# Comprehensive Molecular and Immune Profiling of Triple Negative Invasive Lobular Carcinoma

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## BACKGROUND

Triple-negative invasive lobular carcinoma (TN-ILC) is a rare (0.1-1.4%) breast cancer with prognosis worse than ER positive ILC. Currently there are no targeted therapies or clinical trials specifically for TN-ILC. A comprehensive analysis of the molecular and immune landscape can help identify novel targets and pathways for TN-ILC to improve patient outcomes. Here, we characterized the molecular and immune signature of TN-ILC.

## METHODS

- 395 BC samples (Invasive ductal (ID) TNBC, n=364; TN-ILC, n=31) were analyzed by NGS (592, NextSeq; WES, NovaSeq), WTS (NovaSeq) (Caris Life Sciences, Phoenix, AZ).
- A total of 10 pro-apoptotic (*BAX, BAK1, BID, BAD, BIK, BCL2L11, BMF, HRK, PMAIP1, BBC3*) and 6 anti-apoptotic (*BCL2, BCL2L1, BCL2L2, MCL1, BCL2A1, BCL2L10*) BCL2 family genes were analyzed.
- Tumor mutational burden (TMB) totaled somatic mutations per tumor (high >10 mt/MB) was tested by IHC and NGS.
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Pathway enrichment was determined by GSEA (Broad Inst).
- Statistical significance was determined using chi-square and Mann-Whitney U test with p-values adjusted for multiple comparisons (q < 0.05).



## RESULTS

**Figure 1.** Mutation and copy number alteration analysis of TN-ILC and ID-TNBC

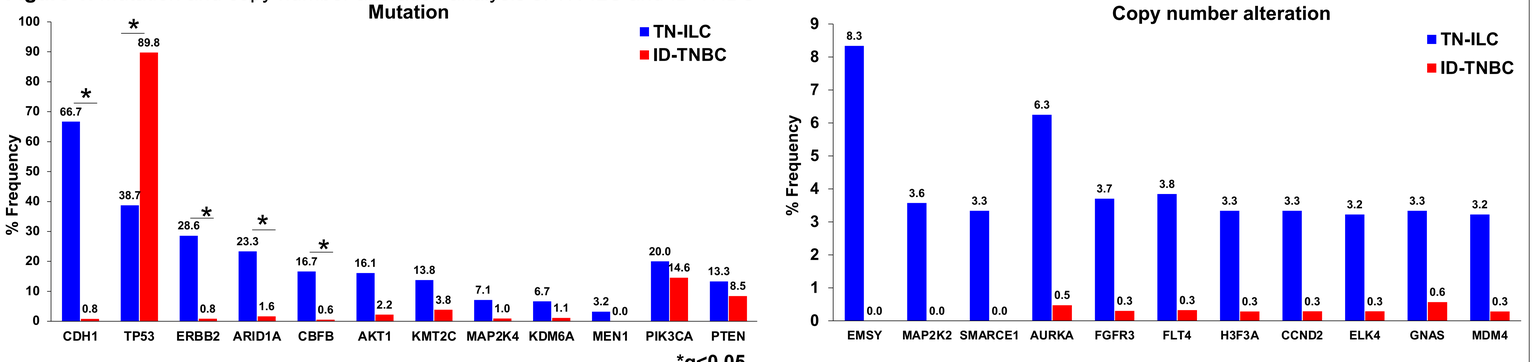



**Figure 2.** Immune cell infiltration in TN-ILC and ID-TNBC

| Cells | TN-ILC | ID-TNBC |
|---|---|---|
| B cells | 4.40 | 4.46 |
| Mφ M1 | 2.17 | 3.46 * |
| Mφ M2 | 5.34 | 2.94 * |
| DC | 3.00 | 3.21 |
| Neutrophils | 4.83 | 2.65 * |
| NK cells | 3.15 | 3.05 |
| T cells CD8 | 0.11 | 0.58 |
| Tregs | 1.51 | 1.99 |

Low Median% High Median%

TN-ILC had higher infiltration of M2 macrophages and neutrophils but lower infiltration of M1 macrophages and CD8 T cells. Median% of monocytes and T cells CD4 were 0.0 in both the groups ***q<0.05.**

**Figure 3.** Immune checkpoint gene expression

| Gene | TN-ILC | ID-TNBC |
|---|---|---|
| CD274 | 2.78 | 4.01 * |
| PDCD1 | 0.24 | 0.52 |
| CTLA4 | 0.82 | 1.61 * |
| PDCD1LG2 | 1.33 | 1.67 |
| FOXP3 | 2.22 | 2.79 |
| HAVCR2 | 14.98 | 19.70 |
| LAG3 | 0.67 | 1.05 * |
| IDO1 | 1.07 | 2.90 * |

Low Median High Median

TN-ILC had decreased *CD274*, *CTLA4*, *FOXP3*, *LAG3* and *IDO1*. ***q<0.05**.

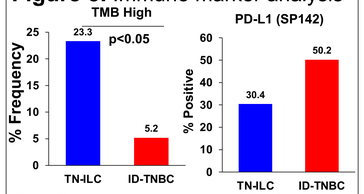
**Figure 4.** BCL2 family gene expression

| | Gene | TN-ILC | ID-TNBC |
|---|---|---|---|
| Pro-apototic | BAX | 22.45 | 32.86 * |
| | BAK1 | 3.85 | 5.16 * |
| | BID | 9.57 | 15.47 * |
| | BAD | 10.59 | 8.59 |
| | BIK | 3.24 | 1.46 * |
| | BCL2L11 | 18.11 | 22.43 |
| | BMF | 6.73 | 7.61 |
| | HRK | 0.00 | 0.11 * |
| | PMAIP1 | 0.27 | 1.70 * |
| | BBC3 | 0.92 | 0.83 |
| Anti-apototic | BCL2 | 2.06 | 2.25 |
| | BCL2L1 | 18.39 | 18.41 |
| | BCL2L2 | 2.48 | 2.30 |
| | MCL1 | 17.08 | 25.86 * |
| | BCL2A1 | 2.22 | 8.42 * |
| | BCL2L10 | 0.20 | 0.38 * |

Low Median High Median

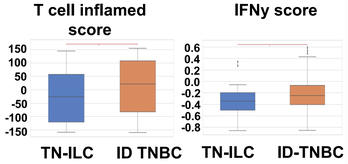
TN-ILC had differential BCL2 family gene expression. ***q<0.05**.

**Figure 5.** Immune marker analysis



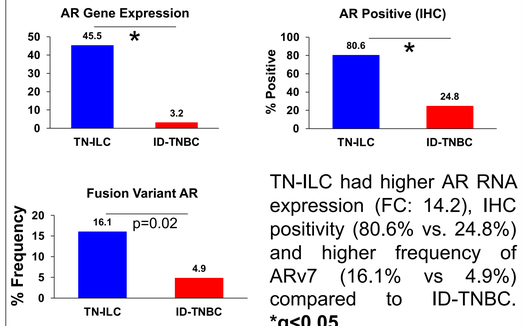

TN-ILC had higher frequency of TMB high (23.3% vs. 5.2%, p<0.05) but there was no difference in PD-L1 (30.4% vs 50.2%, p=06) .

**Figure 6.** T cell inflamed and IFNy score



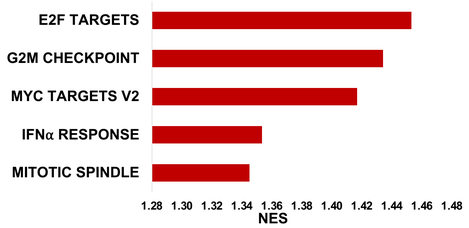

TN-ILC had decreased T cell inflamed score (-0.27 vs 21.5) and IFNy score (-0.34 vs -0.24, **all q<0.05**).

**Figure 7.** AR expression and fusion variant AR analysis




TN-ILC had higher AR RNA expression (FC: 14.2), IHC positivity (80.6% vs. 24.8%) and higher frequency of ARv7 (16.1% vs 4.9%) compared to ID-TNBC. ***q<0.05**.

**Figure 8.** Gene set enrichment analysis (GSEA)




ID TNBC had gene set enrichment of the E2F targets, G2M checkpoint, MYC targets V2, IFNα response and mitotic spindle pathways compared to TN-ILC (**all FDR<0.25**).

## CONCLUSIONS

These data suggest that TN-ILC had higher frequency of *CDH1, ERBB2, AKT1, ARID1A* mutations, higher M2 macrophages and neutrophils and lower M1 macrophages and CD8 T cells infiltration and, lower T cell inflamed signature. High TMB and AR expression can translate into use of immunotherapy (ICI) and AR antagonists in these patients.