Update on Immuno-Oncology Biomarkers

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Outline

- PD-L1 testing in different cancer types
- Expanded role of MSI/MMR status in multiple cancers
- Tumor mutational burden (TMB) as an evolving IO biomarker
- Relationship between TMB and PD-L1
- Expanding use of checkpoint inhibitors to treat different cancer types
- Biomarker landscape
PD-L1 Testing in Different Cancer Types

PD-L1 Basics

Programmed death ligand 1 (PD-L1) is a normal physiologic immune response inhibitor

Programmed death-ligand 1 (PD-L1) is a normal physiologic immune response inhibitor

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Cancers use PD-L1 to inhibit the endogenous anti-tumor immune response

PD-L1 is targeted by infusing antibodies that block PD-L1 function on tumor cells

Antibodies that block PD-L1 inhibit the endogenous anti-tumor immune response

Antibodies that block PD-L1 inhibit the endogenous anti-tumor immune response

Many other immune inhibitor molecules are being targeted but none have shown the efficacy of anti-PD-L1 therapy yet

Immune Checkpoints

Signal 1

Signal 2

PD-L1

CTLA-4

Antigen Presenting Cell

Go

Stop

Go

Stop

Go

Stop

Go

Stop

Go

Stop
Update on Immuno-Oncology Biomarkers

Expression of Targeted Proteins as a Predictive Biomarker

50% 20%

Companion vs. Complementary PD-L1 Testing

Companion diagnostic test
- Used in conjunction with a therapeutic drug to determine its applicability to a specific person.
- Test result **must be positive** in order for the patient to receive the drug.

Complementary diagnostic test
- Identifies patients who respond particularly well to a drug.
- Is **not a pre-requisite** for receiving the drug.

Evaluation of PD-L1 Expression

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cellular Location</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>Cell Membrane</td>
<td>25%</td>
</tr>
<tr>
<td>Tumor cells</td>
<td>Cell Cytoplasm</td>
<td></td>
</tr>
</tbody>
</table>
### Evaluation of PD-L1 Expression

<table>
<thead>
<tr>
<th></th>
<th>Numerator</th>
<th>Denominator</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Proportion Score (TPS)</td>
<td><img src="http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/" alt="Image 1" /></td>
<td><img src="http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/" alt="Image 2" /></td>
<td>50%</td>
</tr>
<tr>
<td>Combined positive score (CPS)</td>
<td><img src="http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/" alt="Image 3" /></td>
<td><img src="http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/" alt="Image 4" /></td>
<td>75%</td>
</tr>
<tr>
<td>Immune Cells (IC)</td>
<td><img src="http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/" alt="Image 5" /></td>
<td><img src="http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/" alt="Image 6" /></td>
<td>50%</td>
</tr>
</tbody>
</table>

**Example: Squamous Lung Cancer**

Images courtesy of Daphne Wang, Johns Hopkins
Example 1: Metastatic Lung Adenocarcinoma

Example 2: Metastatic Lung Adenocarcinoma

Evaluation of PD-L1 Expression

<table>
<thead>
<tr>
<th>Numerator</th>
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<th>Result</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>75%</td>
</tr>
</tbody>
</table>

### Example: Gastric Carcinoma

![Image of Gastric Carcinoma](image_url)

### Evaluation of PD-L1 Expression

<table>
<thead>
<tr>
<th></th>
<th>Numerator</th>
<th>Denominator</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune Cells</td>
<td>IC</td>
<td></td>
<td>50%</td>
</tr>
</tbody>
</table>

http://captodayonline.com/scoring-gastric-gej-cancers-pdl1-expression/

Image courtesy of Dr. Andres Matoso, Johns Hopkins

### Example: Urothelial Carcinoma

![Image of Urothelial Carcinoma](image_url)
Pathologist Performance

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

<table>
<thead>
<tr>
<th>ICC for each antibody in Tumor Cells</th>
<th>TPS</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB N=96</td>
<td>0.832</td>
<td>0.869</td>
</tr>
</tbody>
</table>

Same Cancer, Different PD-L1 Antibodies, Different Cut-Offs for “Positivity”

FDA-approved companion PD-L1 immunohistochemistry

Cut-off for “positivity” = 5%

Cut-off for “positivity” = 10%

PD-L1 Immunohistochemistry: A New Challenge for Pathologists

Test variables
- Which PD-L1 antibody?
- Which staining pattern (TPS, CPS, IC)?
- Which cutoff value?
- Which of the patient’s tumor samples (primary biopsy, post-treatment, metastasis)?
- Which pathologist?

Most challenging IHC interpretation ever!
Case Presentation #1

- 48 year old patient with metastatic gastric cancer
- Oncologist requests microsatellite instability (MSI) status and PD-L1 testing

Statistics: Gastric Cancer

Table 1. Objective Tumor Response

<table>
<thead>
<tr>
<th>Event</th>
<th>No.</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective response (ORR)</td>
<td>30</td>
<td>15.4 (10.1-22.3)</td>
</tr>
<tr>
<td>Disease control (DC; CR+PR+SD; N=30)</td>
<td>30</td>
<td>24.4 (15.3-36.1)</td>
</tr>
<tr>
<td>CR</td>
<td>6</td>
<td>2.3 (0.5-9.5)</td>
</tr>
<tr>
<td>PR</td>
<td>10</td>
<td>9.4 (3.0-17.7)</td>
</tr>
<tr>
<td>SD</td>
<td>30</td>
<td>14.4 (11.5-17.3)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>145</td>
<td>56.9 (51.6-62.1)</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>15</td>
<td>11.5 (5.6-18.3)</td>
</tr>
<tr>
<td>Duration of response (months, ME)</td>
<td>3.4 (CI: 2.4-5.4)</td>
<td></td>
</tr>
</tbody>
</table>

42% PD-L1 negative
6.4% overall response rate

58% PD-L1 positive
15.5% overall response rate
MSI/PD-L1 Testing of the Biopsy

- PCR testing: All 5 microsatellites intact
- FDA-approved companion PD-L1 immunohistochemistry

PD-L1: What We Know Right Now

PD-L1 is an targetable immune inhibitor
- Offers improved response rates in patients with aggressive cancers
- It is an imperfect but useful predictive biomarker
- IHC is used in both companion and complementary tests
- IHC interpretation fraught with variables

Expanded Role of MSI/MMR Status in Multiple Cancers
Tests for MSI and MMR

- Microsatellite testing uses DNA and is performed by PCR
- Mismatch repair proteins are detected by immunohistochemical stains
- Next generation sequencing is an emerging DNA-based test

Landmark Study Results

- 86 patients
- 9/2013 to 9/2016
- 12 tumor types
- ORR 53%
- CRR 21%

Case Presentation #2

- 70-year-old patient with metastatic prostate cancer
  - High-grade, Gleason pattern 5+4
  - Prominent tumor-infiltrating lymphocytes
- Oncologist requests MSI testing
Prostate Cancer and MSI Testing by PCR

MSI/MMR Testing: What We Know Right Now

Oncologists are now requesting this for many different tumors
- Not just colorectal and endometrial cancer

Neither PCR nor NGS is FDA-approved

NGS not widely available

Tumor Mutational Burden (TMB): An Evolving Immuno-Oncology Biomarker
Update on Immuno-Oncology Biomarkers

Tumor Mutational Burden (TMB)

The number of DNA mutations in a cancer cell

DNA sequencing of all coding region (exons) of the entire genome

Calculated value reported as number of mutations per megabase

- >10 mutations/Mb considered “high TMB”

What is a DNA mutation?

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>Alteration in the original DNA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Wild type</td>
</tr>
<tr>
<td>Silent</td>
<td>Synonymous mutation: No change in amino acid sequence</td>
</tr>
<tr>
<td>Missense</td>
<td>Nonsynonymous mutation: Change in amino acid sequence</td>
</tr>
<tr>
<td>Nonsense</td>
<td>STOP</td>
</tr>
</tbody>
</table>

Other types of mutations:
- Insertion
- Deletion
- Duplication
- Repeat expansion

DNA Sequencing Approaches

~180,000 exons in the human genome
30 million bases

- Whole exome sequencing: Evaluates all bases of all coding regions (100% of exons)
- Gene panel: Evaluates only portions of the exome (50% of exons)
**Tumor Mutational Burden (TMB) Testing**

<table>
<thead>
<tr>
<th>Whole Exome Sequencing</th>
<th>Target Gene Panels</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Gold standard</td>
<td>• Uncertainty about minimum necessary coverage</td>
</tr>
<tr>
<td>• Expensive</td>
<td>• Reduced cost</td>
</tr>
<tr>
<td>• Generates a large amount of data</td>
<td>• Generates less data</td>
</tr>
<tr>
<td>• Requires more DNA</td>
<td>• More easily-integrated into hospital labs</td>
</tr>
<tr>
<td></td>
<td>• Requires less DNA</td>
</tr>
</tbody>
</table>

**Tumor Mutational Burden**

• No single definition of which types of mutations to include
• No recommendation on the percentage of exons to sequence
• Multi-stakeholder harmonization effort
  – Government, Academia, Diagnostics, Industry, Operational
• Publish universal best practices defining TMB
• Analytic validation
• Reference standards

**TMB and Response to Immune-Based Therapy**

Correlation Between TMB and Overall Response Rate in 27 Tumor Types


TMB and Immune Response: A Possible Mechanism

Early Evidence for TMB and Clinical Benefit

More Evidence of TMB Correlating with Clinical Benefit

Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden

15.

More Evidence that TMB Might be Useful, continued

But No Difference in Overall Survival
**TMB: What We Know Right Now**

- A mechanism driving anti-cancer immune elimination
- Testing needs standardization
- Predicts progression-free survival
- May not predict overall survival
- Further studies required to define clinical benefit

**Case Presentation #3**

- 57-year-old patient, former smoker, 4.5 cm lung mass on CT scan

  - Pathology
    - Adenocarcinoma with pleural involvement
    - Lymph node positive for metastatic disease
    - Stage IV (T2bN1M1a)

- Oncology referral

**Anticipated Testing**

- Mutational analysis
  - EGFR
  - ALK
  - ROS1
  - BRAF

- PD-L1 immunohistochemistry

- Tumor mutational burden?
Lack of Benefit of PD-1 Blockade vs. Docetaxel in EGFR-Mutant NSCLC (PFS)

<table>
<thead>
<tr>
<th>EGFR Mutation Status</th>
<th>n</th>
<th>Unstratified Hazard Ratio</th>
<th>Progression-free survival, CheckMate-057</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>82</td>
<td>1.46 (0.90-2.37)</td>
<td></td>
</tr>
<tr>
<td>Not detected</td>
<td>340</td>
<td>0.83 (0.65-1.06)</td>
<td></td>
</tr>
<tr>
<td>Not reported</td>
<td>160</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

Subgroup Analysis of PFS

<table>
<thead>
<tr>
<th>EGFR Mutation Status</th>
<th>n</th>
<th>Unstratified Hazard Ratio</th>
<th>Progression Free Survival, Keynote-010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>70/86</td>
<td>1.79 (0.94-3.42)</td>
<td></td>
</tr>
<tr>
<td>Not detected</td>
<td>660/875</td>
<td>0.83 (0.71-0.98)</td>
<td></td>
</tr>
<tr>
<td>Not reported</td>
<td>778/1033</td>
<td>0.85 (0.73-0.98)</td>
<td></td>
</tr>
</tbody>
</table>

* 95% confidence interval

Test Results

- **Mutation analysis**
  - **BRAF**: No mutations detected
  - **EGFR**: No mutations detected
  - **ALK**: No mutations detected
  - **ROS1**: No mutations detected
- **PD-L1 Immunohistochemistry**
  - 90% Tumor Proportional Score
- **Tumor mutational burden**: 18 mutations/Mb

Is this patient a candidate for anti-PD-1/L1 blockade?

TMB: What We Don’t Know Right Now

- **Definition of TMB**
- **How/when do we test?**
  - Validation of testing methods
- **Role of TMB in the presence of other established biomarkers** (eg EGFR)
- **Are TMB and PD-L1 independent complementary biomarkers?**
Update on Immuno-Oncology Biomarkers

Relationship Between TMB and PD-L1

Expanding Use of Checkpoint Inhibitors to Treat Different Cancer Types

**Update on Immuno-Oncology Biomarkers**

**FDA-Approved Checkpoint Inhibitors for Solid Tumors**

<table>
<thead>
<tr>
<th>Checkpoint Inhibitors</th>
<th>FDA Approval Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab (Opdivo)</td>
<td>2013</td>
</tr>
<tr>
<td>Ipilimumab (Yervoy)</td>
<td>2011</td>
</tr>
<tr>
<td>pembrolizumab (Keytruda)</td>
<td>2014</td>
</tr>
<tr>
<td>avelumab (Bavencio)</td>
<td>2016</td>
</tr>
<tr>
<td>atezolizumab (Tecentriq)</td>
<td>2016</td>
</tr>
<tr>
<td>durvalumab (Imfinzi)</td>
<td>2017</td>
</tr>
<tr>
<td>avelumab (Bavencio)</td>
<td>2017</td>
</tr>
<tr>
<td>atezolizumab (Tecentriq)</td>
<td>2018</td>
</tr>
</tbody>
</table>


**Number of PD-1/L1 Combo Trials**

Number of PD-1/L1 Combo Trials Using Common Strategies:
1. Anti-CTLA-4 agents: 253
2. Chemotherapies: 270
3. Radiotherapies: 64
4. Anti-VEGFA agents: 43
5. Chemoradiotherapy combos: 42

**Other Immuno-Oncology Strategies**

- Checkpoint inhibitors
- Engineered/adoptive T-cells
- Immune system modulators
- Cancer vaccines
- Oncolytic virus
Biomarkers in Immuno-Oncology

**Current Clinical Practice**
- PD-L1 immunohistochemistry
- MSI/MMR deficiency
- Tumor infiltrating lymphocytes in breast cancer

**Emerging**
- Tumor mutational burden

**Developing**
- Multiplex immunohistochemistry/immunofluorescence
- Plasma circulating tumor cell/TMB measurements ("Liquid biopsy")
- Multiple gene expression (mRNA) profiles
- Microbiome composition
- Myeloid-derived suppressor cells
- Other immune-modulating molecules (LAG-3, IDO, etc)

**Summary**
- PD-L1 testing in different cancer types
- Expanded role of MSI/MMR status in multiple cancers
- TMB as an evolving immuno-oncology biomarker
- Relationship between TMB and PD-L1
- Expanding use of checkpoint inhibitors to treat different cancer types
- Biomarker landscape