

Concordance of Genomic Alterations by Next-Generation Sequencing in Tumor Tissue versus Circulating Tumor DNA in Breast Cancer



Young Kwang Chae^{1,2,3}, Andrew A. Davis², Sarika Jain^{1,2,3}, Cesar Santa-Maria^{1,2,3}, Lisa Flaum^{2,3}, Nike Beaubier^{3,4}, Leonidas C. Plataniias^{1,2,3,5}, William Gradishar^{2,3}, Francis J. Giles^{1,2,3}, and Massimo Cristofanilli^{1,2,3}

Abstract

While identifying genomic alterations in tumor tissue is the current gold-standard technique for molecular profiling, circulating tumor DNA (ctDNA) represents a noninvasive method of assessing genomic alterations using peripheral blood. The concordance of genomic alterations between two commercially available ctDNA and tissue biopsies was compared in 45 patients with breast cancer using paired next-generation sequencing tissue and ctDNA biopsies. Across all genes, concordance between the two platforms was 91.0% to 94.2%. When only considering genomic alterations in either assay (e.g., excluding wild type/wild type genes), concordance was 10.8% to 15.1% with full plus partial concordance of 13.8% to 19.3%. Concordant mutations were associated with significantly higher variant allele frequency. Over half of mutations detected in either technique were not detected using the other biopsy technique. Including variants of unknown

significance, the average number of alterations per patient was significantly higher for tissue (4.56) compared with ctDNA (2.16). When eliminating alterations not detectable in the ctDNA assay, mean number of alterations for tissue and ctDNA was similar (2.67 for tissue, 2.16 for ctDNA). Across five representative genes (*TP53*, *PIK3CA*, *ERBB2*, *BRCA1*, and *BRCA2*), sensitivity and specificity were 35.7% and 95.0%, respectively. Concordance when genomic alterations was detected in either tissue or ctDNA was low with each technique detecting a significant amount of nonoverlapping mutations. Potential explanations for the lack of concordance include tumor heterogeneity, different sequencing techniques, spatial and temporal factors, and potential germline DNA contamination. The study indicates that both tissue and blood-based NGS may be necessary to describe the complex biology of breast cancer. *Mol Cancer Ther*; 16(7); 1412–20. ©2017 AACR.

Introduction

While treatments for metastatic breast cancer have expanded recently, prognosis remains poor with median survival of about two years (1, 2). The precise characterization of genomic alterations of individual tumors may provide indications for novel treatment strategies. A persistent challenge in defining tumor genomic landscapes is tumor heterogeneity. Intratumor

heterogeneity arises in different locations (spatial heterogeneity) and subclones within a primary tumor, likewise different metastatic sites may exhibit novel genomic alterations (3–7). While tissue-based next-generation sequencing (NGS) remains the gold-standard technique for initial tumor histology, architecture, and genomic characterization, logistic and safety limitations exist for serial biopsies. Existing serum biomarkers for breast cancer, such as carcinoembryonic antigen, cancer antigen 15-3, and cancer antigen 27-29, have limited clinical applicability in most circumstances (8, 9). In contrast, circulating tumor cells (CTC) have shown promise to independently predict progression-free survival and overall survival, as well as response to therapy in patients with metastatic breast cancer (10–12). However, complementary platforms as tools to detect genetic mutations over time with the goal of monitoring tumor clone and subclone evolution are needed to optimally personalize treatment decisions for patients.

Cell-free circulating tumor DNA (ctDNA) has emerged as a potential solution to this problem by detecting small quantities of tumor DNA in the peripheral blood. The goal of this noninvasive technique is to capture molecular and genetic heterogeneity, to identify genetic alterations for targeted therapy, and to monitor tumor evolution and resistance in real time (13). ctDNA can be shed into peripheral blood after tumor cells outgrow their blood supply, become hypoxic, and

¹Developmental Therapeutics Program of Division of Hematology Oncology, Northwestern University Feinberg School of Medicine, Chicago, Illinois. ²Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois. ³Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, Illinois. ⁴Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois. ⁵Department of Medicine, Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois.

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Y.K. Chae and A.A. Davis contributed equally to this article.

Corresponding Author: Young Kwang Chae, 645 North Michigan Avenue, Suite 1006, Chicago IL 60611. Phone: 312-926-4248; Fax: 312-695-0370; E-mail: young.chae@northwestern.edu

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undergo apoptosis or necrosis. ctDNA quantity is on average higher in patients with cancer compared to controls (14, 15). For advanced tumors, ctDNA is variable with some tumor types such as breast cancer expressing higher percentages of ctDNA while others, such as brain cancer, having detectable circulating DNA in less than 50% of patients (16). One study with an estimated 95% of patients having advanced or metastatic disease reported 58% of patients with at least one detectable alteration, which increased to 68% when excluding glioblastoma (17).

In breast cancer, plasma levels of ctDNA have been shown to be higher in cancer patients as compared with benign breast disease and healthy controls (18). In addition, ctDNA was detected in 75% of patients with advanced breast cancer compared with 50% of patients with localized breast cancer and correlated with poorer overall survival (16). In metastatic breast cancer, one study reported that ctDNA was successfully detected in 29 of 30 patients (97%) in whom genomic alterations were identified, and had some correlation with tumor burden and treatment response (19). Early work has also demonstrated potential to predict metastatic relapse in early breast cancer and to identify treatment resistance patterns by detection of *ESR1* mutations (20–22). Potential limitations of ctDNA are based on the quantity (e.g., amount that is accessible in the peripheral blood) or quality (e.g., tumor purity of noncancer cells in the tumor microenvironment) (23).

The goal of our study was to assess concordance across a large number of overlapping genes tested in tissue and ctDNA NGS in paired biopsies performed in patients with breast cancer. The independent predictive value of NGS diagnostic technologies from both tissue and blood has not been completely clarified and the information is critical for selecting molecular tools for planning and disease monitoring. To our knowledge, this is one of the largest samples of breast cancer patients to systematically assess sequencing-specific concordance of genomic alterations across a large number of genes in both NGS platforms.

Materials and Methods

Study design and patients

The Institutional Review Boards of Northwestern University, Feinberg School of Medicine and Thomas Jefferson University approved the retrospective study. Written informed consent from patients was waived per the Institutional Review Boards. The studies were conducted in accordance with the Declaration of Helsinki. Fifty-three patients with commercial ctDNA NGS testing by Guardant360 (Guardant Health) and FoundationOne (Foundation Medicine) were identified. Of these, eight were excluded because the FoundationOne reports were either "qualified" for possible insufficient sample or had "report failure." Therefore, the final sample consisted of 45 patients with both tissue and peripheral blood ctDNA. Clinical and tumor characteristics were obtained retrospectively via patient chart review.

Genes analyzed

The study examined concordance across all genes found in ctDNA that were also tested in tissue biopsy samples. The number of genes tested in the FoundationOne panel (Foundation Medicine) ranged from 236 to 315. The number of genes tested via Guardant360 (Guardant Health) ranged from 54 to 70. Therefore, our analyses examined concordance of between 45 and 67 genes

that were common to both platforms for a particular individual, depending on the exact timeframe of when the testing was performed (Supplementary Tables S1 and S2).

Defining concordance and data analysis

First, concordance with negatives was defined at the gene level as detecting an identical sequencing mutation or not detecting an alteration in a single gene. Therefore, both identical sequencing alterations and lack of an alteration (e.g., double negatives, wild-type/wild-type) in the same individual were considered concordant. In contrast, the finding of different sequencing alterations detected in the same gene when the two platforms were performed was counted as a discordant genomic alteration (e.g., *TP53* R248Q by tissue biopsy and *TP53* R110H by ctDNA; Supplementary Table S3).

Second, concordance on positive mutations was examined for the subset of genes in which a genomic alteration was detected (e.g., eliminating all wild-type/wild-type genes). For this analysis, genes in which mutations were not detected (e.g., wild-type/wild-type *TP53* gene in both assays in the same patient) were excluded from both the numerator and denominator. Concordance was further compared when excluding particular alterations within overlapping genes not sequenced by Guardant360. These included splice site mutations, certain small insertions or deletions, and allelic loss (such as *PTEN*). Partial concordance was defined as having one concordant mutation and at least one discordant genomic alteration in the same gene. Concordance on positives was defined by the total number of fully concordant or partially concordant alterations with the denominator as the total number of DNA alterations in our sample ($N = 232$ genomic alterations or $N = 166$ when excluding alterations not sequenced by the ctDNA assay). Variants of unknown significance (VUS) were included. Synonymous DNA alterations reported by Guardant360 were not included in any concordance analysis because these were not included in FoundationOne reports.

In addition, sensitivity, specificity, and diagnostic accuracy (effectiveness) analyses were performed across five representative genomic alterations (*TP53*, *PIK3CA*, *ERBB2*, *BRCA1*, and *BRCA2*) in the sample. The sample size (N) for this analysis varied depending on the timeframe when the two assays were performed. Youden's J index (sensitivity + specificity – 1) was calculated as an indirect measurement of concordance, as well as an alternative method reflecting diagnostic accuracy (24).

Results

Patient characteristics

Table 1 shows the patient and tumor characteristics of the 45 patients included in the study. Thirty-four patients (75.6%) had inflammatory breast cancer (IBC). At diagnosis, tumor IHC consisted of 20 hormone receptor (HR)⁺/HER2⁻, 8 HR⁺/HER2⁺, 6 HR⁻/HER2⁺, and 11 HR⁻/HER2⁻. The median timeframe between tissue and ctDNA biopsies was 146 days.

Concordance of blood-based ctDNA and tissue-based DNA

Concordance with negatives when comparing the two assays was 91.0% including all genes examined (Table 2). Concordance was high across all patients with range of 81.5% to 97.8%. When excluding particular alterations within overlapping genes not sequenced by Guardant360, concordance was 94.2%. The

Table 1. Characteristics of patients with both tissue and ctDNA NGS testing

	N	Percentage
Age (years)		
Median	55	
Sex		
Female	44	97.8
Male	1	2.2
Breast cancer tissue subtype		
Ductal carcinoma	39	86.7
Lobular carcinoma	1	2.2
Other	5	11.1
Clinical breast cancer type		
Inflammatory	34	75.6
Other	11	24.4
Tumor IHC at diagnosis		
HR ⁺ /HER2 ⁻	20	44.4
HR ⁺ /HER2 ⁺	8	17.8
HR ⁻ /HER2 ⁺	6	13.3
HR ⁻ /HER2 ⁻	11	24.4
Pathologic stage		
I/II/III	14	31.1
IV	31	68.9
Tissue biopsy from site of primary tumor		
Yes	27	60.0
No	18	40.0
Interval between tissue and blood sample collection		
Less than 90 days	19	42.2
More than 90 days	26	57.8

Abbreviation: HR, hormone receptor.

remaining analyses were subset analyses to examine concordance for genes with a genomic alteration present in one or both assays (e.g., excluding double negatives, wild-type/wild-type). Among the subset of genes with reported genomic alterations in either assay ($N = 232$), concordance on positives was 10.8% with full plus partial concordance of 13.8%. When only including alterations detectable in both assays, the full and full plus partial concordance values were 15.1% and 19.3%, respectively. When examining copy number variants (CNV; $N = 86$), concordance was 3.5%. Given the low concordance for CNVs, the data were also analyzed excluding these variants. For single nucleotide changes and indels, concordance with negatives was 95.7% with full and full plus partial concordance of 18.5% and 23.3%, respectively. For patients with available data, no significant differences were found in concordance based on the number of metastatic sites at time of ctDNA biopsy.

Genomic variants were categorized by potential functionality versus VUS (Fig. 1A). The most likely outcome occurring in 53.3% of cases was an alteration detected in tissue, but not ctDNA. In total, 32.5% of alterations were detected exclusively in ctDNA with a higher percentage of VUS (40.5%), as compared with known/likely functional genes (25.2%).

Table 2. Composite data comparing tissue biopsy with ctDNA

Average concordance of genomic analyses when DNA alterations are present or absent	91.0%	94.3% ^a	93.6% ^b
Percentage of tissue alterations found in ctDNA	15.6%	25.6% ^a	31.7% ^b
Percentage of ctDNA alterations found in tissue	33.0%	30.3% ^a	33.0% ^b
Mean and SD of genomic alterations in ctDNA	2.16 (2.31)	2.16 (2.31) ^a	2.85 (2.23) ^b
Mean and SD of genomic alterations in tissue	4.56 (2.98)	2.67 (2.11) ^a	2.97 (2.24) ^b

^aIndicates alterations within overlapping genes not detectable by Guardant360 were excluded.

^bIndicates alterations within overlapping genes not detectable by Guardant360 and 11 cases without any alterations detected in ctDNA were excluded. Variants of unknown significance (VUS) were included. Standard deviation (SD) are included in parentheses.

The sample was also analyzed based on timeframe between biopsies (Supplementary Table S4). Concordance on positives was 12.1% with a full plus partial concordance of 15.3% for paired biopsies less than 90 days apart ($N = 124$). For biopsies more than 90 days apart, concordance on positives was 9.3% with a full plus partial concordance of 12.1% ($N = 108$). When excluding variants not detectable in the ctDNA assay, concordance and full plus partial concordance were 18.1% and 22.9%, respectively, for less than 90 days and 12.0% and 15.6%, respectively, for biopsies greater than 90 days apart. No statistically significant differences were found. Figure 2A demonstrates the landscape of DNA mutations, stratified by indel/point mutation versus CNV, found in both NGS platforms. Large differences were encountered with respect to more mutations detected in *ERBB2* amplifications for tissue and in *MET* amplifications and single nucleotide variants for ctDNA. Figure 2B is an oncoprint chart displaying 10 representative genes across all 45 patients in the sample.

Circulating tumor DNA variant allele frequency

For genes with identical sequencing mutations ($N = 29$), the percent or allele frequency of altered ctDNA was analyzed. The mean variant allele frequency (VAF) of altered ctDNA was 4.3% (SD 6.2%) with median 1.2% (range, 0.3%–21.1%). Mean VAF was greater for concordant mutations (4.30%) as compared with discordant mutations (1.40%; Fig. 1B; $P < 10^{-6}$; Mann–Whitney test). Overall, 79.3% of these identical mutations (23 of 29) were found to have less than 5% allele frequency of altered ctDNA. Concordant gene amplifications ($N = 3$) were excluded from this analysis because no allele frequency was reported. When analyzing ctDNA alterations with VAF greater than 1%, 72.7% (16 of 22) were also detected in tissue.

Average number of DNA alterations

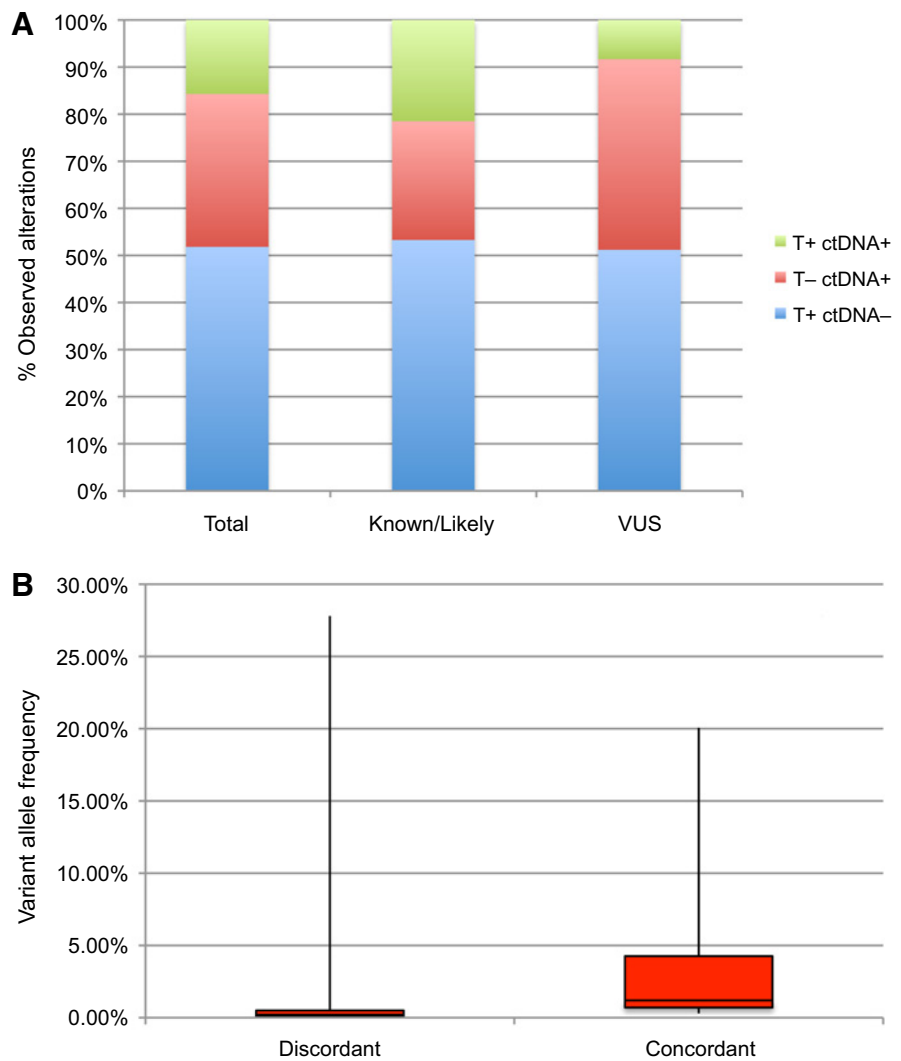
The average number of alterations including VUS per patient was significantly higher for tissue 4.56 (SD 2.98) as compared with ctDNA 2.16 (SD 2.31; $P < 0.0001$, 95% CI, 1.28–3.52; two-sample *t* test; Table 2). More mutations were detected in tissue-based NGS in 38 of 45 (84.4%) patients. When excluding particular alterations within overlapping genes not sequenced by Guardant360, average number of alterations including VUS was not statistically different with 2.67 (SD 2.11) for tissue and 2.16 (SD 2.31) for ctDNA ($P > 0.05$). In addition, when excluding CNVs, mutation number for tissue (1.93) and blood (1.84) was similar.

Diagnostic accuracy analysis

Gene-level sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were analyzed across five representative genes in the sample

Figure 1.

A, Concordance of genomic alterations in tissue and ctDNA categorized by potential functionality. Shown here is tissue (T) and ctDNA. T + ctDNA+, concordant in both platforms. T – ctDNA+: variant found in ctDNA, but not tissue. T + ctDNA–: variant found in tissue, but not ctDNA. There were four instances in which one platform characterized a concordant mutation (T+ctDNA+) mutation as known/likely in one platform, but VUS in the other. These were categorized as “known/likely,” indicating potential functionality. Variants within overlapping genes not tested by Guardant360 were excluded. **B,** Comparing variant allele frequency (VAF) between concordant and discordant variants. Box plots display median VAF with interquartile range and minimum and maximum VAF values. Concordant alterations had a significantly higher VAF ($P < 10^{-6}$; Mann-Whitney test). Copy number variants were excluded.



(Table 3). These genes included *TP53*, *PIK3CA*, *ERBB2*, *BRCA1*, and *BRCA2*. Tissue-based NGS was used as the gold standard for this analysis. Across all five genes examined, when excluding VUS, sensitivity was 35.7%, specificity was 95.0%, and diagnostic accuracy was 79.6% (Table 3). Values were similar when VUS were included with sensitivity of 36.2%, specificity of 92.2%, and diagnostic accuracy of 72.0% (Supplementary Table S5). Diagnostic accuracy for these same genes when excluding CNVs was 84.0%.

Clinical utility of longitudinal circulating tumor DNA testing

Two case reports demonstrate the utility of ctDNA monitoring over time. The first case demonstrated genetic clonal change in peripheral blood in response to treatment. The second case showed how molecular response corresponded to a partial response seen with serial imaging (Fig. 3A and B).

Discussion

Precision oncology aims to make treatment decisions based on the precise genomic profile of the tumor at various time

points. The goal is to enable early treatment decisions based on detecting the emergence of genetic alterations that predict recurrence or resistance to treatment prior to development of clinical symptoms or radiological evidence of disease progression. ctDNA has the potential to improve our understanding of tumor biology in the peripheral blood. This is predicated on ctDNA reliably correlating with the molecular features of the disease likely reflected in the genomic profile of primary and/or metastatic site(s). Therefore, the primary objective of this study was to investigate the concordance rate of genomic alterations using NGS in both tissue and ctDNA biopsies in a sample of patients with breast cancer.

As compared with tissue biopsy, our findings indicated that the ctDNA assay used in our study had high specificity along with diagnostic accuracy of 72% to 80%. When examining all genes, including those without DNA alterations in either assay, concordance with negatives was 91.0% to 94.2%. However, when examining the subset of genes with DNA alterations found in either assay, concordance on positives and full plus partial concordance were low (10.8%–15.1% and 13.8%–19.3%, respectively). Concordance was particularly low for CNVs in our sample (3.5%). Previous research has demonstrated that chromosomal

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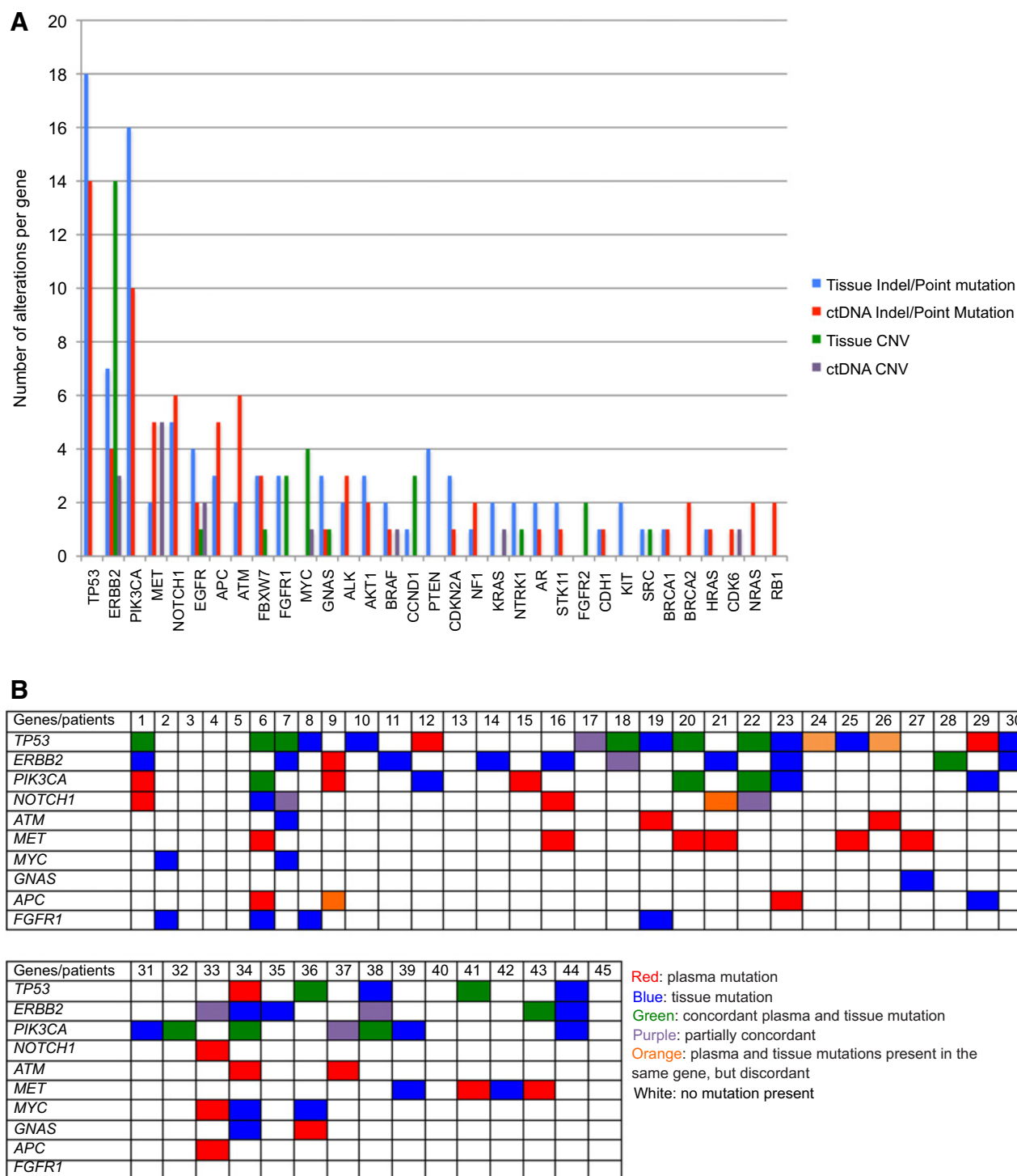


Figure 2. Landscape of genomic alterations in tissue and ctDNA. **A**, Comparing frequency of alterations per gene in tissue and ctDNA for genes with at least two alterations. Variants within overlapping genes not tested by Guardant360 were excluded. The sample was stratified based on indel/point mutation versus copy number variant (CNV) for both tissue and ctDNA. **B**, Oncoprint chart for 10 select genes across all patients. A total of 45 patients were tested for both NGS assays. Variants of unknown significance (VUS) were included and variants within overlapping genes not tested by Guardant360 were excluded.

Table 3. Sensitivity, specificity, and diagnostic accuracy across five genes

ctDNA Mutations	Tissue Mutations		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic Accuracy (%)	Youden's J index
	(+)	(-)						
<i>TP53</i>								
(+)	7	3						
(-)	8	24	46.7	88.9	70.0	75.0	73.8	0.4
<i>PIK3CA</i>								
(+)	3	2						
(-)	9	31	25.0	93.9	60.0	77.5	75.6	0.2
<i>ERBB2</i>								
(+)	4	0						
(-)	10	31	28.6	100.0	100.0	75.6	77.8	0.3
<i>BRCA1</i>								
(+)	1	0						
(-)	0	14	100.0	100.0	100.0	100.0	100.0	1.0
<i>BRCA2</i>								
(+)	0	1						
(-)	0	14	n/a	93.3	0.0	100.0	93.3	n/a
Total positive	15	6						
Total negative	27	114						
Total (positive + negative)	42	120	35.7	95.0	71.4	80.9	79.6	0.3

NOTE: Variants of unknown significance (VUS) were excluded. Variants not tested by Guardant360 were excluded. Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

rearrangements and CNVs can be detected reliably using ctDNA with other techniques including personalized analysis of rearranged ends and digital karyotyping (25–27).

The primary reasons that our concordance for detectable mutations was lower than previously reported studies were that our analyses were not limited to hotspot genes, consisted of more extensive exon sequencing, and included subclones. The similarity between our diagnostic accuracy analyses both with and without VUS indicated that concordance was not significantly affected by whether the detected genes were functional or VUS (Table 3 and Supplementary Table S3). We considered concordant DNA alterations only when the exact same sequencing alteration was present in both biopsies. It is critical to detect specific sequencing variants that may guide resistance patterns to treatment such as endocrine therapy, for example, mutations in *ESR1* (28). Collectively, these data indicate that ctDNA may be best utilized as a predictive tool to identify certain important genomic alterations given the high specificity.

Previous studies have indicated high concordance between tissue-based NGS and ctDNA in studies investigating particular genes, such as *EGFR* alterations in NSCLC, multiple genes in pancreaticobiliary cancers (*KRAS*, *TP53*, *APC*, *FBXW7*, *SMAD4*), exons 12–13 of *KRAS* in colorectal cancer, and *BRAF*V600E and *KIT* mutations in melanoma (29–32). While these studies report high specificity and diagnostic accuracy (greater than 80%–90%) compared with the current gold standard of tissue-based NGS, the high values mostly reflect not detecting DNA alterations in either assay in the same patient. More recently, concordance rates have ranged from 70%–93% for *TP53*, *EGFR*, *PIK3CA*, and *ERBB2* in a mixed sample of advanced solid tumors (33). Another study reported sensitivity of 49.9% and a specificity of 99.8% for patients with advanced or metastatic solid tumors when examining 50 hotspot genes across tissue and ctDNA platforms (34). In breast cancer, high concordance between tissue and ctDNA for *PIK3CA* mutations and *ERBB2* amplifications has been reported, but poor agreement for *TP53* mutations and *EGFR* amplifications (35). Our study indicates the need for more comprehensive analysis of tissue and ctDNA NGS concordance beyond single genes and hotspot regions to

determine utility and potential clinical applications of ctDNA biopsies. Interestingly, studies investigating concordance in biomarker expression between tissue analysis and CTCs, another component of liquid biopsies, have reported a lack of concordance between the two compartments with regards to hormone receptor and *HER2* expression (36–39). These findings clearly demonstrated phenotypic heterogeneity in both primary and metastatic breast cancer.

There are several additional important findings from our study. First, significantly more mutations were detected in tissue (mean 4.56) as compared to ctDNA (mean 2.16). However, when eliminating alterations not detectable with the ctDNA assay used in our study, mean alterations for tissue (2.67) and ctDNA (2.16) were not significantly different. This finding was also validated in our study comparing the same two platforms in a mixed sample of advanced solid tumors with a predominance of lung cancer biopsies (40). While tissue biopsies have spatial limitations and may only target one location, detection of genomic alterations in the peripheral blood has the potential to assess tumor heterogeneity from multiple metastatic sites. However, ctDNA assays only identify mutations after tumor cells outgrow the blood supply, become hypoxic, and undergo apoptosis or necrosis, releasing DNA into the peripheral blood (14). Potential explanations for our findings include inability to capture ctDNA at very low detection thresholds, fewer ctDNA variants released into the peripheral blood, differences in biology of particular cancer types, and different sequencing and detection methods within the genes examined (16, 41).

Second, a large proportion of genomic alterations detected using each platform was not detected in the other NGS platform. Specifically, 25.6% of tissue alterations were found in ctDNA and 30.3% of ctDNA alterations were detected in tissue biopsies. The finding that over half of genomic alterations detected using each technique were not found in the other assay indicates considerable tumor heterogeneity that cannot be fully detected in either biopsy technique alone. A hypothesis that needs further testing is whether concordance varies based on number of metastatic sites and site of tissue biopsy (primary

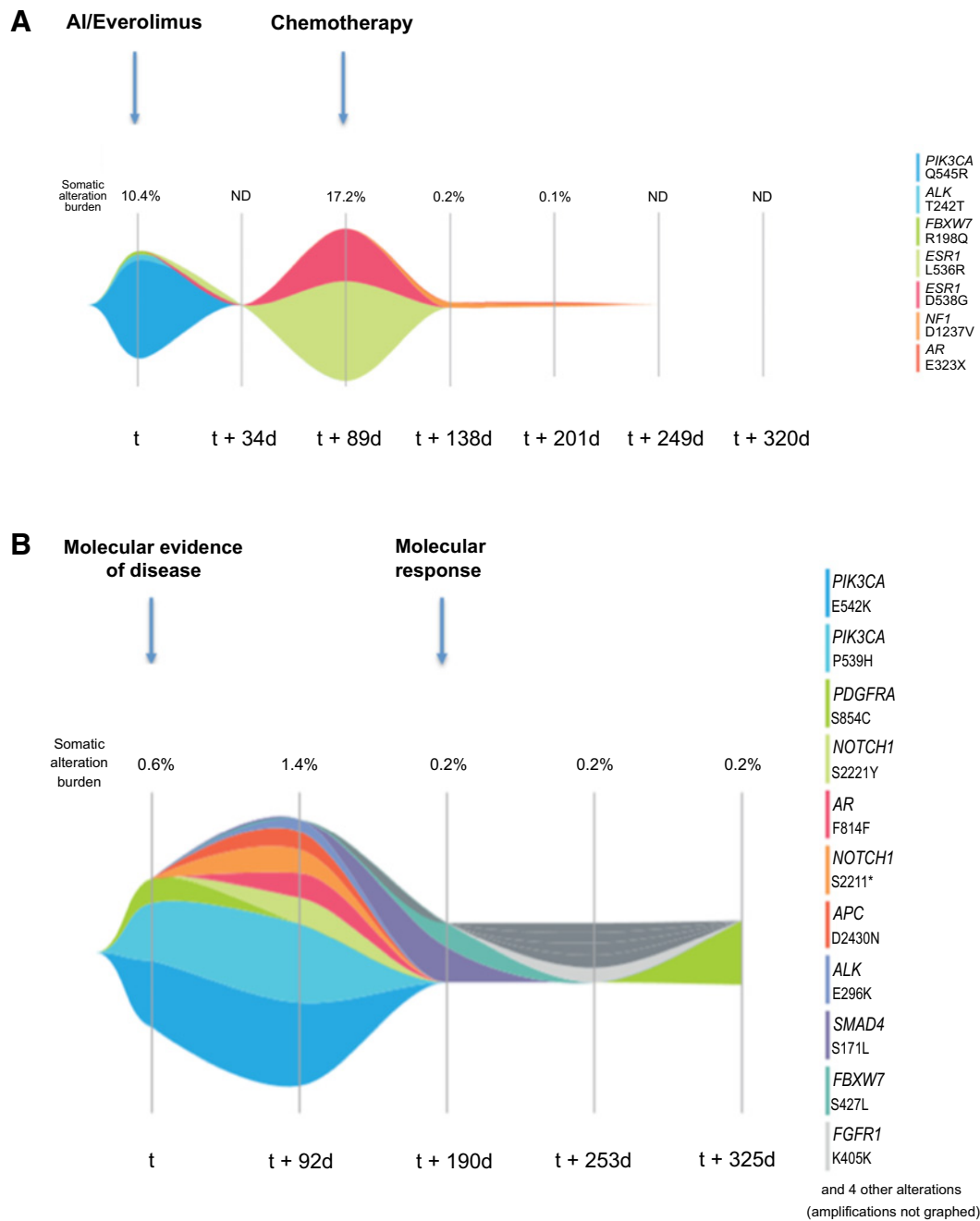


Figure 3.

Clinical utility of longitudinal ctDNA testing. **A**, Identifying disease resistance and actionable mutations for treatment decisions with longitudinal ctDNA monitoring. Shown here is a schematic of allele frequency and somatic alteration burden over time for several genomic alterations generated by Guardant360. The arrow corresponding to exemestane, an aromatase inhibitor (AI), with everolimus indicated initiation of therapy with subsequent disease progression and emergence of two *ESR1* mutations (shown in pink and green). Ixabepilone and capecitabine (chemotherapy) were subsequently started leading to disappearance of *ESR1* mutations, an example of genetic clonal change in peripheral blood in response to treatment. Time interval (t) from baseline and subsequent ctDNA testing included with days indicated by (d). Colors of clones and subclones correspond to specific genomic alterations shown on the right. Somatic alteration burden indicates the maximum percent ctDNA detected at each time point. ND for somatic alteration burden indicates not detected or no alterations found. **B**, Early detection of recurrence using ctDNA for early treatment intervention. Shown here is a schematic of allele frequency and somatic alteration burden over time for several genomic alterations generated by Guardant360. The patient was treated with adjuvant anastrozole. Molecular evidence of disease was demonstrated by the emergence of multiple genomic alterations, but no clinical or radiologic evidence of recurrence. Molecular response corresponded to tumor response after treatment with everolimus, leading to a reduction in the somatic alteration burden and number of genomic alterations. Time interval (t) from baseline and subsequent ctDNA testing included with days indicated by (d). Colors correspond to specific genomic alterations shown on the right. Somatic alteration burden indicates the maximum percentage of ctDNA detected at each time point.

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vs. metastatic site). Previous data have indicated high analytical specificity of 99.9999% for Guardant360 for single nucleotide variants with mutant allele frequency (MAF) greater than 2% and wild-type sequences (42). Specificity at lower allele frequency may indicate a higher potential for false positive variants. Additionally, technical differences between the assays need to be further explored. Overall, our findings indicate a potential complementary role of tissue and ctDNA NGS to accurately characterize sequencing alterations, the overall tumor mutational burden, and to best prognosticate response to therapy for a particular patient.

Third, our data indicate that higher VAF was significantly associated with concordant mutations. Specifically, for ctDNA genomic alterations with VAF greater than 1%, 72.7% were also detected in tissue, indicating greater reliability with tissue. This may indicate that variants detected in smaller quantities may only be present in areas that are missed by tissue biopsies and potentially more difficult to detect in blood biopsies. The association of VAF with concordance was also recently reported in a similar comparison between tissue and ctDNA biopsies in a sample of nine patients (43).

Fourth, we hypothesized that concordance would be higher based on shorter time interval between tissue and ctDNA biopsies. However, we were unable to support this in our study (Supplementary Table S4). Previous work has reported that biopsies with at least one concordant mutation have a shorter timeframe between biopsies, but further work is necessary to analyze this measure across many genes. Larger cohorts are required to test this hypothesis in the future.

Finally, in our study, we characterized the landscape of mutations in the peripheral blood for patients with breast cancer. Through this, there is potential to use this genetic information to identify actionable molecular targets for therapy in the peripheral blood (Supplementary Table S1). While current prospective data are limited in demonstrating improved clinical outcomes using peripheral blood ctDNA across large cohorts of patients, our case examples demonstrate the clinical potential for using ctDNA to identify actionable molecular targets, to detect disease progression, and to longitudinally monitor the genomic profile of tumors (Fig. 3A and B).

To the best of our knowledge, this study was one of the most comprehensive to examine concordance of paired NGS tissue and ctDNA in breast cancer beyond driver mutations and hotspot regions. Our study critically examined gene variant level concordance, and therefore conclusions regarding patient level pathological variables (e.g., treatment response or breast cancer subtypes) were difficult. Limitations of our study include the retrospective nature, a relatively small sample size, and our inclusion of NGS platforms with changing number of genes over time, which limited our characterization of the relative frequencies of genes found in the peripheral blood. Further studies are also needed to

determine how ctDNA concordance and mutational profile may be affected by our inclusion of a large proportion of patients with IBC.

In conclusion, our findings indicated high specificity and concordance when genomic alterations were present or absent. When examining the subset of genes with DNA alterations present, concordance was relatively low. Additionally, while more mutations were detected in tissue biopsies as compared with ctDNA, a considerable amount of nonoverlapping mutations were detected in each platform for paired biopsies. Further independent validation sets are necessary to compare other tissue and ctDNA platforms. Prospective studies are necessary in order to further assess concordance in larger samples, across multiple tumor types, and at various collection intervals between biopsy sampling and treatment, including collecting both tissue and ctDNA at the same time without intervening treatment. In addition, studies assessing whether this information can guide clinical decisions and improve patient outcomes based on the precise molecular signature of the tumor across large cohorts of patients are critical.

Disclosure of Potential Conflicts of Interest

Y.K. Chae is a consultant/advisory board member for Foundation Medicine and Guardant Health. C.A. Santa-Maria reports receiving a commercial research grant from Pfizer and MedImmune. N. Beaubier is director of pathology at Tempus Labs, Inc. M. Cristofanilli is a consultant/advisory board member for Vortex and Dompe. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y.K. Chae, A.A. Davis, S. Jain, F.J. Giles, M. Cristofanilli
Development of methodology: Y.K. Chae, A.A. Davis, F.J. Giles, M. Cristofanilli
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y.K. Chae, S. Jain, L. Flaum, W. Gradishar, F.J. Giles
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y.K. Chae, A.A. Davis, S. Jain, L.C. Platanius, F.J. Giles, M. Cristofanilli
Writing, review, and/or revision of the manuscript: Y.K. Chae, A.A. Davis, S. Jain, C. Santa-Maria, N. Beaubier, L.C. Platanius, W. Gradishar, F.J. Giles, M. Cristofanilli
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y.K. Chae, A.A. Davis
Study supervision: Y.K. Chae, F.J. Giles, M. Cristofanilli

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