Development and Clinical Validation of a Quantitative Mass Spectrometric Assay for PD-L1 Protein in FFPE NSCLC Samples

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OVERVIEW

- Inhibition of PD-1/ PD-L1 signaling appears to have great promise in the clinic. However, the optimal biomarkers have not yet been defined.
- We have developed and clinically validated a quantitative proteomic method to measure PD-L1 in FFPE NSCLC tissue biopsies using mass spectrometry.
- We are currently assessing the utility of the assay to predict clinical response to PD-1 and PD–L1 targeting agents.

BACKGROUND

- Tumor cells express PD-L1 to evade the immune response.

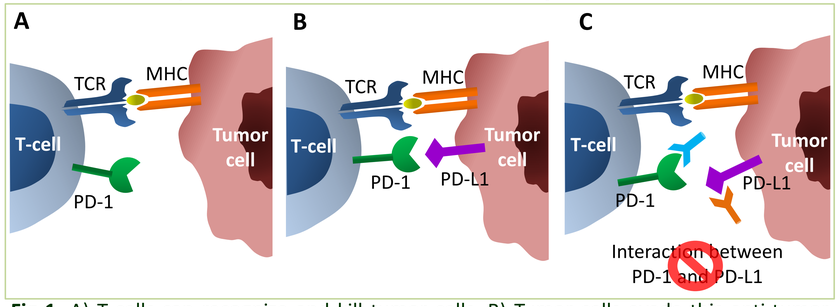

Fig 1. A) T-cells can recognize and kill tumor cells, B) Tumor cells evade this anti-tumor immune response by expressing PD-L1, C) Antibodies targeting PD-1 or PD-L1 can restore the T-cell mediated cytotoxic immune response.

- Tumor expression of PD-L1 is associated with a response to either anti-PD-L1 and anti-PD-1 treatment.

METHODS

- Identification of optimal quantitative peptides using recombinant PD-L1.
- Synthesis of stable isotope-labeled peptides.
- Building standard curves in prokaryotic complex matrix using 5 replicates, and assess linearity of the assay.

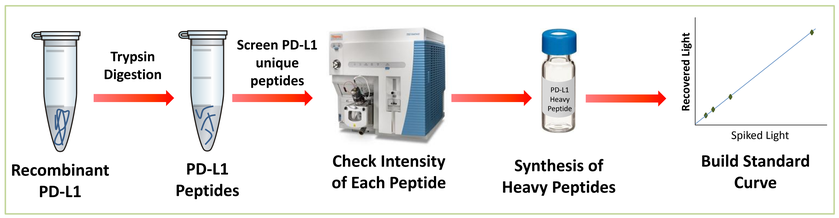

Fig 2. Schematic view of PD-L1 assay development

- Characterization of the assay using 14 formalin fixed cancer cell lines.
- Validate the assay using FFPE sections from normal lung and NSCLC patients.

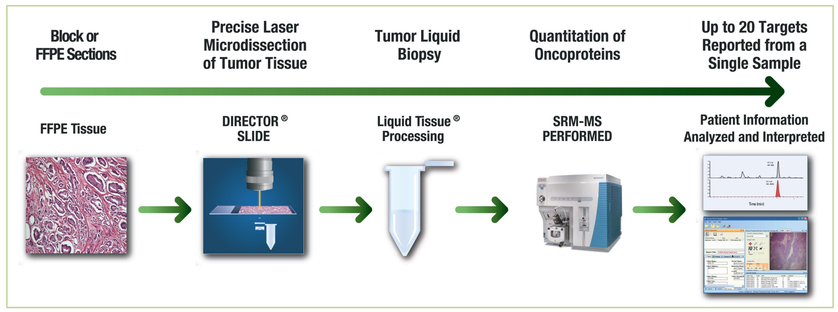

Fig 3. Liquid Tissue® - SRM workflow for protein analysis from FFPE tissue.

RESULTS

- Identification of optimal PD-L1 peptides

Table 1. Tryptic digested PD-L1 peptides.

Sequence	MS/MS spectra found in GPM	Identified in rPD-L1	Identified in Cell line	Region
Sequence 1	YES	YES	ND	Extracellular
Sequence 2	ND	ND	ND	
Sequence 3	YES	YES	YES	
Sequence 4	YES	YES	YES	
Sequence 5	ND	YES	ND	
Sequence 6	YES	YES	ND	
Sequence 7	ND	ND	ND	
Sequence 8	ND	ND	ND	Cytoplasmic

- Assay Range and Linearity

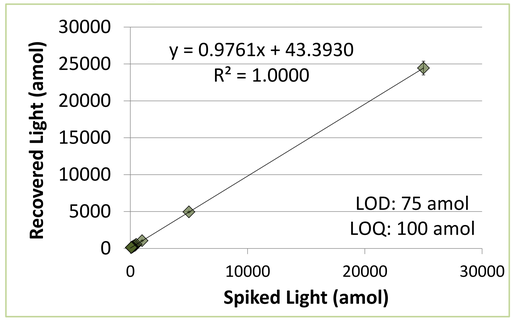

Fig 4. Concentration curve of PD-L1. A constant amount of heavy synthetic peptide and a varying amount of light synthetic peptide were spiked to formalin fixed prokaryotic complex matrix, a surrogate matrix, to build a standard curve.

- Characterization of PD-L1 expression in cell lines

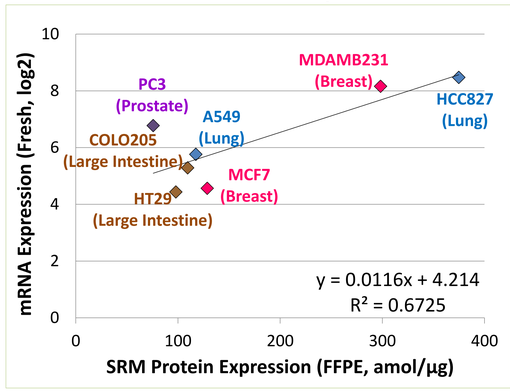

Figure 5. Correlation between PD-L1 protein and PD-L1 mRNA. Fourteen cancer cell lines were screened for PD-L1 protein expression, and compared to mRNA expression (1). PD-L1 protein expression was detected in 7 cell lines with a good correlation with PD-L1 mRNA level.

- Clinical Validation

The PD-L1 assay was run on FFPE sections from 9 normal tissues, 31 early staged (stage 1 and stage 2) and 8 advanced staged (Stage 3) NSCLC patients. NSCLC Samples are classified based on histology (Table 2). PD-L1 expression levels ranged from 98.5 to 369.8 amol/µg (Figure 7).

Table 2. Sample classification and PD-L1 protein expression

	Normal	Early Stage (Stage 1 and 2)		Advanced Stage (Stage 3)	
		Squamous cell carcinoma	Adeno carcinoma	Squamous cell carcinoma	Adeno carcinoma
Sample #	9	25	6	5	3
PD-L1 +	0	6	0	2	1
Percentage	0 %	24 %	0 %	40 %	33 %

- Squamous Cell Carcinoma are more likely to express PD-L1.

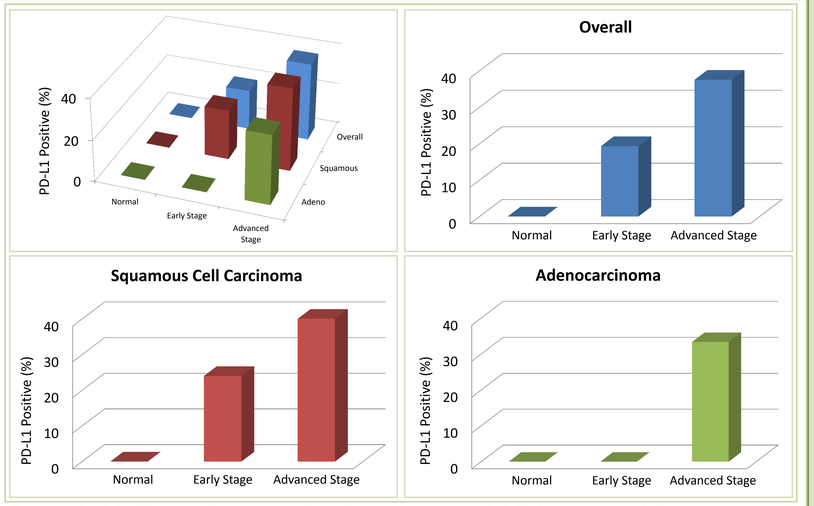

Figure 6. PD-L1 protein expression in normal and NSCLC samples. More advanced NSCLC patients are more likely to be PD-L1 positive compared to early stage NSCLC patients. Early staged patients with squamous cell carcinoma are more likely to express PD-L1.

- PD-L1 expression level is similar between early stage and late stage.

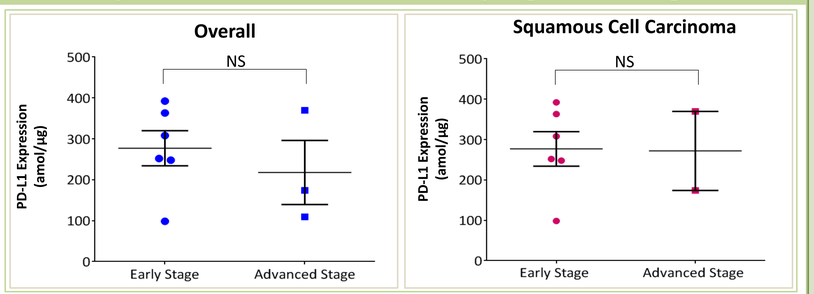

Figure 7. Distribution of PD-L1 protein expression level in NSCLC samples.

DISCUSSION

- This data in squamous is consistent with a recent report (2) in NSCLC.
- The need to characterize expression levels of druggable targets in small NSCLC biopsies is becoming even more critical as new drug targets and biomarkers are identified.
- We have developed a quantitative mass spectrometry based PD-L1 assay for clinical NSCLC biopsies.
- This assay is linear and quantitative, and can be multiplexed with other targets.
- Additional quantitative assays for lymphocyte (CD3, CD8, CD68) and immuno-targets (PD-1, B7-H3) are under development.

REFERENCE

(1) Barretina J et al., The Cancer Cell Line Encyclopedia enables predictive modeling of anticancer drug sensitivity. Nature 2012;483:603-607.
(2) Soria JC et al., Clinical activity, safety and biomarkers of PD-L1 blockade in non-small cell lung cancer (NSCLC): additional analyses from a clinical study of the engineered antibody MPDL3280A (anti-PDL1). ECC 2013 Abstract #3408