Abstract # 14207

NantWorks

IDO and Tim3 Gene Expression is Correlated in NSCLC Patients with Low PDL1 Gene Expression

CONTRIBUTING RESEARCHERS

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BACKGROUND

Tumor cells evade immunosurveillance by expression of immune checkpoint proteins *PD-L1, IDO, and TIM3*, even when tumor-infiltrating lymphocytes (TILs) are present. Combination of checkpoint inhibitors Pembrolizumab(*PD-1*) and Epacadostat (IDOi) failed to show synergy in melanoma. Yet there are several candidate targets for checkpoint inhibition and rational selection of which combinations to explore is lacking. In particular, which checkpoints to target when *PD-L1* is not expressed is largely unexplored. The purpose of this study was to assess the expression of 12 immune checkpoint genes in lung cancer patients with high and low expression of PD-L1.

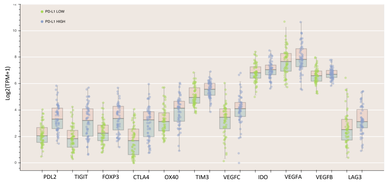
METHODS

- Retrospective analysis of a commercial database of 112 NSCLC patients (Avg. age 63±13.7, 53.6% Female)
- Performed deep whole transcriptomic sequencing (RNA-Seq) (~200x10^6 reads per tumor)
- Cases were categorized as PD-L1-low and PD-L1-high by median splitting
- 12 checkpoint and TME markers were analyzed for enrichment in TMB-high patients
- Immune-cell infiltration was estimated using RNA deconvolution on known immune cell marker genes (Bindea et al. 2013)

RESULTS

Figure 1. **Checkpoints are significantly differentially expressed in PD-L1 high and low settings**: Normalized expression distributions for 12 checkpoint markers in high PD-L1 vs. low PD-L1 patients (left), and two-sided t-test statistics for each comparison (right), with Benjamini-Hochberg corrected p-values for reducing FDR.

All 12 checkpoints and microenvironment markers have increased expression in the PD-L1-high setting, 7 / 12 are significantly over-expressed after FDR correction.



Marker	t	p	adj. p
PDL2	5.63	1.40E-07	8.41E-07
TIGIT	5.10	1.43E-06	5.70E-06
FOXP3	4.99	2.25E-06	6.74E-06
CTLA4	4.66	9.01E-06	2.16E-05
OX40	3.06	2.80E-03	5.61E-03
TIM3	2.96	3.80E-03	5.89E-03
VEGFC	2.95	3.92E-03	5.89E-03
IDO	1.41	1.61E-01	2.15E-01
VEGFA	1.13	2.61E-01	3.13E-01
VEGFB	0.97	3.36E-01	3.67E-01
LAG3	0.88	3.80E-01	3.80E-01

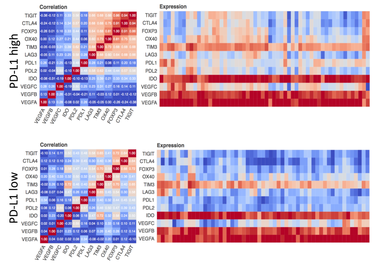
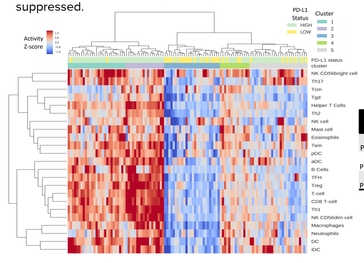


Figure 2. **Checkpoints are more coordinated in the PD-L1 high setting.** Pearson correlation (left) and log2-normalized expression (right) of checkpoints in patients with low and high TMB.

Although PD-L1 itself does not highly correlate with other checkpoint markers, checkpoints are more coordinated in the PD-L1 high setting. Specifically TIGIT, CTLA4, FOXP3, OX40, TIM3 and LAG3 are coexpressed at higher levels.

Figure 3. **Immune infiltration profiles differ between PD-L1 high vs. PD-L1 low:** Immune-cell activity inferred from RNAseq (left). Silhouette analysis revealed 5 clusters. Fisher's exact tests for enrichment of PD-L1 statuses in clusters that achieve significance after FDR-adjustment (right).

High PD-L1 is significantly associated with clusters 1 & 2, which are highly immuno-active. Conversely, low PD-L1 is significantly associated with cluster 3 which is immuno-suppressed.



Comparison	Odds Ratio	p	adj. p
PD-L1 LOW vs. cluster 3	15.24	5.81E-08	5.81E-07
PD-L1 HIGH vs. cluster 2	inf	1.25E-03	6.26E-03
PD-L1 HIGH vs. cluster 1	6.818	3.21E-03	1.07E-02

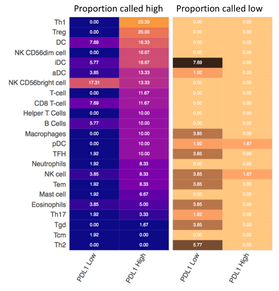


Figure 4. **PD-L1 is a primary driver of immune response:** Percentages of patients with significantly high infiltration (left) or significantly low infiltration (right) for each cell type, split into PD-L1 high and low categories.

Almost all immune cell types are more likely present in PD-L1 high patients than low patients, especially Th1 and Treg cells. Conversely, almost all cell-types are not significantly excluded in PD-L1 patients.

Cell types are significantly excluded from the tumor in some PD-L1 low patients, especially iDC and Th2 cells.

KEY FINDINGS

- **CTLA, TIGIT and FOXP3** showed variable expression and **high correlations** across all 112 samples regardless of PD-L1 expression
- **Expression of all markers is higher among the PD-L1 high** expression group versus the low group
- Within the PD-L1 high expression group, the expression of markers **LAG3, TIM3, OX40, FOXP3, CTLA4 and TIGIT** was both higher and more **highly correlated than in the PD-L1 low group**.
- **Within the PD-L1-low category, IDO1 and TIM3 have relatively high expression and are highly correlated** with each other (R=0.72, p=3.85x10^{-10}).

CONCLUSIONS:

PD-L1 is a primary driver of immune suppression, however when PD-L1 is low there may be some differential role for IDO or TIM3. Combination IDOi and Tim3i should be considered in PD-L1 low patients.

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