Differential ctDNA-based genomic features of Triple-Negative Metastatic Lobular Breast Cancer: insights into a rare and poorly understood disease

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Background

- High throughput genomic technologies such as NGS are enhancing the ability to dynamically characterize MBC but their role in describing combinations of multiple genomic alterations remains unclear.
- Invasive lobular carcinoma (ILC) comprises 10-15% of all breast cancers (BC), predominantly presenting as low grade and hormone receptor positive (HRpos).
- Triple-negative ILC (TN-ILC) is rare, accounting for approximately 2% of all triple-negative BC (TNBC) and 0.1% of all BC. Due to its rarity,.
- TN-ILC is poorly understood and lacks well-established treatment standards.

Aim

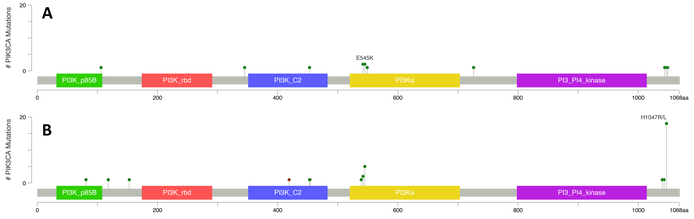
This study aims to investigate the genomic characteristics of TN-ILC through circulating tumor DNA (ctDNA) profiling within a large multi-center consortium.

Methods

- The study analyzed a retrospective cohort of 750 patients (pts) with HER2 negative metastatic breast cancer with ctDNA testing using the Guardant360 NGS panel within a large multicenter academic consortium between 2015 and 2023.
- HR and HER2 status were defined based on the most recent biopsy.
- Pathway classification was defined based on previous work (Sanchez-Vega F et al, Cell. 2018) (i.e., RTK, RAS, RAF, MEK, NRF2, ER, WNT, MYC, P53, cell cycle, notch, PI3K).
- Single nucleotide variations (SNVs) were annotated for their oncogenicity through OncoKB and ClinVar. Only pathogenic variants were included in the models.
- Associations across single nucleotide and copy number variations (SNVs and CNVs), histology, and HR status were tested by multinomial logistic regression in terms of Relative Risk Ratio (RRR), adjusting for significant clinical characteristics (i.e., lines of treatment, metastatic sites).
- Prognosis was analyzed for overall survival (OS) defined from the time of ctDNA collection.

Results

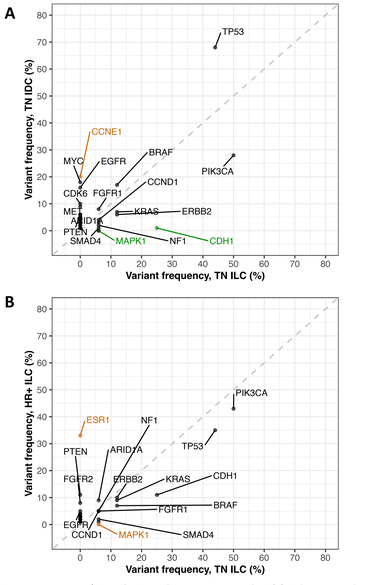
- Among the 750 patients analyzed, the cohort consisted of TN-ILC (N:16, 2%), HRpos-ILC (N:91, 12%), TN-invasive ductal carcinoma (IDC) (N:186, 25%), and HRpos-IDC (N:457, 61%).
- In TN-ILC, the most frequently altered genes were PIK3CA (50%), TP53 (44%), ERBB2 (25%), and CDH1 (25%). Less frequent alterations included BRAF, ALK, ARID1A, MET, RB1, and SMAD4, each occurring in 12-19% of cases. PIK3CA (48%) and TP53 (39%) had a similar alteration frequency in HRpos-ILC, and ESR1 alterations were also common (36%).



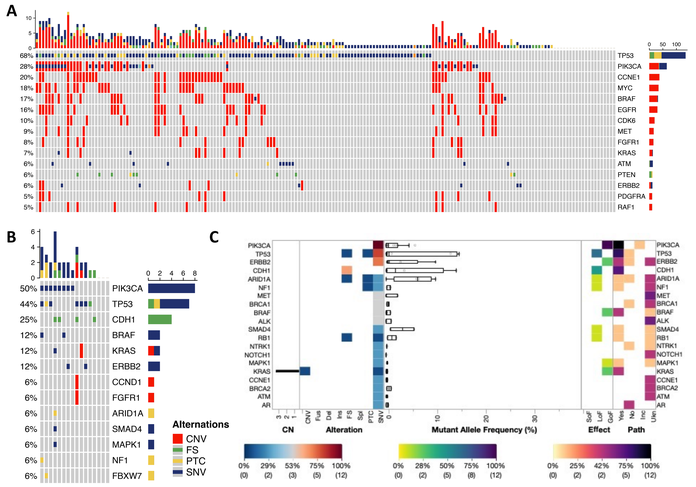
▲ **Figure 1** – Lollipop plot of the PIK3CA variants detected in TN ILC (A) and TN IDC (B)

Variable	TN IDC N = 186[1]	TN ILC N = 16[1]	HR+ IDC N = 457[1]	HR+ ILC N = 91[1]	p-value[2]
Liver Involvement	72 (38.9%)	2 (12.5%)	179 (39.2%)	22 (24.2%)	0.008
Lung Involvement	70 (37.8%)	2 (12.5%)	128 (28.0%)	10 (11.0%)	<0.001
Bone Involvement	89 (48.1%)	14 (87.5%)	333 (72.9%)	73 (80.2%)	<0.001
Node Involvement	90 (48.6%)	4 (25.0%)	183 (40.0%)	20 (22.0%)	<0.001
De Novo Disease	39 (24.5%)	0 (0.0%)	101 (25.4%)	19 (22.9%)	0.14
Soft tissue Involvement	54 (29.2%)	2 (12.5%)	69 (15.1%)	8 (8.8%)	<0.001
Treatment lines					0.109
1	57 (39.0%)	9 (64.3%)	123 (33.6%)	36 (45.0%)	
2	31 (21.2%)	3 (21.4%)	82 (22.4%)	18 (22.5%)	
3	58 (39.7%)	2 (14.3%)	161 (44.0%)	26 (32.5%)	

▲ **Table 1** – Cohort characteristics according to histoype and subtype. [1]n (%), [2]Fisher's exact test



▲ **Figure 2** – Comparison of gene alterations between TN IDC and ILC (A) and HR+ ILC and TN ILC (B). Significant alterations after false discovery rate correction are depicted in green



▲ **Figure 3** – Oncoprint for TN IDC (A) and TN ILC (B). Landscape plot of all detectable aberrations in the TN ILC analyzed (C). Incidence of the single aberrations is represented on the left, the mutant allele frequency (MAF) is showed in the center, pathogenicity and effect on the right. Variants are classified as copy number variants (CNV), fusions (fus), deletions (del), insertions (ins), frameshift (fs), splicing variants (Spl), premature termination codons (PTC), and single nucleotide variants (SNV). Heatmap legend is showed on the right.

- Multivariable multinomial logistic regression, designed with TN-IDC as reference, investigated differences in oncogenic pathway alterations across histologies and subtypes (**Table 1 and 2**).
- In TN-ILC, a significant association was observed for RAS SNVs (RRR = 7.9, p = 0.038) and PI3K SNVs (RRR = 4.32, P = 0.015) (**Table 2**).
- In HRpos-ILC, cell cycle CNVs (RRR = 0.3, P = 0.013) and P53 SNVs (RRR = 0.26, P < 0.001) had a significantly lower prevalence than in TN-IDC. RAS SNVs (RRR = 4.94, P = 0.022) and PI3K SNVs (RRR = 2.6, P = 0.003) were more common (**Table 3**)

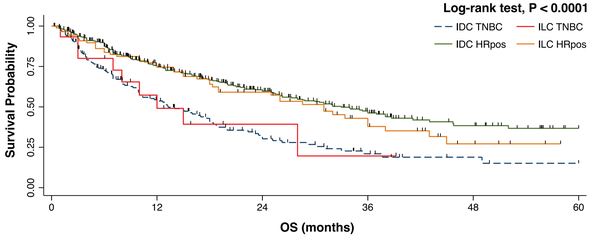
	RRR	95% C.I.		P value
TN ILC				
CNVs cell cycle	0.28	0.03	2.4	0.243
CNVs RTK	0.35	0.04	2.95	0.332
SNVs RAS	7.9	1.12	55.49	0.038
SNVs P53	0.38	0.12	1.23	0.107
SNVs PI3K	4.32	1.33	13.99	0.015
Treatment line				
2	0.57	0.14	2.37	0.435
≥3	0.27	0.05	1.38	0.116
Visceral involvement	0.3	0.08	1.05	0.06

▲ **Table 2** - Multivariable multinomial logistic regression for TN ILC. TN IDC was considered as base subgroup

	RRR	95% C.I.		P value
HRpos ILC				
CNVs cell cycle	0.3	0.12	0.78	0.013
CNVs RTK	0.63	0.27	1.48	0.293
SNVs RAS	4.94	1.26	19.28	0.022
SNVs P53	0.26	0.14	0.49	< 0.001
SNVs PI3K	2.6	1.39	4.88	0.003
Treatment line				
2	0.96	0.45	2.08	0.924
≥3	1.02	0.51	2.02	0.963
Visceral involvement	0.22	0.11	0.41	< 0.001

▲ **Table 3** - Multivariable multinomial logistic regression for HRpos ILC. TN IDC was considered as base subgroup

- Single gene alterations were investigated across significantly altered pathways. PIK3CA SNVs were more common in TN-ILC (50%) compared to TN-IDC (16%), but similar in frequency to HRpos-ILC (42%) (P<0.001).
- PTEN SNVs were higher in HRpos-ILC (11%) than in TN-IDC and TN-ILC (respectively 0% and 6%). KRAS SNVs were more represented in HRpos- and TN-ILC (respectively 6% and 8%), with respect to TN-IDC (1%) (P= 0.017). TP53 SNVs were significantly more detected in TN-IDC (68%) than in HRpos and TN-ILC (respectively 44% and 35%, P<0.001).
- A similarly unfavorable outcome was observed for TN-ILC and TN-IDC (median OS 12 and 13 months, respectively), compared to their HRpos counterparts (median OS 31 and 33 months, respectively) (P<0.001) (**Figure 4**).



▲ **Figure 4** - Kaplan Meier in terms of OS for TN IDC, TN ILC, HRpos ILC and HRpos IDC. A similarly unfavorable prognosis was observed for TN IDC and TN ILC.

Conclusions

- Our study highlights distinct ctDNA-detectable genomic features of triple-negative ILC.
- These findings reinforce the need for improved understanding of TN-ILC to define personalized treatment options for this aggressive and rare subtype.

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