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

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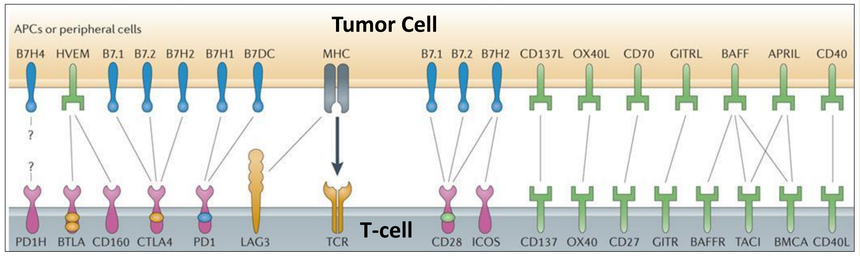
## Background

- Tumor expression of PD-L1 (B7H1) is associated with a response to both anti-PD-L1 and anti-PD-1 therapy.
- Agents that inhibit the PD-1/PD-L1 interaction have been approved in melanoma and are in clinical trials for many other indications, including NSCLC.
- OncoPlexDx offers a quantitative PD-L1 assay to identify PD-L1 positive tumors that might respond to anti-PD-1/PD-L1 therapy.




Yao et al. Nat Rev Drug Discov. 2013 Feb;12(2):130-46

**Figure 1. Immunological Synapse between T-cell and Tumor**

- Besides PD-1/PD-L1 therapy, other immuno-oncology drugs have been approved. Additionally, other protein pairs (Figure 1) that promote tumor survival through immune-system evasion are being evaluated as druggable targets.
- OncoplexDx is currently building an immuno-oncology panel to screen FFPE patient biopsies for expression of druggable immuno targets, which can be assayed simultaneously with our targeted therapy panel.

## Methods


**Figure 2. Liquid Tissue® - SRM workflow for protein analysis from FFPE tissue.**

❖ Optimal quantitative peptides were identified using recombinant PD-L1, and the assay was validated and characterized using 14 formalin fixed cancer cell lines and FFPE clinical tissue.

## Results

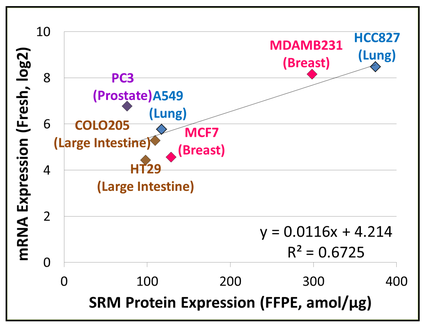



**Figure 3. Correlation between PD-L1 protein and PD-L1 mRNA**. Fourteen cancer cell lines were screened for PD-L1 protein expression, and compared to mRNA expression (Barretina et al., Nature 483:603-607). PD-L1 protein expression was detected in 7 cell lines with a moderate correlation with PD-L1 mRNA levels, suggesting that analysis of mRNA is not representative of protein levels.

## Results (continued)

### Incidence of PD-L1 Expression Increases With Cancer Stage

The PD-L1 assay was run on archival FFPE sections from 9 normal lung tissues, and 31 early staged (stage 1 and stage 2) and 8 advanced staged (Stage 3) NSCLC tumors (Table 1).

**Table 1. 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 |
| % | 0 % | 24 % | 0 % | 40 % | 33 % |

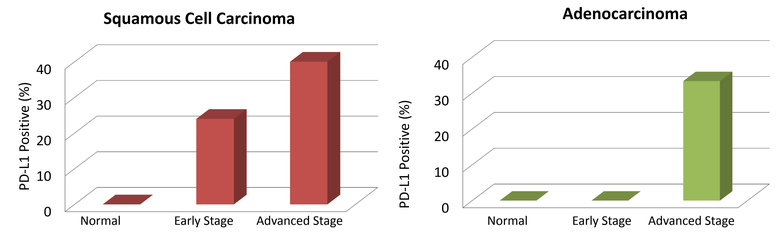



**Figure 4. PD-L1 protein expression in normal and NSCLC samples:** Advanced NSCLC patients are more likely to be PD-L1 positive compared to early stage NSCLC patients.

### Abundance Level of PD-L1 Protein in Archival NSCLC tumors

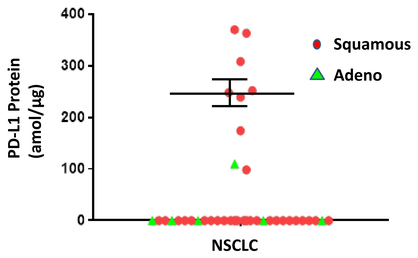
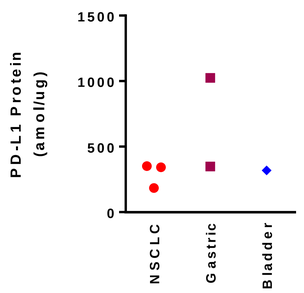



**Figure 4. Distribution of PD-L1 protein expression.** PD-L1 was detected in ~23% of NSCLC samples. The mean(±SD) of the PD-L1 positive samples is demarcated with black bars.

### Identification of PD-L1 Positive Patient Tumors

| Tumor Characteristics | PDL1 amol/µg |
|---|---|
| High grade transitional cell carcinoma of bladder | 318 |
| Poorly differentiated lung adenocarcinoma | 343 |
| Metastatic adenocarcinoma of lung | 351 |
| Malignant neoplasm of lung | 184 |
| Stomach adenocarcinoma | 1025 |
| Metastatic moderately differentiated adenocarcinoma of primary gastric origin | 349 |




## Immuno-Oncology Panel in Development

| Immuno-Oncology (IO) Panel | Tumor Infiltrating Lymphocytes (TIL) Panel |
|---|---|
| PD-L1, PD-L2, B7.1, B7.2, B7H2, B7H3, B7H4, CD70, CD40, CD137L, OX40L, IDO1, GAL9, HVEM | PD-1, CTLA4, CD137, OX40, CD27, CD40L, VISTA, BTLA, CD160, LAG3, TIM3, CD28, CD3, CD8 |

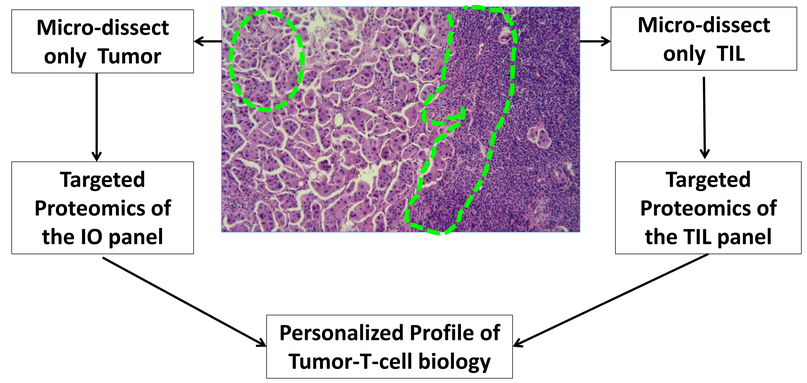



**Figure 5. Diagnostic panel to profile Tumor-TIL interaction**. The panel can be used to make informed decisions about the immuno-oncology based combination therapy. Assays have been developed to proteins highlighted in red. Additional protein targets are currently being evaluated

## Targeted Therapy + Immuno-Therapy

❖ Molecular diagnostics-based combination therapy (targeted therapy & immunotherapy) is used to reduce toxicity and increase effectiveness.
❖ Only targeted proteomics (LT-SRM) can simultaneously quantify multiple proteins in FFPE patient biopsies with limited tissue.
❖ Additional available assays that can help pair therapies are shown in table below.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ALK | ROS1 | RET | EGFR | HER2 | HER3 | HER4 | MET | IGF1R | RON |
| RRM1 | hENT1 | ERCC1 | XRCC1 | TOPO1 | TOPO2A | TS | TUBB3 | SPARC | AR |
| FRalpha | AXL | FGFR1 | FGFR2 | FGFR3 | BRAF | INSR | PSMA | MSLN | IDO1 |

## Discussion

- We have developed a quantitative mass spectrometry based PD-L1 assay for clinical samples. This assay can be multiplexed with other immuno targets to better understand tumor-T-cell biology.
- Targeted proteomics can quantitate multiple proteins from both the tumor and the tumor infiltrating lymphocytes using only a few sections of FFPE tissue.
- The OncoplexDx platform allows for quantitation of multiple actionable protein targets for optimal therapy selection based on tumor biology.