Predicting Response: PD-L1 Biomarker Testing by IHC and RNA-seq

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Introduction

Currently, four FDA-approved biomarker assays are available to screen for PD-L1 to enrich for patient response to immune checkpoint inhibitors (ICI). Each are immunohistochemical (IHC) assays that approximate the percentage of immune or tumor cells expressing PD-L1 using various antibodies, staining and scoring systems. Given this inherent variability, there are concerns whether any single PD-L1 IHC assay, or IHC in general, should be used as a companion or complimentary diagnostic. This is highlighted by the number of individuals, regardless of histology or antibody used, who score below the IHC scoring threshold, but respond to PD-L1 inhibitors. In this study, we assessed PD-L1 (CD274) expression by RNA-seq to determine correlation with IHC and ability to predict clinical benefit in patients treated with checkpoint inhibitors.

RNA-seq Linearity

Linearity of PD-L1 transcript detection by RNA-seq was assessed by comparing the absolute reads and nRPM relative to an input containing 1.5625, 3.125, 6.25, 12.5, 25, and 50 pm RNA library. Transcripts could be detected reliably at levels approaching background.

Expression Correlation

One-way ANOVA demonstrated a significant relationship between PD-L1 gene expression rank (RNA-seq) and PD-L1 protein expression by IHC (p <e-16) across the 436 tumor specimens tested. This relationship of PD-L1 IHC was not influenced by tumor type (p = 0.08) or antibody used (p = 0.76), demonstrating biomarker detection between the two test methodologies was extremely concordant. Ad-hoc Tukey’s HSD comparisons of mean TPS at <1%, 1-4%, and >5% demonstrate significant differences (p < 0.01) between the three groups, consistent with expression rankings (Figure 4).

Response Prediction

To investigate the utility of PD-L1 gene expression as a marker for response to ICI therapy, we calculated the objective response rates (ORR) for subjects with checkpoint blockade response data for both CD274 RNA-seq (Table 1) and PD-L1 IHC (Table 2).

Table 1: ORR for 177 patients based on PD-L1 RNA-seq expression

|Malignancy| PD-L1 0%| 1-5%| 6-25%| 26%| 27-50%| 51-75%| 76-100%| Total| ORR| 95% CI
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<tbody>
<tr>
<td>Melanoma</td>
<td>13</td>
<td>21</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>72</td>
<td>12.2%</td>
<td>9.0% - 16.4%</td>
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<tr>
<td>Renal Cell Carcinoma</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>8</td>
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<td>4</td>
<td>72</td>
<td>12.2%</td>
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Table 2: ORR for 177 patients based on PD-L1 IHC expression

|Malignancy| PD-L1 IHC 0%| 1-5%| 6-25%| 26%| 27-50%| 51-75%| 76-100%| Total| ORR| 95% CI
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</table>
|Renal Cell Carcinoma| 7| 5| 12| 10| 8| 7| 4| 72| 12.2%| 9.0% - 16.4%

Conclusions

1) RNA-seq is a robust, standardized method for determining PD-L1 (CD274) expression in the tumor microenvironment. The assay demonstrates high sensitivity, linearity of expression over a large dynamic range and an objective digital count of expressed transcripts.
2) In 400+ tumors, PD-L1 demonstrates correlated mean expression values when assessed by IHC and RNA-seq.
3) For the ICI treated melanomas with outcomes, PD-L1 IHC ≥1% had a 56% ORR, which improved to >72% ORR using high CD274 gene expression as a predictive cutoff.
4) The results support use of PD-L1 as a predictive biomarker but also demonstrates that assessment of PD-L1 expression by RNA-seq is a reliable alternative with the advantage of measuring several genes simultaneously, including combination immunotherapy targets and control genes for sample quality control purposes.

Methods

Patients and clinical data.

We compared PD-L1 protein expression (IHC) to PD-L1 gene expression (CD274) in 436 tumors, representing metastatic cutaneous melanoma (n = 304), NSCLC (n = 66), renal cell carcinoma (RCC) (n = 58) and head and neck squamous cell carcinoma (HNSCC) (n = 8). All FFPE tissue was collected prior to treatment with ICIs, including 177 individuals with adequate follow-up and evaluable for treatment response by RECIST v1.1.

Immunohistochemical studies. PD-L1 expression of melanoma was assessed by utilizing the Dako Omni platform (Agilent, Santa Clara, CA) and the 28-8 pharmDx antibody, with all other tumor types assessed using the 22c3 pharmDx antibody on Autostainer Link 48 (Agilent, Santa Clara, CA). Tumor proportion scores (TPS) for each histologic type was interpreted as per published guidelines; melanoma and HNSCC TPS 1%, NSCLC TPS 50% and 1%, and RCC TPS 1% (no current guidelines).

RNA-seq profiling.

RNA was extracted from each sample and gene expression (GEX) was evaluated by targeted RNA-seq of 394 immune transcripts, including CD274. Normalized GEX values (nRPM) were ranked from 0 to 100 as compared to a reference population of 167 cases with a broad range of expression in multiple tumor types. A subset of samples representing varying CD274 expression levels were serially diluted to demonstrate sensitivity and linearity of detection.

Data analyses.

One-way ANOVA was performed across all samples for three PD-L1 IHC groups with tumor proportions score of <1%, 1-4% and >5%. Due to significant overall ANOVA p-value, we performed Tukey HSD for multiple pairwise comparison between the means of three IHC groups with p values adjusted for the multiple comparisons. To investigate the relationship between PD-L1 expression and RECIST objective response rates (ORR) for 76 melanoma, 50 NSCLC, 45 RCC and 6 HNSCC cases, gene expression rank ≥75 was considered as high and expression rank ≤25 was considered low.