Molecular evaluation of immunogenicity and genomic alterations in invasive lobular breast cancer

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Introduction

- Invasive lobular carcinoma (ILC) is the second most common type of invasive breast cancer and accounts for 10-15% of all cases.
- Though ILC has distinct clinical, prognostic and molecular features, studies specific to this subtype are limited and include smaller numbers of patients.
- ILCs show a decreased response to neoadjuvant chemotherapy and an increased resistance to endocrine therapy. Thus, there is a great need to identify alternative therapies, such as immunotherapy, that could improve overall survival.
- Success of immunotherapy largely depends on tumor immunogenicity which varies with histologic type. Determination of predictive and prognostic biomarkers for ILC will help determine who can benefit the most.
- Our study investigates canonical markers of immunogenicity – PD-L1 expression and Tumor Mutational Burden (TMB) – in patients with ILC compared to invasive ductal carcinoma (IDC).
- We also analyze differences in immune cell profiles constituting the tumor microenvironment (TME) in ILC and IDC.
- Lastly, we investigated the genomic alterations associated with immunogenicity in ILC

Methods

- A retrospective data analysis was performed on 868 tumor samples to identify breast cancer tumors with ILC or IDC histology profiled at Caris Life Sciences.
- Upon review of the data and discussions with numerous breast clinicians, we decided to exclude triple negative breast cancers (TNBC) given the rarity of the association between TNBC and ILC.
- The VENTANA PD-L1 (SP142) assay was used to score PD-L1 expression on immune cells; and PD-L1 expression in tumor cells was assessed by laboratory developed test using SP142 clone with staining higher than 2+ considered positive.
- TMB was measured by counting somatic non-synonymous missense mutations on the 592 gene panel (Nextseq) next generation sequencing (NGS) assay, and ≥ 10 mutations/megabase (mut/Mb) was considered high.
- Using the whole transcriptome RNA sequencing (NovaSeq) data we analyzed the difference in immune cell profiles constituting the TME using a computational RNA deconvolution approach.
- Next generation sequencing (NGS) was used to identify significant differences in genomic alterations in the tumors.

Tumor receptor status

A

Cancer type	Name	Ductal	Lobular	Grand Total
HR+ Her2-	HR+	601	151	752
HR- Her2+	Her2+	43		43
HR+ Her2+		70	3	73
Grand Total		714	154	868

Tumor sites

B

Tumor site	Ductal	Lobular	Total
Axilla, NOS	23	1	24
bone	11	2	13
Brain	5	2	7
Breast	529	82	611
Chest/Chest wall	28	4	32
Connective Tissue	4	2	6
GI organ	5	18	23
GYN Organs	1	8	9
Liver	34	9	43
Lung	11		11
Lymph Node	44	11	55
Other	10	9	19
Pleura	2		2
Skin	7	6	13
Grand Total	**714**	**154**	**868**

C

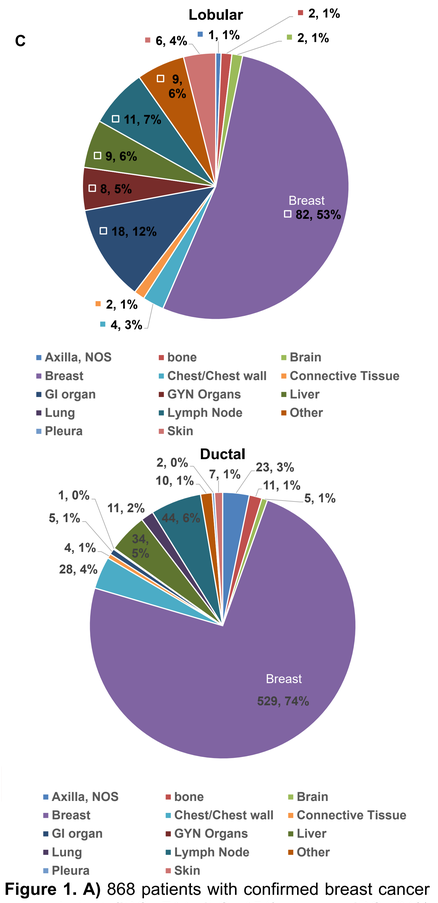

Figure 1. A) 868 patients with confirmed breast cancer were studied (IDC: 714, ILC: 154). Among IDC, 84% were Hormone receptor positive (HR⁺) and 16% Her2⁺, compared to 98% HR⁺ and 2% Her2⁺ in the ILC group. **B)** The samples are further categorized by tumor site with distributions shown in figure **C)** for ILC and IDC

Results

Figure 2: Immune cell profiling of ILC vs. IDC reveals ILC has significantly more M2 macrophages and T regulatory cells suggestive of a more immunosuppressed TME.

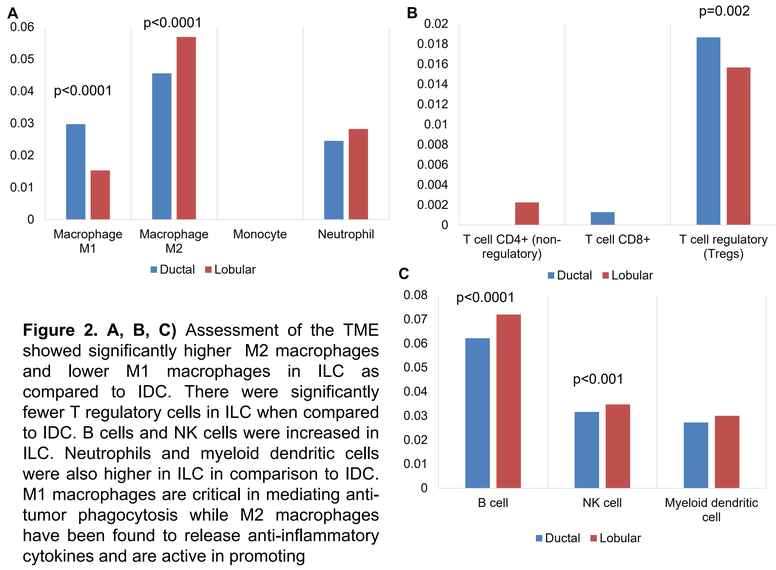

Figure 2. A, B, C) Assessment of the TME showed significantly higher M2 macrophages and lower M1 macrophages in ILC as compared to IDC. There were significantly fewer T regulatory cells in ILC when compared to IDC. B cells and NK cells were increased in ILC. Neutrophils and myeloid dendritic cells were also higher in ILC in comparison to IDC. M1 macrophages are critical in mediating anti-tumor phagocytosis while M2 macrophages have been found to release anti-inflammatory cytokines and are active in promoting Metastasis. As such, we found there to be significantly fewer M1 cells, and significantly more M2 macrophages in ILC as compared to IDC, supportive of a more suppressed TME in ILC.

Figure 3: PD-L1 positivity is higher in IDC as compared to ILC in both immune and tumor cell populations

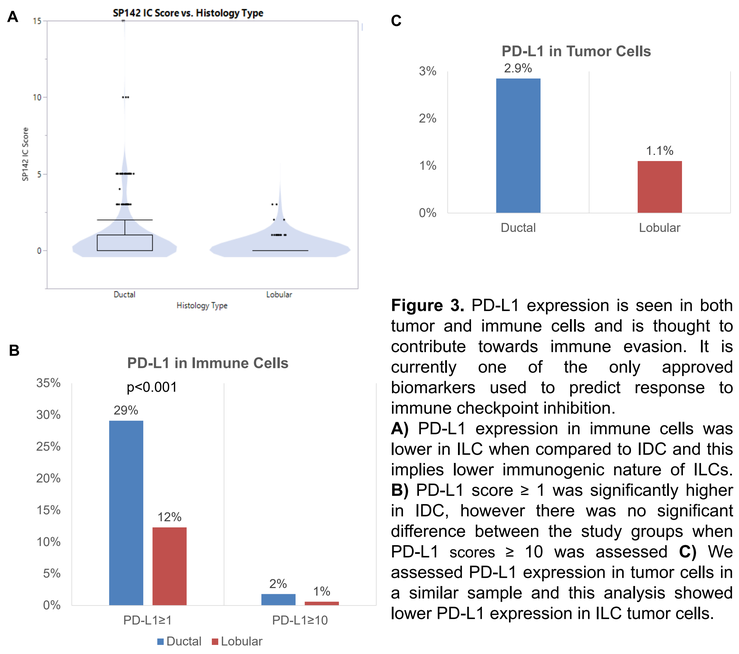

Figure 3. PD-L1 expression is seen in both tumor and immune cells and is thought to contribute towards immune evasion. It is currently one of the only approved biomarkers used to predict response to immune checkpoint inhibition.
A) PD-L1 expression in immune cells was lower in ILC when compared to IDC and this implies lower immunogenic nature of ILCs. **B)** PD-L1 score ≥ 1 was significantly higher in IDC, however there was no significant difference between the study groups when PD-L1 scores ≥ 10 was assessed **C)** We assessed PD-L1 expression in tumor cells in a similar sample and this analysis showed lower PD-L1 expression in ILC tumor cells.

Figure 4: Tumor mutational burden (TMB) is increased in ILC vs. IDC

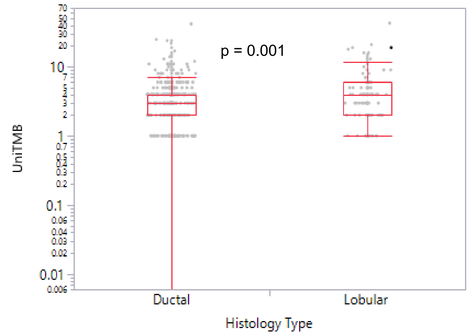

Figure 4. Tumor sites with 10 or more mutations per Mega base were classified as high. ILC was associated with high TMB (≥10mut/Mb).

Figure 5: High TMB in ILC is associated with common mutations such as ATM, RB1 and TP53

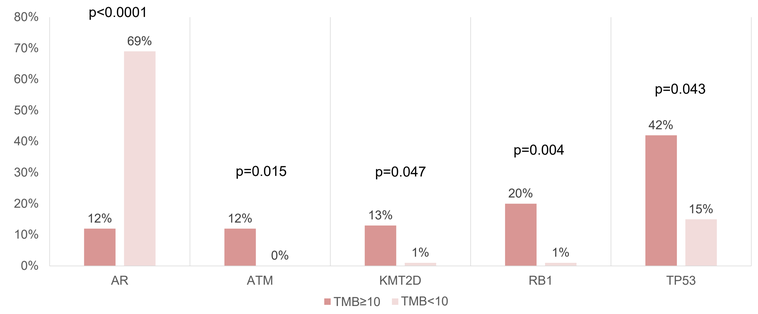

Figure 5. ILC with high TMB was associated with low androgen receptor (AR) expression and had significantly increased frequency of mutations in ATM, KMT2D RB1 and TP53 genes. Immunogenicity in breast cancer is thought to be promoted by TP53 mutations. ATM mutations are shown to be associated with increased response to immunotherapy in bladder cancer and endometrial cancer. So these mutations could be potential biomarkers in predicting response of breast cancer to immune checkpoint therapy. In our study, PD-L1 expression was not significantly associated with any gene mutations.

Summary

- PD-L1 expression in immune cells was lower in ILC, however ILC was associated with significantly higher TMB.
- Immune cell profiling supports a cold or less immunogenic TME for ILC.
- ILC with high TMB was associated with significantly higher genomic alterations, some of these could be potential biomarker to predict response to immune therapy. This needs further investigation in large studies.
- A composite immune biomarker may be able to better characterize immunogenicity of ILC.

References

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Acknowledgements

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