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

Andrew A Davis  
*Washington University School of Medicine in St. Louis*  
Katherine Clifton  
*Washington University School of Medicine in St. Louis*  
Whitney L. Hensing  
*Washington University School of Medicine in St. Louis*  
Nusayba A. Bagegni  
*Washington University School of Medicine in St. Louis*  
Foluso O. Ademuyiwa  
*Washington University School of Medicine in St. Louis*

*See next page for additional authors*

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Authors
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Andrew A. Davis, a Lorenzo Gerratana, b Katherine Clifton, a Arielle J. Medford, d,e Marko Velimirovic, d,e Whitney L. Hensing, b Leslie Bucheit, f Ami N. Shah, b Paolo D’Amico, b Carolina Reduzzi, b Qiang Zhang, b Charles S. Dai, e Elyssa N. Denault, b Nusayba A. Bayegri, b Mateusz Opyrchal, b Foluso O. Ademuyiwa, a Ron Bose, b William J. Gradishar, b Amir Behdad, b Cynthia X. Ma, a Aditya Bardia, d,e and Massimo Cristofanilli a, h

aDivision of Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA
bRobert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL, USA
cDepartment of Medicine, University of Udine, Udine, Italy
dMassachusetts General Hospital, Boston, Massachusetts, USA
eHarvard Medical School, Boston, MA, USA
fGuardant Health, Redwood City, CA, USA
gIndiana University School of Medicine, Indianapolis, IN, USA
hWeill Cornell Medicine, New York, NY, USA

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, NRF, RB1, ESR1, FGFR2) (Benjamini–Hochberg Procedure, all q < 0.05).


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Keywords: Invasive lobular carcinoma; Circulating tumour DNA; Genomics; Metastatic breast cancer

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. 1 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. 3 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
TBX3 were more commonly detected compared to pa-
and FGF19 remain controversial with some studies suggesting
addition, the clinical outcomes of patients with ILC
observed in patients with ILC.10

In metastatic breast cancer, circulating tumour DNA (ctDNA)
has emerged as a tool to detect targetable alterations and to
monitor disease resistance. Despite the advances in precision
medicine, characterisation of ctDNA differences across breast
cancer histologies, including invasive lobular carcinoma (ILC)
and invasive ductal carcinoma (IDC), is limited. We explored
prior work using PubMed with the following terms: “invasive
lobular carcinoma,” “circulating tumour DNA,” “ctDNA,” and
“metastatic breast cancer.” At the time of our search, no
studies existed that explicitly evaluated differences in ctDNA
across breast cancer histologies.

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Evidence before this study
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“metastatic breast cancer.” At the time of our search, no
studies existed that explicitly evaluated differences in ctDNA
across breast cancer histologies.

Added value of this study
Here, we present our analyses of a clinically annotated dataset
of nearly 1000 patients at three sites with metastatic breast
cancer who underwent ctDNA evaluation. We report the
differences in genomic alterations across ILC, IDC, and mixed
histologies. Evaluation of ctDNA define genomic mutations
that are more common in ILC (e.g., CDH1, ERBB2, and PTEN)
and oncogenic pathways that are more frequently
dysregulated (e.g., PI3K pathway) compared to IDC and mixed
histologies. In an independent cohort of nearly 7000 patients
with uniform ctDNA testing, we defined co-mutations
associated with CDH1 with implications for targeted
treatment and disease resistance.

Implications of all available evidence
These data define differences in genomic profiles across
histologies in patients with metastatic breast cancer with
implications for future treatment strategies and clinical trial
design. Further studies are needed to define precision
medicine treatment approaches for patients with ILC with
defined histopathologic and ctDNA genomic profiles.

lower frequency of lung and liver metastases.5 Despite
the distinct pattern of metastatic spread, unique strat-

gies 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 pa-
tients with ILC are in CDH1, PIK3CA, TP53, CCND1,
and FGF19 genes.6 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.6-8 For patients with
metastatic ILC, mutations in CDH1, NF1, PIK3CA, and
TBX3 were more commonly detected compared to pa-
tients with metastatic IDC.6 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.6 In
addition to mutational differences, differences in

immune infiltration and copy number changes have been
observed in patients with ILC.6-12

Despite emerging genomic differences between ILC
and IDC, clinically, treatment for both histologic sub-
types remains similar despite suggestions of lower ef-
cicacy of chemotherapy for patients with ILC.13-14 In
addition, the clinical outcomes of patients with ILC
remain controversial with some studies suggesting
worse long-term outcomes for patients with ILC,15-16
while other studies have reported better or similar
long-term outcomes.17-22 Due to this uncertainty, recent
work has attempted to characterise specific alterations
and gene signatures associated with prognosis.22 Treat-
ment strategies incorporating immune checkpoint
inhibitors and other novel agents are currently under
investigation.19,20

The evaluation of ctDNA represents a less invasive
technique compared to tissue biopsies to assess
genomic alterations and clonal evolution.23-24 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.25 A potential advantage of
ctDNA testing is the ability to capture spatial and tem-
poral heterogeneity.26,27 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 under-
went 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 fre-
cquency, copy number alterations, and oncogenic path-
ways across histologic subtypes. These data have
important implications for characterising genomic dif-
ferences across histologies using ctDNA for future
precision medicine approaches for patients with meta-
static ILC.

Methods
Patient selection and study design
This retrospective cohort study included data from pa-
tients 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 (in/dels), gene fusions/rearrangements, and copy number variants (CNVs).31 Mutations were annotated using OncoKB to define pathogenic variants.25 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).25 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.


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 MXD 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 MXD, respectively; P = 0.847). When combining pathogenic mutations and CNVs, the most common alterations in the MXD histology cohort is also shown (Fig. 1c).

Across all patients, genomic differences were compared among patients with ILC, IDC, and MXD (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).

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**Table 1:** Clinical pathological comparisons across patients with IDC, ILC, and mixed (MXD) histologies.

Abbreviations: MXD, mixed; HR, hormone-receptor; TNBC, triple negative breast cancer; CNS, central nervous system; N = 980.
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 show on the right. The frequency of alterations is reflected as the number in parenthesis of the colour scale bar. N = 980.
cancer in ILC compared to other histologies, we then restricted our analysis to only HR+ HER2 negative patients. Based on this analysis, ILC was significantly associated with mutations in CDH1, ERBB2, FGFR2, NF1, and PTEN (logistic regression, all P < 0.05). Multivariable analysis confirmed significant associations with mutations in CDH1 (OR 9.4, [95% CI 3.3–27.2]), ERBB2 (OR 3.6, [95% CI 1.6–8.2]), and PTEN (OR 2.5, [95% CI 1.05–5.8]) (logistic regression, all P < 0.05).

Mixed histology
A total of 67 patients were classified as MXD histology, including 56 with HR+ HER2 negative breast cancer. For comparisons across histologies, analyses were restricted to patients with HR+ HER2 negative breast cancer. Given the smaller sample size of patients with MXD histology, alterations were included regardless of mutation frequency. Among all three histologies, there were significant differences in CDH1, ERBB2, FGFR2, IDH2, NF1, PGD FRA, PTEN, RB1, and RHOA alterations (Fisher’s exact test, P < 0.05) (Supplemental Table 2). Notably, no patients with MXD histology had alterations in CDH1 (0/56, 0%) or PTEN.

Oncogenic pathways
Based on prior work defining canonical oncogenic pathways, the following pathways were compared across ILC and IDC: RTK, RAS, RAF, MEK, NFR2, ER, WNT, MYC, P53, cell cycle, notch, and PI3K.31 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, Supplemental 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 history 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, ER51, 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.33 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.11 In contrast to previous tissue-based studies, the frequency of CDH1 mutations in our clinically annotated cohort was lower than expected. Prior studies sequencing tissue in patients with ILC have reported the frequency of CDH1 mutations as ranging from approximately 53%–77% with CDH1 mutation frequency similar in primary and metastatic ILC.2,6–8,36,37 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.21 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, 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 define 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.

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Table 3: Oncogenic pathways and clinical characteristics associated with overall survival in patients with ILC versus IDC.
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 de-identified our mixed cohort. Our data extend prior work in tissue across histologies. Further, the mutational profiling 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, WHL, LB, ANS, PD
Quality control of data and algorithm: AAD, LG, KC, AJM, MV, WHL, 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 AstraZenea. 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, NeolImmuneTech, RNA diagnostics, and Astellas. She reports fees from Teladoc Health, Pfizer, AstraZeneca, QED Therapeutics, Immunomedics, Cardinal Health, Athenex, and Biotheranostics. L. Buchté 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 support from Daiichi Sankyo, Seattle Genetics Inc., Sermonix, 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 Thermofisher 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 Gilead. 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.

References
19 Sikora MJ, Cooper KL, Bahreini A, et al. Invasive lobular carcinoma cell lines are characterized by unique estrogen-mediated gene