with PARPi in BRCA-proficient cancers, including those that have become BRCA proficient due to reversion events (54–57).

The study has several limitations. Because this is a retrospective review of clinically ordered cfDNA test results, the cohort may be biased toward patients more likely to have aggressive disease and a poor prognosis, including those harboring BRCA and other resistance mutations. Detailed treatment and germline testing history were not available for most of the cohort. Consequently, we are unable to estimate the prevalence of reversion mutations according to type and duration of prior treatments, the overall sensitivity of plasma detection of BRCA mutations compared with standard validated germline or tissue assays, or confirm the germline status assigned by the bioinformatics algorithm for all cases. Our study may have underestimated the prevalence of BRCA mutations as detection of indels larger than approximately 50–70 bp is challenging in plasma and the presence of low shedding tumors (either due to biological factors or the timing of blood collection following various therapeutic interventions) impacts sensitivity. However, other recent studies have evaluated the performance of the Guardant360 assay in these regards. BRCA reversion mutations were found in the cfDNA of 18% of platinum-refractory, 13% of platinum-resistant, and 2% of platinum-sensitive recurrent BRCA+ ovarian cancer samples with cfDNA detected and in 20% of patients progressing on a PARPi (33). Furthermore, in patients experiencing disease progression the assay detected 67 of 71 (94.4%) of BRCA mutations that were identified using a validated germline assay and 30 of 41 (73.2%) somatic loss-of-function mutations detected using a validated tissue NGS assay. Similarly, while the only clinical information from test request forms that was used and reported in this study is the cancer type, which we believe is likely to be accurate, we cannot rule out the possibility of some misclassification. Finally, this study focused only on BRCA1/2. Other HR pathway genes may also prove to be useful in predicting response to PARPi and were not evaluated in this study, but could be considered in a future analysis of cfDNA data.

In summary, we demonstrate that plasma-based cfDNA NGS testing can identify three clinically relevant classes of BRCA1/2 mutations, germline, somatic loss-of-function, and reversions, in a significant proportion of patients with advanced-stage breast, ovarian, pancreatic, and prostate cancer. Using a single blood test, cfDNA NGS may identify patients with targetable loss-of-function BRCA mutations while simultaneously evaluating for the presence of reversions indicating those likely to be resistant to PARPi or platinum-based therapies. Routine cfDNA testing in patients with advanced cancer may help identify potential candidates for appropriate genotype-directed therapy.

Disclosure of Potential Conflicts of Interest
T.A. Rich, J. Yen, and T. Nance are employees/paid consultants for Guardian Health. O. Sartor is an employee/paid consultant for Advanced Accelerator Applications, Astellas, AstraZeneca, Bayer, Blue Earth Diagnostics, Bavarian Nordic, Bristol-Myers Squibb, Clovis, Constellation, Dendreon, EMD Serono, Myriad, Nexcelpharm, Novartis, Progenics, Janssen, Merck, Pfizer, and Sanofi; reports receiving commercial research grants from AstraZeneca, Bayer, Dendreon, Endocyte, Innocin, Invitae, Janssen, Merck, Sanofi, and SOTIO; holds ownership interest (including patents) in PSMA Therapeutics; and has received other remuneration from Sanofi. A. Hardin, V.M. Raymond, and S.R. Fairclough are employees/paid consultants for and hold ownership interest (including patents) in Guardant Health. M.B. Lilly reports receiving speakers bureau honoraria from Guardant Health. S.P. Patel is an employee/paid consultant for Guardant, Illumina, AstraZeneca, Lilly, Nektar, Bristol-Myers Squibb, and Novartis. A.M. Brutkay is an employee/paid consultant for Myriad and Guardant. B.A. Parker is an employee/paid consultant for Bia LAST Inc. and EMD Serona; reports receiving commercial research grants from Oncernal Inc., Novartis, GlaxoSmithKline, and Pfizer; holds ownership interest (including patents) in Merck; and reports receiving other remuneration from NCCN and SD Komen Board. N.
Agarwal is an employee/paid consultant for Pfizer, Novartis, Merck, Genentech, Eisai, Exelixis, Clovis, EMD Serono, Bristol-Myers Squibb, Astra Zeneca, Astellas, Lilly Lilly, Bayer, and Pharmaceuticalcs. B. L. Maughan is an employee/paid consultant for Exelixis, Astellas, Bayer Oncology, Bristol-Myers Squibb, Janssen Oncology, Tempus, and Peloton Therapeutics, and reports receiving commercial research grants from Bavar- ian Nordic, Bristol-Myers Squibb, Exelixis, and Clovis. B. R. Lanman is an employee/ paid consultant for Guardant Health, Inc., and holds ownership interest (including patents) in Guardant Health, Inc., Forward Medical, Inc., and Biosoil. A. Bardia is an employee/paid consultant for Immunomedics, Novartis, Radius Health, Pfizer, Genentech, Sanofi, Merck, Duichi/Astra Zeneca, and Phillips. M. Cristofanilli is an employee/paid consultant for CytoDyn, Pfizer, Foundation Medicine, Lilly, Genen- tech, Novartis, Sermonix, and GI Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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Routine Plasma-Based Genotyping to Comprehensively Detect Germline, Somatic, and Reversion BRCA Mutations among Patients with Advanced Solid Tumors

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