Biomarkers for PD-1 checkpoint inhibition

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Clinical activity of PD-1 and PD-L1 blocking antibodies validates this pathway as a target for cancer therapy

**ASCO 2014**: New evidence for activity in advanced **bladder cancer** (ORR 25%, MPDL), **SCCHN** (20%, MK-3475; 14%, MEDI4736); **ovarian cancer** (17%, nivo)
Clinical activity of PD-1 and PD-L1 blocking antibodies validates this pathway as a target for cancer therapy.

- MK-3475
- Nivolumab
- BMS-936559
- MPDL3280A

**Objective response rate (%)**

<table>
<thead>
<tr>
<th></th>
<th>Melanoma</th>
<th>NSCLC</th>
<th>RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-3475</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nivolumab</td>
<td></td>
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<tr>
<td>BMS-936559</td>
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<tr>
<td>MPDL3280A</td>
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<td></td>
</tr>
</tbody>
</table>

nr, not reported

Anti-PD-1

Anti-PD-L1
Role of PD-1 pathway in suppressing antitumor immunity

**Activation**
(cytokines, lysis, prolifer., migration)

**Inhibition**
( anergy, exhaustion, death)
Factors potentially influencing response to PD-1 pathway blockade

1) T cell specificity

2) T cell migration/proliferation

3) Tumor cell PD-L1 expression
Mutational heterogeneity in cancer creates opportunities for immune recognition

- Does tumor mutational density predict response to immunotherapy (pre-Rx marker)?
- Is successful immunotherapy associated with reactivity against specific mutant tumor antigens (on-Rx marker)?

Lawrence et al., Nature 2013
Cancer exome-guided on-treatment analysis of effects of ipilimumab:
Evolution of dominant CD8+ T cell response against melanoma-associated mutation in a responding patient

1075 non-synonomous somatic mutations

Adapted from van Rooij et al., JCO 2013
On-treatment biomarkers: selective infiltration of CD8+ T cells precedes melanoma response to anti-PD-1

Intermittent dosing regimen of nivolumab (Brahmer et al., JCO 2010)
Preliminary correlation of PD-L1 expression in pre-treatment tumor biopsies, with clinical response to anti-PD-1 therapy

49 patients include 20 with melanoma, 13 NSCLC, 7 colon, 6 kidney, and 3 prostate cancer (adapted from Topalian et al., NEJM 2012)
### PD-L1 IHC methods currently in testing

<table>
<thead>
<tr>
<th></th>
<th>JHU</th>
<th>BMS</th>
<th>Merck</th>
<th>Roche</th>
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<tbody>
<tr>
<td>mAb clone</td>
<td>5H1</td>
<td>28-8</td>
<td>22C3</td>
<td>SP142</td>
</tr>
<tr>
<td>Automated</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Staining location scored</td>
<td>Membrane</td>
<td>Membrane</td>
<td>Membrane</td>
<td>Membrane</td>
</tr>
<tr>
<td>Cell type(s) scored</td>
<td>Tumor cells</td>
<td>Tumor cells</td>
<td>Tumor and/or infiltrating imm. cells</td>
<td>Infiltrating immune cells</td>
</tr>
<tr>
<td>Positive cutoff</td>
<td>≥ 5%</td>
<td>≥ 5%</td>
<td>≥ 1%</td>
<td>≥ 1% to ≥ 10% (“IHC 1-2-3”)</td>
</tr>
</tbody>
</table>

- **Note:** these assays are still under development pending additional clinical correlative data
# Intra-tumoral PD-L1 expression and response to PD-1/PD-L1 blockade

<table>
<thead>
<tr>
<th></th>
<th>Nivolumab Solid Tumors</th>
<th>Nivolumab Melanoma</th>
<th>MPDL3280a Solid Tumors</th>
<th>MPDL3280a Melanoma</th>
<th>Pembrolizumab Melanoma</th>
<th>Pembrolizumab NSCLC</th>
<th>Pembrolizumab Bladder</th>
<th>Pembrolizumab Head &amp; Neck</th>
<th>Pembrolizumab Melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n=</strong></td>
<td>42</td>
<td>44</td>
<td>34</td>
<td>94</td>
<td>30</td>
<td>53</td>
<td>113</td>
<td>129</td>
<td>65</td>
</tr>
<tr>
<td><strong>Response Rates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unselected</td>
<td>21%</td>
<td>32%</td>
<td>29%</td>
<td>22%</td>
<td>23%</td>
<td>23%</td>
<td>40%</td>
<td>19%</td>
<td>26%</td>
</tr>
<tr>
<td>PD-L1 +</td>
<td>36%</td>
<td>67%</td>
<td>44%</td>
<td>39%</td>
<td>27%</td>
<td>46%</td>
<td>49%</td>
<td>37%</td>
<td>43%</td>
</tr>
<tr>
<td>PD-L1 –</td>
<td>0%</td>
<td>19%</td>
<td>17%</td>
<td>13%</td>
<td>20%</td>
<td>15%*</td>
<td>13%</td>
<td>11%</td>
<td>11%</td>
</tr>
</tbody>
</table>

Presented by: Margaret Callahan
Variable expression of PD-L1 among melanoma lesions from individual patients receiving anti-PD-1 therapy.

**“PD-L1+ tumor”**: ≥ 5% tumor cells with cell surface PD-L1 expression

**“PD-L1+ patient”**: patient in whom any tumor is/was PD-L1+

*(Topalian et al., NEJM 2012)*
Pitfalls for PD-L1 biomarker: focal expression in some tumors

“Marker negative” specimen or sampling error???

Invasive primary melanoma, nodular subtype. 10% of tumor cells express PD-L1.
Association of TILs with tumor PD-L1: TILs are necessary but \textit{not} sufficient for PD-L1 expression in melanoma

\begin{center}
\begin{tikzpicture}
\begin{axis}[
    width=\textwidth,
    ybar,
    bar width=10pt,
    ymin=0,
    ymax=100,
    ylabel={\% of cases},
    xtick=data,
    xticklabels={PD-L1(+), n=57, PD-L1(-), n=93},
    xticklabels style={/pgf/number format/1000 sep=, /pgf/number format/fixed},
    xticklabel style={/pgf/number format/1000 sep=, /pgf/number format/fixed},
    ytick={0,20,40,60,80,100},
    yticklabels={0,20,40,60,80,100},
    yticklabel style={/pgf/number format/1000 sep=, /pgf/number format/fixed},
    legend style={at={(0.5,0.15)}, anchor=south, draw=none, fill=none},
    legend cell align={left},
    legend columns=-1,
]
\addplot[blue, fill=blue!50] coordinates {
    (PD-L1(+), n=57, 100)
    (PD-L1(-), n=93, 20)
};
\addplot[red, fill=red!50] coordinates {
    (PD-L1(+), n=57, 0)
    (PD-L1(-), n=93, 80)
};
\addlegendentry{TILs}
\addlegendentry{No TILs}
\end{axis}
\end{tikzpicture}
\end{center}

\textbf{Hypothesis: } functional differences in TILs explain differences in PD-L1 expression by melanoma cells

\textit{Taube et al., Science Transl Med 2012}
Enhanced IFN-γ expression in PD-L1(+) vs. (-) melanomas (qRT-PCR)

(Taube et al., STM 2012)
2 Mechanisms for PD-L1 up-regulation in tumors

**Innate Resistance**

Constitutive tumor signaling induces PD-L1 on tumor cells

**Adaptive Resistance**

PD-L1 expression reflects immune reaction
PD-L1 expression by tumor cells is the strongest single predictor of response to anti-PD-1 therapy

PD-L1 expression by tumor cells is the strongest single predictor of response to anti-PD-1 therapy

J Taube, R Anders, et al., CCR 2014
PD-L1 expression by tumor cells is the strongest single predictor of response to anti-PD-1 therapy

PD-L1 expression by tumor cells is the strongest single predictor of response to anti-PD-1 therapy.

**J Taube, R Anders, et al., CCR 2014**

**Chart Details:**
- Objective response rate (%)
- Bars represent positive and negative categories.
- Tumor PD-L1 (n=41), TIL PD-1 (n=37), Imm cell PD-L1 (n=41), TILs present (n=41)
- p-values:
  - Tumor PD-L1: p=0.025
  - TIL PD-1: p=0.067
  - Imm cell PD-L1: p=0.142
  - TILs present: p=0.410
Question: Why do some patients with PD-L1+ tumors not respond to anti-PD-1 therapy?

Approach: from 26 patients with RCC treated at a single institution, screen 35 tumor specimens for PD-L1 expression.

- 21 assessed with IHC for PD-L1 protein expression
- 14 specimens not available for study
- 14 specimens (67%) from 13 patients were PD-L1(+)
- 7 specimens PD-L1(-)
- 8 primary tumors
- 6 metastases
- RNA extraction, gene expression analysis
Whole genome microarray: distinct gene profile associated with RCC *non-response* to anti-PD-1 therapy

Supervised cluster analysis based on 234 genes derived from $p \leq 0.01$ and fold change $\geq 1.5$, R vs. NR

(M. Ascierto et al., unpublished)
CONCLUSIONS

- The immune system is dynamic and its interactions with cancer vary over space (anatomy) and time (chronology of metastasis), posing challenges for biomarker development.

- PD-L1 expression in pre-treatment tumor biopsies (IHC) is currently the strongest single predictor of clinical response to PD-1 pathway blockade.
  - Responders in the “marker negative” population raise concerns for clinical application on a per-patient basis.
  - PD-L1 expression may identify cancer types amenable to PD-1 blockade.

- Current limitations in tissue availability for in-depth biomarker studies may be addressed with innovative clinical trials (neo-adjuvant, rapid autopsy).
Thanks to collaborating clinical trial centers.
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