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Comparative Clinical Utility of Tumor Genomic Testing and Cell-Free DNA in Metastatic Breast Cancer

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Abstract

Purpose—Breast cancer metastases differ biologically from primary disease; therefore, metastatic biopsies may assist in treatment decision-making. Commercial genomic testing of both tumor and circulating tumor DNA have become available clinically, but utility of these tests in breast cancer management remains unclear.

Methods—Patients undergoing a clinically indicated metastatic tumor biopsy were consented to the ongoing METAMORPH registry. Tumor and blood were collected at time of disease progression before subsequent therapy, and patients were followed for response on subsequent treatment. Tumor testing (n=53) and concurrent cell-free DNA (n=32) in a subset of patients was performed using CLIA-approved assays.

Results—The proportion of patients with a genomic alteration was lower in tumor than in blood (69% vs 91%; p=0.06). After restricting analysis to alterations covered on both platforms, 83% of tumor alterations were detected in blood, while 90% of blood alterations were detected in tumor. Mutational load specific for the panel genes was calculated for both tumor and blood. Time to progression on subsequent treatment was significantly shorter for patients whose tumors had high

panel-specific mutational load (HR 0.31, 95%CI 0.12–0.78) or a *TP53* mutation (HR=0.35, 95%CI 0.20–0.79), after adjusting for stage at presentation, hormone receptor status, prior treatment type and number of lines of metastatic treatment.

Conclusions—Treating oncologists must distinguish platform differences from true biological heterogeneity when comparing tumor and cfDNA genomic testing results. Tumor and concurrent cfDNA contribute unique genomic information in metastatic breast cancer patients, providing potentially useful biomarkers for aggressive metastatic disease.

Keywords

metastatic breast cancer; liquid biopsy; cell-free DNA; genomic testing; massively parallel sequencing

INTRODUCTION

Breast cancer is the most common malignancy and the second leading cause of cancer death in women[1]. Approximately 20% of women diagnosed with the disease ultimately recur and over 40,000 women die annually of metastatic breast cancer (MBC)[1]. Standard treatment is guided by expression of hormone receptors (HR) or the epidermal growth factor receptor-2 (Her2), with sequential endocrine therapies initially in most HR+ disease, chronic anti-Her2 therapy (with or without chemotherapy) in Her2+ disease, and sequential chemotherapy in triple negative and endocrine-resistant disease[2].

Receptor expression and genetic changes can differ between the primary and metastases[3], as tumors continue to evolve both stochastically and in response to treatment; therefore, analyzing biomarkers of metastatic tumors has potential clinical utility. Currently, there are numerous commercial assays that can detect genomic alterations in tumors and shed tumor DNA in the circulation (cfDNA). A number of studies have reported on the spectrum of mutations identified in primary and metastatic breast cancer[4–7], and similar recurrent genomic alterations have been identified both in tumor and in blood[8–13]. However, the clinical utility of this information in treatment decision-making has not been established, despite the theoretical potential to improve prognostication, expand therapeutic targets[14–16], or enable tracking of therapeutic response[17].

In order to gain insight into the potential clinical utility of commercial CLIA-approved tumor and cfDNA genomic assays in the management of metastatic breast cancer in the clinic, we sought to evaluate the hypotheses that (1) cfDNA could be used in place of tumor biopsy to identify therapeutic and response biomarkers and (2) specific mutations or panel-specific measures of mutational burden or heterogeneity would be associated with response to subsequent therapy. We analyzed data from patients undergoing concurrent assays at the time of clinical confirmatory biopsy. The aims of the current analysis were to (1) determine concordance of genomic alterations in shared genes on different clinical tumor and cfDNA panels, (2) identify reasons for discordance that impact clinical interpretation by treating oncologists, and (3) identify potential genomic alterations that could be useful in predicting treatment response.

METHODS

Patients, Informed Consent, and Study Design

Patients undergoing metastatic tumor biopsy for clinical reasons (either to confirm histology or receptor status) were prospectively identified as per inclusion criteria (Supplementary Methods) for the METAMORPH (“Metastatic Markers of Recurrent Tumor Phenotype”) Study, which is a registry for commercial testing and additional collection of research tumor and blood samples. The protocol was approved by the Institutional Review Board (IRB) at the University of Pennsylvania. Tumor and blood samples were collected from identified patients within one month of each other and prior to the start of the patient’s subsequent treatment (Supplementary Figure 1). Patients subsequently underwent standard-of-care treatment determined by their treating physician. Physicians and patients received results of the genomic assays approximately 2 – 3 weeks after biopsy and collection, and were not mandated to use this information for treatment selection. Patients were tracked prospectively with data collected regarding date of last treatment, reason for discontinuing treatment, and best response (based upon clinical and radiographic clinical determinations).

Tumor Genomic Testing

Genomic tumor testing was performed at the Center for Personalized Diagnostics (CPD) at the University of Pennsylvania Department of Pathology using a CLIA-approved targeted gene panel. Formalin-fixed paraffin embedded (FFPE) tumor blocks were obtained. A Hematoxylin and Eosin stained slide was used to select areas containing at minimum 10% tumor for macrodissection and isolation of DNA. Tumor DNA was analyzed on one of two clinically available Illumina amplicon based massively parallel sequencing panels (San Diego, CA). When possible, the “CPD_Full” panel was performed, which contained 203 amplicons covering mutational hotspots of 47 cancer related genes (Illumina TruSeq Cancer Panel). If insufficient DNA was obtained for the full panel, the “CPD_PPP” was performed, which contains a subset of 77 amplicons covering portions of 20 cancer related genes (Supplementary Table 1–2). Samples were sequenced on an Illumina miSeq to a minimum mean coverage of 1000X[18]. Data were analyzed through an in-house bioinformatics pipeline to identify single nucleotide variants (SNVs), insertion/deletion (indels) and amplifications[18]. The minimum allele frequency (AF) detectable by the tumor assay was 4%. Forty-four patients had CPD_Full and nine patients had CPD_PPP tumor testing.

cfDNA Genomic Testing

Whole blood was sent to GuardantHealth,, a CLIA-certified, College of American Pathologists-accredited laboratory (Redwood City, CA) for the Guardant360 cfDNA test in a subset of thirty-five patients who were included in a 10-patient pilot study (funded by Guardant Health) or enrolled on the Guardant Health registration study. When the Guardant study ended, samples were no longer sent for evaluation to avoid having patients incur clinical cost. cfDNA was isolated for digital sequencing as previously described[19]. Hotspots for targeted genes were sequenced. Over the course of the study, the panel underwent changes; “GH_v1” contained genomic regions of 54 cancer related genes and “GH_v2” contained the genomic regions of 68 cancer genes (Supplementary Tables 1–2). Samples were sequenced using a proprietary ultra-deep sequencing assay to a mean

coverage of 15000X[19]. Data were analyzed through a custom bioinformatics pipeline to identify SNVs and, in selected genes, indels, amplifications and fusions[19]. The minimum AF detectable by the cfDNA was 0.1%. Twenty-eight patients had GH_v1 testing and seven had GH_v2 testing.

Analysis of sequencing data

The tumor genomic assay reported SNVs, indels, and amplifications classified as variants of uncertain significance (VUS) or pathogenic in all covered genes. The cfDNA assay reported all identified SNVs and amplifications in *EGFR*, *ERBB2*, and *MET*. All variants were classified according to American College of Medical Genetics and Genomic guidelines[20,21]. cfDNA variants not reported in the tumor were examined in the raw sequencing data by the study team. Tumor variants not reported in cfDNA assay were examined by the Guardant Health team. Only the variants in 18 shared genes on both CPD assays for patients with tumor results and the 46 shared genes on both cfDNA assays for patients with cfDNA results were analyzed.

As small hotspot panels are unlikely to accurately determine overall mutational burden as measured by whole genome or whole exome sequencing, “panel-specific mutational loads” were calculated for both tumor and cfDNA data. Tumor panel-specific mutational load was calculated by summing the total number of variants in the 18 shared genes found on both the CPD_PPP and CPD_Full panels for each sample. cfDNA panel-specific mutational load was calculated by summing the total number of variants in the 46 shared genes found on both the GH_v1 and GH_v2 panels for each sample. We determined the average number of variants in these genes per subject in The Cancer Genome Atlas (TCGA) primary breast tumors (n=963). On average, TCGA subjects had 1 ± 1 variants in the shared genes on the tumor assay and 2 ± 1 variants in the shared genes on the cfDNA assay (Supplementary Table 3). We then classified patients in this study as having a high panel-specific mutational load if they had mutational loads in shared genes two standard deviations above the mean on the particular assay, i.e., three or more tumor variants and/or four or more cfDNA variants. Mutational heterogeneity was defined as the presence of a mutation that would be covered and reported by both assays but only found by one assay in a patient.

Statistical analyses

Means of continuous variables were compared between analysis groups using a two-tailed Student's t-test. Outliers were excluded based on Grubb's test (extreme studentized deviate test). Comparisons of rates in different groups were conducted using a Fisher's exact test of significance. Time to progression (TTP) was defined as the number of days from the start of treatment to the first indication of progressive disease, by either scan or clinical assessment. Kaplan Meier curves were calculated to estimate TTP; comparison of TTP between analysis groups used the logrank test. Cox proportional hazards models were constructed to assess the relationship between TTP and genomic variables of interest, adjusting for other determinants of clinical outcome, including receptor status, type of treatment and numbers of lines of therapy. All tests were two-sided and conducted in Stata (version 11) and R (version 3.3.1) at a significance level of 0.05. Results are reported in accordance with REMARK guidelines.

RESULTS

Study population

Seventy patients were enrolled and tumor biopsies were obtainable in 66 (94%) (Supplementary Figure 2, Table 1). Receptors were obtained for 62 patients. Eight of the patients (20%) had discordant receptor status between the primary and metastatic tumor (Supplementary Table 4). Five patients lost HR expression, two gained HR expression, and one lost Her2 expression. The average time between primary and metastatic diagnosis was significantly longer in patients with discordant receptors compared to those with concordant receptors (128 versus 73 months, $p=0.002$). Fifty-three (80%) had successful tumor genomic testing, and 35 had successful cfDNA testing. The characteristics of those with cfDNA were similar to those in the overall cohort (Table 1).

Mutation and variant spectra of tumor biopsies and cfDNA

Within the 53 patients in the tumor cohort, 65 alterations in 14 genes were found on the tumor genomic report (Table 2, Figure 1). Among the 32 patients with concurrently measured tumor and cfDNA (tumor/cfDNA cohort), 39 alterations in 6 genes were reported from the tumor assay; 93 alterations were reported in 27 genes from the cfDNA assay (Table 2, Supplementary Figure 3). *TP53* and *PIK3CA* were the most commonly mutated genes identified in each assay. Addition of the cfDNA assay did not increase the number of patients identified to have a *PIK3CA* mutation or *ERBB2* (HER2) amplification. However, it did identify two additional patients with *TP53* mutations, one additional patient with an activating *ERBB2* mutation, three patients with *JAK2* mutations and one patient with *EGFR* and *MET* co-amplification. The cfDNA did not identify six variants found in tumor only – two *TP53* mutations, two *PIK3CA* mutations, an *ERBB2* amplification and a *SMAD4* VUS. At least one pathogenic mutation was identified in 69% and 72% of patients on the tumor and cfDNA assays, respectively. Thus, the addition of the cfDNA assay to tumor genomic testing resulted in a negligible increase in the number of patients with a pathogenic mutation. When all variants were considered, 69% of patients had at least one variant identified in tumor and this increased to 91% with the addition of the cfDNA assay. As small genomic panels likely do not accurately identify whole genome reported mutational burden(ref), panel-specific mutational load was determined and high versus low cutoffs determined by analysis of TCGA data for the panel-specific genes. Using this measure, 9% of the tumor cohort and 21% of the tumor/cfDNA cohort were classified as having a high panel-specific mutational load on one of their assays. Only two patients in the tumor/cfDNA cohort had a high panel-specific mutational load in the tumor alone, and the cfDNA assay identified six additional patients with a high panel-specific mutational load.

Concordance between tumor and cfDNA assays

Among the tumor/cfDNA cohort, 100 variants in total were found. Using data available on clinical reports, only 29 of 100 variants (29%) were concordantly identified by both assays: four amplifications in *ERBB2* and *EGFR* and 25 missense mutations in *PIK3CA*, *TP53*, and *ERBB2* (Figure 2a). A substantial fraction (53 of 71, 75%) of discordant results occurred due to differences in test coverage or variant reporting practices of the clinical laboratory. Forty-five variants identified in cfDNA were in genomic regions not covered by the tumor

assay (45% of identified variants) (Figure 2a). It is important to note that some variants were in genes reported on the coverage list of each assay but in exons or introns that were not covered for that gene via discussion with the clinical laboratory. Eight variants were covered and identified by both assays but of a variant class not clinically reported by the clinical laboratory's reporting practices (Figure 2a).

Ultimately, 47 variants were covered and reportable by both assays performed in a given patient and 18 variants were only detected by one assay, giving a nontechnical discordance rate of 38% (18 of 47 variants). Six variants were identified only in tumor, including one *ERBB2* amplification and pathogenic mutations or VUSs in *PIK3CA* (2), *TP53* (2), and *SMAD4* (1). Twelve variants were only identified in cfDNA: *EGFR* and *MET* amplifications, three pathogenic mutations in *TP53*, three *JAK2* p.V617F mutations, and one VUS each in *EGFR*, *JAK3*, *NPM1*, and *RET*. Fifteen of 32 patients had mutational heterogeneity between assays. Four patients with mutational heterogeneity had excess mutations in tumor, and twelve patients with mutational heterogeneity had excess mutations in cfDNA.

Allele frequency differences between concordant and discordant variants

Allele frequencies (AF) in tumor-only genomic testing are affected by stromal and inflammatory cell DNA and in cfDNA testing by normal leukocyte DNA; therefore, we would not expect similar AF in tumor and cfDNA. However, we evaluated the relative AF to identify potential factors that may contribute to discordance in identification of alterations. For concordant variants, 89% were identified at a higher AF in tumor versus cfDNA (Figure 2b). Four variants were found at similar AF: a *SMAD4* VUS at approximately 50% AF in both assays, and one *TP53* and two *PIK3CA* hotspot mutations found at 20–30% AF in both tumor and cfDNA. Considering all variants covered by both assays, the AF of concordant and discordant tumor variants ranged from 8.0–87.7% and 32.5–49.5%, respectively. In comparison, the AF of concordant and discordant cfDNA variants ranged from 0.4–50.4% and 0.2–0.8%, respectively (Figure 2b). The AF were not significantly different between concordant and discordant tumor variants; however, the AF of discordant cfDNA variants was significantly lower than concordant variants (Figure 2c). Given this observation, the raw tumor sequencing data were reanalyzed, and evidence of the cfDNA variant was found in at least five reads for eight of the ten discordant SNVs with AF ranging from 0.03–0.45% in the tumor (Supplementary Table 5). For amplifications, all four concordant amplifications were found at similar levels in both tumor and cfDNA (Figure 2b). The two discordant cfDNA amplifications were very low level, below 3-fold, whereas the discordant tumor amplification was a high-level amplification (Figure 2c). There was no evidence of the two amplifications in the tumor testing. Overall, therefore, incorporating reanalysis of raw sequencing data, the nontechnical discordance rate between tumor and cfDNA was 21%.

Effects of genomic profiles on response to subsequent therapy

We examined whether genomic alterations were associated with outcomes from conventional treatment of MBC patients by determining time to progression (TTP) for the therapy immediately following genomic assessment. Treatment decisions were not based upon the results of these assays. Adjusted for stage, hormone receptor status, and prior

treatment type and number of lines, patients with high panel-specific mutational load had a significantly shorter TTP than those with a low mutational load (HR=0.31 95%CI 0.12–0.78, $p=0.0112$) (Figure 3a, Supplementary Table 6). For tumor alterations, the low versus high panel-specific mutational load groups had 0.9 (± 0.8) versus 3.3 (± 0.6) variants ($p<10^{-6}$). For cfDNA alterations, the low versus high panel-specific mutational load groups had 1.3 (± 0.9) variants versus 6.1 (± 3.2) variants ($p<10^{-11}$). In addition, the presence of a *TP53* mutation in either the tumor or cfDNA was associated with a significantly shorter TTP (HR=0.35, 95%CI 0.20–0.79, $p=0.00374$) (Figure 3b, Supplementary Table 6). No association was seen with *PIK3CA* mutation status (Supplementary Figure 4a). Finally, for patients in the tumor/cfDNA cohort, there was no association between mutational heterogeneity and TTP (Supplementary Figure 4b).

DISCUSSION

Commercial CLIA-approved assays for genomic testing of tumors and cfDNA are clinically available for patients with breast cancer, though there is little data evaluating the interpretation of different genomic assays performed in a clinical oncology setting. In the METAMORPH study, we systematically evaluated results of two different commercially available clinical testing panels from tumors compared to cfDNA obtained concurrently in a subset of MBC patients. We delineate a number of purely technical aspects of genomic profiling that resulted in a high rate of discordant results. True (nontechnical) discordance was shown for 21% of variants detected, and in both directions, with 10% of tumor variants not detected in cfDNA and 17% of cfDNA variants not detected in tumor. These results suggest that neither assay captures the full range of potentially actionable mutations for an individual patient. Finally, for conventional, non-targeted therapy, a high panel-specific mutational load measured by a small targeted panel or a *TP53* mutation were associated with a shorter TTP of therapy in MBC patients, suggesting that such patients will ultimately exhaust therapeutic options sooner for this incurable condition.

Prior studies of concordance between tumor and cfDNA in metastatic cancer patients have been mainly restricted to analysis of pathogenic mutations and/or evaluation of identical genomic assays in both tissue sources. Studies evaluating pathogenic mutations identified by the same panel in metastatic breast cancer patients have found concordance rates above 70% [22–24], and a recent prospective study using different clinically available panels confirmed these findings[9]. Studies in mixed tumor types have shown both lower[25,26] and higher[27,28] rates of concordance. For example, in the prospective MOSCATO trial of 283 patients, only 50% of variants identified by the same panel were concordant between tumor and cfDNA. In this study we wished to evaluate non-identical assays that are clinically available, and we initially show only a 29% concordance rate between tumor and cfDNA. A large proportion of the discordance was not biological and due to technical reasons such as differences in test coverage of genes, laboratory variant reporting practices, variant classification, and allele frequency thresholds for detection based on total sequencing depth. These factors are extremely important for practicing oncologists to understand when ordering and interpreting the results of such tests in order to avoid erroneous conclusions about potential therapeutic targets, or the gain or loss of specific mutations or overall changes in mutational burden under the pressure of therapy.

The six variants identified in five patients' tumors that were not found in cfDNA were all high allele frequency mutations and therefore it is possible these results are due to differences in the shedding of tumor DNA in those individuals. However, three of these five patients had other variants that were concordant between tumor and cfDNA, suggesting differences in ability to shed DNA between either different clonal populations of a tumor or different metastatic tumor sites. Reanalysis of cfDNA variants initially felt to represent true discordance showed that the majority of discordant cfDNA SNVs were due a lower variant allele frequency detection cutoff of the cfDNA assay. Given the challenges of using next generation based sequencing for copy number analysis, one must be careful to make this assumption for the low-level amplifications, of which no evidence was seen in the raw tumor sequencing data. Furthermore, of the low frequency mutations identified in cfDNA only, three were in *JAK2* and three in *TP53*, both of which are clonal hematopoiesis genes[29] and therefore could be due to mutated DNA from clonal lymphocyte populations.

Few tools exist to guide optimal treatment selection or sequence in MBC patients. Antigens, such as CA 27–29, and circulating tumor cells[30], while recommended within the ASCO guidelines to assist in decision-making[31], are of limited clinical value. The clinical utility of genomic assays have further been called into question by the recent IMAGE trial which was closed due to protocol-specified interim futility analysis[9]. Our finding that a high panel-specific mutational load in the *metastatic* lesion in either the cfDNA (occurring in 25% of patients) or tumor (occurring in 9% of patients) is predictive of poor response to conventional therapy independent of receptor status is novel and should be confirmed. Supporting this finding is a recent analysis of 560 *primary* ER+ tumors showed that 29% of the patients had a high mutational load and this was associated with decreased overall survival[32]. While it is unlikely that the mutational burden on these small captures will be fully concordant with whole genome or whole exome calculated mutational burden, our results suggest that clinically useful information on mutational burden can still be identified using panel-specific cutoffs. Prior studies have also suggested that *TP53* mutations in primary breast cancer predict poor overall survival[33,34], and our data confirm this observation in MBC patients as well. Therefore, analysis of mutational load or *TP53* mutations in tumors and/or cfDNA may be a useful clinical biomarker of therapy response in MBC patients.

There are a number of caveats to our study. First, the data reflect a small number of patients; however, these data provide the first in-depth analysis of clinically obtained and evaluated genomic tumor profiling with concurrent cfDNA analysis in a real-world clinical oncology setting. In addition, tumor genomic profiling was performed on FFPE derived samples using two different genomic panels, which may bias results as compared to analysis using fresh frozen tissue or identical panels; however, as this was a clinical utility study, we wished to perform genomic profiling as is standardly done clinically.

In summary, our data provide new insights into the differences in mutation detection between tumor and cfDNA in metastatic breast cancer patients that are important to the interpretation and application of these commonly used assays, and identifies panel-specific measures of mutational load as a potentially useful biomarker in patient management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Mutational spectra in the tumor/cfDNA cohort. Heat map showing the alterations identified by metastatic tumor testing and cell-free DNA (cfDNA) testing in the tumor/cfDNA cohort. Individual patient identifiers (ID) listed with annotations for receptor status of primary (if applicable) and of the biopsied metastasis. Total number of alterations identified by each assay; tumor assay reports pathogenic mutations and variants of uncertain significance (VUSs) and cfDNA assay reports all variants. Time elapsed between (b.) collection of tumor and blood for respective assays shown in weeks. Only genes shared between both versions of the respective assays are shown (i.e. shared genes on CPD_Full and CPD_PPP for tumor genes and shared genes on GH_v1 and GH_v2 for cfDNA genes)

Concordance between tumor and cfDNA assays. (a) Bar plot depicting the number of variants concordant (covered, reportable and detected by both assays) in both assays and discordant between tumor and cfDNA assays. Discordant variants plotted within three classes of discordance by the assay by which they were identified. The three types of discordance were variants only covered by one assay, variants covered by both assays but only reportable by one assay, and variants covered and reportable by both assays but only detected by one assay. (b) Plot of paired allele frequencies and paired fold amplifications of individual concordant variants as reported by the tumor and cfDNA assays. (c) Summary of allele frequencies and fold amplifications of discordant variants only identified by tumor or cfDNA assays versus concordant variants. Means of continuous variables compared by a Student's t test

Fig. 3.

Analysis of time to progression on standard treatment stratified by genomic biomarkers. **a** Patients were stratified into a high mutational load or low mutational load group. Time from initiation of treatment to first progression was analyzed using a Cox proportional-hazard model and adjusting for receptor status of the metastatic biopsy, number of lines of therapy and hormonal treatment versus chemotherapy. **b** Patients were stratified into a *TP53* mutation positive or *TP53* mutation negative group. Time from initiation of treatment to first progression was analyzed using a Cox proportional-hazard model and adjusting for receptor

status of the metastatic biopsy, number of lines of therapy, and hormonal treatment versus chemotherapy

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Table 1

Characteristics of Study Population

	Tumor Cohort		Tumor/cfDNA Cohort (subset)	
Total number	53		32	
Age (Median, range)	56	31–79	56	33–79
	<i>n</i>	%	<i>N</i>	%
Race				
White	41	77%	26	81%
Black	9	17%	6	19%
Other/Unknown	3	6%	0	0%
Stage at Diagnosis				
Stage 0 (DCIS)	3	6%	2	6%
Stage I	9	17%	5	16%
Stage II	16	30%	10	31%
Stage III	12	22%	8	25%
Stage IV	13	25%	7	22%
Receptor Status of Primary¹ (total n=40/25)				
HR+/Her2– or Her2 unknown	23	58%	15	60%
HR+ or HR–/Her2+	7	18%	6	24%
HR–/Her2– or Her2 unknown	10	25%	4	16%
Receptor Status of Metastases (total n=53/32)				
HR+/Her2– or Her2 unknown	31	58%	19	59%
HR+ or HR–/Her2+	7	13%	5	16%
HR–/Her2– or Her2 unknown	15	28%	8	25%
Adjuvant Therapy				
Received adjuvant hormone ²	21	78%	14	74%
Received adjuvant chemotherapy ³	29	97%	16	94%
	<i>Median</i>	<i>Range</i>	<i>Median</i>	<i>Range</i>
# prior lines therapy for stage IV disease¹				
HR+/Her2– or Her2 unknown	3	0–14	3	0–14
HR+ or HR–/Her2+	1	0–11	1	0–6
HR–/Her2– or Her2 unknown	2	0–9	2	0–3
Disease-free Interval¹				
HR+/Her2– or Her2 unknown	122	35–502	152	50–502
HR+ or HR–/Her2+	26	9–387	266	69–387
HR–/Her2– or Her2 unknown	70	25–761	39	25–51
Time to progression on subsequent therapy⁴				
HR+/Her2– or Her2 unknown	10	0–36	12	3–34
HR+ or HR–/Her2+	16	4–40	26	6–40
HR–/Her2– or Her2 unknown	4	1–24	4	1–16

¹ Stage 0–III tumors only, HR status based on primary

²Diagnosed Stage 0–III and any HR+ tumors, HR status based on primary; total n=27 and 19

³Diagnosed Stage 0–III and any HR–Her2, any Her2+, and N1–N3 HR+Her2–; HR status based on primary; total n=30 and 17

⁴HR status based on metastatic lesion

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Table 2

Mutations identified in the tumor and cDNA assays

	Tumor cohort (n=53)		Tumor/cDNA cohort (subset, n=32)	
	Tumor assay	Tumor assay	Tumor assay	cDNA assay
Total # alterations in # genes (all genes) ^{1,2}	65 in 14 genes	39 in 6 genes	93 in 27 genes	
Total # alteration in # genes (shared genes) ^{1,2}	58 in 9 genes	35 in 4 genes	90 in 24 genes	
Pathogenic mutations identified in shared genes ^{1,2}	<i>TP53</i> (21), <i>PIK3CA</i> (19), <i>ERBB2</i> (11), <i>EGFR</i> (2), <i>AKT1</i> (1), <i>KIT</i> (1), <i>PDGFRA</i> (1), <i>PTEN</i> (1)	<i>PIK3CA</i> (15), <i>TP53</i> (13), <i>ERBB2</i> (6), <i>EGFR</i>	<i>PIK3CA</i> (13), <i>TP53</i> (12), <i>ERBB2</i> (6), <i>JAK2</i> (3), <i>EGFR</i> (2), <i>MET</i> (1)	
Nonpathogenic alterations identified in shared genes ^{1,2}	<i>PIK3CA</i> (1)	None	<i>EGFR</i> (7), <i>BRAF</i> (6), <i>MET</i> (6), <i>ERBB2</i> (5), <i>PIK3CA</i> (5), <i>TP53</i> (4), <i>FBXW7</i> (2), <i>FGFR2</i> (2), <i>NOTCH1</i> (2), <i>RET</i> (2) ³	
	# of patients	% of cohort (n=53)	# of patients	% of cohort (n=32)
<i>TP53</i> mutation ⁴	19	36%	11	34%
<i>PIK3CA</i> mutation ⁵	18	34%	14	44%
<i>ERBB2</i> amplification	8	15%	4	13%
<i>ERBB2</i> mutation ⁶	2	4%	1	3%
<i>EGFR</i> amplification	2	4%	1	3%
1 pathogenic mutation	37	70%	22	69%
No variants identified	16	30%	10	31%
High mutational load ⁷	5	9%	2	6%
			8	25%

¹ Eighteen genes were found on both tumor assay versions (CPD_Full and CPD_PPP). CPD_PPP had two genes not found on CPD_Full, and CPD_Full had 28 genes not found on CPD_PPP. Variants found in non-shared genes included pathogenic mutations in *ATM* (1), *RBI* (1), and *STK11* (1) and nonpathogenic variants in *SMAD4* (3), and *SMO* (1).

² Forty-six genes were found on both cDNA assay versions (GH_v1 and GH_v2). GH_v1 had seven genes not found on GH_v2, and GH_v2 had 22 genes not found on GH_v1. Variants found in non-shared genes included one pathogenic mutation in *CDK6* and nonpathogenic variants in *BRCA1* (1) and *CCNE1* (1).

³ Additionally, one nonpathogenic variant was found in each of the following genes found on both GH_v1 and GH_v2: *ALK*, *AR*, *FGFR1*, *JAK3*, *KIT*, *KRAS*, *MYC*, *NF1*, *NPM1*, *PDGFRA*, *PROC*, *SMAD4*, and *SMARCB1*.

⁴ Pathogenic *TP53* mutations identified in individual patients included p.K132R, p.V173L, p.R175G, p.R175H, p.C176F, p.P190L, p.E204X, p.R213X, p.Y234N, p.S241F, p.R248Q (2), p.R248W (2), p.E285K, p.E285X, p.V272L, p.V272M, p.R337C, p.R342G, and three frameshift mutations

⁵ Pathogenic *PIK3CA* mutations in individual patients included p.G106S, p.E542K, p.E545K (3), p.H1047R (12), p.H1047L, p.H1047Q

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⁶ *ERBB2* mutations in individual patients included p.D769H, p.V777L (2)

⁷ High mutational load is greater than 2 variants in tumor or greater than 3 variants in cDNA.