

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*, *NF1*, *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.⁴ 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

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Research in context**Evidence before this study**

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.

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 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 *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.^{7–9} For patients with metastatic ILC, mutations in *CDH1*, *NF1*, *PIK3CA*, and *TBX3* were more commonly detected compared to patients 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.^{10–12}

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.^{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.^{13,17} Due to this uncertainty, recent work has attempted to characterise specific alterations and gene signatures associated with prognosis.¹⁸ Treatment strategies incorporating immune checkpoint

inhibitors and other novel agents are currently under investigation.^{2,19,20}

The evaluation of ctDNA represents a less invasive technique compared to tissue biopsies to assess genomic alterations and clonal evolution.^{21–24} 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 temporal 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 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).²⁹ Mutations were annotated using OncoKB to define pathogenic variants.³¹ 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 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 metastatic ILC were in *PIK3CA*, *TP53*, *ESR1*, *ERBB2*, and *ARID1A* (Fig. 1a) and *TP53*, *PIK3CA*, *ESR1*, *EGFR*, and *FGFR1* for IDC (Fig. 1b). Among pathogenic SNVs only, *CDH1* was the fifth most common mutation observed in ILC patients (Supplemental Fig. S1). No CNVs in *CDH1* were detected. The landscape of 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*, *IDH2*, *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

	IDC	ILC	MXD	P value
Total (N: 980)	792	121	67	
Subtype (N: 973)				<0.001
HR positive HER2 negative	523	105	56	
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)				<0.001
No	533	105	49	
Yes	257	16	18	
Liver metastasis (N: 978)				0.031
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	617	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	
≥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	

Abbreviations: MXD, mixed; HR, hormone-receptor; TNBC, triple negative breast cancer; CNS, central nervous system. N = 980.

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

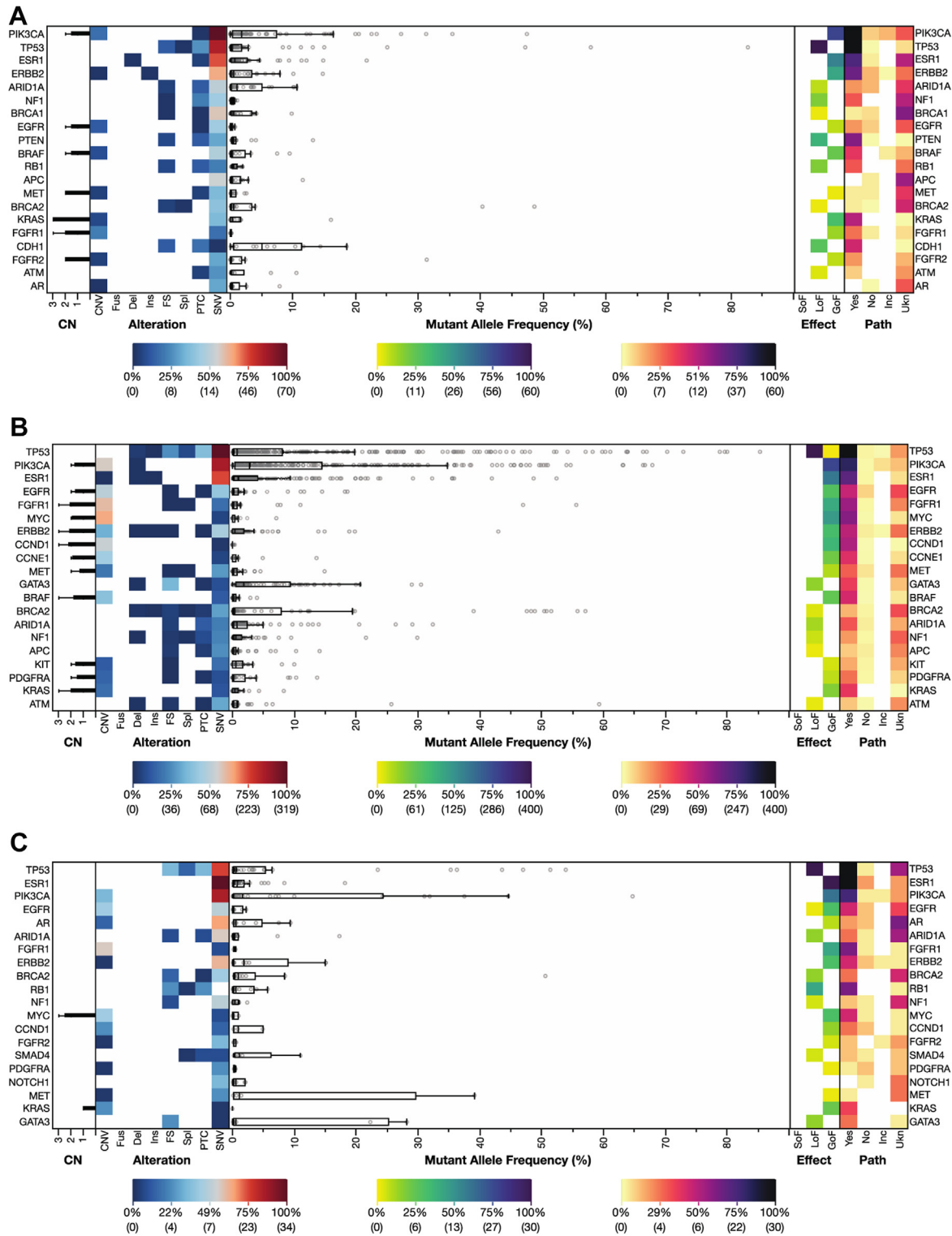


Fig. 1: Landscape of detectable alterations in ctDNA in patients with ILC (a), IDC (b), and mixed (MXD) histologies (c). Incidence of alterations [copy number variations (CNV), fusions (Fus), deletions (Del), insertions (Ins), frameshift (FS), splicing variants (Spl), premature termination codons (PTC) 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.

	N	OR	95% C.I.	P value	OR
Gene alterations					
NF1 SNVs					
Wild type	953	1.00			0.173
Mutated	27	1.99	0.74	5.33	
PTEN SNVs					
Wild type	937	1.00			0.089
Mutated	43	1.97	0.90	4.29	
CDH1 SNVs					
Wild type	962	1.00			<0.001
Mutated	18	12.63	4.50	35.40	
RB1 SNVs					
Wild type	951	1.00			0.116
Mutated	29	2.22	0.82	6.00	
FGFR2 SNVs					
Wild type	880	1.00			0.063
Mutated	100	2.62	0.95	7.24	
Oncogenic pathways					
PI3K SNVs					
Wild type	630	1.00			0.006
Mutated	350	1.76	1.18	2.64	
Cell cycle CNVs					
Wild type	805	1.00			0.123
Amplified	175	0.60	0.31	1.15	
RTK CNVs					
Wild type	766	1.00			0.009
Amplified	214	0.44	0.24	0.81	
Abbreviations: SNVs, single nucleotide variations; CNVs, copy number variations. N = 980.					
Table 2: Gene alterations and oncogenic pathways altered in ILC versus IDC.					

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*, *PGDFRA*, *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, NRF2, ER, WNT, MYC, P53, cell cycle, notch, and PI3K.³² 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 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*, *ESR1*, 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

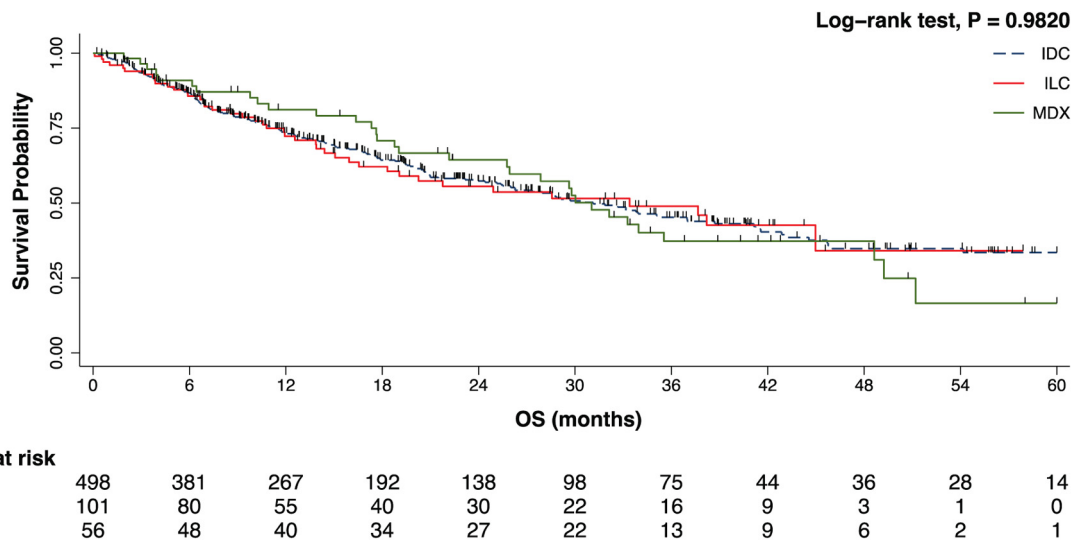


Fig. 2: Overall survival based on histology for patients with HR+ HER2 negative metastatic breast cancer. Overall survival (OS) was compared for patients across each histology. No significant differences were observed across patients with ILC, IDC, and MXD histologies (log-rank test; $P = 0.98$). $N = 655$.

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.⁶

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.^{34,35} 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%.⁷ While there were no clearly definable genomic patterns that were unique to patients with mixed

	N	HR	95% C.I.		P value
ILC (N = 82)					
RAS SNVs					
Wild type	70	1.00			
Mutated	12	1.60	0.55	4.65	0.39
RAF SNVs					
Wild type	73	1.00			
Mutated	9	5.79	1.16	28.98	0.033
P53 SNVs					
Wild type	54	1.00			
Mutated	28	1.44	0.57	3.61	0.438
RTK CNVs					
Wild type	71	1.00			
Amplified	11	2.18	0.57	8.36	0.254
RAF CNVs					
Wild type	76	1.00			
Amplified	6	0.43	0.07	2.88	0.387
PI3K SNVs					
Wild type	48	1.00			
Mutated	34	1.28	0.56	2.91	0.553
Treatment line					
1	32	1.00			
2	18	1.94	0.69	5.46	0.212
3	9	1.16	0.30	4.52	0.828
≥4	23	2.54	0.95	6.80	0.063
Lung involvement					
No	72	1.00			
Yes	10	0.81	0.21	3.17	0.76
CNS involvement					
No	78	1.00			
Yes	4	34.94	8.08	151.04	<0.001
IDC (N = 402)					
RTK SNVs					
Wild type	314	1.00			
Mutated	88	2.09	0.95	4.59	0.066
RAS SNVs					
Wild type	366	1.00			
Mutated	36	1.71	0.95	3.07	0.072
RAF SNVs					
Wild type	378	1.00			
Mutated	24	0.44	0.10	1.85	0.260
P53 SNVs					
Wild type	273	1.00			
Mutated	129	1.83	1.33	2.52	<0.001
Cell cycle SNVs					
Wild type	337	1.00			
Mutated	65	1.22	0.67	2.24	0.511
RTK CNVs					
Wild type	324	1.00			
Amplified	78	0.45	0.19	1.10	0.081
RAS CNVs					
Wild type	389	1.00			
Amplified	13	0.73	0.30	1.79	0.49
RAF CNVs					
Wild type	383	1.00			
Amplified	19	1.46	0.28	7.61	0.655

(Table 3 continues on next column)

	N	HR	95% C.I.		P value
(Continued from previous column)					
ER CNVs					
Wild type	397	1.00			
Amplified	5	1.47	0.43	5.05	0.544
MYC CNVs					
Wild type	358	1.00			
Amplified	44	1.60	0.96	2.67	0.071
Cell cycle CNVs					
Wild type	344	1.00			
Amplified	58	1.33	0.70	2.53	0.378
PI3K CNVs					
Wild type	367	1.00			
Amplified	35	1.22	0.68	2.18	0.501
ER SNVs					
Wild type	285	1.00			
Mutated	117	1.19	0.84	1.69	0.321
PI3K SNVs					
Wild type	255	1.00			
Mutated	147	1.17	0.83	1.65	0.377
Treatment line					
1	195	1.00			
2	63	2.45	1.37	4.40	0.003
3	35	4.32	2.45	7.63	<0.001
≥5	195	3.86	2.29	6.52	<0.001
De novo disease					
No	305	1.00			
Yes	97	1.24	0.87	1.75	0.231
Liver involvement					
No	214	1.00			
Yes	188	2.02	1.46	2.79	<0.001
Bone involvement					
No	92	1.00			
Yes	310	1.43	0.95	2.14	0.085
Soft tissue involvement					
No	335	1.00			
Yes	67	1.50	1.02	2.23	0.042
CNS involvement					
No	376	1.00			
Yes	26	3.02	1.71	5.33	<0.001

Abbreviations: CNVs, copy number variations; SNVs, single nucleotide variations; HR, hazard ratio; CI, confidence interval.

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

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

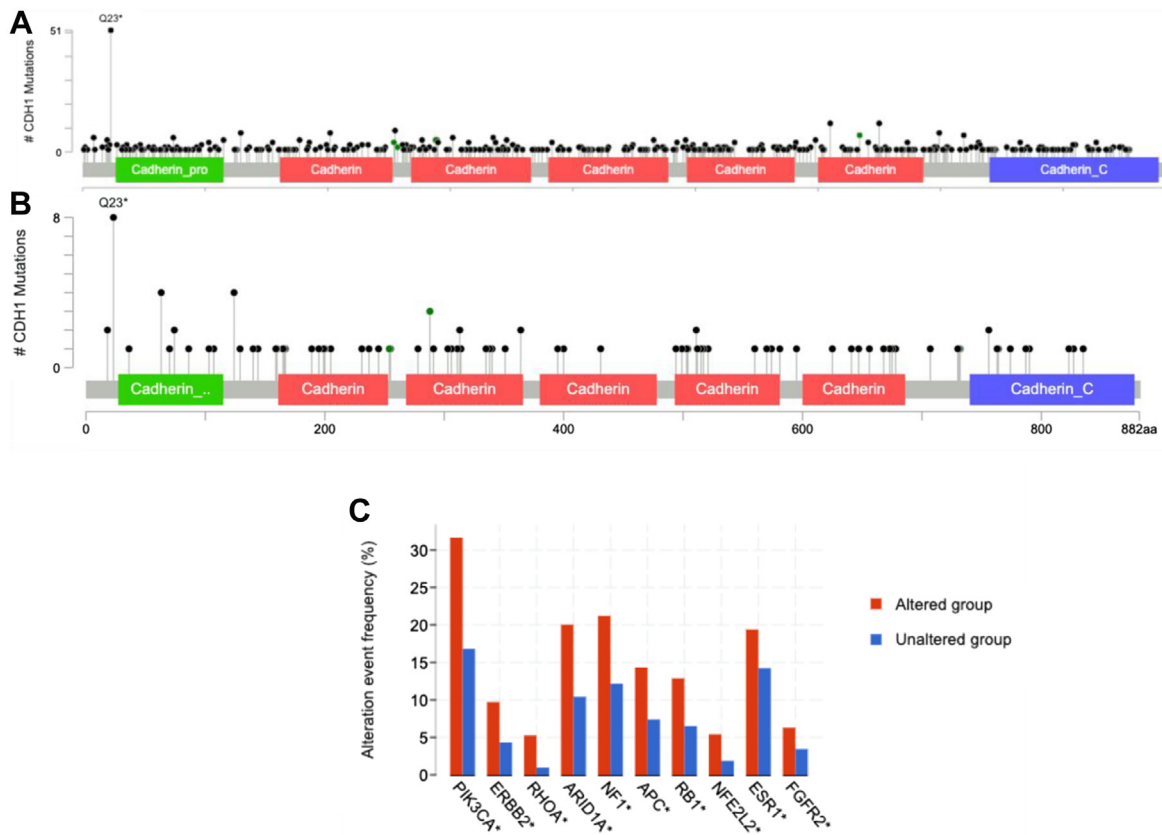


Fig. 3: Landscape of *CDH1* mutations in blood and tissue and *CDH1* co-mutations in blood. *CDH1* mutations were assessed in nearly 7000 breast cancer patients using the 83-gene Guardant360™ with an observed frequency of 10.8% in blood (a) and 11.0% in tissue based on TCGA (b). Splice mutations were 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.

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.

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

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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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