

Utilization of a tissue-based PD1_PD-L1 in situ proximity ligation assay in lung adenocarcinoma and pleural mesothelioma

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Background

The use of immunohistochemistry (IHC) to assess expression of programmed death-ligand 1 (PD-L1) as a biomarker for therapeutic strategies targeting the programmed cell death protein 1 (PD1) / PD-L1 pathway is controversial possibly due to the inability of the approach to determine if the signaling pathway is engaged within the tumor.

In this study, we aimed to determine the frequencies of PD1_PD-L1 interaction in lung adenocarcinoma (LUAD) and pleural mesothelioma, its correlation with PD-L1 IHC and the impact of this interaction on the composition of the tumor microenvironment (TME).

Methods

Four tissue microarrays (TMA) of 34 FFPE surgically resected Stage I/II LUAD and 15 pleural mesotheliomas were evaluated for PD1_PD-L1 interaction, PD-L1 IHC and PD1 & PD-L1 DSP (GeoMx Digital Spatial Profiler, DSP). Clinicopathological data (**Table 1**) was available for all samples.

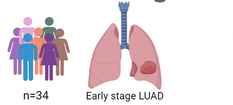
1. PD1_PD-L1 interaction and scoring: Using chromogenic *in situ* proximity ligation assay (Naveni® PD1/PD-L1 BOND RX HRP, Navinci Diagnostics), fully automated on the autostainer Leica BOND RX. The Scoring are using two analysis regions (AR): tumor and TME were evaluated with 2 methodologies:

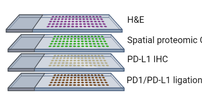
- Standard Microscopy (SM): regions were considered positive when clusters of dots were observed among cells.
- Digital image analysis (DIA): we evaluated the number of dots per cell in Tumor and TME. The average of PD1_PD-L1 dots per cell, optical density, H-score, and percentage of positivity, using thresholds from 1+ to 4+ were reported.

2. PD-L1 IHC scoring: Using SM, we estimated the tumor proportion score (TPS) and immune cells (IC) score. Regions with ≥1% were considered positive.

3. GeoMx® DSP Assay: immune protein information was available from a serial section of LUAD TMA. Mann-Whitney, One-way ANOVA and Spearman r correlations were performed.

Experimental Design







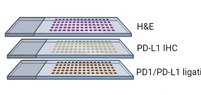
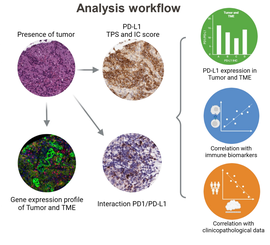


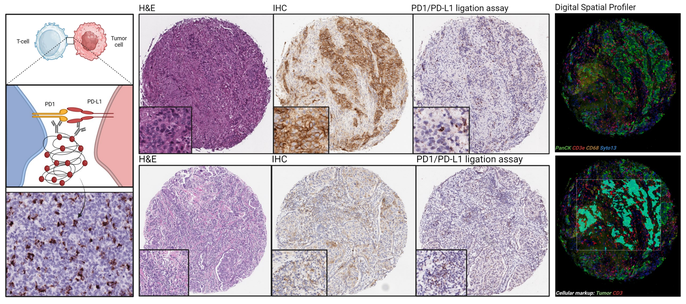
Table 1.- Clinicopathological characteristics

Characteristics	LUAD N, %	Mesothelioma N,%
All samples	34	15
Age (Range, median)	(38-85 y; 68.5 y)	(48-76 y; 68 y)
Sex		
Female	23, 68%	6, 40%
Male	11, 32%	9, 60%
Smoking status		
No	8, 24%	8, 53%
Yes	26, 76%	7, 47%
Pathology stage (AJCC 8th edition)		
Stage I	18, 53%	
Stage II	16, 47%	

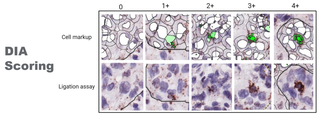
Notes: LUAD, Lung adenocarcinoma; y, years; AJCC, American Joint Committee on Cancer.



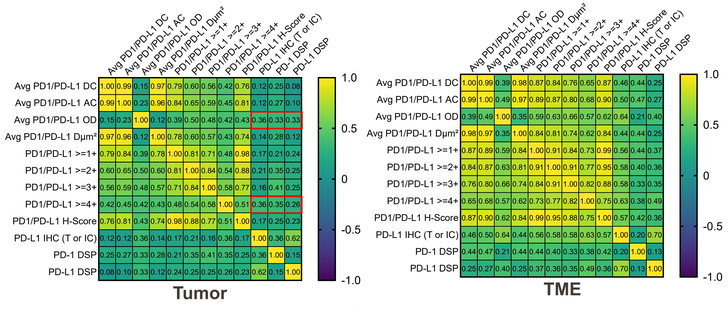
PD1_PD-L1 interaction and PD-L1 expression in LUAD and Mesothelioma



Feature	Assay	Analysis region	Parameter	Scoring
Expression of positivity	PD1/PD-L1 ligation assay	Tumor/TME	DIA	0-4 (+)
H-score	PD1/PD-L1 ligation assay	Tumor/TME	DIA	0-400
Optical density	PD1/PD-L1 ligation assay	Tumor/TME	DIA	Average
Dots per cell	PD1/PD-L1 ligation assay	Tumor/TME	DIA	Average
PD1_PD-L1 interaction	PD1/PD-L1 ligation assay	Tumor/TME	SM	0-100% (Neg/Pos; cutoff, >=1%)
Tumor proportion score	PD-L1 IHC	Tumor	SM	0-100% (Neg/Pos; cutoff, >=1%)
Immune cell score	PD-L1 IHC	TME	SM	0-100% (Neg/Pos; cutoff, >=1%)
Protein biomarker	PD1	Tumor/TME	DSP	DSP counts
Protein biomarker	PD-L1	Tumor/TME	DSP	DSP counts



Correlation of PD1_PD-L1 with PD-L1 (IHC/DSP) and PD1 DSP

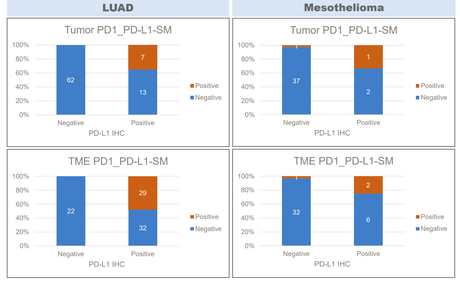


Notes: DC: dots per cell, AC: area per cell (μm²), OD: Optical Density, Dμm²: Dots Per μm², T: tumor

PD1_PD-L1 Interaction and PD-L1 IHC expression

PD1_PD-L1 Interaction by Core (SM)

PD1_PD-L1	LUAD	Mesothelioma
Negative	129 (78.18%)	77 (93.9%)
Positive	36 (21.82%)	5 (6.1%)
Total	165	82



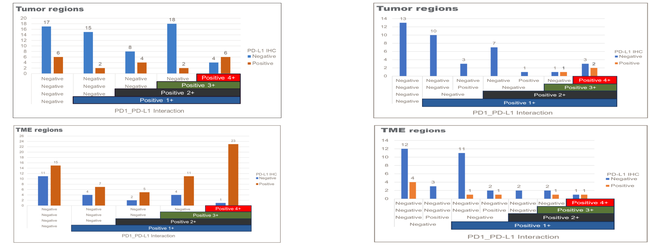
PD1_PD-L1 Interaction by Core (DIA)

LUAD

PD1_PD-L1	>1+	>=2+	>=3+	>=4+
Negative	49 (29.7%)	77 (46.67%)	96 (58.18%)	131 (79.39%)
Positive	116 (70.3%)	88 (53.33%)	69 (41.82%)	34 (20.61%)
Total	165	165	165	165

Mesothelioma

PD1_PD-L1	>1+	>=2+	>=3+	>=4+
Negative	32 (39.02%)	57 (69.51%)	64 (78.05%)	74 (90.24%)
Positive	50 (60.98%)	25 (30.49%)	18 (21.95%)	8 (9.76%)
Total	82	82	82	82



Immune biomarkers correlated to PD1_PD-L1 interaction

Immune biomarkers (DSP)	r	95% confidence interval	P (two-tailed)
Tumor region			
PD-L2	0.2504	0.009344 to 0.4640	0.0365
CD44	0.2515	0.01050 to 0.4649	0.0357
PD-L1	0.2632	0.02300 to 0.4746	0.0277
FAP-alpha	0.2768	0.03765 to 0.4859	0.0204
Ki-67	0.3102	0.07407 to 0.5133	0.009
PD1	0.3516	0.1201 to 0.5468	0.0028
IDO1	0.3667	0.1372 to 0.5588	0.0018
CD34	-0.281	-0.4894 to -0.04214	0.0185
Fibronectin	-0.261	-0.4727 to -0.02048	0.0292
Stroma region			
CD127	0.2464	0.003160 to 0.4621	0.0413
CD11c	0.2731	0.03180 to 0.4843	0.0232
PD-L2	0.3221	0.08535 to 0.5244	0.007
Beta-2-microglobulin	0.3662	0.1348 to 0.5597	0.002
CD44	0.3739	0.1436 to 0.5658	0.0016
PD1	0.3759	0.1459 to 0.5674	0.0015
CD14	0.3913	0.1635 to 0.5795	0.0009
VISTA	0.4091	0.1840 to 0.5934	0.0005
FAP-alpha	0.4747	0.2615 to 0.6437	<0.0001
CD40	0.482	0.2703 to 0.6493	<0.0001
PD-L1	0.4923	0.2827 to 0.6570	<0.0001
CD34	-0.555	-0.7034 to -0.3604	<0.0001
FOXP3	-0.294	-0.5012 to -0.05414	0.0143
STING	-0.27	-0.4817 to -0.02840	0.0249
Tim-3	-0.267	-0.4791 to -0.02504	0.0267
Fibronectin	-0.256	-0.4699 to -0.01311	0.034

Results

In LUAD, PD1_PD-L1 interaction was observed in 7/20 (35%) tumor regions that were positive for PD-L1-TPS and in 29/61 (48%) TME regions positive for PD-L1-IC. In mesothelioma, PD1_PD-L1 interaction was observed in 1/3 (33.33%) PD-L1-TPS positive tumor regions and 2/8 (25%) PD-L1-IC positive TME regions.

In LUAD, PD1_PD-L1 interaction evaluated by DIA (optical density) positively correlated with PD-L1 IHC in Tumor (r=0.36, p=0.0009) and TME (r=0.64,p<0.0001).

Notably, PD1_PD-L1 correlated with proliferation in the tumor and specific myeloid biomarkers in TME.

Amongst with the clinicopathological data available, only tumor size (as assessed by imaging) showed a negative correlation with PD1_PD-L1 interaction (r=-0.38, p=0.028), median tumor size of 3 cm (1.7-5 cm).

Conclusions

- This study demonstrates the importance of receptor-ligand engagement driving distinct profiles within the tumor and TME.
- Importantly, while PD1_PD-L1 correlated with PD-L1 IHC, not all PD-L1 positive tumors were also positive for PD1_PD-L1.
- As expected, we found lower frequencies of interaction of PD1_PD-L1 in mesothelioma compared to LUAD samples.
- Additional studies are underway to define specific transcriptional programs and cell types that correlate with and drive engagement of this axis in both LUAD and pleural mesothelioma.

Acknowledgements

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