Circulating tumour DNA characterisation of invasive lobular carcinoma in patients with metastatic breast cancer

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Summary

Background Limited data exist to characterise molecular differences in circulating tumour DNA (ctDNA) for patients with invasive lobular carcinoma (ILC). We analysed metastatic breast cancer patients with ctDNA testing to assess genomic differences among patients with ILC, invasive ductal carcinoma (IDC), and mixed histology.

Methods We retrospectively analysed 980 clinically annotated patients (121 ILC, 792 IDC, and 67 mixed histology) from three academic centers with ctDNA evaluation by Guardant360™. Single nucleotide variations (SNVs), copy number variations (CNVs), and oncogenic pathways were compared across histologies.

Findings ILC was significantly associated with HR+ HER2 negative and HER2 low. SNVs were higher in patients with ILC compared to IDC or mixed histology (Mann Whitney U test, P < 0.05). In multivariable analysis, HR+ HER2 negative ILC was significantly associated with mutations in CDH1 (odds ratio (OR) 9.4, [95% CI 3.3–27.2]), ERBB2 (OR 3.6, [95% confidence interval (CI) 1.6–8.2]), and PTEN (OR 2.5, [95% CI 1.05–5.8]) genes. CDH1 mutations were not present in the mixed histology cohort. Mutations in the PI3K pathway genes (OR 1.76 [95% CI 1.18–2.64]) were more common in patients with ILC. In an independent cohort of nearly 7000 metastatic breast cancer patients, CDH1 was significantly co-mutated with targetable alterations (PIK3CA, ERBB2) and mutations associated with endocrine resistance (ARID1A, NFI, RB1, ESR1, FGFR2) (Benjamini–Hochberg Procedure, all q < 0.05).

Interpretation Evaluation of ctDNA revealed differences in pathogenic alterations and oncogenic pathways across breast cancer histologies with implications for histologic classification and precision medicine treatment.

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Introduction

Invasive lobular carcinoma (ILC) represents the second most common histology of breast cancer, accounting for approximately 10–15% of cases.1,2 With an estimated 281,550 cases of breast cancer in women diagnosed in 2021, ILC may represent over 30,000 cases per year.3 Patients with ILC are characterised by loss of E-cadherin in the vast majority of cases resulting in a linear, single-cell appearance on histology.1 This histologic pattern leads to a clinical phenotype of patients with metastatic ILC having a greater frequency of bone, gastrointestinal, omental, and ovarian metastases and a...
lower frequency of lung and liver metastases. Despite the distinct pattern of metastatic spread, unique strategies for treating patients with ILC remain limited.

Prior studies evaluating ILC using tissue-based sequencing have defined a distinct genomic profile compared to patients with invasive ductal carcinoma (IDC). The most common genomic alterations for patients with ILC are in CDH1, PIK3CA, TP53, CCND1, and FGFr19 genes. In primary tissue, alterations in AKT1, CDH1, FOXA1, HER2, HER3, TBX3 and PTEN loss were associated with luminal ILC, while GATA3 mutation was associated with IDC. For patients with metastatic ILC, mutations in CDH1, NF1, PIK3CA, and TBX3 were more commonly detected compared to patients with metastatic IDC. Tumour mutational burden (TMB) also appeared higher in patients with ILC compared with IDC, particularly when metastatic sites were biopsied, suggesting increased genomic complexity of metastatic sites for patients with ILC. In addition to mutational differences, differences in immune infiltration and copy number changes have been observed in patients with ILC.

Despite emerging genomic differences between ILC and IDC, clinically, treatment for both histologic subtypes remains similar despite suggestions of lower efficacy of chemotherapy for patients with ILC. In addition, the clinical outcomes of patients with ILC remain controversial with some studies suggesting worse long-term outcomes for patients with ILC, while other studies have reported better or similar long-term outcomes. Due to this uncertainty, recent work has attempted to characterise specific alterations and gene signatures associated with prognosis. Treatment strategies incorporating immune checkpoint inhibitors and other novel agents are currently under investigation.

The evaluation of ctDNA represents a less invasive technique compared to tissue biopsies to assess genomic alterations and clonal evolution. Prior studies have demonstrated a relatively high concordance between blood and tissue next-generation sequencing (NGS) with expected differences due to biological factors and sampling variability. A potential advantage of ctDNA testing is the ability to capture spatial and temporal heterogeneity. However, few studies have explored differences in ctDNA for patients across breast histologies.

In this study, we characterised differences in ctDNA based on histology using a large, multi-institutional cohort of metastatic breast cancer patients who underwent clinical testing using a uniform ctDNA NGS assay. We hypothesised that differences in ctDNA could be detected across breast cancer histologies. The study objectives were to define differences in mutation frequency, copy number alterations, and oncogenic pathways across histologic subtypes. These data have important implications for characterising genomic differences across histologies using ctDNA for future precision medicine approaches for patients with metastatic ILC.

Methods

Patient selection and study design
This retrospective cohort study included data from patients with metastatic breast cancer combined under a data use agreement and approved by the institutional review boards (IRB) of three sites: Washington...
University School of Medicine (St. Louis, MO; IRB#202101147), Northwestern University (Chicago, IL; IRB#STU00214133) and Massachusetts General Hospital (Boston, MA; IRB#2013P000848). The requirement for informed consent was waived by the IRB for this de-identified analysis. Data were shared using a data use agreement that was signed by the principal investigator from each site. The study was performed in concordance with the Health Insurance Portability and Accountability Act and the Declaration of Helsinki. All patients included in the study had ctDNA testing with plasma-based genotyping performed by Guardant360™ (Redwood City, CA). A total of 980 patients were included in the analysis with one plasma sample analysed per patient. Plasma samples were collected at baseline (prior to any treatment initiation) or at the time of clinical progression prior to initiation of the next line of therapy. At each site, manual chart review was performed to review clinical, pathological, treatment, and outcome data. Sex was self-reported by study participants. Histological classification was defined based on review of original pathology reports from the primary tumour or from breast biopsies of patients with de novo metastatic breast cancer. ILC cases were classified based on standard pathological criteria including classic ILC and special ILC subtypes (e.g. pleomorphic ILC). Cases with both ductal and lobular features were classified as mixed histology (MXD) per standard pathological criteria.

ctDNA sequencing and analysis
ctDNA from each academic site was evaluated using the commercially available Guardant360™ assay (Guardant Health, Inc., Redwood City, CA) to evaluate up to 74 cancer-related genes as previously described.28–30 The NGS testing was performed as part of standard clinical care in a CLIA-certified and College of American Pathologists accredited laboratory. Blood was collected in two 10 mL Streck tubes and processed plasma was evaluated for single-nucleotide variants (SNVs), insertions-deletions (indels), gene fusions/rearrangements, and copy number variants (CNVs).31 Mutations were annotated using OncoKB to define pathogenic variants.32 Oncogenic pathways (RTK, RAS, RAF, MEK, NRF2, ER, WNT, MYC, P53, cell cycle, notch, PI3K) were defined based on prior work generated using The Cancer Genome Atlas (TCGA).32 To validate co-mutations identified with CDH1 in the academic site cohort described above, genomic results from an independent cohort of advanced, non-overlapping breast cancer patients testing clinically using the Guardant360™ 83-gene panel were retrospectively analysed from an IRB-approved protocol (Pro00034566/CR00218935) with a waiver of patient consent. These samples were sequenced from October 2021–March 2022 and included patients with mutational data but without histological classification.

Statistical analysis
Clinical and pathological variables were reported using descriptive analyses through frequencies for categorical variables or medians and interquartile range (IQR) for continuous variables. Mutational profiles were compared using Fisher’s exact test to assess differences in alteration frequency across histologic subtypes. Differences between the CDH1-altered and unaltered groups were calculated using the Benjamini–Hochberg test with q-values generated to correct for multiple testing. Univariable and multivariable logistic regression for features associated with ILC was performed to determine odds ratios (OR) and 95% confidence intervals (CI). Genes with at least 5 pathogenic alterations in both ILC and IDC were analysed through univariable models and selected for multivariable regression when significant.

Overall survival (OS) was defined from the time of baseline ctDNA collection to death from any cause with data censored at last follow-up if the patient was still alive. Lines of therapy and sites of disease were included in the multivariable model. Differences in OS were assessed using the log-rank test and Cox regression models and displayed using Kaplan–Meier plots. The proportional-hazards assumption was tested based on Schoenfeld residuals. Gene alterations were separately classified as CNVs and SNVs. Only pathogenic mutations based on OncoKB were included in the logistic and Cox regression models.

Statistical analysis was performed using STATA (StataCorp. (2019) Stata Statistical Software: Release 16.1. College Station, TX: StataCorp LP), JMP (SAS Institute Inc. (2019), version 16. Cary, NC), and R (R Core Team (2019), version 4.1.0. R Foundation for Statistical Computing, Vienna, Austria).

Role of funders
The funders had no role in the study design, data collection, data analyses, interpretation, or writing of the report.

Results
Cohort characteristics
The combined cohort from Washington University School of Medicine, Northwestern University, and Massachusetts General Hospital consisted of 980 patients with metastatic breast cancer who underwent uniform ctDNA testing at the time of diagnosis (e.g. de novo metastatic breast cancer) or at the time of clinical or radiographic progression prior to initiation of the next line of therapy. The cohort consisted of 121 patients with ILC (12.4%), 792 with IDC (80.8%), and 67 with MXD histology (6.8%) (Table 1). Patients with ILC were significantly more likely to be hormone-receptor positive (HR+) HER2 negative as compared to patients with IDC.
or MXD histology (89.0% vs. 66.4% vs. 83.6%, respectively, chi square, P < 0.001). There were 4 patients with HR negative HER2 positive ILC (3.3%) and 9 patients with triple negative breast cancer and ILC histology (7.4%). HER2 low subtype was significantly more common in patients with ILC (P = 0.001). Patients with ILC were significantly more likely to have bone metastasis and significantly less likely to have lung, liver, or lymph node metastasis (chi square, all P < 0.05).

ctDNA alterations

Alterations detected in ctDNA were evaluated in patients with ILC, IDC, and MDX histology. 20% of patients (238) had no detectable ctDNA alterations. No differences were observed in the percentage of patients with detectable alterations (e.g. ctDNA positivity) across histologies (80.2%, 79.3% and 82.1% for ILC, IDC, and MDX, respectively; P = 0.847). When combining pathogenic mutations and CNVs, the most common alterations in the MXD histology cohort is also shown (Fig. 1c).

The total number of ctDNA pathogenic alterations were compared among patients with ILC, IDC, and MDX (Supplemental Fig. S2). No differences were observed in terms of MAF of the dominant clone or when combining pathogenic mutations and CNVs. However, patients with ILC had a significantly higher number of pathogenic mutations compared to the IDC and mixed histology cohorts (median 3 [IQR 1–6] vs. 2 [IQR 0–4] vs. 2 [IQR 0–5]) and a significantly lower number of CNVs (Mann–Whitney U test, P < 0.05).

Across all patients, genomic differences were compared across patients with ILC and IDC. In univariable modeling, significant differences were detected in the SNVs of the following genes: CDH1, FGFR2, IDH1, MYC, NF1, PDGFRA, RB1, TERT (Fisher’s exact test; all P < 0.05) and the CNVs of the following genes: CCNE1, ERBB2, MYC, PDGFRA (Fisher’s exact test; all P < 0.05). Next, univariable logistic regression was performed limited to genes with at least 5 detected alterations in each subtype to ensure model stability. Mutations in genes that were significantly associated with ILC included CDH1, FGFR2, NF1, PTEN, RB1 (logistic regression, all P < 0.05) (Supplemental Table 1).

In multivariable logistic regression, CDH1 mutations were significantly more common in ILC (OR 12.6, [95% CI 4.5–35.4]) (Table 2). Given differences in the proportion of patients with HR+ HER2 negative breast cancer; CNS, central nervous system. N = 980.

| Total (N: 980) | IDC  | ILC  | MXD  | P value |
|---------------|------|------|------|---------|
| HR positive HER2 negative | 523  | 105  | 56   | <0.001  |
| HER2 positive | 120  | 4    | 4    |         |
| TNBC          | 145  | 9    | 7    |         |
| HER2 status   | 0.001|
| Negative      | 155  | 23   | 23   |         |
| Low           | 255  | 43   | 30   |         |
| Positive      | 120  | 4    | 4    |         |
| Lung metastasis (N: 978) | 5.33 | 105  | 49   | <0.001  |
| No            | 257  | 16   | 18   |         |
| Liver metastasis (N: 978) | 0.931|
| No            | 477  | 88   | 40   |         |
| Yes           | 313  | 33   | 27   |         |
| Bone metastasis (N: 978) | 0.001|
| No            | 286  | 22   | 14   |         |
| Yes           | 504  | 99   | 53   |         |
| Node metastasis (N: 978) | 0.008|
| No            | 451  | 87   | 38   |         |
| Yes           | 339  | 34   | 29   |         |
| Soft tissue metastasis (N: 978) | 0.589|
| No            | 647  | 98   | 50   |         |
| Yes           | 173  | 23   | 17   |         |
| CNS metastasis (N: 978) | 0.099|
| No            | 725  | 114  | 66   |         |
| Yes           | 65   | 7    | 1    |         |
| De novo metastatic disease (N: 841) | 0.832|
| No            | 507  | 80   | 51   |         |
| Yes           | 165  | 24   | 14   |         |
| Line of therapy (N: 769) | 0.469|
| 1             | 181  | 39   | 21   |         |
| 2             | 122  | 20   | 14   |         |
| 3             | 91   | 12   | 9    |         |
| 2-4           | 215  | 26   | 19   |         |
| Previous chemotherapy | 0.001|
| No            | 289  | 59   | 42   |         |
| Yes           | 298  | 33   | 20   |         |
| Previous endocrine therapy | 0.740|
| No            | 233  | 34   | 22   |         |
| Yes           | 354  | 58   | 40   |         |
| Previous mTOR inhibitors | 0.869|
| No            | 508  | 79   | 55   |         |
| Yes           | 79   | 13   | 7    |         |
| Previous PI3K inhibitors | 0.352|
| No            | 554  | 90   | 58   |         |
| Yes           | 33   | 2    | 4    |         |
| Previous CDK4/6 inhibitors | 0.133|
| No            | 383  | 52   | 35   |         |
| Yes           | 204  | 40   | 27   |         |

Table 1: Clinical pathological comparisons across patients with IDC, ILC, and mixed (MXD) histologies.
Fig. 1: Landscape of detectable alterations in ctDNA in patients with ILC (a), IDC (b), and mixed (MXD) histologies (c). Incidence of alterations [copy number variations (CNV), fusions (Fus), deletions (Del), insertions (Ins), frameshift (FS), splicing variants (Spl), premature termination codons (PTC) and single nucleotide variation (SNV)] is represented on the left with ordered frequency based on the sum of all variants in a particular gene. The mutant allele frequency (MAF) of each mutation is shown in the middle. Effect [gain of function (GOF), loss of function (LOF) and switch of function (SOF)] and pathogenicity [yes, no, unknown (Ukn) and inconclusive (Inc)] of all the detected alterations are shown on the right. The frequency of alterations is reflected as the number in parenthesis of the colour scale bar. N = 980.
### Table 2: Gene alterations and oncogenic pathways altered in ILC versus IDC.

| Gene alterations | N  | OR  | 95% C.I. | P value | OR  |
|------------------|----|-----|----------|---------|-----|
| NFE2L2 SNVs     |    |     |          |         | 1.73|
| Wild type        | 953| 1.00|          |         |     |
| Mutated          | 27 | 0.99| 0.74     | 5.33    |     |
| PTEN SNVs        |    |     |          | 0.089   |     |
| Wild type        | 937| 1.00|          |         |     |
| Mutated          | 43 | 0.97| 0.90     | 4.29    |     |
| CDK1 SNVs        |    |     |          | <0.001  |     |
| Wild type        | 962| 1.00|          |         |     |
| Mutated          | 18 | 12.63| 4.50   | 35.40 |     |
| RB1 SNVs         |    |     |          | 0.116   |     |
| Wild type        | 951| 1.00|          |         |     |
| Mutated          | 29 | 2.22| 0.82     | 6.00    |     |
| FGFR2 SNVs       |    |     |          | 0.063   |     |
| Wild type        | 880| 1.00|          |         |     |
| Mutated          | 100| 2.62| 0.95     | 7.24    |     |
| Oncogenic pathways | | | | | |
| PI3K SNVs        |    |     |          | 0.006   |     |
| Wild type        | 630| 1.00|          |         |     |
| Mutated          | 350| 1.76| 1.18     | 2.64    |     |
| Cell cycle CNVs  |    |     |          | 0.123   |     |
| Wild type        | 805| 1.00|          |         |     |
| Amplified        | 175| 0.60| 0.31     | 1.15    |     |
| RTK CNVs         |    |     |          | 0.009   |     |
| Wild type        | 766| 1.00|          |         |     |
| Amplified        | 214| 0.44| 0.24     | 0.81    |     |

Abbreviations: SNVs, single nucleotide variations; CNVs, copy number variations. N = 980.

### Oncogenic pathways

Based on prior work defining canonical oncogenic pathways, the following pathways were compared across ILC and IDC: RTK, RAS, RAF, MEK, NRF2, ER, WNT, MYC, P53, cell cycle, notch, and PI3K. Patients with ILC were significantly more likely to have oncogenic alterations for SNVs in the PI3K pathway and a lower likelihood of CNVs in the RTK and cell cycle pathways. In multivariable analysis, significant associations were confirmed for SNVs in PI3K (OR 1.76 [95% CI [1.18–2.64]) and CNVs in RTK (OR 0.44; 95% CI [0.24–0.81]) (Table 2).

### Survival analysis

OS was evaluated in the subset of patients with HR+ HER2 negative metastatic breast cancer. Within this cohort of 684 patients (69.8% of the total dataset), outcome data were available for 655 patients (101 ILC, 498 IDC, 56 mixed), which consisted of 96% of evaluable patients. No significant differences in outcomes were observed across the three cohorts (log-rank test, P = 0.98) (Fig. 2). In addition, single mutations that were associated with ILC (CDH1, ERBB2, and PTEN) did not appear to impact survival, although sample size was limited (Supplemental Fig. S3). When analysing oncogenic pathways, in multivariable analysis including lines of therapy and sites of disease, alterations in the RAF pathway (HR 5.79, [95% CI 1.16–28.9]) were associated with worse survival for patients with ILC (Table 3, Supplemen tally Table 3). In multivariable analysis for patients with IDC, SNVs in the P53 pathway (HR 1.83, [95% CI 1.33–2.52]) was associated with worse OS (Table 3). The proportional-hazards assumption was met for both multivariable models (Schoenfeld residuals, P = 0.1430 and P = 0.9980 for ILC and IDC, respectively).

### Independent validation cohort

To assess co-mutations with CDH1, genomic results from an independent cohort of nearly 7000 patients with metastatic breast cancer who underwent testing using the Guardant360™ 83-gene panel was analysed. Median age of this cohort was 64 [range 23–98] with 99% female and 1% male patients. No histology or other clinical data were available for these patients. The frequency of CDH1 alterations in this cohort was 10.8%. CDH1 was significantly co-mutated with multiple genes including: PIK3CA, ERBB2, RHOA, ARID1A, NF1, APC, RB1, NFE2L2, ESRI, and FGFR2 (Benjamini–Hochberg test, all q < 0.05) (Fig. 3).

### Discussion

Defining novel therapeutic strategies for patients with metastatic ILC has been challenging due to...
discrepancies in pathology classification and inconsistent reporting of histology in clinical trials. To our knowledge, no previous studies have explored differences in ctDNA profiles among patients with ILC, IDC, and MXD histologies. We hypothesised that we could detect differences in the genomic landscape of ctDNA across breast cancer histologies, and we explored the impact of individual alterations and oncogenic pathways on overall survival.

Using a large, multi-institutional, clinically annotated ctDNA dataset, our findings confirmed the feasibility of detecting differences in alterations across histologies. Our cohort had the anticipated proportion of patients with ILC histology (12.4%) and, as expected, the vast majority of patients with ILC were HR+ HER2 negative (≈90%). Notably, patients with ILC had a significantly higher number of pathogenic SNVs in ctDNA and a lower number of CNVs as compared to patients with IDC and MXD histology. Based on this finding and prior work evaluating TMB in tissue, there is a potential to explore blood-based tumour mutational burden as a biomarker for response to immune checkpoint inhibitors for patients with ILC.

For patients with HR+ HER2 negative metastatic breast cancer, we observed higher frequencies of CDH1, ERBB2, and PTEN mutations in patients with ILC compared to IDC with ERBB2 as a promising drug target in patients who are HER2 non-amplified. In our cohort, CDH1 was the fifth most common pathogenic SNV in patients with ILC with CDH1 mutations only detected in 9% of patients, as compared with 1% of IDC patients. Our frequency of detecting CDH1 mutations appear similar to data from plasmaMATCH that reported 2–3% of patients with CDH1 mutations detected in a cohort of approximately 1000 patients using the same assay, although these patients were not stratified by histology. Based on expanded coverage of CDH1 in the Guardant360™ 83-gene panel, the 11% frequency of CDH1 alterations across all patients was similar to that observed based on tissue data from TCGA, although no histology data were available for this independent ctDNA validation cohort. In addition, this analysis allowed us to validate multiple important co-mutations with CDH1 including targetable mutations (PIK3CA and ERBB2) and mutations associated with endocrine resistance (ARID1A, NF1, RB1, ESR1, and FGFR2).

Comparing concurrent blood and tissue biopsies for patients with CDH1 mutations including known histology is warranted to investigate this question in the future.

In both clinical practice and research studies, mixed histology patients remain a challenge to accurately characterise, and therefore we evaluated differences based on ctDNA profiling. Of note, we observed no CDH1 mutations in the mixed histology cohort, while prior studies evaluating patients with mixed histology have reported mutations frequencies of approximately 14%. While there were no clearly definable genomic patterns that were unique to patients with mixed histology...
histology, the mixed histology cohort had some genomic differences observed in higher frequencies in ILC versus IDC. Therefore, future studies should combine both pathologic and multiomic assessments, including genomic, transcriptomic, and proteomic differences, to further delineate patients with mixed histology.

To assess the impact of previously characterised oncogenic pathways in tissue, we assessed these pathway-based alterations including both SNVs and CNVs.

### Table 3: Oncogenic pathways and clinical characteristics associated with overall survival in patients with ILC versus IDC.

| Pathway          | Wild type (N = 82) | Mutated (N = 10) | HR 95% C.I.         | P value |
|------------------|--------------------|------------------|---------------------|---------|
| RAS SNVs         | Wild type 70       | 1.00             | 0.55 4.65           | 0.39    |
|                  | Mutated 12         | 1.60             | 0.55 4.65           | 0.39    |
| RAF SNVs         | Wild type 73       | 1.00             | 5.79 28.98          | 0.033   |
|                  | Mutated 9          | 1.16             | 28.98 0.033         |         |
| EGFR SNVs        | Wild type 54       | 1.00             | 1.44 3.61           | 0.43    |
|                 | Mutated 28         | 0.57             | 3.61 0.43           |         |
| RTK CNVs         | Wild type 76       | 1.00             | 2.18 8.36           | 0.254   |
|                  | Amplified 11       | 0.43             | 8.36 0.254          |         |
| RAF CNVs         | Wild type 76       | 1.00             | 0.43 2.88           | 0.387   |
|                  | Amplified 6        | 0.43             | 2.88 0.387          |         |
| PI3K SNVs        | Wild type 48       | 1.00             | 1.28 2.91           | 0.533   |
|                 | Mutated 34         | 0.56             | 2.91 0.533          |         |
| Treatment line   | 1                  | 32               | 1.00                |         |
|                  | 2                  | 18               | 1.24 4.40           | <0.001  |
|                  | 3                  | 9                | 0.81 3.17           | 0.76    |
| Lung involvement | No                 | 72               | 1.00                |         |
|                  | Yes                | 10              | 0.81 3.17           | 0.76    |
| CNS involvement  | No                 | 78               | 1.00                |         |
|                  | Yes                | 4                | 34.94 151.04        | <0.001  |
| IDC (N = 402)    | RTK SNVs           | Wild type 344    | 1.00                |         |
|                  | Mutated 88         | 0.69             | 5.46 0.212          |         |
| RAS SNVs         | Wild type 366      | 1.00             | 0.95 4.59           | 0.066   |
|                  | Mutated 36         | 0.95             | 4.59 0.066          |         |
| RAF SNVs         | Wild type 378      | 1.00             | 0.44 1.85           | 0.260   |
|                  | Mutated 24         | 0.40             | 1.85 0.260          |         |
| EGFR SNVs        | Wild type 273      | 1.00             | 1.83 2.52           | <0.001  |
|                 | Mutated 129        | 1.33             | 2.52 <0.001         |         |
| Cell cycle CNVs  | Wild type 337      | 1.00             | 1.22 2.24           | 0.521   |
|                  | Mutated 65         | 0.67             | 2.24 0.521          |         |
| RTK CNVs         | Wild type 324      | 1.00             | 0.45 1.10           | 0.081   |
|                  | Amplified 78       | 0.19             | 1.10 0.081          |         |
| RAS CNVs         | Wild type 389      | 1.00             | 0.73 1.79           | 0.49    |
|                  | Amplified 13       | 0.30             | 1.79 0.49           |         |
| RAF CNVs         | Wild type 383      | 1.00             | 1.46 7.61           | 0.655   |
|                  | Amplified 19       | 0.28             | 7.61 0.655          |         |

(Continued from previous column)

| Pathway          | Wild type (N = 417) | Amplified (N = 104) | HR 95% C.I.         | P value |
|------------------|---------------------|--------------------|---------------------|---------|
| ER CNVs          | Wild type 397       | 1.00               | 0.43 5.05           | 0.544   |
|                  | Amplified 5         | 1.47               | 5.05 0.544          |         |
| MYC CNVs         | Wild type 358       | 1.00               | 0.96 2.67           | 0.071   |
|                  | Amplified 44        | 0.96               | 2.67 0.071          |         |
| Cell cycle CNVs  | Wild type 344       | 1.00               | 0.70 2.53           | 0.378   |
|                  | Amplified 58        | 0.70               | 2.53 0.378          |         |
| PI3K CNVs        | Wild type 367       | 1.00               | 0.68 2.18           | 0.501   |
|                  | Amplified 35        | 0.68               | 2.18 0.501          |         |
| ER SNVs          | Wild type 285       | 1.00               | 0.84 1.69           | 0.321   |
|                  | Amplified 117       | 0.84               | 1.69 0.321          |         |
| PI3K SNVs        | Wild type 255       | 1.00               | 0.83 1.65           | 0.377   |
|                  | Amplified 147       | 0.83               | 1.65 0.377          |         |
| Treatment line   | 1                   | 195               | 1.00                |         |
|                  | 2                   | 63                | 2.45 4.40           | 0.003   |
|                  | 3                   | 35                | 2.45 7.63           | <0.001  |
|                  | 4                   | 35                | 2.45 7.63           | <0.001  |
| Liver involvement| No                  | 305               | 1.00                |         |
|                  | Yes                 | 97                | 1.24 1.75           | 0.231   |
| Bone involvement | No                  | 214               | 1.00                |         |
|                  | Yes                 | 188               | 2.02 2.16           | <0.001  |
| Soft tissue involvement | No | 335 | 1.00 |         |
|                  | Yes                 | 67                | 1.50 2.23           | 0.042   |
| CNS involvement  | No                  | 376               | 1.00                |         |
|                  | Yes                 | 26                | 3.02 5.33           | <0.001  |

Abbreviations: CNVs, copy number variations; SNVs, single nucleotide variations; HR, hazard ratio; CI, confidence interval.
CNVs. We found that patients with ILC were enriched in SNVs associated with the PI3K pathway. The association of ILC with PI3K alterations is consistent with prior work in tissue and emphasises that these driver mutations are critical for disease pathogenesis and progression.⁷,⁸ Our finding of commonly mutated alterations in the PI3K pathway supports the exploration of both PIK3CA and AKT inhibitors to explore differential sensitivity for patients with ILC versus IDC.

We further assessed potential differences in survival for patients with metastatic ILC, IDC, and MXD histologies. Our data demonstrated no significant differences based on histology for HR+ HER2 negative patients treated with standard-of-care therapies and single mutations associated with ILC (e.g. CDH1, ERBB2, and PTEN) did not appear to impact survival. In contrast, alterations grouped by oncogenic pathways appeared to have a differential impact on OS with SNVs in RAF pathway associated with shorter OS for patients with ILC, while mutations in the TP53 pathway were associated with shorter OS for patients with IDC. Further studies are necessary to explore how treatments targeting these pathways may have a histology-specific impact on prognosis and how these alterations change with serial assessment of ctDNA in patients with ILC versus IDC.

There were several limitations to our study. First, our study did not perform central pathology review to confirm histology. However, our inclusion of three sites promotes the generalisability of our findings and primary pathology was reviewed by subspecialists in breast pathology at each academic site. Second, we did not have concurrent tissue and blood assessments for patients limiting our ability to compare differences in detection of particular mutations (e.g. CDH1) across tissue and blood. Third, while our independent validation cohort consisted of nearly 7000 patients, no histology data were available for these patients. Fourth, there may have been selection bias in the study given differences in ordering and clinical testing of ctDNA in different parts of the world.

fig. 3: Landscape of CDH1 mutations in blood and tissue and CDH1 co-mutations in blood. CDH1 mutations were assessed in nearly 7000 breast cancer patients using the 83-gene Guardant360™ with an observed frequency of 10.8% in blood (a) and 11.0% in tissue based on TCGA (b). Splice mutations were not included in the lollipop plots. The 10 most significant co-mutated alterations with CDH1 are shown (c). Synonymous alterations, variants of unknown significance, and germline alterations were removed for this analysis. Only the first ctDNA test was included for patients with multiple samples.
Collectively, our findings demonstrate the feasibility of detecting ctDNA differences in patients with metastatic breast cancer across histologies. We defined mutations that were more commonly detected in ILC, assessed co-mutations with CDH1, and identified oncogenic pathways that were differentially dysregulated across histologies. Further, the mutational profile of mixed histology patients was defined and consisted of genomic features characteristic of both ILC and IDC, but notably there were no CDH1 mutations observed in our mixed cohort. Our data extend prior work in tissue to define patients with metastatic ILC using ctDNA. Our findings may have implications for the design of future studies and implementation of precision medicine-based approaches for patients with ILC based on ctDNA.

Contributors

Study concept and design: AAD, LG, MC
Data acquisition: AAD, KC, AJM, MV, WLH, LB, ANS, PD
Quality control of data and algorithm: AAD, LG, KC, AJM, MV, WLH, ANS
Interpretation of data, approval and editing of the manuscript: all authors
AAD and LG have verified the underlying data. All authors have read and approved the final version of the manuscript.

Data sharing statement

De-identified data of the 980 patients will be available upon reasonable request of the authors as the data are currently shared under a data use agreement among the principal investigators of the three institutions with multiple ongoing analyses and manuscripts.

Declaration of interests

A. A. Davis reports participating in a scientific advisory board for Pfizer, Inc. L. Gerratana reports consulting fees from Eli Lilly & Co, Novartis, and AstraZeneca. K. Clifton reports research funding from the Cancer and Aging Research Group Pilot Grant outside this work and consulting fees from Biotheranostics. F. O. Ademuyiwa reports research funding from Pfizer, Immunomedics, NeoImmuneTech, RNA diagnostics, and Astellas. She reports fees from Teladoc Health, Pfizer, AstraZeneca, QED Therapeutics, Immunomedics, Cardinal Health, Athenex, and Biotheranostics. L. Bucheit is an employee of Guardant Health and has received stock/stock options in the company. A. Shah reports serving on an advisory board for AstraZeneca. P. D’Amico reports grants or contracts from Roche and the American Italian Cancer Foundation and planned employment with Merck. N. Bagegni reports institutional research funding from Daiichi Sankyo, Seattle Genetics Inc., Seronoix, Xcovery Holding Company LLC, Pfizer, Inc., AstraZeneca, Sarah Cannon Development Innovations, Ambrx, and Novartis Pharmaceuticals outside this work. She has also received honoraria from OncLive. M. Opyrchal reports receiving grants from Bayer and Eli Lilly & Co outside this work and consulting fees from AstraZeneca and Novartis. R. Bose reports institutional research funding from Puma and consulting fees from Genentech. A. Behdad reports serving as a speaker or receiving honoraria from Foundation Medicine, China, Lilly, Bayer, and Thermo Fisher Scientific. He also reports serving on an advisory board and receiving honoraria from Leica. C.X. Ma holds consultant or advisory roles from Puma, Pfizer, Seattle Genetics, AstraZeneca, Natera, Onco-signal, Olaris, Athenex, Eisai, Philips Electronics, Agendia, Biovia, Jacobo, Invitada, Sanofi, Bayer HealthCare, Eli Lilly, and Gildeas. She has received honoraria from PlusOne Health GmbH and UpToDate. She has also received research funding from Pfizer and Puma Biotechnology. A. Bardia reports consulting or advisory roles with Genentech, Novartis, Pfizer, Merck, Sanofi, Radius Health, Immunomedics/Gilead, AstraZeneca/Daiichi Sankyo, and Eli Lilly. He reports research funding from Pfizer, Novartis, Genentech, Merck, Radius Health, Immunomedics/Gilead, AstraZeneca/Daiichi Sankyo, Phillips, Eli Lilly & Co, and Foundation Medicine outside this work. M. Cristofanilli reports consulting fees from AstraZeneca, Eli Lilly & Co, Ellipses, and Menarini. He has received honoraria from Pfizer and participated in advisory boards for Merck and AstraZeneca. The remaining authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2022.104316.

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