The Promises and Potential of Predictive Biomarkers for IO

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• Stock Options
  – Adaptive Biotech, Amphivena, Intensity
Educational Objective

After participation in this symposium, participants will be better able to:

• Describe recent advances in the field of predictive biomarkers for immunotherapy
Which of the Following Predictive Biomarkers Were Used as Companion Diagnostics Leading to Approvals for Treatment with Single Agent Anti-PD-1 or Anti-PD-L1?

1. Number of T lymphocytes found in tumor biopsy
2. PD-1 expression within the tumor microenvironment
3. PD-L1 expression on tumor cells or immune cells within tumor
4. Tumor mutation burden
5. Interferon-gamma gene signature within tumor
6. Mismatch repair deficiency/microsatellite-high
7. 2 and 4
8. 3 and 6
9. All of the above
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7. 2 and 4
8. 3 and 6
9. All of the above

Audience Response:
- 1. Number of T lymphocytes found in tumor biopsy: 3%
- 2. PD-1 expression within the tumor microenvironment: 13%
- 3. PD-L1 expression on tumor cells or immune cells within tumor: 21%
- 4. Tumor mutation burden: 0%
- 5. Interferon-gamma gene signature within tumor: 3%
- 6. Mismatch repair deficiency/microsatellite-high: 0%
- 7. 2 and 4: 3%
- 8. 3 and 6: 41%
- 9. All of the above: 18%
## Spectrum of PD-1/PD-L1 Antagonist Activity

### Active
- Melanoma
- Renal cancer (clear cell)
- NSCLC: adenocarcinoma and squamous cell
- Head and neck cancer
- MMR-repair deficient tumors (colon cancer, cholangiocarcinoma)
- Bladder
- Hodgkin lymphoma
- Merkel cell
- Gastric and gastroesophageal junction cancer
- Hepatocellular carcinoma
- SCLC
- Renal cancer (non-clear cell)
- Triple-negative breast cancer
- Ovarian cancer
- Thymoma
- Mesothelioma
- Cervical cancer
- Diffuse large-cell lymphoma
- Follicular lymphoma
- T-cell lymphoma (cutaneous T-cell lymphomas, peripheral T-cell lymphoma)

### Minimal to no activity
- Prostate cancer
- MMR\(^+\) (MSS) colon cancer
- Myeloma
- Pancreatic cancer

### Approved anti-PD-1 agents\(^2\)
- Nivolumab
- Pembrolizumab

### Approved anti-PD-L1 agents\(^2\)
- Atezolizumab
- Durvalumab
- Avelumab

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NSCLC, non-small-cell lung cancer; MMR, mismatch repair; MSS, microsatellite stable; SCLC, small-cell lung cancer.

1. Personal communication M. Sznol.
Conditions Necessary for Tumor Response to Single-Agent Anti-PD-1 or Anti-PD-L1

- Tumor antigens
- Preserved antigen-presentation machinery
- Tumor antigen-specific T cells within the tumor microenvironment
- T cell priming and activation

T cell

- Antigen recognition/activation
- TCR binds to peptide/MHC on tumor or antigen-presenting immune cell

T cells upregulate PD-1

IFNγ

- Tumor or immune cell upregulation of PD-L1

T cell function

IFNγ, interferon-gamma; MHC, major histocompatibility complex; TCR, T cell receptor.

Personal communication M. Sznol.
Potential Predictors for Clinical Response to Anti-PD-1/PD-L1 Pathway Blockade

- PD-L1 expression: (tumor, tumor-infiltrating immune cells)
- Presence and location of T cells: (IHC for CD3⁺, or CD3+CD8⁺, or CD3+CD8⁺ granzymeB⁺, peripheral versus stromal, or gene signature)
- PD-1⁺ T cells or PD-1⁺/CTLA-4⁺ CD8 T cells
- Proximity of PD-L1 expression to CD8⁺PD-1⁺ T cells (IHC)
- Presence of interferon-gamma gene signature
- T-cell clonality
- Tumor mutation burden (DNA sequencing or RNA sequencing)
  - MMR deficiency or microsatellite-high
- Clinical factors: high LDH, high CRP, high neutrophil/lymphocyte ratio, presence of liver metastases, “high” tumor burden

CD, cluster of differentiation; CRP, C-reactive protein; IHC, immunohistochemistry; LDH, lactate dehydrogenase.

Personal communication M. Sznol.
PD-L1 expression is heterogeneous and associated with TILs

In melanocytes, PD-L1 expression correlates strongly with the presence of TILs.

<table>
<thead>
<tr>
<th>Histology</th>
<th>PD-L1+</th>
<th>PD-L1-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign nevi</td>
<td>TIL+ 100, TIL- 0</td>
<td>TIL+ 15, TIL- 85</td>
</tr>
<tr>
<td>Primary melanoma (in situ or invasive)</td>
<td>TIL+ 100, TIL- 0</td>
<td>TIL+ 43, TIL- 57</td>
</tr>
<tr>
<td>Metastases</td>
<td>TIL+ 96, TIL- 4</td>
<td>TIL+ 22, TIL- 78</td>
</tr>
</tbody>
</table>

TIL, tumor-infiltrating lymphocyte.

Comparison of PD-L1 Platforms
NSCLC, 13 pathologists, 90 slides


Tumor cells positive at cut points
- Percent positive > 50%
- Percent positive > 1%

Immune cells positive at cut points
- Percent positive > 10%
- Percent positive > 1%

Assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>22c3</th>
<th>28-8</th>
<th>SP142</th>
<th>E1L3N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells positive (%)</td>
<td>&lt;50%</td>
<td>&gt;50%</td>
<td>&gt;50%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Immune cells positive (%)</td>
<td>&lt;10%</td>
<td>&gt;10%</td>
<td>&gt;10%</td>
<td>&gt;10%</td>
</tr>
</tbody>
</table>

Scale bars = 200 μm
Which of the Following is True Regarding the Use of PD-L1 as a Predictive Biomarker in Metastatic Melanoma and NSCLC?

1. The higher the expression level of PD-L1, the higher the response to anti-PD-1
2. Patients with < 5% tumor expression of PD-L1 would not benefit from anti-PD-1 single agent treatment in metastatic melanoma
3. In the randomized study of Ipilimumab-nivolumab or nivolumab versus Ipilimumab, improved OS for the ipi/nivo arm compared to nivo alone arm was observed in tumors with PD-L1 expression ≥ 1%
4. Pembrolizumab as a single agent is approved for first-line treatment of NSCLC in patients with PD-L1 expression ≥ 1%

OS, overall survival.
Which of the Following is True Regarding the Use of PD-L1 as a Predictive Biomarker in Metastatic Melanoma and NSCLC?

1. The higher the expression level of PD-L1, the higher the response to anti-PD-1
   - Audience Response: 36%

2. Patients with < 5% tumor expression of PD-L1 would not benefit from anti-PD-1 single agent treatment in metastatic melanoma
   - Audience Response: 14%

3. In the randomized study of Ipilimumab-nivolumab or nivolumab versus Ipilimumab, improved OS for the ipi/nivo arm compared to nivo alone arm was observed in tumors with PD-L1 expression ≥ 1%
   - Audience Response: 32%

4. Pembrolizumab as a single agent is approved for first-line treatment of NSCLC in patients with PD-L1 expression ≥ 1%
   - Audience Response: 18%

OS, overall survival.
Objective Response to Anti-PD-1 by PD-L1 Expression

- Anti-PD-L1 IHC (22C3 antibody); MEL score ≥ 2 is considered PD-L1-positive
Objective Response to Pembrolizumab by PD-L1 Expression Level (22C3 antibody assay) in Melanoma

- Response to pembrolizumab treatment correlated with PD-L1 expression levels

ORR, overall response rate; PFS, progression-free survival.

Response to Nivolumab vs Dacarbazine by PD-L1 Status in Melanoma

Patients benefit from Nivolumab regardless of PD-L1 expression

<table>
<thead>
<tr>
<th></th>
<th>NIVO ≥ 5% (N = 59)</th>
<th>NIVO &lt; 5% (N = 127)</th>
<th>DTIC ≥ 5% (N = 61)</th>
<th>DTIC &lt; 5% (N = 118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, mos (95% CI)</td>
<td>NR (NR–NR)</td>
<td>NR (16.6–NR)</td>
<td>9.7 (6.7–13.5)</td>
<td>11.6 (9.3–13.0)</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.56 (0.32–0.98)</td>
<td>1.16 (0.79–1.68)</td>
<td>p = 0.0399</td>
<td>p = 0.451</td>
</tr>
</tbody>
</table>

Hazard ratios expressed as PD-L1 ≥ 5% over PD-L1 < 5%.
CI, confidence interval; DTIC, dacarbazine; HR, hazard ratio; mos, months; NIVO, nivolumab; NR, not reached.

CA209-067: Ipi/Nivo or Nivo versus Ipi
Post-Hoc Analyses of OS by PD-L1 Status

- Survival advantage appears to be in PD-L1 negative group

PD-L1 Expression in NSCLC and Response to Pembrolizumab

- ORR with pembrolizumab correlated with PD-L1 expression levels in patients with NSCLC

PS, percentage of neoplastic cells with membranous PD-L1 staining.
PS, proportion score.

Pembrolizumab vs Chemotherapy in NSCLC, PD-L1 > 50%:
PFS and OS in the ITT Population

- First-line pembrolizumab significantly improved PFS and OS in PD-L1+ NSCLC patients

HR for disease progression or death, 0.50 (95% CI 0.37–0.68)  
p < 0.001

HR for death, 0.60 (95% CI, 0.41–0.89)  
p = 0.005

ITC, intention to treat.

First-Line Nivolumab vs Chemotherapy for Stage IV or Recurrent NSCLC (PD-L1 ≥ 5%) Did Not Improve OS


<table>
<thead>
<tr>
<th></th>
<th>Nivolumab n = 211</th>
<th>Chemotherapy n = 212</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, months</td>
<td>14.4 (11.7–17.4)</td>
<td>13.2 (10.7–17.1)</td>
</tr>
<tr>
<td>1-year OS rate, %</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>HR = 1.02 (95% CI 0.80–1.30)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 60% in the chemotherapy arm had subsequent nivolumab therapy
- 44% in the nivolumab arm had subsequent systemic therapy

All randomized patients (≥ 1% PD-L1+): HR = 1.07 (95% CI 0.86–1.33)
Nivolumab Plus Ipilimumab in First-Line Treatment of NSCLC: Efficacy Across Tumor PD-L1 Expression Levels

- ORR with the combination of nivolumab + ipilimumab correlated with tumor PD-L1 expression

Combination data based on a February 2016 database lock; monotherapy data based on a March 2015 database lock.
q2w, every 2 weeks; q6/12w, every 6 or 12 weeks.

PD-L1+ Tumor-Associated Immune Cells Are More Predictive of Nivolumab Outcome in SCCHN than Tumor PD-L1 expression

[data not available]

SCCHN, squamous cell carcinoma of the head and neck; TAIC, tumor-associated immune cells.

Higher somatic nonsynonymous mutation burden was associated with clinical efficacy of pembrolizumab.

DCB, durable clinical benefit; NDB, no durable benefit.

Impact of TMB on the Efficacy of First-Line Nivolumab in Stage IV or Recurrent NSCLC


TMB, tumor mutation burden.
Pembrolizumab in MMR-Deficient Cancer

<table>
<thead>
<tr>
<th>Type of response</th>
<th>MMR-deficient colorectal cancer (N = 10)</th>
<th>MMR-proficient colorectal cancer (N = 18)</th>
<th>MMR-deficient non-colorectal cancer (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response, n (%)</td>
<td>0</td>
<td>0</td>
<td>1 (14)a</td>
</tr>
<tr>
<td>Partial response, n (%)</td>
<td>4 (40)</td>
<td>0</td>
<td>4 (57)b</td>
</tr>
<tr>
<td>Stable disease at Week 12, n (%)</td>
<td>5 (50)</td>
<td>2 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Progressive disease, n (%)</td>
<td>1 (10)</td>
<td>11 (61)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Could not be evaluated, n (%)c</td>
<td>0</td>
<td>5 (28)</td>
<td>0</td>
</tr>
<tr>
<td>Objective response rate, % (95% CI)</td>
<td>40 (12–74)</td>
<td>0 (0–19)</td>
<td>71 (29–96)</td>
</tr>
<tr>
<td>Disease control rate, % (95% CI)d</td>
<td>90 (55–100)</td>
<td>11 (1–35)</td>
<td>71 (29–96)</td>
</tr>
<tr>
<td>Median duration of response, weeks</td>
<td>NR</td>
<td>NAa</td>
<td>NR</td>
</tr>
<tr>
<td>Median time to response, weeks (range)</td>
<td>28 (13–35)</td>
<td>NAa</td>
<td>12 (10–13)</td>
</tr>
</tbody>
</table>

- Objective responses according to RECIST criteria

\(^a\) One patient had a partial response at 12 weeks, which then became a complete response at 20 weeks.

\(^b\) One patient had a partial response at 12 weeks.

\(^c\) Patients could not be evaluated if they did not undergo a scan at 12 weeks because of clinical progression.

\(^d\) The rate of disease control was defined as the percentage of patients who had a complete response, partial response, or stable disease for ≥ 12 weeks.

\(^e\) The median time to response was not applicable (NA) because no responses were observed among patients with MMR-deficient colorectal cancer.

NA, not applicable.


OR to pembrolizumab correlated with total somatic mutations

Objective response  Stable disease  Progressive disease

• Somatic mutations per tumor

0 1,000 2,000 3,000 4,000 5,000

MMR-deficient tumors  MMR-proficient tumors

p = 0.007  p = 0.02

Somatic mutations per tumor
Patient Survival and Clinical Response to Pembrolizumab Across 12 Different Tumor Types With MMR Deficiency

- **Change from baseline (%)**
  - −100
  - −50
  - 0
  - 50

- **Change from baseline SLD (%)**
  - 0
  - 50
  - 100

- **PFS (%)**
  - 0
  - 50
  - 100

- **Time (months)**
  - 0
  - 6
  - 12
  - 18
  - 24
  - 30
  - 36

- **OS (%)**
  - 0
  - 50
  - 100

- **Time (months)**
  - 0
  - 6
  - 12
  - 18
  - 24
  - 30
  - 36

- **Patients enrolled on study**
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50
  - 60

- **Best response**
  - 0
  - 10
  - 20

- **20-week response rate**
  - 0
  - 10
  - 20

- **Deceased**
  - 0
  - 20
  - 40

- **Off treatment**
  - 0
  - 20
  - 40

- **Progression**
  - 0
  - 10
  - 20

**Tumor Types:**
- Ampula of vater
- Cholangiocarcinoma
- Colorectal
- Endometrial cancer
- Gastroesophageal
- Neuroendocrine
- Osteosarcoma
- Pancreas
- Prostate
- Small intestine
- Thyroid
- Unknown primary

SLD, sum of longest diameters.

IFNy and Expanded Immune Signatures Correlate With Response to Pembrolizumab in Melanoma

Preliminary IFNy (10 gene)  
Preliminary expanded immune (28 gene)  
Correlation with response in the validation set

<table>
<thead>
<tr>
<th>Signature</th>
<th>BOR by RECIST</th>
<th>PFS by RESIST</th>
<th>OS</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary IFNγ</td>
<td>p = 0.047</td>
<td>p = 0.016</td>
<td>p</td>
<td>51</td>
</tr>
<tr>
<td>Preliminary expanded immune</td>
<td>p = 0.027</td>
<td>p = 0.015</td>
<td>p</td>
<td>62</td>
</tr>
</tbody>
</table>

* Development of the expanded immune signature was performed in an unsupervised manner by individuals blinded to response data. Nominal one-sided p value from logistic regression (for BOR per RECIST v1.1) or Cox regression (for PFS and OS).

BOR, best overall response.

PFS and OS in Patients With Melanoma and IFNγ Signature Score Above and Below the Cutoff

Which of the Following Does NOT Predict for Response or Resistance to Single-Agent Anti-PD-1 or Anti-PD-L1?

1. Mesenchymal–angiogenesis gene signature
2. Myeloid cell gene signature
3. Gut microbiome
4. Patterns of proteins present in peripheral blood
5. Tumor cell signaling pathways
6. Defects in tumor antigen presentation machinery (beta-2-microglobulin, loss of MHC molecules, antigen-loss variants)
7. Oral microbiome
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5. Tumor cell signaling pathways
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7. Oral microbiome

Audience Response:
- Mesenchymal–angiogenesis gene signature: 6%
- Myeloid cell gene signature: 0%
- Gut microbiome: 0%
- Patterns of proteins present in peripheral blood: 13%
- Tumor cell signaling pathways: 10%
- Defects in tumor antigen presentation machinery: 3%
- Oral microbiome: 68%
Transcriptome Map of Angiogenesis and Immune-Associated Genes in RCC Tumors

- **T-effector<sup>High</sup> subpopulation**
- **Myeloid inflammation<sup>Low</sup>**
- **Myeloid inflammation<sup>High</sup>**

RCC, renal cell carcinoma.

Addition of Bevacizumab to Atezolizumab in First Line Was Associated With Improved Benefit in the T-Effector$^{High}$ Myeloid Inflammation$^{High}$ Subgroup


Atezo, atezolizumab; Bev, bevacizumab.

Loss of PTEN Promotes Resistance to T-Cell-Mediated Immunotherapy

PTEN, phosphatase and tensin homolog.

PTEN status by IHC

<table>
<thead>
<tr>
<th>TIL growers</th>
<th>TIL nongrowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>9</td>
</tr>
<tr>
<td>Present</td>
<td>72</td>
</tr>
<tr>
<td>Total PTEN</td>
<td>9/81</td>
</tr>
<tr>
<td>absent (%)</td>
<td>(11)</td>
</tr>
<tr>
<td>Present</td>
<td>31</td>
</tr>
<tr>
<td>Total PTEN</td>
<td>11/42</td>
</tr>
<tr>
<td>present (%)</td>
<td>(26)</td>
</tr>
</tbody>
</table>

p = 0.0405

CD8+ at tumor site (%)

PTEN absent

PTEN present

p < 0.001

Association of the Diversity and Composition of the Gut Microbiome Are Associated With Enhanced Responses and Improved PFS in Metastatic Melanoma Patients on Anti-PD-1 Therapy


TME, tumor microenvironment.
Predictive Biomarkers Are Critical for Development of Novel Immune Therapies

- But possibly complicated and expensive and may require baseline and early post-treatment sampling and adaptive treatment changes

**Patient**
- Multi-parameter tumor IHC
- DNA sequencing and RNA sequencing (tumor and immune cells)
- Microbiome
- Blood-based protein profiles

**Baseline**
- Integrated analysis

**Treatment**
- Continue treatment
- Treatment + X
- New treatment

**Early Post-Treatment**
- Multi-parameter tumor IHC
- DNA sequencing and RNA sequencing (tumor and immune cells)
- Blood-based protein profiles

Personal communication M. Sznol.
Early On-Treatment Changes in Peripheral B Cells May Predict for Severe ICI Toxicity

ICI, immune checkpoint inhibitor; IRAE, immune-related adverse event.

Conclusions

- Predictive biomarkers are critical to optimize activity and reduce toxicity and cost of immunotherapies.
- PD-L1 and testing for dMMR/MSI-H are used in current practice to select for single-agent anti-PD-1 or anti-PD-L1 treatment.
- Most PD-L1 assays produce similar results, with the exception of SP142.
- Multiple other biomarkers (clinical, in tumor cells or tumor immune cells, blood-based or microbiome) could further select for anti-PD1/PD-L1 single-agent or combination therapies or no immune therapies.
- Combinations of biomarkers may prove superior, but interplay between biomarkers remains undefined.
- Most biomarkers do not provide critical information on an optimal combination partner for anti-PD-1 or anti-PD-L1: may require an individualized approach.
- Pre- and early post-treatment biopsies might better distinguish responders from nonresponders.
- Biomarkers to predict toxicity are a major unmet need.

dMMR, deficient MMR; MSI-H, MSI-high.