Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

David L. Rimm MD-PhD
Professor
Departments of Pathology and Medicine (Oncology)
Director, Yale Pathology Tissue Services
Disclosures for David L. Rimm MD-PhD

• In the last 12 months I have been engaged in the following relationships:

• I am a Consultant/Advisor to Astra Zeneca, Biocept, BMS, Cell Signaling Technology, Merck, Novartis, PAIGE, Perkin Elmer and Ultivue

• Astra Zeneca, Cepheid, NavigateBP, NextCure, Lilly, Perkin Elmer, and Ultivue fund research in my lab.
Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

• Companion vs Complementary Diagnostic Tests
• Immunohistochemistry
• Genomic testing (targeted and TMB)
• Expression (mRNA) signatures
• Multiplex Fluorescence
• cfDNA and other circulating markers (NLR, LIPI)
PD-L1 Assay Terminology:

- Companion Diagnostic Test (Cdx)
  - **Test result required for prescription of the drug.**
  - Specified on the Drug Label
  - Often, this category is typically used when the test is an inclusion criteria for the trial (but not always – see gastro-esophageal)

- Complementary Diagnostic Test
  - **Test result is predictive, but not required for prescription of the drug**
  - Nice to have, but not need to have – No clear message on reimbursement
  - Term attributed to Liz Mansfield when she was at the FDA
  - Mostly, this category is used when the assay is integrated into the trial, but not used for inclusion criteria (All-comers trials)
<table>
<thead>
<tr>
<th>Test</th>
<th>Scoring System</th>
<th>Indication</th>
<th>Drug</th>
<th>Date of Decision or Notice</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1 IHC 22c3 PharmDx</td>
<td>TPS (0, 1-49, &gt;50)</td>
<td>Lung Cancer</td>
<td>Pembrolizumab</td>
<td>10/24/2016</td>
</tr>
<tr>
<td>PD-L1 IHC 22c3 PharmDx</td>
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<td>6/12/2018</td>
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<tr>
<td>Ventana ALK D5F3 CDx</td>
<td>+/-</td>
<td>Lung Cancer</td>
<td>Ceritinib or Crizotinib</td>
<td>6/12/2015</td>
</tr>
<tr>
<td>Dako EGFR PharmDx Kit</td>
<td>+/-</td>
<td>Colorectal Cancer</td>
<td>Cetuximab or panatumumab</td>
<td>9/27/2006</td>
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<tr>
<td>Dako C-Kit PharmDx</td>
<td>+/-</td>
<td>GIST</td>
<td>Imatinib</td>
<td>11/02/2012</td>
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<tr>
<td>Pathway Anti-HER2 (4B5)</td>
<td>0-3+</td>
<td>Breast Cancer</td>
<td>trastuzumab</td>
<td>4/9/2014</td>
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<tr>
<td>Bond Oracle HER2 IHC system</td>
<td>0-3+</td>
<td>Breast Cancer</td>
<td>trastuzumab</td>
<td>4/18/2012</td>
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<tr>
<td>Herceptest (Dako)</td>
<td>0-3+</td>
<td>Breast Cancer</td>
<td>Trastuzumab, pertuzumab and adotraszumab emtansine</td>
<td>9/25/1998</td>
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## Current Companion Dx for PD-L1

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Assay Comparison Literature:

PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project

Fred R. Hirsch, MD, PhD, a,b,* Abigail McElhinny, PhD, c Dave Stanforth, MBA, d James Ranger-Moore, PhD, e Malinika Jansson, MA, f Karina Kulangara, PhD, f William Richardson, BA, f Penny Towne, BS, MBA, f Debra Hanks, MD, f Bharathi Vennapusa, MD, g Amita Mistry, MD, h Rasika Kalamegham, PhD, i,s Steve Averbuch, MD, j James Novotny, PhD, k Eric Rubin, MD, l Kenneth Emancipator, MD, m Ian McCaffery, PhD, n J. Andrew Williams, PhD, o Jill Walker, PhD, p John Longshore, PhD, q Ming Sound Tsao, MD, r Keith M. Kerr, MB, FRCPath s

A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer

David L. Rimm, MD, PhD; Gang Han, PhD; Janis M. Taube, MD; Eunhee S. Yi, MD; Julia A. Bridge, MD; Douglas B. Flieder, MD; Robert Homer, MD, PhD; William W. West, MD; Hong Wu, MD; Anja C. Roden, MD; Junya Fujimoto, MD; Hui Yu, MD; Robert Anders, MD; Ashley Kowalewski, MS; Christopher Rivard, PhD; Janaa Rehman, MD; Cory Batenchuk, PhD; Virginia Burns, PhD; Fred R. Hirsch, MD, PhD; Ignacio I. Wistuba, MD, PhD
Example of PD-L1 Tumor Expression

Yale School of Medicine
**BLUEPRINT-1:**
- 39 Cases – no outcome data
- 3 pathologists – from Dako and Ventana
- 4 Assays – FDA/IUO
- Not statistically powered
- By agreement of 6 companies (BMS, Merck, Genentech, AZ, Dako, Ventana)

**NCCN:**
- 90 Cases - no outcome data
- 13 pathologists – from 7 academic sites
- 4 Assays – 3FDA/IUO and 1 LDT (E1L3N on Leica Bond)
- Prescribed, powered, statistical protocol for ICC between pathologists, assays and localization.
- Led by NCCN, sponsored by BMS
Comparison of Immune Cell Scores

BLUEPRINT-1:

NCCN:
PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project

Ming Sound Tsao, MD, Keith M. Kerr, MD, Mark Kocke, MD, PhD, Mary-Beth Beasley, MD, Alain C. Borczuk, MD, Johan Botling, MD, Lukas Buchendorf, MD, Lucian Chiriac, MD, Gang Chen, MD, Teh-Ying Chou, MD, PhD, Jin-Haeng Chung, MD, PhD, Sanja Dacic, MD, PhD, Sylvie Lantuejoul, MD, Mari Mino-Kenudson, MD, Andre L. Moreira, MD, Andrew G. Nicholson, DM, Masayuki Noguchi, MD, PhD, Giuseppe Pelosi, MD, Claudia Polter, MD, Prudence A. Russell, MD, Jennifer Sauter, MD, Erik Thunnissen, MD, PhD, Ignacio Wataba, MD, PhD, Hui Yu, MD, PhD, Muny W. Wynes, PhD, Melania Pintilie, MSc, Yasushi Yatabe, MD, PhD, Fred R. Hirsch, MD, PhD
25 pathologists reading 81 cases (including some cytology specimens) after a 1.5 day training course

Yale School of Medicine

M. Tsao et al, IASLC presentation at WCLC 2017
# PD-L1 immunohistochemistry (IHC) assays

<table>
<thead>
<tr>
<th>Drug</th>
<th>PD-L1 IHC Assay</th>
<th>PD-L1 scoring</th>
<th>Cut-offs reported in clinical trials</th>
<th>FDA Diagnostic Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>28-8</td>
<td>Tumor cells</td>
<td>1%, 5%, 10%</td>
<td>Complementary</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>22C3</td>
<td>Tumor cells (TPS)</td>
<td>1%, 50%</td>
<td>Companion</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>SP142</td>
<td>Tumor cells (TC)</td>
<td>1%, 5%, 50%</td>
<td>Complementary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune cells (IC)</td>
<td>1%, 5%, 10%</td>
<td></td>
</tr>
<tr>
<td>Durvalumab</td>
<td>SP263</td>
<td>Tumor cells</td>
<td>25%</td>
<td>Unknown</td>
</tr>
<tr>
<td>Avelumab</td>
<td>73-10</td>
<td>Tumor cells</td>
<td>1%, 50%, 80%</td>
<td>Unknown</td>
</tr>
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TPS: tumor proportional score; TC: staining on tumor cell; IC: staining on immune cells
**Strong reliability among all pathologists on tumor cell scoring**

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<th>NSCLC tissue only</th>
<th>Cytology only</th>
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<tr>
<td><strong>DIGITAL</strong></td>
<td></td>
<td></td>
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<tr>
<td>22C3</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
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<tr>
<td>28-8</td>
<td>0.86</td>
<td>0.88</td>
<td>0.77</td>
</tr>
<tr>
<td>SP-142</td>
<td>0.81</td>
<td>0.85</td>
<td>0.76</td>
</tr>
<tr>
<td>SP-263</td>
<td>0.90</td>
<td>0.93</td>
<td>0.82</td>
</tr>
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<td>73-10</td>
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<tr>
<td><strong>All assays</strong></td>
<td>0.91</td>
<td>0.93</td>
<td>0.84</td>
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**GLASS SLIDE**

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<td>0.89</td>
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ICC: **>0.90** excellent  
**0.75-0.9** good

*Koo TK & Li MY. J Chiropr Med 2016:15:155-63*
Blueprint 2 results similar to NCCN study

M. Tsao et al, IASLC presentation at WCLC 2017
Poor reliability among all pathologists on immune cell scoring

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<td>0.28</td>
<td>0.23</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>28-8</td>
<td>0.19</td>
<td>0.14</td>
<td>0.29</td>
<td>0.19</td>
</tr>
<tr>
<td>SP-142</td>
<td>0.36</td>
<td>0.28</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>SP-263</td>
<td>0.25</td>
<td>0.13</td>
<td>0.17</td>
<td>0.10</td>
</tr>
<tr>
<td>73-10</td>
<td>0.17</td>
<td>0.10</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>All assays</td>
<td>0.19</td>
<td>0.11</td>
<td>0.21</td>
<td>0.13</td>
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Fleiss Kappa statistics:
- 0.40-0.59: weak
- 0.20-0.39: minimal
- <0.01-0.20: slight/none
Two statistically powered, multi-institutional studies (NCCN and Blueprint 2) and a number of smaller studies have shown

1. The 22c3, 28-8 and SP263 assays are practically equivalent, while the SP142 assay shows uniformly lower scores for both tumor cells and immune cells.

2. Cytology specimens, although not included in the label for the FDA approved assay, are practically equivalent to surgical biopsy specimens although there is just slightly lower concordance in the TPS scoring.

3. Pathologist can read TPS (tumor proportion scores) with high concordance, but even with training, are not concordant in reading of immune cell scores.

4. ICC is higher when assessing higher percentages of cells.
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STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in KRAS-Mutant Lung Adenocarcinoma


RESEARCH ARTICLE

HR for disease progression or death 4.8 (95% CI, 2.0–11.1) $P = 0.00012$, log-rank test

Group mPFS
STK11/LKB1$^\text{MUT}$ 13.7m
STK11/LKB1$^\text{WT}$ 19.3m

HR for death 14.3, 95% CI (3.4–66.7) $P < 0.0001$, log-rank test

Group mOS
STK11/LKB1$^\text{MUT}$ 11.1m
STK11/LKB1$^\text{WT}$ 26.8m

Progression-free survival (%) at 36 months:
- Red: STK11/LKB1$^\text{MUT}$
- Blue: STK11/LKB1$^\text{WT}$

Overall survival (%) at 36 months:
- Red: STK11/LKB1$^\text{MUT}$
- Blue: STK11/LKB1$^\text{WT}$
Checkmate 26 – the most promising TMB data

Carbone et al, NEJM 2017
TMB does not predict Overall Survival

Supplementary Appendix
In BMS 568 TMB and PD-L1 Identify Distinct and Independent Populations of NSCLC

- No association between PD-L1 expression and TMB levels was observed

$r = -0.16$

$P = 0.13$
Inverse Relationship between PD-L1 and TMB in the literature

86 NSCLC cases analyzed with MSK-IMPACT panel (341-468 genes)
Rizvi H. et al., 2017, JCO

49 NSCLC cases analyzed with whole exome sequencing
Gettinger et al., 2018, Nat Comm (in press)
PD-L1 Expression and TMB are complementary

C

D

H. Rizvi et al JCO 2018
29 of 312 (9%) cases discordant assignment

Figure S5. Total Exome Mutations Versus Genes in FoundationOne Panel

*Based on in silico analysis filtering on 315 genes in FoundationOne comprehensive genomic profile (Foundation Medicine, Inc., Cambridge, MA, USA)
1. TMB is a biomarker for immunotherapy and it is complementary to PD-L1
2. TMB is PREDICTIVE for outcome
3. TMB testing is NOT standardized (Biggest Challenge for TMB)
4. TMB may be associated with PFS but not OS
5. Sensitivity and Specificity (AUC) no better than existing tests
6. TMB costs about 5-10X IHC (actual cost, not charge) and uses 10x as much tissue
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Four Areas of Immune Biology are Represented in the Tissue Inflammation Signature

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<td>CD8A</td>
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<tr>
<td>CD27</td>
<td>LAG3</td>
</tr>
<tr>
<td>CXCR6</td>
<td>PD-L1</td>
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<tr>
<td>IDO1</td>
<td>PD-L2</td>
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<td>HLA-E</td>
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<td>NKG7</td>
<td>HLA-DQA1</td>
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<tr>
<td>CMKL1</td>
<td>HLA-DRB1</td>
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TIS has been clinically verified in HNSCC, gastric, TNBC, urothelial, anal, biliary, colorectal, esophageal, and ovarian cancer.

<sup>13</sup> The Tumor Inflammation Signature Assay (TIS) for use on the nCounter Dx Analysis System is for investigational use only. Limited by United Stated law to investigational use.
An RNA-based Signature?

**IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade**


1Merck & Co. Inc., Kenilworth, New Jersey, USA. 2University of Washington, Seattle, Washington, USA. 3University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
4University of Chicago, Chicago, Illinois, USA. 5UCLA, Los Angeles, California, USA.

**RESEARCH ARTICLE**

The Journal of Clinical Investigation

**Graph:**
- **18-gene T cell-inflamed GEP score:** AUC = 0.75
- **PD-L1 by IHC:** AUC = 0.95

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- Most samples yield >20 ng of usable RNA per slide
- 50 ng of RNA required for 1 assay
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Association between TILs and Response to PD-1 blockade

Tumeh et al., 2014 Nature
Testing CD4, CD8 and CD20 for Prediction for Response to Immunotherapy in Melanoma

QIF:

- **CD4**
  - HI (n = 27)
  - LO (n = 67)
  - P = 0.12

- **CD8**
  - HI (n = 31)
  - LO (n = 63)
  - P = 0.0002

- **CD20**
  - HI (n = 21)
  - LO (n = 73)
  - P = 0.19

Cell counts:

- CD4
  - HI (n = 28)
  - LO (n = 66)
  - P = 0.47

- CD8
  - HI (n = 30)
  - LO (n = 63)
  - P < 0.0001

- CD20
  - HI (n = 34)
  - LO (n = 60)
  - P = 0.043

Pok Fai Wong et al, in review
Predictive performance of CD8 evaluated by receiver operating characteristic (ROC) curves in Melanoma

Quantitative Fluorescence

Cell counts

Pok Fai Wong et al, in review
PD-L1/PD-1 interaction to predict response

Johnson, Bordeaux...Dakappagari, AACC 2016 and CCR in press
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Prospective Clinical Evaluation of Blood-Based Tumor Mutational Burden (bTMB) as a Predictive Biomarker for Atezolizumab in 1L NSCLC: Interim B-F1RST Results

Vamsidhar Velcheti, Edv Phillip Stella, Vincent Sh Cindy Y

1Taussig Cancer Institute, Cleveland Clir  2Florida Hospital Cancer In  3St. Joseph Mercy Hospital

Maximum SLD Reduction From Baseline by bTMB Subgroup in the Interim Analysis Population

Only patients with post-baseline target lesion measurements are shown in plot (n = 70).
10 patients had MEDP < 1% 14 patients without valid sample.

Yale School of Medicine
Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer

Laura Mezquita, MD; Edouard Auclin, MD; Roberto Ferrara, MD; Melinda Charrier, PharmD, PhD; Jordi Remon, MD; David Planchard, MD; David Kaikati, MD; Paul Schumacher, MD, PhD; Santiago Gromeo, MD; Luis Paz-Ares, MD, PhD; Laura Lévy, MD; Clarisse Audigier-Valette, MD; Enrique Pilar Garrido, MD, PhD; Solenn Brosseau; Catherine Léveillé, MD; Caroline Caramela, MD; Jihene Lahmar; Jean Charles Soria, MD, PhD; Benjamin Lévy, MD; and Pascal Sebag-Montefiore, MB, BS, PhD
Questions – during panel discussion