Associations of the immunomodulatory molecule Siglec-15 gene and protein expression to immune gene signatures as a potential biomarker for Siglec-15 targeted therapy in non-small cell lung cancer

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Abstract #: 219

BACKGROUND

Despite the recent success of immunotherapies against cancer, not all patients benefit from treatment. Biomarkers capable of predicting therapeutic response may improve patient selection and increase the number of patients that benefit from treatment. Siglec-15 is a novel immunosuppressive protein expressed on tumor associated macrophages (TAMs) and tumor cells in many indications and is currently under clinical investigation (PYX-106, NCT05718557) (**Figure 1**). In patients where disease tissue is not readily accessible such as non-small cell lung cancer (NSCLC), blood-based biomarkers should be explored. Here the biology and expression patterns of Siglec-15 and PD-L1 in matched blood and tumor samples were investigated.

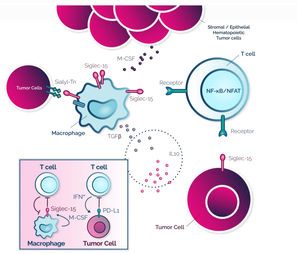


Figure 1. Mechanism of Action. Immuno-suppressive biology of Siglec-15 in the TME[1,2].

METHODS

Table 1. Sample information for commercially sourced donor specimens.

Donor	Tissue	Diagnosis	Location	AJCC/UICC Stage Group
12	Lung	Lung Cancer, Adenocarcinoma	Primary	II-B
37	Lung	Squamous cell carcinoma	Primary	II-B
60	Lung	Adenocarcinoma, papillary predominant	Primary	I-B
77	Lung	Lung Cancer, Large Cell	Primary	III-A
13	Lung	Lung Cancer, Adenocarcinoma	Primary	II-B

Matched peripheral blood mononuclear cells (PBMCs) and formalin-fixed, paraffin-embedded (FFPE) tumor samples from 5 treatment naïve patient donors with NSCLC were commercially sourced (**Table 1**). Immunohistochemistry (IHC) was performed for Siglec-15 (proprietary antibody; SITC2024-Poster#521[3]) and PD-L1 (22C3 pharmDx) in tumors. Protein expression was scored by a board-certified pathologist. The IO360 Nanostring panel, with customized genes, was used to measure bulk gene expression from PBMCs and tumors (**Figure 2**).

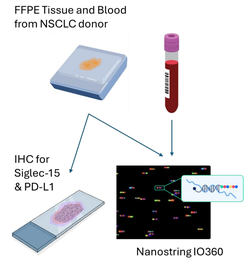


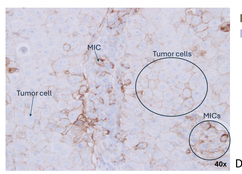
Figure 2. Methods. Donor samples were analyzed by IHC and Nanostring.

PD-L1 gene expression does not correlate with protein measured by IHC in tumor samples

Table 2. Tumor sample scores for PD-L1 IHC staining.

Donor	% at 0 (no staining)	% PD-L1 Staining @ 1+	% PD-L1 Staining @ 2+	% PD-L1 Staining @ 3+	TPS# (%)	Estimated Tumor Cells&	MICs&	MIDS (0-4)
12	100	0	0	0	0	107000	980	1
37	100	0	0	0	0	152000	14000	2
60	100	0	0	0	0	3000	15	1
77	100	0	0	0	0	68000	15000	3
13	65	35	0	0	35	105000	1900	2

#NSCLC; &Mononuclear Inflammatory Cells (MICs - estimate number); Mononuclear Inflammatory Density Score (MIDS)



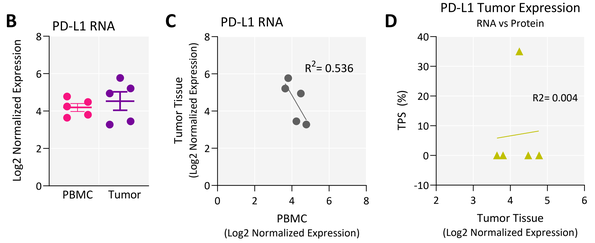
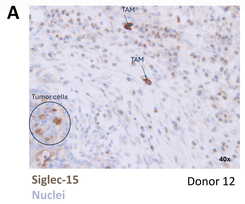


Figure 3. Expression of PD-L1 in blood and tumor samples on NSCLC donors. A Representative image of IHC staining for PD-L1 expression. PD-L1 expression was found on only one sample by Tumor Proportion Score (TPS), which does not account for immune cell staining that may be picked up by RNA analysis. **B** PD-L1 gene expression was low in both PBMC and tumor samples. **C** There was no correlation in the RNA expression of PD-L1 in PBMCs vs tumor tissues. **D** Gene expression of PD-L1 did not correlate with TPS scores for protein expression by IHC which may be due to PD-L1 expression on immune cells or larger portions of tissue being tested.

Siglec-15 gene expression correlates with Siglec-15+ TAMs in tumor samples

Table 4. Number of Siglec-15+ tumor cells and TAMs in each tumor sample as measured by IHC.

Donor	% Positive Tumor Cells	Average # of Siglec-15+ TAMs/20x field
12	75	133
37	0	34
60	70	2
77	0	91
13	30	126



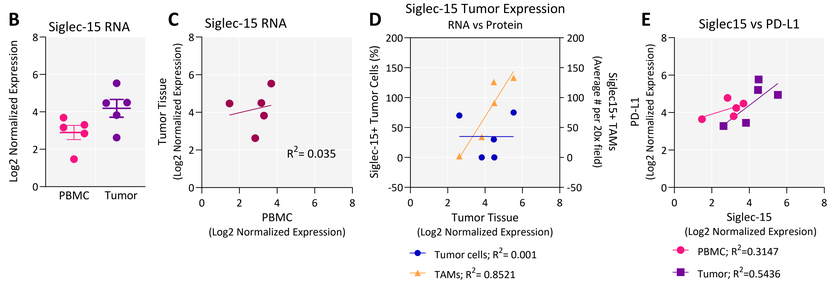


Figure 4. Expression of Siglec-15 in blood and tumor samples from NSCLC donors. A Representative images of IHC staining for Siglec-15 expression. Siglec-15 was expressed on tumor cells in 3 of 5 samples. Siglec-15+ Tumor Associated Macrophages (TAMs) were present in all samples. **B** Siglec-15 RNA is expressed at slightly higher levels in tumor samples than in PBMCs. **C** There is no correlation of Siglec-15 expression between PBMC and tumor samples. **D** Gene expression of Siglec-15 correlated with the average number of Siglec-15+ TAMs in a 20x field but not with the percent of Siglec-15+ tumor cells. **E** In this small sample set, RNA expression for PD-L1 and Siglec-15 did not have a significant correlation.

Siglec-15 and PD-L1 gene expression correlations with ROSALIND immune cell scores in PBMCs and tumor samples

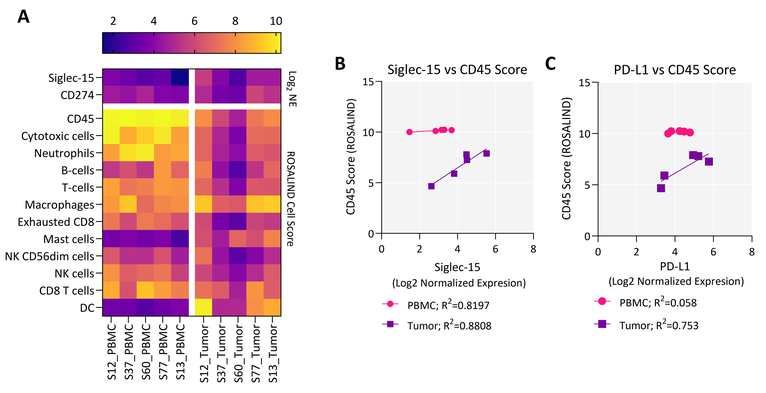


Figure 5. Correlation of immune cell density and expression of Siglec-15 and PD-L1 RNA. A Cell scores are generated by ROSALIND using a Nanostring algorithm. **B** Siglec-15 gene expression correlated with increased immune cell scores in both PBMC and tumor samples. **C** PD-L1 gene expression correlated with increased immune cell scores in tumor but not PBMC samples.

Siglec15 gene expression in tumor samples correlates with an immunosuppressive TME

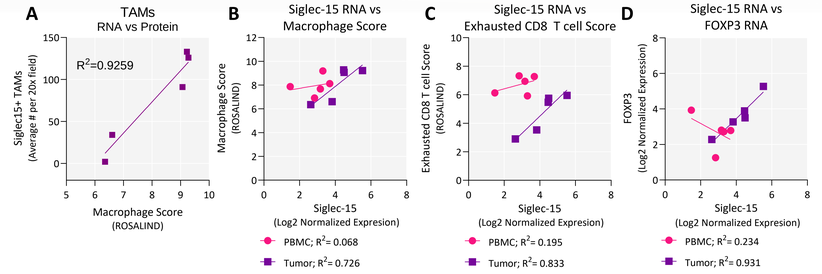


Figure 6. Correlation of Siglec-15 RNA expression with immune parameters in tumor samples. A The macrophage score calculated by ROSALIND correlated with the number of Siglec-15+ TAMs on the tumor samples. **B** The macrophage score from ROSALIND correlates with Siglec-15 RNA expression in tumor samples but not PBMCs. **C** Siglec-15 gene expression also correlates with the ROSALIND score for exhausted CD8+ T cells in tumor samples but not PBMCs. **D** A significant correlation exists between the RNA expression of Siglec-15 and FOXP3 (a marker for regulatory T cells) but not PBMCs.

LRRC4C gene expression correlates with Siglec-15 gene expression and Siglec-15+ TAMs in tumor samples

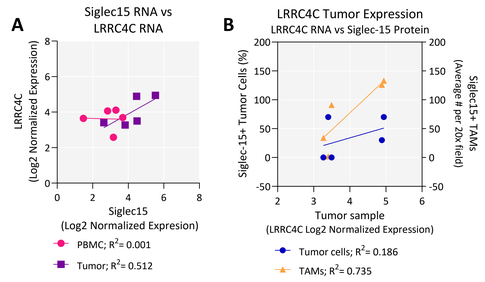


Figure 7. Correlation of Siglec-15 expression to possible binding partner, LRRC4C, in tumor samples. A LRRC4C gene expression correlated with Siglec-15 gene expression in tumor tissues but not in PBMCs. **B** LRRC4C gene expression correlated with Siglec-15+ TAMs in tumor tissue as measured by IHC, but did not correlate with Siglec-15 protein expression measured as percent Siglec-15+ tumor cells.

CONCLUSIONS

In this limited data set, expression of Siglec-15 and PD-L1 were detected in matched PBMCs and tumors by Nanostring and in tumors by IHC. Correlations were observed between Siglec-15 gene expression and immune cell scores in tumors. This analysis may inform the utilization of Siglec-15 expression by IHC and gene expression of Siglec-15 and immune cells as potential biomarkers to identify indications for Siglec-15 targeted therapy. The co-expression of Siglec-15 and PD-L1 in tumors may inform indication selection for combination therapies. Future studies will expand this data set for NSCLC and evaluate other indications.

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