Comprehensive Genomic Profiling (CGP) of metastatic Invasive Lobular Carcinomas reveals heterogeneity in immune biomarkers and resistance alterations across biopsy sites

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ABSTRACT

Introduction
Metastatic breast cancer is a clinically challenging disease with poor outcomes. Invasive lobular carcinoma (ILC) is a rarer subtype of breast cancer with distinct patterns of metastasis, including frequent gastrointestinal (GI) and female reproductive (FR) metastases (mets). Due to their relative rarity, little is known about the genomic characteristic of ILC across met sites. Here we explore the genomic landscape of 1909 ILC specimens, stratified by met site, with an examination of immune checkpoint inhibitor (ICPI) biomarkers, therapeutic alterations, and resistance mutations.

Results
High TMB and PD-L1 IC staining may predict response to ICPI. ILC mets overall had a greater rate of high TMB (>=10 muts/mb) relative to IDC mets (21% v 9%, p = 7E-25) and breast-biopsied (breast) ILC (21% v 10%; p = 9E-15) with the highest frequency in GI (23%) and skin (21%). PD-L1 IC+ rates were lower in ILC mets (18%) relative to IDC mets (34%) and breast ILC (31%), but were variable across sites, with relatively high rates of positivity in GI (48%), skin (29%), and FR (18%) mets, and no positive staining in bone mets (0/37).

Alterations in *PIK3CA*, which may be targetable with kinase inhibitors, were higher in ILC mets (58%) relative to IDC mets (34%) and generally exhibited a similar frequency across ILC met sites, with modestly lower prevalence in skin (48%, p = 0.005). Pathogenic alterations in BRCA1/2 were observed in 4.8% of ILC mets overall, with a lower frequency in gi mets (1.3%, p = 0.03).

A comparison of ILC breast biopsies to ILC mets revealed 19 genes with higher prevalence in at least one ILC met site, most with known roles in therapy resistance (eg *ESR1, NF1, RB1, KRAS, ERBB2, BRAF*), though significant heterogeneity was observed across sites. Met-enriched (ME) alterations were highest in ILC from the liver (71%) and lowest in FR (33%). *ERBB2* mutations, which are may be targetable with HER2 kinase inhibitors, were predominantly found in liver mets (21%) with significantly lower prevalence in skin (11%), bone (10%), GI (3%), and FR (3%). *ESR1* alterations were common in most ILC sites, with the highest prevalence in liver (26%) and low frequency in FR (4%). While FR ILC harbor few ME alterations, the rare alterations are primarily found in *NF1* (5%) and *NCOR1* (5%).

Conclusions
CGP revealed significant heterogeneity in ILC mets across tissues. ICPI biomarkers were variable across sites with the highest frequency in ILC GI mets, potentially offering additional treatment avenues for these tumors. Potentially targetable alterations in *PIK3CA* were common in ILC mets with high prevalence across sites, suggesting potential broad utility for PIK3CA inhibitors. Therapy-resistance alterations were common in ILC mets but varied across sites. Notably, *ERBB2* alterations were most prevalent in ILC liver mets, but less common at other sites. The high prevalence of therapeutic and resistance alterations suggests value in profiling metastatic lesions.

MATERIALS AND METHODS

Comprehensive genomic profiling (CGP) workflow

CGP was carried out at Foundation Medicine with hybrid capture for exons from up to 395 cancer-related genes plus select introns from up to 31 genes (PMID 24142049). Tumor mutational burden (TMB) was determined on 0.8–1.1 Mb (PMID: 28420421). Ventana PD-L1 IC staining (SP142; positive >= 1% staining) was available for a subset of samples. 1071 breast-biopsied and 1909 met-biopsied ILC and 6926 breast-biopsied and 1901 met-biopsied IDC were available for analysis.

RESULTS

Figure 1. Biomarkers of immune checkpoint inhibitor response differ across ILC met sites. (A) Fraction of patients with tumor mutation burden greater or equal to 10 mutations/mb is shown for all IDC mets and ILC metastases stratified by site. (B) VENTANA SP142-IC PDL1 positivity rates are shown across sites. The highest biomarker prevalence is observed in GI and skin ILC metastases.

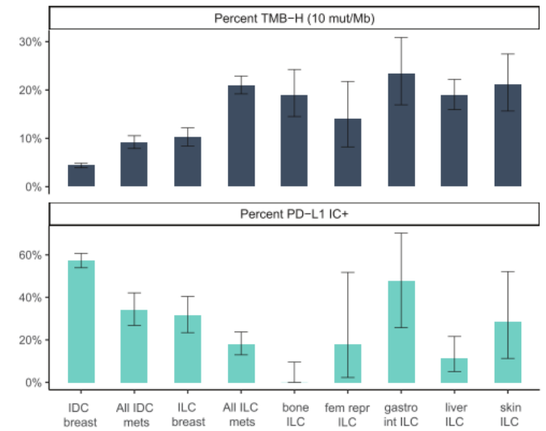


Figure 2. Prevalence of potentially actionable alterations across sites. (A) *PIK3CA* short variant alteration prevalence is similarly high across met sites. (B) *BRCA1/2* prevalence differs across sites with the highest prevalence in female reproductive ILC mets and lowest prevalence in gi ILC mets.

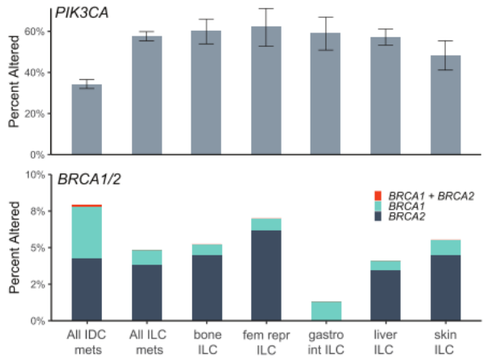


Figure 3. Longtail of alteration prevalence in local ILC (breast biopsied) and metastatic ILC. Shown are all genes alterations that are significantly enriched in metastatic ILC overall or in at least one metastatic site, relative to breast-biopsied ILC.

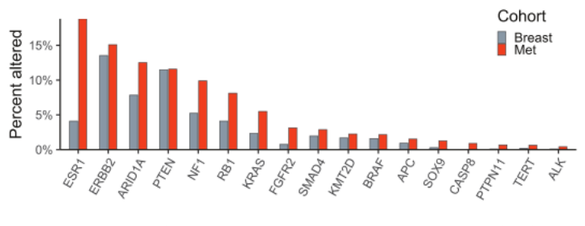


Table 1. Prevalence of met-enriched alterations in breast-biopsied ILC/IDC, metastatic IDC/ILC and ILC metastases broken down by site. Shown are all genes alteration classes that are significantly enriched in metastatic ILC overall or in at least one met site, relative to breast ILC. Mut indicates short variant alterations and RE indicates rearrangement/fusion events. * indicates significance vs breast ILC for each site.

	breast_ILC	met_ILC	breast_IDC	met_IDC	GI	liver	FR	bone	skin
sample count (N)	1071	1909	6926	1901	154	639	114	268	199
Met-Enriched									
ESR1_mut	3.8%	18.3%	2.2%	17.0%	20.8% *	25.5% *	3.5%	11.9% *	7.5% *
ERBB2_mut	9.7%	12.5%	2.0%	2.7%	3.2%	20.7% *	2.6%	9.7%	11.1%
ARID1A_mut	7.5%	12.2%	4.0%	5.5%	14.3% *	11.4% *	7.9%	9.0%	10.6%
NF1_mut	4.4%	8.7%	3.5%	4.2%	5.8%	8.5% *	5.3%	10.4% *	8.0% *
RB1_mut	2.7%	6.3%	4.3%	3.5%	7.8% *	5.9% *	2.6%	4.5%	11.6% *
KRAS	2.3%	5.5%	3.9%	3.6%	5.2%	6.7% *	2.6%	6.3% *	2.0%
PTEN_del	3.1%	4.1%	5.7%	4.7%	1.9%	4.7%	4.4%	1.9%	8.0% *
FGFR2	0.7%	3.1%	2.9%	2.6%	1.3%	4.1% *	1.8%	4.1% *	2.0%
NCOR1_mut	1.2%	2.9%	1.1%	1.1%	3.2%	3.0% *	5.3% *	1.9%	1.0%
SMAD4	2.0%	2.9%	1.2%	1.9%	1.9%	4.7% *	1.8%	2.6%	1.5%
BRAF	1.6%	2.1%	1.2%	1.4%	0.0%	1.4%	2.6%	4.5% *	2.5%
FOXP1_mut	0.4%	1.6%	0.3%	0.3%	2.6% *	1.1% *	0.0%	1.9% *	1.0%
APC_mut	0.9%	1.4%	1.1%	1.1%	0.6%	0.6%	1.8%	3.4% *	2.0%
SOX9	0.3%	1.3%	0.5%	0.4%	0.0%	1.1% *	0.0%	1.5% *	0.5%
CASP8_mut	0.0%	0.8%	0.5%	0.5%	0.0%	1.1% *	0.9%	1.1% *	1.0% *
PTPN11_mut	0.1%	0.7%	0.2%	0.3%	0.0%	0.6%	1.8% *	1.5% *	1.0%
TERT_mut	0.2%	0.6%	0.4%	0.7%	2.6% *	0.0%	0.0%	1.1%	0.5%
ALK_mut	0.1%	0.4%	0.2%	0.4%	2.6% *	0.0%	0.9%	0.4%	0.5%
KMT2D_RE	0.1%	0.3%	0.4%	0.3%	1.9% *	0.2%	0.0%	0.0%	0.5%
any_ME	32.5%	60.1%	30.1%	42.4%	53.9% *	71.4% *	33.3%	53.4% *	52.3% *

Figure 4. Frequency of metastasis-enriched alterations across sites. Plotted are the fraction of samples with at least one metastasis-enriched (ME) alteration (Table1). Female reproductive tumors exhibited the lowest prevalence of ME alterations, while liver mets had the highest prevalence.

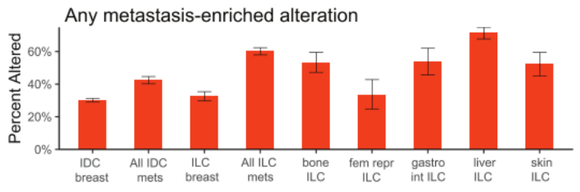
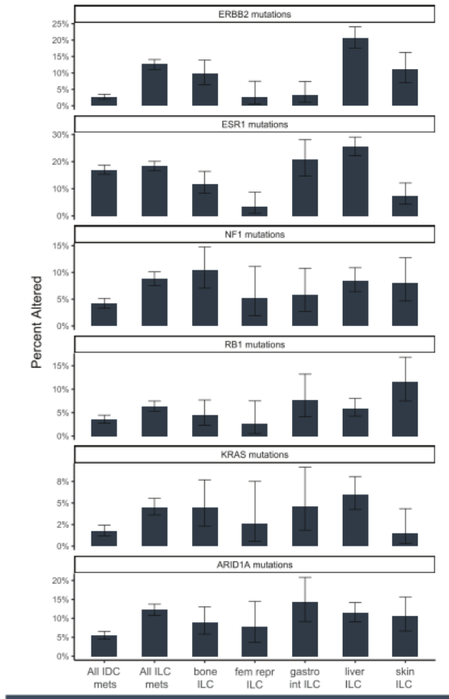


Figure 5. Prevalence of select metastasis-enriched alterations across ILC metastatic sites. Notable heterogeneity is observed in *ERBB2, ESR1, NF1,* and *RB1.*



CONCLUSIONS

- ICPI biomarkers were variable across sites with the highest frequency in ILC gastrointestinal mets, potentially offering additional treatment avenues
- Alterations in *PIK3CA* were common in ILC mets with high prevalence across sites, suggesting potential broad utility for PIK3CA inhibitors.
- Therapy-resistance alterations were common in ILC mets but varied across sites, with *ERBB2* mutations most prevalent in ILC liver mets.
- RB1 mutations are associated with CDK4/6i resistance and were highest in gastrointestinal and skin sites
- High prevalence of therapeutic and resistance alterations suggests value in profiling metastatic lesions.