Learning Objectives

1. To understand the changes in lung cancer testing and treatment happening at a rapid pace

2. To be educated on the current lung cancer guidelines and recommendations, including the role of immunotherapy and PD-L1 testing

3. Understand the molecular marker testing of ALK, EGFR and ROS1 status in treatment decisions

4. Role and limitations of NGS testing and liquid biopsies (cf-DNA) testing in lung cancers

Introduction

• “Multi-disciplinary” approach to treatment of lung cancer has been the standard of care to improve outcomes

• One of the most exciting advancements in lung cancer medicine in recent times has been “targeted therapies”

• Surgery, radiation, chemotherapy, targeted treatments and immunotherapy—alone or in combination—are used to treating lung cancer

• Patient’s lung tumors that express certain biomarkers such as EGFR, ALK1 and ROS1 will receive targeted drug alone or in combination with chemotherapy
Introduction, cont.

• Targeted drugs include erlotinib (Tarceva) crizotinib (Xalkori), ceritinib (Zykadia), gefatinib (Iressa), etc.

• Immunotherapy has recently emerged as a new treatment option for certain lung cancers

• Treatment of lung cancer is complex; the guidelines emerged and provide the tools for oncologists to choose appropriate therapies

• Numerous recommendations have been added to the updates recently in regards to the molecular diagnostics at a rapid pace

Highlights of the New 2017 Recommendations

• New recommendations for molecular testing were published in 2017 based on new evidence gathered from targetable genes and tumor types for testing, etc.

Key recommendations for 2017 include:

• Pathologists can use either cell blocks or other cytology specimens for molecular testing

• Recommend against EGFR immunohistochemistry (IHC) testing
Highlights of the New 2017 Recommendations

• Immunohistochemistry (IHC) as an alternate methodology for ALK and/or ROS1 testing

• Alternate gene testing for ERRB2, RET, BRAF, KRAS and MET have been included

• Testing for EGFR T790M mutations in patients with secondary resistance to EGFR inhibitors has been approved

Role of Immunotherapy and PD-L1 Immunohistochemistry

• Immunotherapy, with anti-PD-1/PD-L1 drugs, has changed the standard of care in advanced stage cancers such as melanoma, lung cancer, renal cancer and Hodgkin lymphoma

• Pembrolizumab (Keytruda), FDA approved as first-line therapy in advanced stage non-small cell lung cancers

• 45% response rate in tumors with immunohistochemistry expression of >50% PD-L1
**Harnessing the Immune System to Fight Cancer**

New drugs and methods of altering a patient's own immune cells are helping some cancer patients — but not all — even when standard treatments fail.

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**Keynote Oncology Clinical Trials**

Keynote is a series of clinical trials to determine whether an investigational immunotherapy may help in the treatment of cancer. The investigational immunotherapy is pembrolizumab (MK-3475).

We have investigational clinical trials under way or planned in multiple cancer types. We are currently studying the types of cancer listed below. We encourage you to speak with your physician to determine if a Keynote clinical trial may be right for you.

<table>
<thead>
<tr>
<th>Bladder</th>
<th>Breast</th>
<th>Colorectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophageal</td>
<td>Gastric</td>
<td>Head &amp; Neck</td>
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<tr>
<td>Hematology</td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>Lung</td>
<td>Melanoma</td>
<td>Ovarian</td>
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<tr>
<td>Pediatric</td>
<td>Prostate</td>
<td>Solid Tumors</td>
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</table>
# Active Clinical Trials

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Keynote Trial</th>
<th>Condition</th>
<th>Phase and Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of Single Agent Pembrolizumab Versus Single Agent Chemotherapy for Metastatic Triple Negative Breast Cancer</td>
<td>119</td>
<td>Metastatic Triple Negative Breast Cancer</td>
<td>Phase 3 Active, not recruiting</td>
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<tr>
<td>Study of Pembrolizumab Plus Chemotherapy vs. Placebo Plus Chemotherapy for Previously Untreated Locally Recurrent Inoperable or Metastatic Triple Negative Breast Cancer</td>
<td>355</td>
<td>Triple Negative Breast Cancer (TNBC)</td>
<td>Phase 3 Recruiting</td>
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<tr>
<td>Study of Pembrolizumab Monotherapy for Metastatic Triple-Negative Breast Cancer</td>
<td>166</td>
<td>Breast Cancer</td>
<td>Phase 2 Active, not recruiting</td>
</tr>
<tr>
<td>Safety and Efficacy Study of Pembrolizumab in Combination With Chemotherapy as Neoadjuvant Treatment for Participants With Triple Negative Breast Cancer</td>
<td>173</td>
<td>Triple Negative Breast Neoplasms</td>
<td>Phase 1 Recruiting</td>
</tr>
<tr>
<td>Study of Pembrolizumab Plus Chemotherapy vs Placebo Plus Chemotherapy as Neoadjuvant Therapy and Pembrolizumab vs Placebo as Adjunct Therapy in Participants With Triple Negative Breast Cancer</td>
<td>522</td>
<td>Triple Negative Breast Neoplasms</td>
<td>Phase 3 Recruiting</td>
</tr>
</tbody>
</table>

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# Active Clinical Trials, cont.

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Keynote Trial</th>
<th>Condition</th>
<th>Phase and Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of Platinum+Pemetrexed Chemotherapy With or Without Pembrolizumab in Participants With First Line Metastatic Non-squamous Non-small Cell Lung Cancer</td>
<td>189</td>
<td>Non-Small-Cell Lung Carcinoma</td>
<td>Phase 3 Active, not recruiting</td>
</tr>
<tr>
<td>A Study of Carboplatin-Pemetrexed Neb-Pemetrexed Chemotherapy With or Without Pembrolizumab in Adults With First Line Metastatic Squamous Non-small Cell Lung Cancer</td>
<td>407</td>
<td>Non-small Cell Lung Cancer</td>
<td>Phase 3 Recruiting</td>
</tr>
<tr>
<td>Study of Pembrolizumab in Participants With Progressive Locally Advanced or Metastatic Carcinoma, Melanoma, or Non-small Cell Lung Carcinoma</td>
<td>501</td>
<td>Cancer, Solid Tumor</td>
<td>Phase 1 Active, not recruiting</td>
</tr>
<tr>
<td>Study of Two Doses of Pembrolizumab Versus Docetaxel in Previously Treated Participants With Non-Small Cell Lung Cancer</td>
<td>510</td>
<td>Non Small Cell Lung Cancer (NSCLC)</td>
<td>Phase 2/Phase 3 Active, not recruiting</td>
</tr>
<tr>
<td>Study of Pembrolizumab Monotherapy in Advanced Solid Tumors and Pembrolizumab Combination Therapy in Advanced Non-small Cell Lung Cancer/ Extensive-disease Small Cell Lung Cancer</td>
<td>511</td>
<td>Solid Tumor</td>
<td>Phase 1 Recruiting</td>
</tr>
<tr>
<td>Study of Pembrolizumab in Participants With Advanced Non-small Cell Lung Cancer</td>
<td>525</td>
<td>Non-small Cell Lung Cancer</td>
<td>Phase 1 Completed</td>
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<tr>
<td>A Study of Pembrolizumab in Combination With Chemotherapy or Immunotherapy in Participants With Lung Cancer</td>
<td>521</td>
<td>Non-small Cell Lung Carcinomas</td>
<td>Phase 1/Phase 2 Active, not recruiting</td>
</tr>
</tbody>
</table>
**PD-1/PD-L1 Checkpoint Inhibitors: In Normal Epithelial Cell**

- PD-1 (receptor) expressed on cytotoxic T-cells and other immune cells
- PD-L1 (protein) expressed on some macrophages and epithelial cells (other normal cells)
- PD-1/PD-L1 interaction protects normal cells against immune recognition by inhibiting the action of cytotoxic T-cells
- Inactivation of cytotoxic T-cells down regulates the immune response
- Inactive T-cell are exhausted, ceases to divide; eventually die by programmed cell death or apoptosis

**PD-1/PD-L1 Checkpoint Inhibitors: In Tumor Cells**

- Some carcinoma cells up-regulate PD-L1 as mechanism to evade the immune checkpoint
- Activated T-cells recognize PD-L1 on carcinoma cells, similar to that of a normal cells, rendering T-cells inactive
- Carcinoma cells escape the immune checkpoint, continue to avoid detection for elimination and proliferate
Performance of PD-L1 Immunomarker

- Biomarkers such as EGFR mutations, ALK and ROS1 rearrangements predict response rates reported up to 70% when the mutations are present.
- The selective performance of these biomarkers is therefore perceived as excellent.
- Biology, therapeutic effect of PD-L1 inhibition, and the nature of this biomarker testing are completely different.

Anti PD-1 Therapy: Mechanism

- Monoclonal drugs that bind to PD-1 (do not activate) on cytotoxic T cells.
- Prevents tumor cells that express PD-L1 from binding to the PD-1 receptor on T cells.
- Allows for cytotoxic T-cells to recognize and kill tumor cells.
PD-L1 Therapy for NSCLC

<table>
<thead>
<tr>
<th>Assay</th>
<th>Pembrolizumab</th>
<th>Nivolumab</th>
<th>Atezolizumab</th>
</tr>
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<tbody>
<tr>
<td>Indication</td>
<td>1st</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>PD-L1 required</td>
<td>≥50%</td>
<td>No</td>
<td>≥1%</td>
</tr>
<tr>
<td>Regimen</td>
<td>Single agent</td>
<td>With chemo*</td>
<td>Single agent</td>
</tr>
</tbody>
</table>

* With carboplatin and pemetrexed for adenocarcinomas only
* Responses enriched when positive

Terms to be Familiar With: FDA IHC Diagnostic Tests

FDA introduced two terms in attempt to regulate the Laboratory Developed Tests (LDTs)

Companion diagnostics:
- Typically linked to a specific drug within it's approved label
- Identifies patients who have significant benefit from the drug
- Example: HER2

Complementary diagnostics:
- Are not required for drug use
- Provides additional info for physicians regarding use of drug on which the patients can benefit from therapy
### PD-1–PD-L1 Inhibitors and Tests

<table>
<thead>
<tr>
<th>Drug</th>
<th>Line Tx</th>
<th>Clone</th>
<th>FDA status</th>
<th>Platform</th>
<th>Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab (Opdivo)</td>
<td>&gt;2</td>
<td>Dako 28-8</td>
<td>Complementary</td>
<td>Autostainer</td>
<td>≥1% TC</td>
</tr>
<tr>
<td>Keytruda (Pembrolizumab)</td>
<td>&gt;1</td>
<td>Dako 22C3</td>
<td>Companion</td>
<td>Autostainer LINK48</td>
<td>&gt;50% or none TC</td>
</tr>
<tr>
<td>Tecentriq (Atezolizumab)</td>
<td>&gt;2</td>
<td>Ventana SP142</td>
<td>Complementary</td>
<td>Benchmark Ultra</td>
<td>&gt;50% TC, &gt;10% IC</td>
</tr>
<tr>
<td>Durvalumab (Imfinzi)</td>
<td>-</td>
<td>Venana SP263</td>
<td>-</td>
<td>Benchmark Ultra</td>
<td>&gt;25% tumor cells</td>
</tr>
<tr>
<td>Avelumab (Bavencio)</td>
<td>-</td>
<td>Dako 73-10</td>
<td>-</td>
<td>DAKO</td>
<td>≥1% TC</td>
</tr>
</tbody>
</table>

### PD-L1 Immunomarker

- Tissue fixation, handling, and processing, and treatment of sections being prepared for IHC, including antigen-retrieval steps, may influence the test outcome.

- Pathologists reading these tests must learn what constitutes true staining or artifact for each particular assay, and how to apply any scoring algorithm.

- Assessment should be carefully controlled, executed, and monitored.
The PD-L1 protein is reported - present or absent

Protein expression is a continuous variable

Absent, low to intermediate to high levels of expression

Expression is heterogeneous among tumor cells
Keynote 001 Study

- Favorable response to pembrolizumab seen in the 50% or greater score cohort is mostly driven by scores well above 50%

- The cohort of patients with tumor proportion score 1% to 49% had outcomes not statistically different from those patients with tumors scoring less than 1%

- Predictive power of the PD-L1 IHC score is not linear across the spectrum of expression
At Least 4 Drugs, At Least 4 Biomarkers?

PD-L1 Immunohistochemistry: Challenges for Labs

- Two assays developed by Dako (Carpinteria, California), are based upon the 28-8 clone for nivolumab and the 22C3 clone for pembrolizumab.

- These tests are validated for use only with Dako IHC autostainer.

- For atezolizumab and durvalumab, different anti–PD-L1 IHC clones, SP142 and SP263 were developed as biomarker assays by Roche Ventana use only on Ventana-automated IHC platforms.
Key Points for Successful Implementation of PD-L1 in Labs

- Communication as multi-disciplinary team approach
- Adequate tissue acquisition: cell block, etc.
- PD-L1 IHC: tissue fixation, epitope stability and processing could impact the outcome
- Validation

PD-L1 Immunomarker

- PD-L1 IHC as a predictive assay for selecting patients for anti–PD-L1 therapy is expected to be complex
- Better understanding of the assay and realistic expectations of its predictive performance need to be established
- Heterogeneity of the biomarker confounds its use as a predictive marker
- Pathology and oncology communities will continue to wrestle with delivery and interpretation of the PD-L1 IHC assays for at least some of the patients being considered for these therapies
Role of Next Generation Sequencing (NGS) Testing for Lung Cancer Testing

- Next-generation sequencing (NGS) with a multi-gene panel is now available for patients with lung adenocarcinoma

- NGS includes “must test genes” (EGFR, ALK, ROS1) as well as thousands of genes that might potentially be useful for understanding cancer biology and potential therapy

- NGS using large targeted gene panel shown successful performance in 94% of small biopsy and cytology samples compared to 100% of resections

- NGS testing has also shown high sensitivity in comparison to single gene testing in numerous published studies

Role of Next Generation Sequencing (NGS) Testing for Lung Cancer Testing

- NGS technology demonstrated high potential for detecting gene mutations that can be treated with targeted agents and resistance genes when patients show resistance to some agents in lung carcinomas

- Limitations to NGS exist such as inconsistencies between NGS results and clinical observations

- Additional studies are needed to evaluate the large number of mutations observed by NGS for development of effective therapeutic targets

- Tumor heterogeneity and detection of low-level mutations challenges the accurate analysis and reliability of the information achieved by NGS
Role of Circulating DNA (cfDNA) for Lung Cancer Testing

• The recommendation in the new guidelines states the use of cfDNA to "rule in" targetable mutations when tissue is limited or difficult to obtain.

• Studies have shown the cfDNA methods have high specificity with very low false positive rates (<5%-20%).

• Due to low sensitivity of testing (60-70%), absence of mutations don’t exclude the possibility of a mutation.

• In a recent published joint review from CAP/ASCO, they found significant discordance between cfDNA vs tissue results.

• The study focused on methodologies, interpretation of cfDNA testing found that there is limited evidence/data to support the wide clinical use of cfDNA testing.

Final Thoughts

• New guidelines with evidence based recommendations offer guidance to pathologists/oncologists in better testing and treating the advanced stage lung cancer patients.

• The first “must test” to include EGFR, ALK and ROS1 have become standard of care in advanced lung cancer patients.

• NGS technology has rapidly advanced and expected to become widely used in the clinic as the technology improves, leading to the early diagnosis of NSCLC and identification of greater number of driver genes and potential drug targets.

• Larger studies with data and outcomes with NGS testing and results of outcomes in patients with immunotherapy will change further the management of lung cancer patients in future.
Thank You!

ASCP Scientific Updates: Molecular Marker Testing Recommendations for Lung Cancer Treatment

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