LAG3+ Tumor Infiltrating Lymphocytes Predict Outcome in Treatment Naïve Triple Negative Breast Carcinoma

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Significance:

Triple negative breast carcinoma (TNBC), characterized by lack of estrogen (ER) and progesterone receptors (PR) and absence of Her2/neu (Her2) receptor amplification, typically follows an aggressive course that includes high relapse rates, rapid progression, and poor outcome. Advances in treatment have been limited by molecular heterogeneity within TNBCs and lack of effective molecular targets. Although a subset of TNBC associated with tumor infiltrating lymphocytes (TILs) is more immunogenic than other breast cancers, response to immunotherapy has been variable and reported in only 10-40% of cases. Identification of prognostic immune biomarkers will identify novel therapeutic targets and facilitate appropriate patient selection for therapy.

Aim:

- To analyze the immune gene expression profile in TNBC and identify a prognostic marker.

Material and Methods:

- **Targeted mRNA sequencing:** 30 formalin fixed paraffin embedded whole sections from treatment naïve TNBC with TILs were sequenced using the HTG Edge Seq Assay (HTG Molecular Diagnostics, Inc. Tucson AZ). Differential expression (DE) of 1392 immune-related genes in the Precision Immuno-Oncology Panel was analyzed.
  - 15 cases had favorable outcome (recurrence free overall survival (OS) ≥ 5 yrs)
  - 15 cases had unfavorable outcome (never disease free or dead of disease in < 5 yrs)
- **Validation** of the top 40 up- and down- regulated DE genes with adjusted p-value of < 0.001 was performed against the TNBC mRNA profiles in The Cancer Genome Atlas database. Four genes showed significant DE in both data sets of which LAG3 was chosen for further evaluation by immunohistochemistry.
- **LAG3 immunohistochemistry** (IHC) (rabbit monoclonal AntiLAG3 antibody, Abcam plc.) was performed on whole sections from 56 consecutive treatment naïve TNBC patient specimens. LAG3 IHC score for each TNBC was based on the average number of LAG3+ immune cells per high power field in 3-5 hot spots (<1cell positive=*0;* 1-10 cells positive=*1+;* 11-20 cells positive=*2+*; >20 cells positive, focal=*3+*, >20 cells positive, diffuse=*4+*).
- **Patients**: All were female, age 29-89 years (median 55 yrs) with 0.6 to 9.0 cm (median 2.6 cm) TNBCs and a median Ki67 of 42%. 26 patients had metastatic carcinoma in 1-17 (median 2) axillary lymph nodes. None had distant metastases at presentation. Disease free survival (DFS) varied from 0 to 114.8 months (median 81.4 mos.) and overall survival (OS) varied from 7.6 to 114.8 months (median 81.5 mos.).
- **Statistical analysis:** The student's t-test was used to correlate median LAG3 expression with DFS and OS. A Kaplan Meier plot was constructed to analyze 5 year OS in TNBC patients. Log rank test was used to stratify the patients by LAG3 IHC score.

Results:

DE analysis of our data set yielded 242 statistically significant differentially expressed immune related genes, one of which was LAG3. A similar analysis of the TCGA TNBC data set also revealed significant differential expression of LAG3.

LAG3 immunoexpression was seen only in immune cells and was heterogeneously distributed in the carcinoma. LAG3 IHC scores were 0 in 9, low (1-3+) in 24 and high (4+) in 23 cases. Median DFS was 35.8 months in TNBC with LAG3 IHC0, 81 months in LAG3 IHC1-3+ and 82 months in LAG3 IHC4+ cases. Median DFS was significantly different in LAG3 IHC0 versus IHC4+ cases (p=0.045). Median OS was 50 months in LAG3 IHC0, 62 months in LAG3 IHC1-3+ and 81.4 months in LAG3 IHC4+ cases. OS was significantly different in LAG3 IHC0 versus LAG3 IHC4+ cases (p=0.037) and in LAG3 IHC1-3+ versus IHC4+ cases (p=0.043).

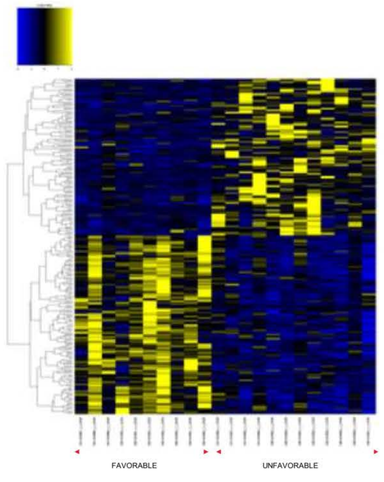


Figure 1: Heatmap showing the relative expression levels of transcripts differentially expressed with adjusted p-values less than 0.05 between TNBC patient groups with favorable and unfavorable outcomes

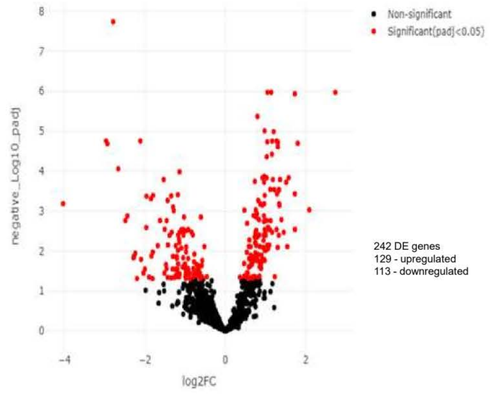


Figure. 2: Volcano plot of immune related gene expression change in TNBC with favorable and unfavorable outcomes. Significantly changed genes are defined with adjusted p < 0.05.

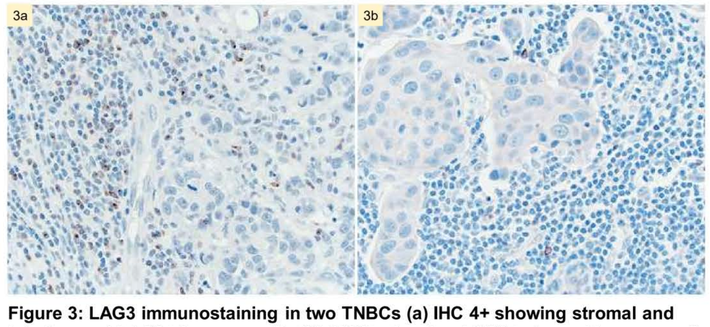
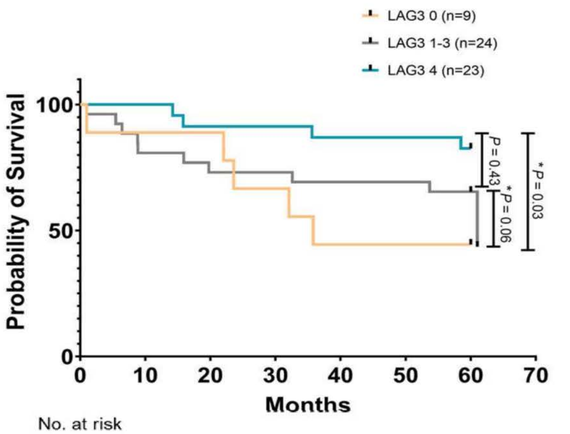


Figure 3: LAG3 immunostaining in two TNBCs (a) IHC 4+ showing stromal and intratumoral LAG3+ immune cells (b) IHC1+ showing LAG3+ stromal immune cells



No. at risk

	0	20	40	60
LAG3 0	9	8	5	4
LAG3 1-3	24	20	19	17
LAG3 4	23	22	21	19

Figure 4: Kaplan Meier plot of 5 year overall survival in 56 patients stratified by LAG3 IHC expression level in TNBC.

Conclusions

1. **LAG3 is a prognostic marker in TNBCs.**
2. **LAG3 is expressed in immune cells and shows heterogenous expression in the TNBC.**
3. **TNBC with high LAG3 immunoexpression had longer median DFS than those showing low LAG3 expression.**
4. **TNBC with high LAG3 immunoexpression had longer OS than those showing no or low LAG3 immunoexpression, suggesting that cases with high LAG3 immunoexpression might require less aggressive therapy compared to the others.**
5. **Confirmation of these results requires a larger TNBC cohort.**

Acknowledgements

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