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Longitudinal Shifts of Solid Tumor and Liquid Biopsy Sequencing Concordance in Metastatic Breast Cancer

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PURPOSE Tissue-based next-generation sequencing (NGS) in metastatic breast cancer (mBC) is limited by the inability to noninvasively track tumor evolution. Cell-free DNA (cfDNA) NGS has made sequential testing feasible; however, the relationship between cfDNA and tissue-based testing in mBC is not well understood. Here, we evaluate concordance between tissue and cfDNA NGS relative to cfDNA sampling frequency in a large, clinically annotated mBC data set.

METHODS Tempus LENS was used to analyze deidentified records of mBC cases with Tempus xT (tissue) and xF (cfDNA) sequencing results. Then, various metrics of concordance were assessed within overlapping probe regions of the tissue and cfDNA assays (104 genes), focusing on pathogenic variants. Variant allele frequencies of discordant and concordant pathogenic variants were also compared. Analyses were stratified by mBC subtype and time between tests.

RESULTS Records from 300 paired tissue and liquid biopsies were analyzed. Median time between tissue and blood collection was 78.5 days (standard deviation = 642.99). The median number of pathogenic variants/patient was one for cfDNA and two for tissue. Across the cohort, 77.8% of pathogenic tissue variants were found in cfDNA and 75.7% of pathogenic cfDNA variants were found in tissue when tests were ≤ 7 days apart, which decreased to 50.3% and 51.8%, respectively, for > 365 days. Furthermore, the median patient-level variant concordance was 67% when tests were ≤7 days apart and 30%-37% when > 30 days. The median variant allele frequencies of discordant variants were generally lower than those of concordant variants within the same time frame.

CONCLUSION We observed high concordances between tissue and cfDNA results that generally decreased with longer durations between tests. Thus, cfDNA NGS reliably measures tissue genomics and is likely beneficial for longitudinal monitoring of molecular changes in mBC.

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BACKGROUND

Despite advancements in the early detection and treatment of breast cancer, a portion of cases still recur as metastatic disease, and metastatic breast cancer (mBC) is considered incurable. The main challenge of effective mBC treatment is tumor heterogeneity, which may be evaluated by using next-generation sequencing (NGS).[1-3] Although tissue biopsies remain the gold standard for NGS-based genomic characterization,[4] they are invasive and carry the risk of surgical complications.[5-7] Therefore, tissue-based NGS is difficult to repeat in clinical practice and impractical for periodic monitoring of treatment response. Furthermore, a small piece of tissue does not necessarily represent the entire tumor composition in primary and metastatic sites.[1-3,8]

Alternatively, liquid biopsies are noninvasive and easily repeated, enabling the monitoring of dynamic diseases via cell-free DNA (cfDNA).[9] Since circulating tumor DNA (ctDNA) is shed into the blood from different disease sites, liquid biopsy can offer a more comprehensive picture of malignancies.[8,10] For example, in a recent comparison of postprogression ctDNA versus solid-tumor DNA in gastrointestinal cancer, clinically relevant resistance alterations in ctDNA were not found in the matched tumor biopsy in 78% of cases.[3] Although liquid biopsy is clinically useful, there are still limitations. Low concentrations of ctDNA in the sample can lead to false negatives.[11,12] Conversely, gene alterations associated with clonal hematopoiesis of indeterminate potential (CHIP) may be mistaken for oncogenic drivers, resulting in false positives.[13,14] Finally, small protocol deviations during blood collection, storage, or processing may complicate detection.[15]

A possible solution to the challenges posed by both methods is concurrent tissue and cfDNA analysis to characterize the genomic landscape and monitor

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Tracking the molecular composition of metastatic breast cancer (mBC) with liquid and/or solid biopsy can improve targeted therapy approaches, but the relationship between cell-free DNA (cfDNA) and tissue-based testing is not well understood. We evaluated concordance between tissue and cfDNA next-generation sequencing (NGS) results in relation to cfDNA sampling frequency in a large, real-world mBC data set.

Knowledge Generated

Longitudinal comparisons from multiple perspectives revealed high concordances between tissue and cfDNA results that decreased with longer durations between tests. In some cases, cfDNA NGS detected variants that were missing from paired tissue NGS results and vice versa. Furthermore, the median variant allele frequencies of discordant variants were generally lower than those of concordant variants within the same time frame.

Relevance

cfDNA NGS reliably measures genomic variants when tissue is not available and is likely beneficial for longitudinal monitoring of molecular changes in mBC. Nonetheless, combined testing may detect variants missed by either method alone.

tumor evolution. Previous studies show that more than half of the mutations detected in either tissue or liquid biopsy are not detected using the other technique, indicating potential complementary roles.[16,17] In fact, the addition of plasma testing to tissue increases therapeutic target detection for patients with metastatic non–small-cell lung cancer by 15%.[18] Moreover, positive concordance among frequent alterations varies across genes and decreases with longer time intervals between the collection of tissue and blood.[19]

To expand upon these findings and guide future applications of concurrent liquid and solid biopsy NGS testing in mBC, a better understanding of their relationship is needed. Here, we assess the concordance and temporal relationship between tissue and cfDNA NGS data in paired solid and liquid biopsies from a large, real-world mBC data set.

METHODS

All analyses were completed using the Tempus LENS platform, which aggregates deidentified data from samples tested with the Tempus Platform and enables real-time cohort identification and analysis. Tissue and liquid biopsy samples were collected and processed as previously described, with cfDNA analyzed by using the Tempus xF assay and tissue by using the Tempus xT assay.[17,20] All xT versions were included, with the current version targeting 648 genes and previous iterations 596 genes. All versions detect single-nucleotide variants (SNVs), insertions/deletions (indels), copy-number variants, and select chromosomal rearrangements. The xF assay identifies SNVs and indels in a targeted panel of 105 genes from peripheral blood, copy-number variants in six genes, and chromosomal rearrangements in seven genes.

Within Tempus LENS, we randomly selected 300 deidentified records of patients with a stage IV mBC diagnosis. Each record required results from at least one tissue and one cfDNA test, along with known collection times and tissue biopsy sites. Only records from the same bioinformatic pipeline versions of each assay were selected. When records included multiple tissue or cfDNA tests, the pair with the shortest time interval between collection dates was chosen.

Variants were classified on the basis of recommendations from the AMP/ASCO/CAP/ClinGen Somatic working group and ACMG guidelines using proprietary software and databases. CHIP variants were not specifically excluded, nor tissue germline variants if identified as pathogenic in cfDNA (included 25 variants across the data set). We focused on concordance of detected pathogenic variants (SNVs and indels) in overlapping genomic regions, resulting in 104 genes. Variants detected at variant allele frequencies (VAFs) below their respective assay's limit of detection (LOD) were filtered out (xT LODs: SNV = 0.05 and indel = 0.1; xF LODs: SNV = 0.0025 and indel = 0.005).[17,20]

The frequencies of pathogenic variants from each assay were compared for individual patient records and among the entire data set. Then, various metrics of concordance were assessed (Fig 1A), including gene-level variant concordance, concordance of variants present in more than three patients (> 1% of the cohort, deemed reoccurring variants), patient-level variant concordance, and cohort-matched variant concordance, as defined below.

- Gene-level variant concordance: all pathogenic variants detected within a particular gene for a given patient.
- Mutually exclusive gene-level concordance: genes with alterations detected in both tissue and cfDNA, but never the same variant.
- Partial gene-level concordance: at least one variant of a given gene was detected in both assays but additional variants in the same gene were mutually exclusive.
- Patient-level variant concordance: the total number of unique pathogenic variants in both tissue and cfDNA,

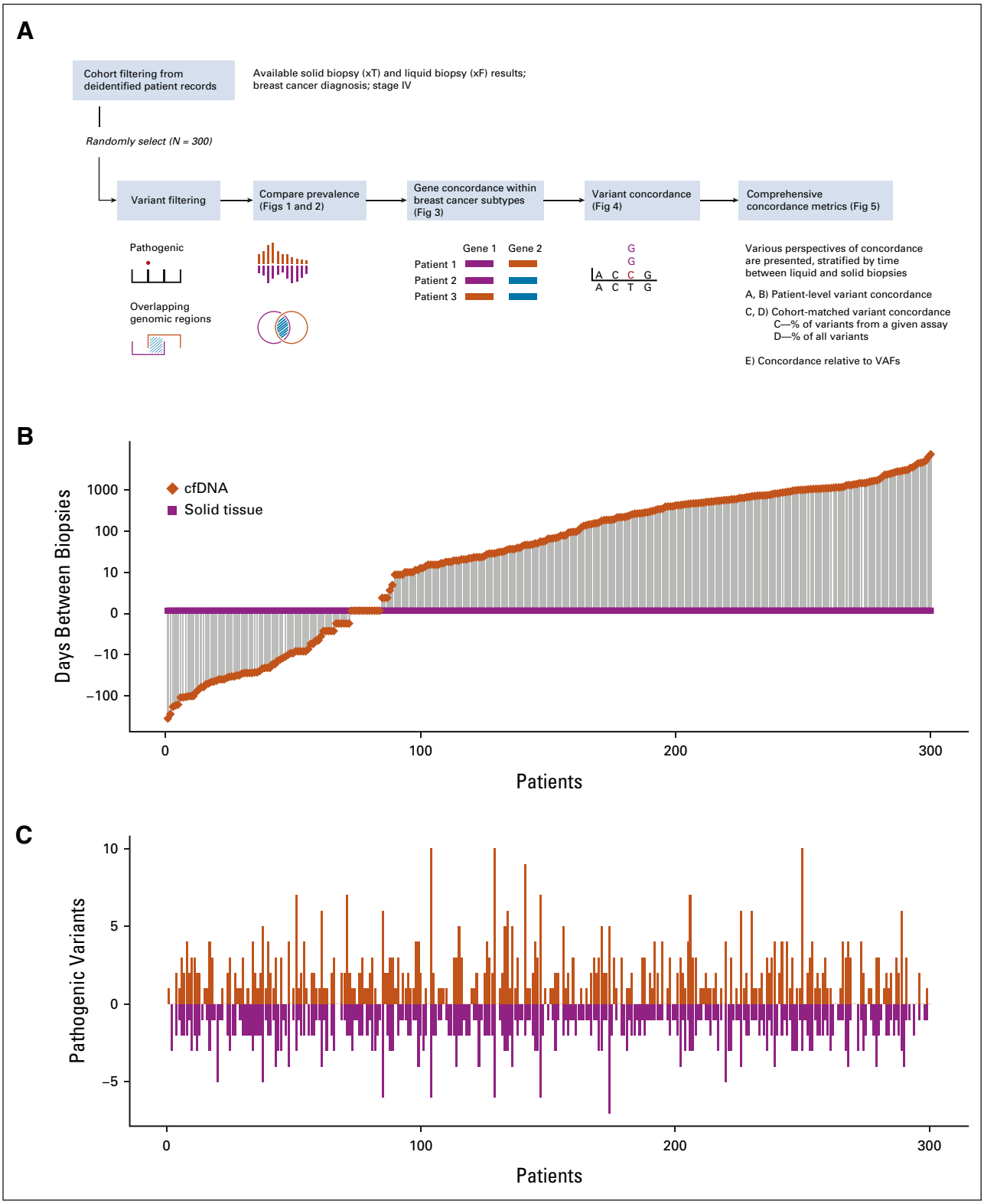

FIG 1. (A) A schema representing cohort selection and analysis using the Tempus LENS platform, along with an overview of the comparisons between solid and liquid biopsy NGS presented in the study. (B) Time between tissue (purple square) and liquid (orange diamond) biopsies in 300 patients with metastatic breast cancer. (C) Pathogenic variants identified in tissue (purple) or cfDNA (orange) within overlapping genomic regions of the assays. cfDNA, cell-free DNA; NGS, next-generation sequencing; VAF, variant allele frequency.

divided by all unique pathogenic variants identified on either test for each patient record. Variants were considered concordant if reported by both tissue and cfDNA assays for a given record, and discordant if only reported in one assay.

- Cohort-matched variant concordance: the number of intrapatient concordant variants divided by either all variants across the data set for each patient record or all variants from a given assay for each patient record.

Patient records with no pathogenic variants were excluded from patient-level and cohort-matched variant concordance calculations. VAFs from each assay were stratified by concordance and time between tests and plotted for comparison. *P* values are from two-sided Wilcoxon Rank-Sum tests, with the exception of the cohort-matched variant concordance comparisons between time intervals which are from two-sided Fisher's Exact tests.

RESULTS

Data Set Characteristics

Using the Tempus LENS platform, we analyzed 300 patients with mBC who underwent sequencing with at least one tissue and one cfDNA assay during their routine clinical care (Fig 1A). Clinical characteristics from patient records included in the final data set are presented in Table 1.

Use Patterns of Liquid Biopsy and Tissue NGS Testing in mBC

We first investigated patterns of tissue and cfDNA sequencing use among clinicians (Fig 1B), with tissue collection date considered time point zero. Most cfDNA tests were follow-ups to tissue sequencing. Median time between tissue collection and blood draw was 78.5 (standard deviation [SD] = 642.99) days (Fig 1B). For more than half (52%) of the submitted samples, the time interval between solid and liquid biopsies was < 100 days (Table 1, Fig 1B). Although concordance analyses were restricted to pairs of one cfDNA and one tissue test per patient, 14% of the data set (n = 43/300) had multiple cfDNA tests (median = 2, SD = 0.70, of patients with multiple tests) and 9% (n = 28/300) had multiple tissue tests (median = 2, SD = 0.26, of patients with multiple tests).

Landscape and Prevalence of Pathogenic Variants Detected in Tissue and Cell-Free DNA

The median number of pathogenic variants reported per record in overlapping probe regions was two for tissue and one for cfDNA (range = 0-7 and 0-10, respectively; Figures 1C, 2A, and 2B). Furthermore, only 6% of patient records (n = 19) had no alterations detected in either test, meaning most (94%) mBC cases had at least one pathogenic alteration in either tissue, cfDNA, or both (Fig 1C). Overall, the distributions of pathogenic variant frequencies in tissue and cfDNA were similar across the data set (Fig 2A), although relatively more patients had no pathogenic variants detected in cfDNA (28%, n = 84) compared with tissue (11.7%, n = 35). Conversely, more patients had ≥ 5 pathogenic variants in cfDNA (7%, n = 21) compared with tissue (2.7%, n = 8). Likewise, the landscape and prevalence of the 25 most frequently identified mutations were generally similar between tissue and cfDNA (Fig 2C), with some small deviations. Across results from the data set, the five most frequently detected pathogenic variants were *TP53* (44% of tissue, 40% of cfDNA, and 52% of patients when combining results from either test), *PIK3CA* (29% of tissue, 24% of cfDNA, and 33% of either), *ESR1* (15% of tissue, 16% of cfDNA, and 22% of either), *CDH1* (11% of tissue, 9% of cfDNA, and 12% of either), and *GATA3* (10% of tissue, 7% of cfDNA, and 11% of either).

Gene-Level and Reoccurring Variant Concordances

The data set was then subdivided by mBC subtypes to assess gene-level variant concordance and prevalence. Figure 3 displays gene-level variant concordances in the most frequently mutated genes within each subtype (patients without variants in these genes are not displayed; top to bottom = descending frequency order). *TP53* was the most frequently mutated gene across all subtypes, followed by *PIK3CA*. Although *ESR1* was third most frequent for both luminal A and luminal B samples, neither human epidermal growth factor receptor 2-enriched nor triple-negative had *ESR1* in the most frequently mutated (Figs 3A-3D). Notably, all *ESR1* mutations in luminal B cancer were detected by liquid biopsy–only (Fig 3B). Among triple-negative cases with *BRCA1* (n = 6) or *PIK3CA* variants (n = 13) in either assay (Fig 3D), all *BRCA1* results were concordant, as were most *PIK3CA* (n = 8 perfectly concordant, n = 9 including partially concordant). Appendix Figure A1 shows results from the incomplete category.

After assessing gene-level concordance by subtype, we focused on only variants identified in > 3 patients (> 1% of the cohort). Across all records, concordances in these reoccurring pathogenic variants were largely gene-dependent (Fig 4). For example, detection of variants in *PIK3CA* were predominantly concordant, besides p.His1047Leu where more patients had the variant in tissue-only (n = 4) compared with liquid biopsy–only (n = 1) or both (n = 2). Contrastingly, all *ESR1* variants were more likely to be identified when analyzing cfDNA (cfDNA-only or concordant) than tissue samples alone.

Patient-Level Variant Concordance and Temporal Patterns of Tissue and Cell-Free DNA Tests

Among all records, 81% of results had at least one concordant variant or no pathogenic variants identified in either test when samples were taken ≤ 7 days apart. This concordance dropped to 72% when > 7 but < 30 days apart, and further decreased to 56%-64% when > 30 days apart (Fig 5A). When evaluating patient-level variant concordance, which excluded those with concordance due to lack of pathogenic variants from Figure 5A, we observed high concordances between tissue and cfDNA results that generally decreased with longer intervals between testing.

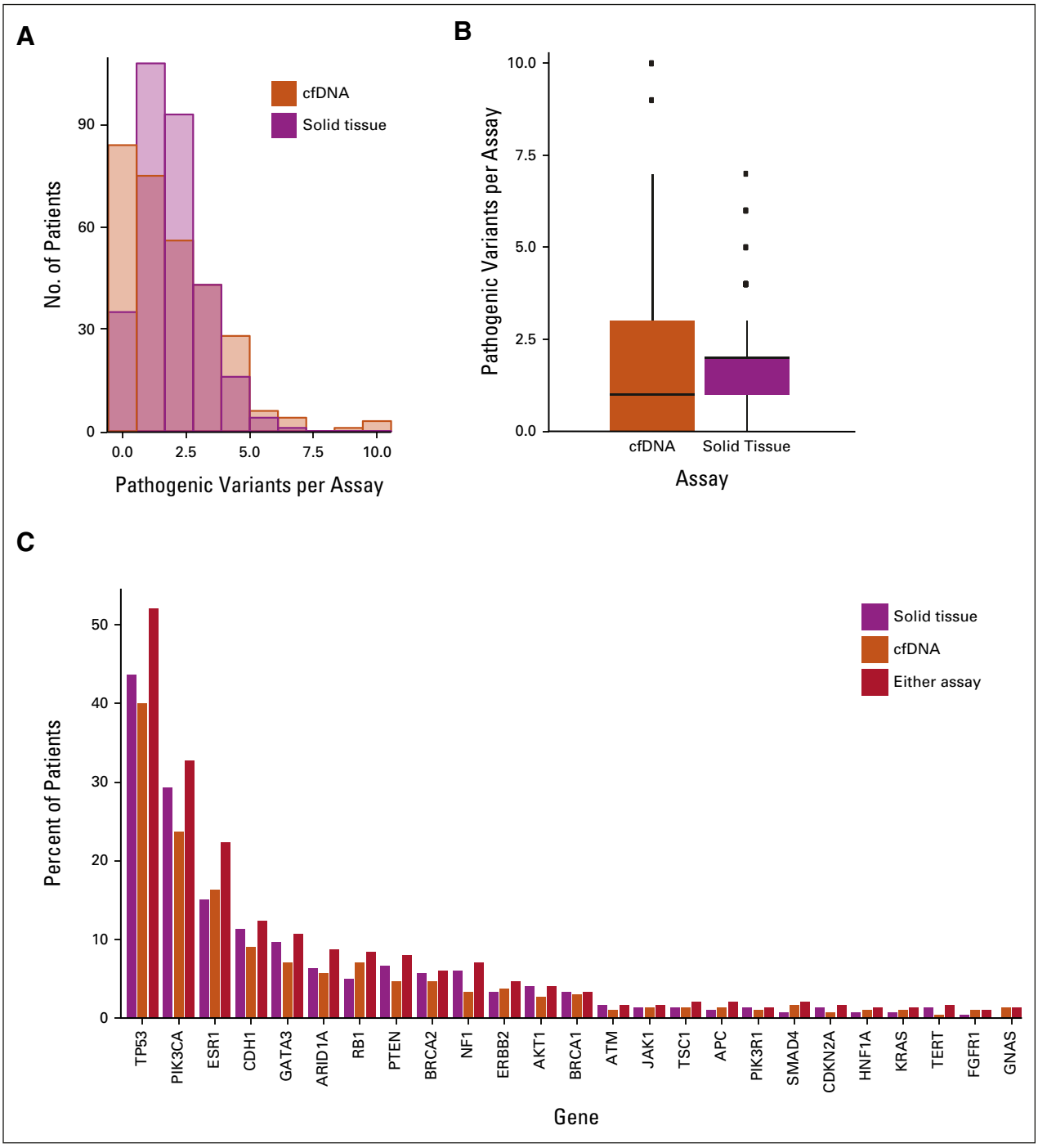

FIG 2. (A) The distribution of pathogenic variant frequencies detected within overlapping genomic regions of the tissue (purple) and cfDNA (orange) assays. (B) The median number of pathogenic variants detected per patient record in cfDNA (orange) and tissue (purple). (C) The top 25 most frequently detected pathogenic variants in cfDNA (orange), tissue (purple), and either test (red). cfDNA, cell-free DNA.

Median patient-level variant concordance was 67% when tests were ≤ 7 days apart, 50% when > 7 but < 30 days, and 30%-37% when > 30 days apart (Fig 5B). Only patient-level variant concordances between ≤ 7-day and > 365-day testing intervals were significantly different after multiple-test correction (false discovery rate-adjusted $P = .015$).

Cohort-Matched Variant Concordance and Temporal Patterns of Tissue and Cell-Free DNA Results

Next, cohort-matched variant concordance was assessed, wherein pathogenic variants from each patient record relative to all pathogenic variants identified in the cohort were compared between tissue and cfDNA. When tissue and blood collection were ≤ 7 days apart, 77.8% of

TABLE 1. Clinical Characteristics of the Data Set (N = 300)

Category	Classification or Value	Patients, No. (%)
Sex	Female	296 (98.67)
	Male	4 (1.33)
Self-reported race	American Indian or Alaska Native	1 (0.33)
	Asian	8 (2.67)
	Black or African American	32 (10.67)
	Native Hawaiian or Other Pacific Islander	1 (0.33)
	Not reported	81 (27.00)
	Other race	12 (4.00)
	White	165 (55.00)
Tissue biopsy site	Liver	83 (27.67)
	Breast/nipple	78 (26.00)
	Lung/thorax/pleura	41 (13.67)
	Lymph nodes	36 (12.00)
	Bone	18 (6.00)
	Brain	16 (5.33)
	Abdomen/peritoneum/ trunk	12 (4.00)
	Other (n = 1 per site)	8 (2.67)
	Head, face, or neck	6 (2.00)
	Colon	2 (0.67)
Smoking status	Current smoker	16 (5.33)
	Ex-smoker	58 (19.33)
	Never smoker	151 (50.33)
	Unknown	75 (25.00)
Subtype	Luminal A	156 (52.00)
	Triple-negative	57 (19)
	Luminal B	16 (5.00)
	HER2-enriched	12 (4.00)
	Incomplete	59 (20)
Days between solid and liquid biopsy	≤ 7 days	42 (14.00)
	> 7 and ≤ 30 days	68 (22.67)
	> 30 and ≤ 100 days	46 (15.33)
	> 100 and ≤ 365 days	59 (19.67)
	> 365 days	85 (28.33)
Median age (SD) at tissue biopsy, years	57.351 (12.601)	300 (100)
Median time (SD) between tissue and cfDNA biopsies, days	78.5 (642.99)	300 (100)

Abbreviations: cfDNA, cell-free DNA; HER2, human epidermal growth factor receptor 2; SD, standard deviation.

pathogenic tissue variants were found within each patient's cfDNA (n = 56 concordant/72 total tissue variants) and 75.7% of pathogenic cfDNA variants were found within each patient's tissue (n = 56 concordant/74 total cfDNA variants; Fig 5C), with 62% of all variants detected in both assays, 20% in cfDNA-only, and 18% in tissue-only (Fig 5D). Like patient-level variant concordance, cohort-matched variant concordance generally decreased as the time between tests increased (Fig 5C and Fig 5D). When tests were > 365 days apart, for example, 50.3% of pathogenic tissue variants were found within each patient's cfDNA (n = 73 concordant/145 total tissue variants) and 51.8% of pathogenic cfDNA variants were found within each patient's tissue (n = 73 concordant/141 total cfDNA variants; Fig 5C), with 34% of all variants detected in both assays, 32% in cfDNA-only, and 34% in tissue-only (Fig 5D).Furthermore, this decrease in concordance over time was statistically significant when comparing ≤ 7 days to all other time intervals except for ≤ 30 days (> 30 but ≤ 100 Days: P = 2.6e-03, > 100 but ≤ 365 Days: P = 1.7e-04, and > 365 days: P = 8.2e-06).

Variant Allele Frequencies of Discordant and Concordant Variants

To investigate discordances between tissue and cfDNA, we next examined the relationship between VAF and concordance. In almost all cases, the median VAFs of discordant variants were lower than those of concordant variants for both tissue and cfDNA (Fig 5E). Across all time periods, the cfDNA VAFs were statistically different between concordant and cfDNA-only variants (≤ 7 days: P = .026, > 7 but ≤ 30 days: P = 1.89e-13, > 30 but ≤ 100 days: P = 1.04e-4, > 100 but ≤ 365 days: P = 1.79e-7, and > 365 days: P = 7.65e-6). Furthermore, only the three longest time periods had significantly different tissue VAFs between concordant and tissue-only variants (> 30 but ≤ 100 days: P = 7.53e-04, > 100 but ≤ 365 days: P = 4.12e-5, and > 365 days: P = 1.6e-3). Interestingly, the differences in tissue VAFs between concordant and tissue-only variants for both ≤ 7 days and > 7 but ≤ 30 days were not significantly different.

DISCUSSION

Liquid biopsy NGS has the potential to overcome the issue of tumor heterogeneity and improve our understanding of changes in tumor biology over time, ultimately enhancing precision oncology approaches in breast cancer. cfDNA may be particularly useful in metastatic settings, as circulating tumor cell levels increase alongside progression[21,22] and mBCs may harbor newly developed alterations not reflected in the baseline breast sample.[23] In this real-world data set, assay use patterns suggested interest in concurrent plus longitudinal testing among clinicians who order both cfDNA and tissue NGS for mBC, as 14% of biopsy pairs were collected within 7 days of each other and most cfDNA tests were ordered as follow-up to tissue sequencing. These use patterns confirm the importance of understanding the tissue and cfDNA NGS relationship to interpret concurrent and temporally distant results in clinical settings. Compared with more targeted approaches such as droplet digital polymerase chain reaction, however,

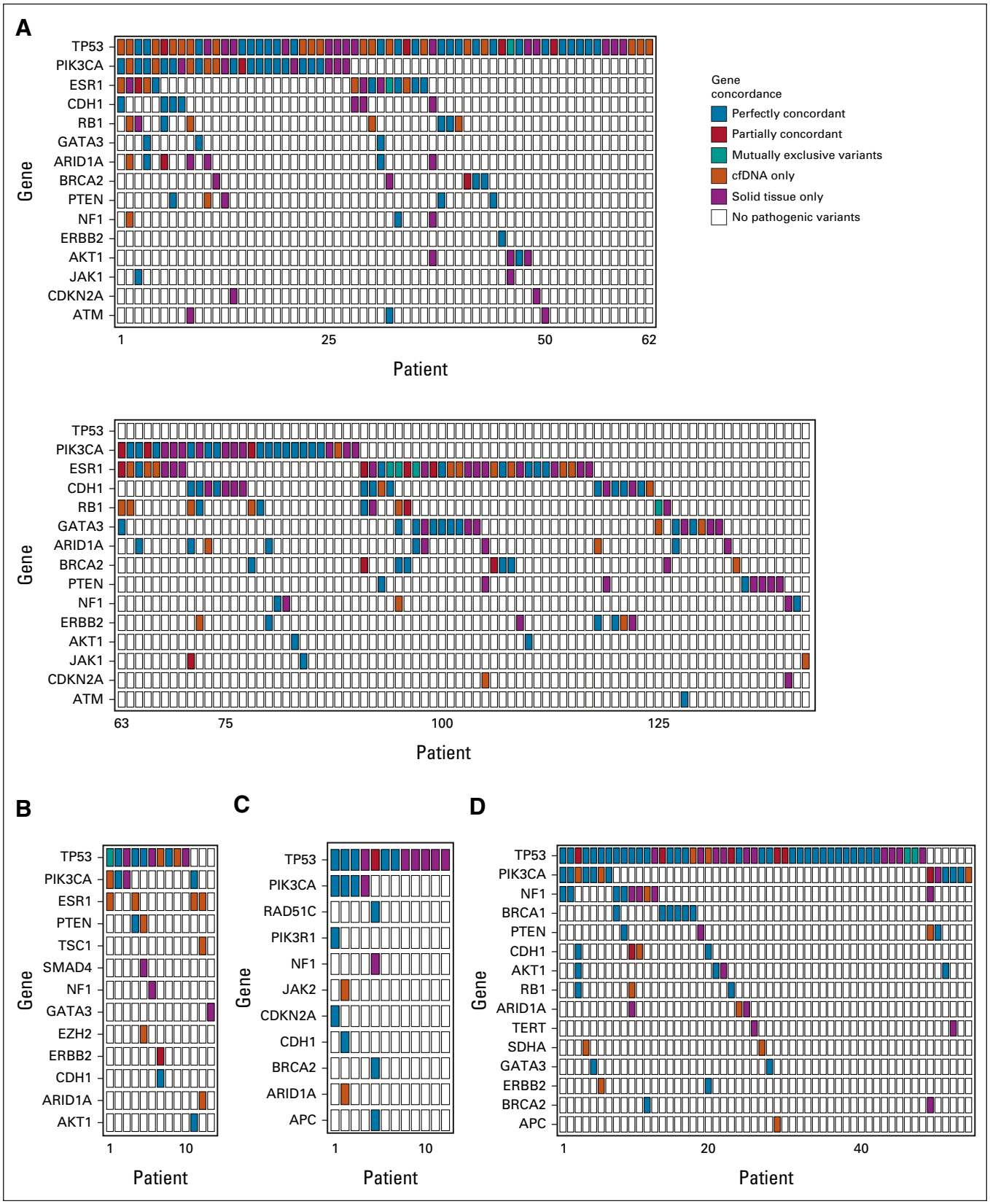

FIG 3. Pathogenic variant detection and gene-level concordance in the top detected variants by breast cancer subtype: (A) luminal A (n = 156), (B) luminal B (n = 16), (C) HER2 enriched (n = 12), or (D) triple-negative (n = 57). The results for each gene are classified as concordant (blue), partially concordant (red), mutually exclusive (teal), cfDNA-only (orange), or tissue-only (purple). All other variants were not detected (white). Patients with no pathogenic variants within the displayed genes are not shown. cfDNA, cell-free DNA; HER2, human epidermal growth factor receptor 2.

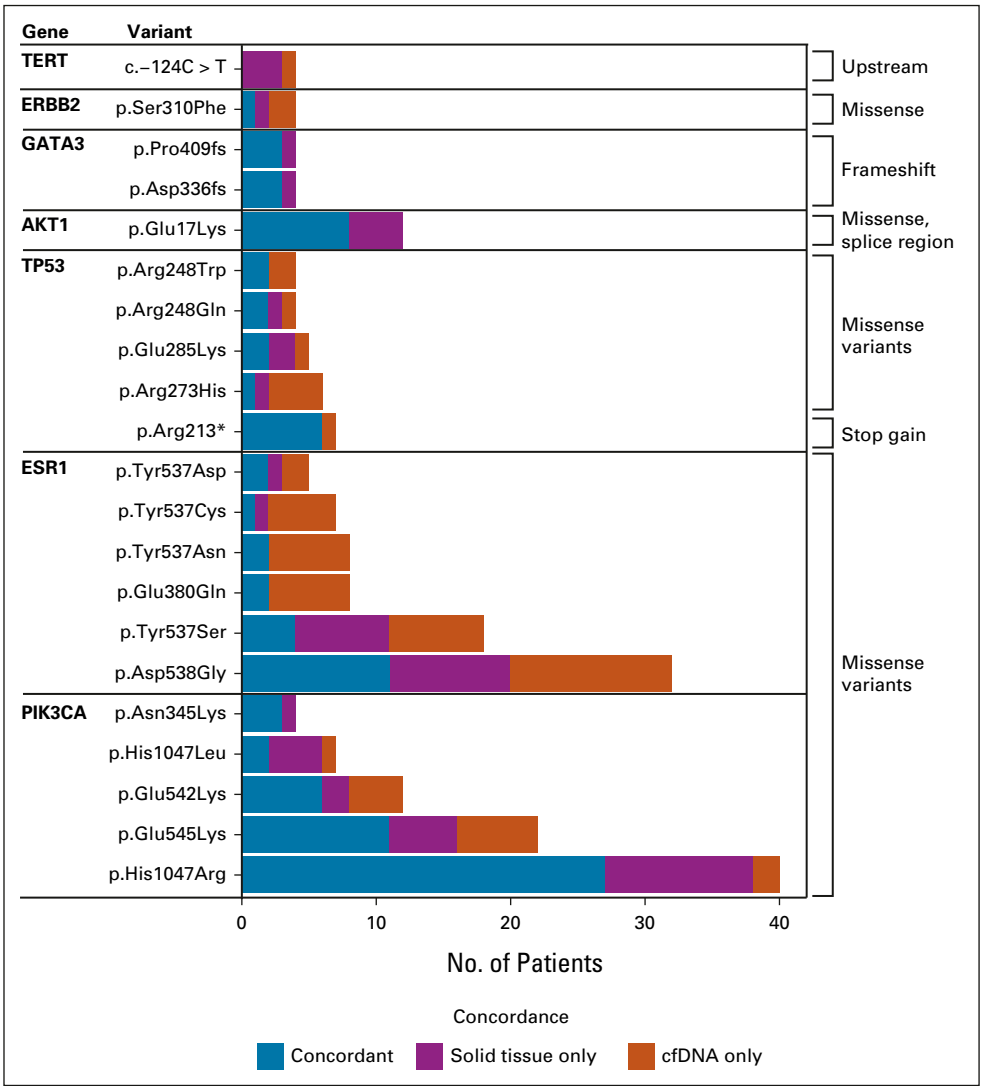

FIG 4. Concordance of variants identified in > 1% of patients (>3) across the data set, depicting the number of patients with concordant (blue), tissue-only (purple), or cfDNA-only (orange). The functional impact of these variants (missense, stop gain, missense in splicing regions, frameshift, or upstream) are annotated on the right. cfDNA, cell-free DNA.

studies specifically showing the reliability of ctDNA NGS against tissue results in mBC are scarce.[24] Previous efforts were limited by small sample sizes and lack of standardization between NGS panels over time, which ultimately restricted conclusions regarding concordance and temporal effects.[9,25-28] To facilitate a more comprehensive analysis of tissue and cfDNA concordance in mBC here, we selected a large, real-world cohort with known time intervals between matched biopsies, including only comparable versions of each assay and restricting analyses to overlapping genomic regions.

When broadly assessing the prevalence and landscape of pathogenic variants, SNVs/indels were similar between assays and reflected the known landscape of mBC.[29] Subtype-specific variant prevalence also followed expected trends where, for example, *ESR1* was highly prevalent in estrogen receptor–positive but not estrogen receptor–negative subtypes. This survey of the pathogenic landscape implies the reliability of cfDNA NGS to capture variants detected in tissue, as frequencies of top variants followed the same pattern in tissue and cfDNA. For all genes with pathogenic variants in >5% of patients, however, the percentage of patients with detected variants was highest when considering the results from either assay. Thus, combined testing may detect variants missed by either method alone.

Further supporting this conclusion was the distribution of pathogenic variants detected per patient by each assay. The median number of variants identified per patient record was higher in tissue than cfDNA, similar to previous reports although less disparate,[9,25] and relatively more patients had zero pathogenic variants identified in cfDNA. Contrarily, more patients had cfDNA results with > 5 variants detected compared with tissue. Given the cohort entirely comprised patients with metastatic disease, this could be due to a broader scope of tumor heterogeneity achievable with cfDNA NGS as an aggregate of detectable ctDNA from all metastatic lesions.[30] Another possible explanation is emerging mutations, as most cfDNA tests were conducted as follow-ups to tissue sequencing. The

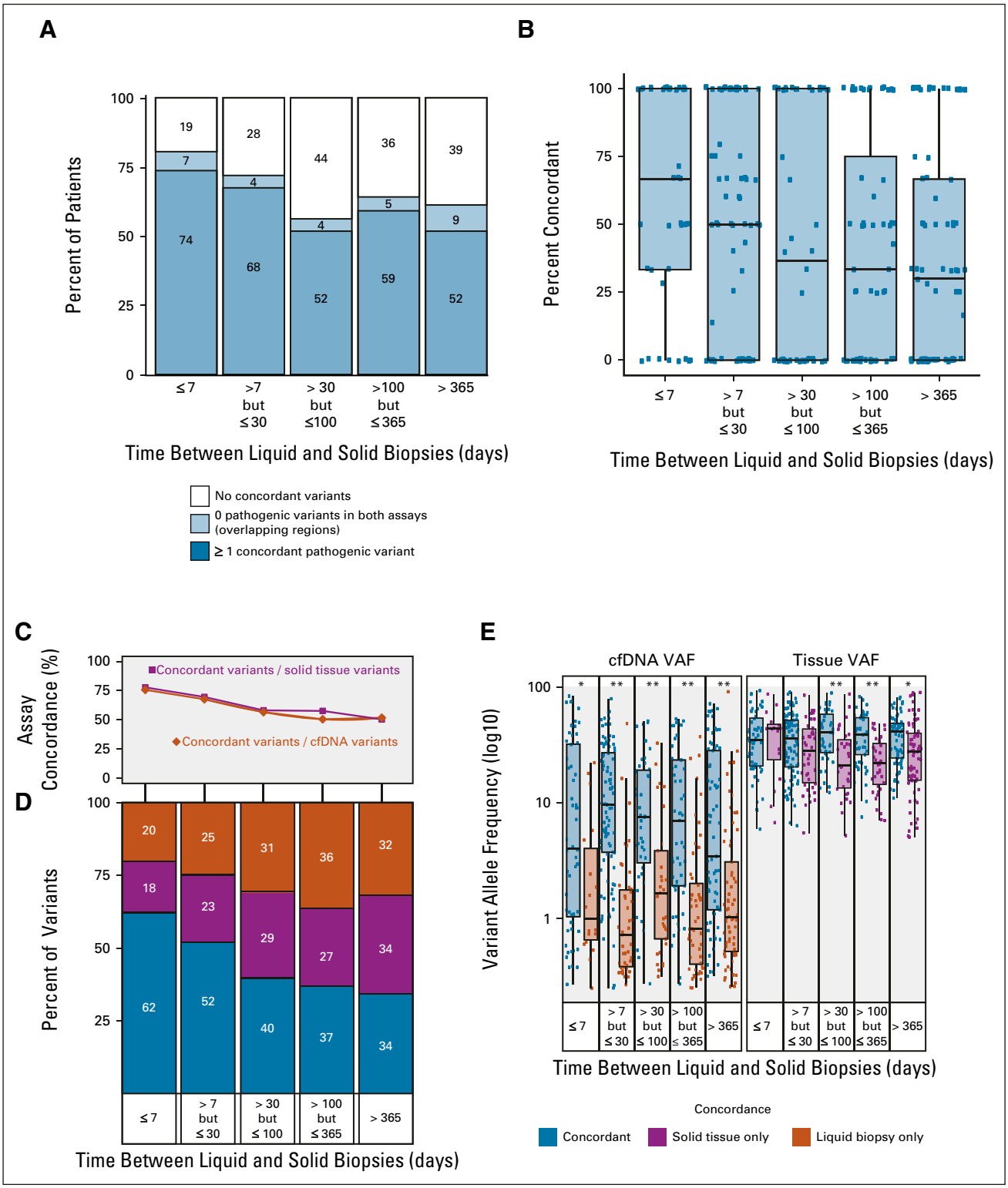

FIG 5. (A) The percent of patients with concordant tissue and cfDNA results over time. Cases are classified as discordant (white), concordant because of the presence of at least one pathogenic variant identified in both tests (dark blue), or concordant because of both tests resulting in no identified pathogenic variants within the overlapping genomic regions (light blue). (B) Patient-level variant concordance showing the percentage of concordant results per patient record, excluding concordance because of no pathogenic variants identified in either test, separated by days between tissue and cfDNA tests. Each dot represents a single patient record. Cohort-matched variant concordance over time by (C) pathogenic tissue variants (purple) or pathogenic cfDNA variants (orange), and (D) all variants that are concordant (blue), only identified in the patient's tissue (purple), or only identified in the patient's cfDNA (orange). (E) Concordance between tissue and cfDNA VAFs over time, separated by concordant variants (blue), tissue-only variants (purple), or cfDNA-only variants (orange). $*P < .05$; $**P < .001$. cfDNA, cell-free DNA; VAF, variant allele frequency.

possibility of emerging mutations was also evident in the gene-dependent concordances observed, where all *ESR1* variants were more likely to be detected in cfDNA than tissue. Additionally, all *ESR1* mutations in luminal B samples were exclusively detected by using cfDNA sequencing. These results are particularly interesting as *ESR1* mutations confer endocrine resistance and may carry treatment implications.[31] Conversely, certain variants in *PIK3CA* were detected at higher rates in tissue than cfDNA. Again, these gene-dependent concordances varying by assay suggest an advantage of combined tissue and cfDNA testing compared with either alone.

The main purpose of this study was to thoroughly assess the relationship between patient results from tissue and cfDNA by examining concordance from multiple perspectives. Although a valuable metric for comparing detection of variants within genes, previous studies show that gene-level comparison alone may portray higher concordance than broader scopes.[9] Other previous efforts have included all variants, not only pathogenic, or removed cases with no pathogenic variants detected in cfDNA even when found in tissue, both of which could cause overestimates of concordance.[16] To avoid potential overestimation, we focused on overlapping regions and showed patient results regardless of the number of identified pathogenic variants, and included a cohort-matched concordance to provide the most comprehensive analysis possible. Cumulatively, we observed high concordances between tissue and cfDNA results that decreased with longer intervals between sample collection. This result was similar to the findings of Schwaederle et al,[19] although here we evaluated a larger cohort and used a different NGS platform. The high concordances observed demonstrate that cfDNA NGS reliably represents tissue genomics, which was primarily of interest, but the decrease in concordance over time also suggests a possible evolution of tumor genomics measurable by cfDNA NGS.

Finally, we confirmed prior observations of significantly lower VAFs in discordant compared with concordant variants,[9,32] where VAFs were statistically different between concordant and cfDNA-only variants for all intertest time spans. However, tissue VAFs between concordant and tissue-only variants were not significantly different within the two shortest time frames but were for samples collected $>$ 30 days apart. One possible explanation is the presence of CHIP variants, which appear more frequently in cfDNA analyses relative to tissue,[33] or the increased possibility for false-positive calls at low VAFs.[13,14] Alternatively, these results may indicate that liquid biopsy can identify subclonal variants within primary tumors or metastatic lesions, and discordant variants may be due to the loss or emergence of new subclones. The lack of significant differences between concordant and tissue VAFs within the two shortest time frames may also highlight the ability of liquid biopsy to sample molecular heterogeneity across multiple metastatic sites at the same time. This interpretation is further supported by our observation that the median VAFs of concordant liquid biopsy variants were higher than those of discordant variants across all time points.

Although illuminating as an initial assessment of liquid biopsy NGS feasibility in mBC, the retrospective design here is limiting. Prospective studies incorporating treatment and outcomes data are necessary to fully evaluate utility. Nonetheless, these results suggest that concurrent testing with tissue and liquid biopsies, along with sequential cfDNA NGS, are informative for monitoring molecular changes in mBC. Primarily, the findings demonstrate that cfDNA is a reliable metric of tumor genomics and may provide additional insights when combined with tissue sequencing.

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Stock and Other Ownership Interests: Thorne HealthTech
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Consulting or Advisory Role: NanoString Technologies

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Stock and Other Ownership Interests: Loxo, Celcuity
Consulting or Advisory Role: Horizon Discovery, Loxo, Casdin Capital, Jackson Laboratory for Genomic Medicine, Celcuity, Sermonix Pharmaceuticals, Hologic, EQRx
Research Funding: AbbVie, Pfizer, GE Healthcare, Lilly
Patents, Royalties, Other Intellectual Property: Royalties paid through inventions at Johns Hopkins University by Horizon Discovery Ltd
Travel, Accommodations, Expenses: Lilly, Loxo
Uncompensated Relationships: Tempus

No other potential conflicts of interest were reported.

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APPENDIX

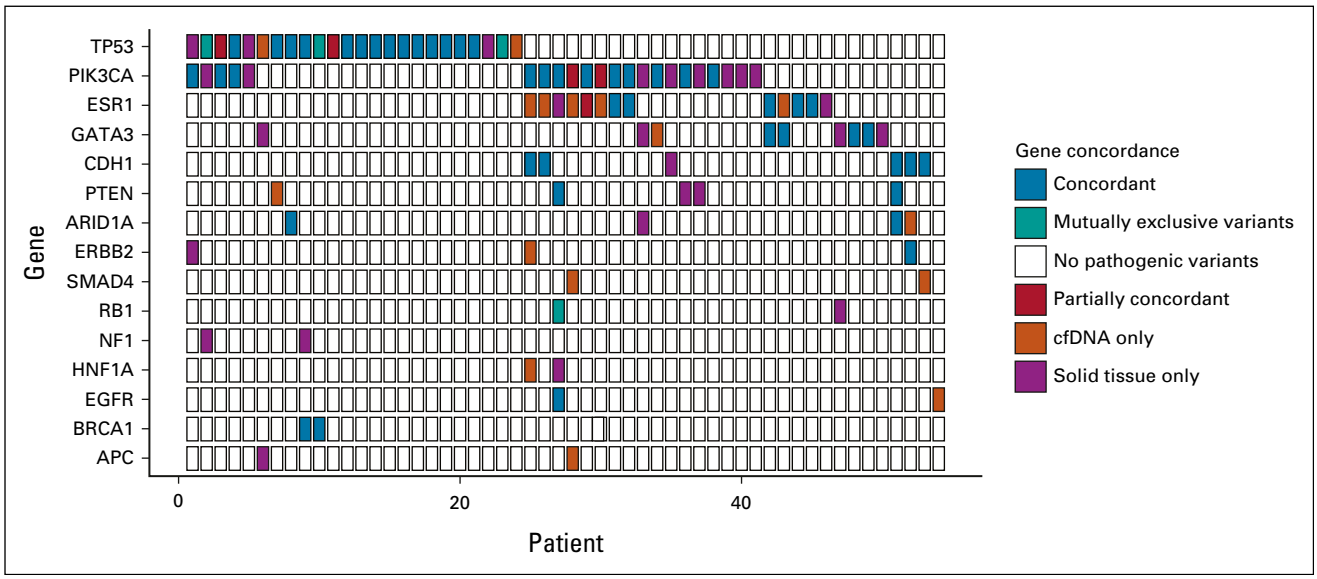

FIG A1. Pathogenic variant detection and gene-level concordance in the top 15 most frequently detected variants among breast cancer subtypes categorized as incomplete. The category incomplete (20%, n = 59) consists of patient records with insufficient receptor status data to define breast cancer subtypes, including statuses such as ER–/PR–/HER2+/HER2– (n = 2), ER+/ER–/PR–/HER2– (n = 2), or ER+ (n = 14). The results for each gene are classified as concordant (blue), partially concordant (red), mutually exclusive (teal), cfDNA-only (orange), or tissue-only (purple). All other variants were not detected (white). Patient records with no pathogenic variants within the displayed genes are not shown. cfDNA, cell-free DNA; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; VAF, variant allele frequency.