PD-L1 Testing

The What, Why and How in a Growing and Complicated Testing Environment

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The RAMS ARE BACK?
And for you Dodger Fans!

I set my DVR to record 'The Biggest Loser' and it keeps recording Dodger games.

someecards user card

YOU ONLY LIVE ONCE.
DON'T WASTE IT BEING A...
DODGERS FAN
Objectives

1. Review the biology of PD-L1/ PD-1 interaction in tumor biology and principles of immune checkpoint inhibitor therapy.

2. Review the current drug approvals, companion/complementary PD-L1 test assays and scoring criteria.

3. Discuss scoring with the Dako 22C3 PharmDx assay and associated pitfalls.
Principles of Immune Checkpoint Therapy

PD-L1 binds to PD-1 and inhibits T cell killing of tumor cell

Blocking PD-L1 or PD-1 allows T cell killing of tumor cell

Tumor cell death
Principles of Immune Checkpoint Therapy

A. Without Immunotherapy
- APC
- MHC Peptide
- TCR
- Inactivation of T-Cell
- Tumor escape

B. With Immunotherapy
- Anti-CTLA-4 antibody
- MHC Peptide
- TCR
- Activation of T-Cell
- Elimination of tumor cells

B. Without Immunotherapy
- APC
- MHC Peptide
- TCR
- Inactivation of T-Cell
- Tumor escape

B. With Immunotherapy
- Anti-PD-1
- PD-L1
- PD-1
- Activation of T-Cell
- Elimination of tumor cells

http://dx.doi.org/10.1136/gutjnl-2018-316948
Immune Checkpoint Therapy Timeline

1991
- B7-CTLA-4 interaction (Peter Linsley) [25]

1992
- B7 applied to cancer immunotherapy (Lieping Chen) [28]
- PD-1 gene cloning (Tasuku Honjo) [58]

1999
- B7-H1 gene cloning and T cell function (Lieping Chen) [47]
- PD-1 gene null mice (Tasuku Honjo) [60]

2000
- B7-H1-PD-1 interaction, B7-H1 renamed PD-L1 (Clive Wood and Tasuko Honjo) [57]
- PD-L1 expression by tumors & targeting PD-L1 for cancer immunotherapy (Lieping Chen) [56]

2002
- Anti-PD-L1 or PD-1 gene deletion inhibits tumor growth in mice (Minato) [43]
- Both T cells and PD-L1 expression are required for PD-L1 directed therapy (Scott Strome and Lieping Chen) [67]

2004
- PD-L1 gene null mice (Lieping Chen) [68]
- Anti-PD-L1 & anti-PD-1 elicits similar anti-tumor activities; “Molecular Shield” mechanism (Lieping Chen) [79]

2005
- First anti-PD-1 trial result (Suzanne Topalian) [73]
- First anti-PD-L1 trial result (Julie Brahmer) [75]

2006
- First anti-PD clinical trial initiated (Suzanne Topalian, Julie Brahmer, Lieping Chen, etc.)

2010
- First Anti-PD-1 antibody approved by FDA for cancer [77]

2012
- Cancer immunotherapy cited as the breakthrough of the year by the Science magazine [1]

2014
- First anti-PD-L1 antibody approved by FDA for cancer [82, 83]

2016
- First anti-PD-L1 antibody approved by FDA for cancer [82, 83]
Principles of Immune Checkpoint Therapy
Other Pathways

A simplified view of co-stimulatory and co-inhibitory ligand-receptor pairs that regulate T cell activity

T cell activating interactions
- 4-1BBL
- OX40L
- GITRL
- ICOSL
- B7.1, B7.2
- CD-28

T cell inhibitory interactions
- CTLA-4
- PD-1
- GITR
- ICOS
- CD-28
- B7.1, B7.2
- PD-L1, PD-L2
- HVEM
- VISTA-R?
- GAL-9
- MHC II

Note that some binding partners involving some molecules, such as VISTA, are still being explored. Many additional co-stimulatory and co-inhibitory molecules (not shown) are involved in T cell activity and in the tumor microenvironment.

Original figure modified for this publication. Callahan MK, Postow MA, Wolchok JD. Immunomodulatory therapy for melanoma: ipilimumab and beyond. Clin Dermatol 2013; 31:191. Illustration used with the permission of Elsevier Inc. All rights reserved.
1. Meta-analysis of a number of trials report better response rates and progression free survival than chemotherapy in most solid tumors to date.

2. Responses are best in advanced melanoma, especially in first line therapy with combined nivolumab and ipilimumab (39% and 58% 3 year PFS and OS; better in BRAF mutated tumors).

3. Rates of pseudoprogresion (response after initial period of progression) range from 1.3 to 8%.

4. Rates of hyperprogresion (rapid progression after initiation of therapy) range from 4-29%.
1. Indications for immunotherapy continue to evolve with new cancers acquiring FDA approval for treatment with pembrolizumab and other drugs on a regular basis.

2. Certain immunotherapy drug approvals are tied specific FDA approved PD-L1 IHC assays, either as companion diagnostics (required for treatment) or complementary diagnostics (optional) or not at all.

3. Determining which test assay to implement and how to validate has been a source of confusion and difficulty for laboratories.
### Immunotherapy drug approvals

- **anti-PD-1**
- **anti-PD-L1**
- **anti-CTLA-4**

+Med. DLBCL

Cervical ca

Small cell lung ca

Triple negative breast ca

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**Table 1. Anti-PD-1/PD-L1 Immunotherapies, Indications and diagnostic assay requirement**

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Drug(s)</th>
<th>Drug target(s)</th>
<th>Indications in US</th>
<th>Indications in EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Pembrolizumab</td>
<td>PD-1</td>
<td>Unresectable or metastatic</td>
<td>Unresectable or metastatic with low tumour PD-1 expression</td>
</tr>
<tr>
<td></td>
<td>Nivolumab</td>
<td>PD-1</td>
<td>Metastatic disease with progression or/after platinum-based chemotherapy or after FDA-approved treatment if EGFR+ or ALK+</td>
<td>Locally advanced or metastatic disease after prior chemotherapy in adults</td>
</tr>
<tr>
<td></td>
<td>Nivolumab + ipilimumab</td>
<td>PD-1, CTLA-4</td>
<td>If progression after chemotherapy or after targeted treatment if EGFR+ or ALK+</td>
<td>If progression after chemotherapy or after targeted treatment if EGFR+ or ALK+</td>
</tr>
</tbody>
</table>

**Non-small-cell lung cancer**

- **Pembrolizumab**
  - PD-1
  - 1<sup>st</sup> line with pemetrexed & carboplatin
  - 1<sup>st</sup> line monotherapy if EGFR-/ALK-
  - 2<sup>nd</sup> line monotherapy if progression on/after platinum-based chemotherapy or after FDA-approved treatment if EGFR+ or ALK+

- **Nivolumab**
  - PD-1
  - 1<sup>st</sup> line with pemetrexed & carboplatin
  - 1<sup>st</sup> line monotherapy if EGFR-/ALK-
  - 2<sup>nd</sup> line monotherapy if progression on/after platinum-based chemotherapy or after FDA-approved treatment if EGFR+ or ALK+

**Renal cell carcinoma**

- **Nivolumab**
  - PD-1
  - Advanced disease after prior anti-angiogenic therapy

**Classical Hodgkin lymphoma**

- **Nivolumab**
  - PD-1
  - Relapsed or progressed disease after auto-HSCT and BV, or 3 or more lines of therapy including auto-HSCT

- **Pembrolizumab**
  - PD-1
  - With refractory disease or who have relapsed after 3 or more prior lines of therapy

**Bladder cancer**

- **Atezolizumab**
  - PD-L1
  - Locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy

- **Nivolumab**
  - PD-1
  - Locally advanced or metastatic urothelial carcinoma after prior platinum-based chemotherapy or considered cisplatin ineligible

**Head and neck cancer**

- **Pembrolizumab**
  - PD-1
  - Locally advanced or metastatic urothelial carcinoma not eligible for cisplatin-containing chemotherapy or who have disease progression during or following platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy

**Lung cancer**

- **Nivolumab**
  - PD-1
  - Recurrent or metastatic squamous cell carcinoma with disease progression on or after platinum-based therapy

**Kidney cell carcinoma**

- **Avelumab**
  - PD-L1
  - Metastatic disease

**Gastric cancer**

- **Pembrolizumab**
  - PD-1
  - Recurrent locally advanced or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma with disease progression on or after two or more prior lines of therapy including: fluoropyrimidine- and platinum-containing chemotherapy and/or: HER2/neu targeted therapy

**Liver cancer**

- **Nivolumab**
  - PD-1
  - Hepatocellular carcinoma previously treated with sorafenib

**MSI-H or dMMR-deficient solid tumours**

- **Pembrolizumab**
  - PD-1
  - Unresectable or metastatic solid tumours that have progressed following prior treatment and who have no satisfactory alternative treatment options, or colorectal cancer that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan

**MSI-H or dMMR-deficient colorectal tumours**

- **Nivolumab**
  - PD-1
  - Metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan
<table>
<thead>
<tr>
<th>Drug</th>
<th>Indications</th>
<th>Diagnostic Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab (Opdivo)</td>
<td>NSCLC- 2nd line</td>
<td>Dako 28-8-complement.</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>Dako 28-8-complement.</td>
</tr>
<tr>
<td></td>
<td>Bladder</td>
<td>Dako 28-8-complement.</td>
</tr>
<tr>
<td></td>
<td>Colorectal-2nd line</td>
<td>MMR IHC/ MSI PCR</td>
</tr>
<tr>
<td>Atezolizumab (Tecentriq)</td>
<td>Bladder- 1st or 2nd</td>
<td>Vent. SP142- companion.</td>
</tr>
<tr>
<td></td>
<td>NSCLC- 2nd line</td>
<td>No specific test required.</td>
</tr>
<tr>
<td></td>
<td>Met/ unresect. triple negative breast ca</td>
<td>FDA approved test. (Vent)</td>
</tr>
<tr>
<td>Durvalumab (Imfinzi)</td>
<td>Bladder- 2nd line</td>
<td>Vent. SP263-complement.</td>
</tr>
<tr>
<td>Avelumab (Bavencio)</td>
<td>Merkel cell- 1st line</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>Bladder- 2nd line</td>
<td>Dako 73-10 FDA approved</td>
</tr>
<tr>
<td>Ipilimumab (Yervoy)</td>
<td>Melanoma</td>
<td>None</td>
</tr>
<tr>
<td>Drug</td>
<td>Indications</td>
<td>Diagnostic Test</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>NSCLC- 1st line</td>
<td>Dako 22C3-companion*</td>
</tr>
<tr>
<td>(Keytruda)</td>
<td>NSCLC- 2nd line</td>
<td>Dako 22C3-companion#</td>
</tr>
<tr>
<td></td>
<td>Bladder ca-2nd line</td>
<td>Dako 22C3-companion@</td>
</tr>
<tr>
<td></td>
<td>GE junc.-gastric</td>
<td>Dako 22C3-companion@</td>
</tr>
<tr>
<td></td>
<td>Cervical- 2nd line</td>
<td>Dako 22C3-companion@</td>
</tr>
<tr>
<td></td>
<td>Head &amp; neck SCC</td>
<td>Optional</td>
</tr>
<tr>
<td></td>
<td>Hodgkin- 2nd line</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>Prim. Med. NHL</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>Liver-HCC-2nd line</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>Merkel cell ca</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>Lung-small cell ca</td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td>MMR def-MSI</td>
<td>MMR IHC/ MSI PCR</td>
</tr>
<tr>
<td></td>
<td>solid tumors 2nd line</td>
<td></td>
</tr>
</tbody>
</table>

*TPS \(\geq 50\%\)  #TPS \(\geq 1\%\)  @CPS \(\geq 1\)
Commercially available anti-PD-L1 clones

E1L3N, E1J2J, SP142, 28-8, 22C3, SP263, 73-10, EPR1161-2, 7G11

Blueprint study 1: 28-8, 22C3 and SP263 were found to be equivalent across the case set (n=39).

Blueprint 2: added clone 73-10, slightly superior (n=81). SP142 detected fewer positive cases than the others.

8 commercial clones tested on 259 NSCLC’s: E1L3N, E1J2J, SP142, 28-8, 22C3, and SP263 found to be equivalent with SP263 showing the best performance. (Para ER et al, Appl Immunohistochem Mol Morphol. 2018 Feb;26(2):83-93).
Options for PD-L1 expression testing

1. Bring in the most commonly used assay (Dako 22C3) for the most prescribed drug (pembrolizumab) and validate for only those indications. Send out for the others.

2. Bring in one assay and validate for all approved drug-tumor combinations (i.e. E1L3N; not attractive for most labs given the additional validation requirements as a non-FDA approved LDT).

3. Bring in all 4 FDA approved assays (Dako 22C3, Dako 28-8, Ventana SP142 and Ventana SP263).

SQL/LSA chose to option #1 for the immediate future.
Considerations before testing

It is imperative to know which immunotherapy drug is being considered for use and the indications for its use.

For example, advanced NSCLC with pembrolizumab.

For PD-L1 testing using the Dako 22C3 PharmDx assay, currently the only test offered by SQL, the indications are:

- Advanced NSCLC: testing required (CD)
- Urothelial cancer: testing required
- Gastric or GE junction: testing required
- Cervical cancer: testing required
- Head & Neck SCC, Melanoma, Hodgkin, Med. DLBCL, Merkel, HCC, small cell lung ca: optional
Tumors for which the DAKO 22C3 PharmDx is **not** required to be used to guide immunotherapy

Bladder, lung cancer, triple neg. breast ca treated with atezoluzimab (Tecentriq, Vent. SP142 for bladder /breast required- companion diagnostic)

Bladder cancer treated with durvalumab (Imfinzi, Vent. SP263 complementary (optional) or avelumab (no test).

**Melanoma, NSCLC, bladder** or colorectal treated with nivolumab (**first 3: Dako 28.8 complementary**, colorectal: MMR/MSI) or ipilumimab (none required).

All other solid tumors or lymphomas (other than Hodgkin or Med. DLBCL) considered for 2nd/3rd line pembrolizumab (Keytruda) therapy require MMR deficient or MSI +
No PD-L1 test approved to guide therapy (4/1/19)

Breast ca other than triple negative and Prostate carcinomas
Pancreatic and biliary carcinomas
Small intestinal carcinomas
Endocrine malignancies
Non-squamous, non-melanoma head and neck cancers
Neuroendocrine carcinomas including pulmonary small cell
Non-Hodgkin lymphoma other than primary Med. DLBCL
Renal cell and adrenal cortical carcinomas
Gynecologic malignancies other than cervical
Primary CNS tumors
Soft tissue and bone sarcomas
Cutaneous malignancies other than melanoma and Merkel cell
Most pediatric malignancies unless noted in slides 3 and 4

Any solid tumor failing standard treatment may be treated with pembrolizumab if proven MMR deficient/ MS unstable
For Non-small cell lung cancer: Tumor Proportion Score:

\[
\frac{\text{# viable tumor cells labeling}}{\text{total # viable tumor cells}} = 0-100\%
\]

Labeling must be membranous, partial or complete.

Do not score cytoplasmic only, crushed or necrotic cells or immune cells.

<1% is negative; 1-49% is positive low expression; \geq 50% is positive, high expression.
Cell line- positive control-Dako- PharmDx-22C3
Cell line-negative control-Dako- PharmDx-22C3

One set of positive and neg. cell line controls are performed for each run.
Tissue control-Dako- PharmDx- 22C3

Immune cells and rare tumor cells are immunoreactive
Immune cells and tumor cells are immunoreactive
Immune cells and tumor cells are immunoreactive
Met lung ca-pl. fluid-Dako- PharmDx- 22C3
Met lung adenoca-pleural fluid - TPS 100%
Met lung adenocarcinoma-soft tissue-TPS 80%
Met lung adenoca-soft tissue-TPS 80%
Oral cavity SCCA - TPS 70-80%
Oral cavity SCCA - TPS 70-80%
Nasal SCCA-TPS 0%: Intratumoral lymphs reactive
Nasal cavity SCCA - TPS <1%
Intratumoral lymphs reactive
Nasal cavity SCCA-TPS 0%
Intratumoral lymphs reactive

CD45

PD-L1
Parallel CD45 can be useful
Oropharyngeal SCCA - TPS 1%
Intratumoral immune cells also reactive
Oropharyngeal SCCA- TPS 1%
1% tumor cells- intratumoral lymphs reactive
Oropharyngeal SCCA - TPS 1%
Peritumoral immune cells also reactive
Oropharyngeal SCCA - TPS 1%
Oropharyngeal SCCA - TPS 1%
Lung-adenoca-core-TPS 15%
Lung-adenoca-core-TPS 15%
Lung-adenoca-core-TPS 15%- area 1
Intratumoral immune cells also reactive
Lung-adenoca-core-TPS 15%- area 2
Lung-adenoca-core-TPS 15%- area 3
Lung-SCCA-core-TPS <1%
Intratumoral immune cells also reactive
Lung-SCCA-core-TPS <1%
immune cells also reactive
Liver-met lung adenocarcinoma - TPS 0%
Intratumoral immune cells reactive
Lung-SCCA-core-TPS <1%

Intratumoral immune cells also reactive
Lung-SCCA-core-TPS 0%
Lung-SCCA-core-TPS <1%
Intratumoral immune cells also reactive
Lung-adenoca-TBbx
Intratumoral immune cells
Lung-adenoca-TBbx- ?TPS
Lung-adenoca-TBbx-TPS <1%
Intratumoral immune cells reactive
Lung-adenoca-TBbx-TPS <1%
Intratumoral immune cells reactive
For bladder, gastric/GE, cervical cancer:
Combined Proportion Score:

\[ \frac{\text{# viable tumor + immune cells labeling}}{\text{total # viable tumor cells}} \times 100 = 0-100 \] (100 is the maximum reportable score)

Tumor labeling must be membranous, partial or complete.

Do not score cytoplasmic only, crushed or necrotic cells.

Score of greater than or equal to 1 (\( \geq 1 \)) is positive.

For H\&N SCCA, both TPS and CPS have been used.
What else can we add?

For small cell carcinoma:

Though PD-L1 testing by IHC is not required for treatment in the FDA approval:

**Combined Proportion Score of >1% or labeling of adjacent tumor stromal inflammatory cells if present.**

Phase Ib KEYNOTE-028 Study. JCO; VOLUME 35 • NUMBER 34 • DECEMBER 1, 2017
Distal esophageal adenocarcinoma
Distal esophageal adenocarcinoma
Distal esophageal adeno-CPS 70
Intratumoral immune cells also reactive
Distal esophageal adeno-CPS 70
Intratumoral immune cells also reactive
Distal esophageal adeno-CPS 70
Intratumoral immune cells also reactive
Do not score surface/ non-invasive tumor
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative
Cytoplasmic blush- do not score
Pancreatic cancer - negative but should not be reported. Not approved by PD-L1 IHC
Companion vs complementary diagnostic assays vary by drug and tumor site (i.e. pembrolizumab, 22C3 vs nivolumab, 28-8). For some indications there is no specified assay or it states “an FDA approved assay”.

Considering using the assay in the trial that led to the approved indication.

Scoring criteria also vary: TPS, CPS, stromal compartment. Minimum number of viable cells (100).

Most assays on the market are equivalent with exception of SP142.

Ongoing Blueprint studies may allow for testing with one kit that is equivalent.
References


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