Disclosure

- Pathologist Consultant for Inivata, Ltd. (Liquid Biopsy):
- No other disclosures
Objectives

• Review Tier 1 / Actionable Mutations in mNSCLC

• Discuss Emerging NSCLC Biomarkers

• Understand the pathologist role
Lung Cancer

• Leading cause of cancer death in the US
  - ~85% are smoking related
  - NSCLC: 18% 5-year survival overall

• Most are non-small cell carcinoma (~85%)
  - 40% Adenocarcinoma
  - 30% Squamous
  - 15% Large cell

• Several 1st line targeted therapies depend upon molecular analysis
Odds for Targetable Mutation

MORE Likely in Never Smoker

- 75% of never smokers
- 15% of smokers

MORE Likely in Younger Age*

20% Never
7% Never

ASCO Post 2017:

Staging is still important

Guidelines are currently aimed at advanced / unresectable disease

In lung cancer, most present at Stage III / IV

Low stage-specific survival; (?smoking comorbidities)
Tiers of Clinical Importance

1. Strong clinical significance
2. Potential clinical significance
3. Unknown clinical significance (“VUS”)
(4. Likely benign)
Tier 1 Mutations in NSCLC

• Up to 50% of patients will demonstrate an actionable mutation

• Minimum testing recommendations (sensitivity at least 20% at diagnostic sample and 5% at recurrence / suspected resistance):
  • CAP: 1. EGFR, 2. ALK, 3. ROS1
  • NCCN: 4. BRAF, 5. PD-L1

• Additional recommendations (NCCN):
  MET
  RET
  ERBB2
  KRAS
  NTRK 1, 2, 3
SENSITIZING EGFR MUTATION POSITIVE

FIRST-LINE THERAPY

EGFR mutation discovered prior to first-line systemic therapy:
- Osimertinib (category 1)
- Erlotinib (category 1)
- Afatinib (category 1)
- Gefitinib (category 1)
- Dacomitinib (category 1)

EGFR mutation discovered during first-line systemic therapy:
- Complete planned systemic therapy, including maintenance therapy, or interrupt, followed by
- Osimertinib (preferred)
- Erlotinib
- Afatinib or gefitinib or dacomitinib

Progression:
- See Subsequent Therapy (NSCL-20)
- See Subsequent Therapy (NSCL-19)

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.
## Practical Progress: 15 sites in NJ and MD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>PCR/FISH Testing Attempted</th>
<th>NGS Attempted</th>
<th>Successfully Tested for Both EGFR + ALK</th>
<th>Successfully Tested for All 7 NCCN Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients, all years</td>
<td>814 (100)</td>
<td>471 (58)</td>
<td>73 (9)</td>
<td>479 (59)</td>
<td>63 (8)</td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>295 (36)</td>
<td>172 (58)</td>
<td>7 (2)</td>
<td>153 (52)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>2014</td>
<td>226 (28)</td>
<td>145 (64)</td>
<td>19 (8)</td>
<td>153 (68)</td>
<td>19 (8)</td>
</tr>
<tr>
<td>2015</td>
<td>293 (36)</td>
<td>154 (53)</td>
<td>47 (16)</td>
<td>173 (59)</td>
<td>38 (13)</td>
</tr>
</tbody>
</table>

Data presented as n (%).
Abbreviations: ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; FISH = fluorescent in situ hybridization; NCCN = National Comprehensive Cancer Network; PCR = polymerase chain reaction.
Variants to Test

1. EGFR
2. ALK
3. ROS1
4. BRAF
5. PD-L1
6. NTRK1, 2, 3

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1. KRAS
2. MET
3. ERBB2

Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival between responders and nonresponders. Exp, experimental; HR, hazard ratio.
Testing – what test for what

1. **EGFR** – insertions / deletions, amplification, SNVs
2. **ALK** – rearrangements, SNVs
3. **ROS1** – rearrangements, SNVs
4. **BRAF** - SNVs
5. **PD-L1** – overexpression (IHC)
6. **NTRK1, 2, 3** - rearrangements

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1. **KRAS** – SNVs, copy number changes
2. **MET** – SNVs, intronic changes, amplification (7q31.2)
3. **RET** – rearrangements
4. **ERBB2** – insertions, amplification (17q12)
Samples
Sample

- Tumor – FFPE, Fresh, FNA
  - DIAGNOSIS
  - Molecular testing: NGS, FISH, etc

- Plasma
  - NOT diagnostic
  - HIGH specificity
  - Up to 30% false negative rate
  - NCCN guidelines:
    - Patient unfit for invasive sampling
    - Have tumor diagnosis, but sample insufficient for molecular testing
The small sample - triage

**Initial materials:** PD-L1 and NGS assay / FISH
Cut extra unstained if FISH is protocol (avoid re-facing block)
Each biopsy in separate cassette / Shallow facing to preserve tissue deeper in block
(avoid aggressive initial block facing)

Use your cytology smears (validate these for any in house assay)

Prioritize PD-L1 / consider blood based test for NGS
1. EGFR

- 1. EGFR sensitizing mutations (7-23% Western; 30-50% Asian)
  - Most are either exon 19 deletion or exon 21 L858R mutation
  - At Diagnosis – EGFR tyrosine kinase inhibitor (TKI) therapy (e.g. osimertinib)
    - First line treatment
      - Longer progression-free survival
      - Higher overall response rates
Patient progression on EGFR TKI

• Upon Progression - resistance mechanism
  • 1st / 2nd generation TKIs - ~50% develop EGFR T790M mutation
  • 3rd generation → clinical trial / chemoimmunotherapy / ?

Other mechanisms of acquired resistance (many !!)
  MET amp
  ERBB2 amp
  EGFR amp
  EGFR C797S
  KRAS
  RET fusion
  Small cell transformation
EGFR - What test?

IHC – not recommended
Sequencing
  Sanger
  NGS
  Mass Array

What about amplifications?
What about at recurrence?
Chemoresistance and the chronic disease theory of cancer
2. ALK

- (D) Computed Tomography Scan of This Patient With the EML4-ALK Variant E17; Ins89A20 Before and After Crizotinib Treatment, Showing a Good PR After Second-Line Crizotinib Treatment

ALK Rearrangement – What test?

- IHC is OK
- FISH is common
- Sequencing is possible: DNA / RNA / cDNA sequencing
3. ROS1

- ROS1 rearrangement (1-2%) – IHC screen ONLY (low specificity); FISH confirm
  - At Diagnosis – crizotinib (70% response rate)

![Break-apart FISH probe for ROS1 gene](image)
ROS1 Rearrangement – What test

IHC not recommended
FISH is common
Sequencing is possible
4. BRAF

- BRAF V600E (1-2%)
  - At Diagnosis – combination BRAF and MEK inhibitor therapy
BRAF SNV – What test?

Sequencing:
  Sanger (20%)
  NGS (5%)
5. PD-L1

- PD-L1 expression (23-28%)
  - >50% cells – 1st line treatment (pembrolizumab)
    - Note that PD-L1 testing is not needed for other inhibitors (e.g. nivolumab, atezolizumab)
  - >1% cells – 2nd line
- Harmonization of IHC ??

Figure 5

Immunooncology predictive markers

PD-L1 IHC
Microsatellite instability
Tumor mutational burden
KRAS / STK11 mutation
NCCN Guidelines Version 3.2019
Non-Small Cell Lung Cancer

TARGETED THERAPY FOR ADVANCED OR METASTATIC DISEASE

Monitoring During Initial Therapy
- Response assessment after 2 cycles, then every 2–4 cycles with CT of known sites of disease with or without contrast or when indicated.

Monitoring During Subsequent Therapy
- Response assessment with CT of known sites of disease with or without contrast every 6–12 weeks. Timing of CT scans within parameters is a clinical decision.

Sensitizing EGFR Mutation Positive
- First-line therapy
  ▶ Afatinib
  ▶ Erlotinib
  ▶ Dacomitinib
  ▶ Gefitinib
  ▶ Osimertinib
- Subsequent therapy
  ▶ Osimertinib

ALK Rearrangement Positive
- First-line therapy
  ▶ Alectinib
  ▶ Brigatinib
  ▶ Ceritinib
  ▶ Crizotinib
- Subsequent therapy
  ▶ Alectinib
  ▶ Brigatinib
  ▶ Ceritinib
  ▶ Lorlatinib

ROS1 Rearrangement Positive
- First-line therapy
  ▶ Ceritinib
  ▶ Crizotinib

BRAF V600E Mutation Positive
- First-line therapy
  ▶ Dabrafenib/trametinib
- Subsequent therapy
  ▶ Dabrafenib/trametinib

NTRK Gene Fusion Positive
- First-line/Subsequent therapy
  ▶ Larotrectinib

PD-L1 ≥50%
- First-line therapy
  ▶ Pembrolizumab
  ▶ Carboplatin or cisplatin/pemtrexed/pembrolizumab (non-squamous)
  ▶ Carboplatin/paclitaxel/bevacizumab/atezolizumab (non-squamous)
  ▶ Carboplatin or cisplatin/(paclitaxel or albumin-bound paclitaxel)/pembrolizumab (squamous)
NTRK1, 2, 3 Rearrangement

- TRK = tropomyosin receptor kinase
- TRK inhibitor
  - FDA-approved drug, November 2018, Larotrectinib
- Into NCCN guidelines January 2019
- 0.23% of NSCLC cases
- PAN-CANCER indication

Lung adenocarcinoma with SQSTM1-NTRK1 fusion

Fig. 2
Responses to larotrectinib in clinical trials. a (baseline) and b (after three cycles of larotrectinib) are scans of a 2-year-old female patient with a previously treated, non-resectable infantile fibrosarcoma harboring an ETV6-NTRK3 fusion. The patient was referred for surgery after four cycles of larotrectinib and a pathological complete response with clear margins was confirmed. c (baseline) and d (after four cycles of larotrectinib) are scans of a 45-year-old female with a metastatic lung adenocarcinoma harboring an SQSTM1-NTRK1 fusion, and pulmonary hypertrophic osteoarthropathy. The patient had evidence of disease progression following treatment with multiple cycles of platinum and pemetrexed. After starting on larotrectinib 100 mg BID, a durable partial response was achieved.

Why do I care about KRAS??
KRAS Plus

KRAS plus STK11 mutations
LKB1 modulates STING (stimulator of interferon genes) expression in KRAS-mutant lung cancer.


Note: LKB1 = STK11
Emerging Biomarkers

### NCCN Guidelines Version 3.2019
Non-Small Cell Lung Cancer

#### EMERGING BIOMARKERS TO IDENTIFY NOVEL THERAPIES FOR PATIENTS WITH METASTATIC NSCLC

<table>
<thead>
<tr>
<th>Genetic Alteration (ie, Driver event)</th>
<th>Available Targeted Agents with Activity Against Driver Event in Lung Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-level ( MET ) amplification or ( MET ) exon 14 skipping mutation</td>
<td>Crizotinib&lt;sup&gt;1-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>( RET ) rearrangements</td>
<td>Cabozantinib&lt;sup&gt;6&lt;/sup&gt;,&lt;sup&gt;7&lt;/sup&gt; Vandelanib&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>( ERBB2 ) (( HER2 )) mutations</td>
<td>Ado-trastuzumab emtansine&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tumor mutational burden (TMB)*</td>
<td>Nivolumab + ipilimumab&lt;sup&gt;10&lt;/sup&gt; Nivolumab&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*TMB is an evolving biomarker that may be helpful in selecting patients for immunotherapy. There is no consensus on how to measure TMB.*
MET Amplification
Crizotinib: Subcarinal lymph node

FISH showing amplification

https://doi.org/10.1097/JTO.0b013e31821528d3
Computed tomographic scans obtained after 4 weeks of crizotinib treatment in a 73-year-old man with metastatic squamous cell lung cancer and MET exon 14 skipping.

MET Exon 14 skipping

Rebecca S. Heist et al. The Oncologist 2016;21:481-486
Summary of clinical characteristics of all patients identified with MET exon 14.

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (minimum, maximum), yr</td>
<td>43, 84</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>20</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Adeno/pleomorphic carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Stage I</td>
<td>13</td>
</tr>
<tr>
<td>Stage II</td>
<td>3</td>
</tr>
<tr>
<td>Stage III</td>
<td>3</td>
</tr>
<tr>
<td>Stage IV</td>
<td>7</td>
</tr>
<tr>
<td>Never-smoker</td>
<td>12</td>
</tr>
<tr>
<td>Former smoker</td>
<td>14</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0</td>
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<tr>
<td>0 pack-years</td>
<td>12</td>
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<tr>
<td>1–20 pack-years</td>
<td>9</td>
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<tr>
<td>&gt;20 pack-years</td>
<td>5</td>
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<tr>
<td>Coexisting alterations</td>
<td></td>
</tr>
<tr>
<td>Classic EGFR sensitizing mutations</td>
<td>0⁸</td>
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<tr>
<td>ALK rearrangement</td>
<td>0</td>
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<tr>
<td>ROS1 rearrangement</td>
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<tr>
<td>PI3K</td>
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<td>P53</td>
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<td>CTNNB</td>
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<td>PTEN</td>
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<tr>
<td>CDKN2A</td>
<td>1</td>
</tr>
<tr>
<td>SMAD4</td>
<td>1</td>
</tr>
</tbody>
</table>

Unless otherwise noted, values are the numbers of patients.

*Although no sensitizing EGFR mutations were detected, one patient had an Arg277Gln mutation in EGFR of unknown significance, and two patients had EGFR amplification.

*Although no ROS1 rearrangements were detected, one patient had a Lys2033Gln mutation of unknown significance.

Rebecca S. Heist et al. The Oncologist 2016;21:481-486
The genomic position of METex14 alterations.
 RET Rearrangement

Treatment options:
“multi tyrosine kinase inhibitors” (MKIs) with anti-RET activity (i.e. not crizotinib or gefinitib) such as cabozantinib / vandetanib

  High toxicity / lower response rates

RET-selective inhibitors – clinical trial (e.g. LOXO-292, BLU-667)
ERBB2

Exon 20 Insertion - sensitive

Amplification - resistance to EGFR TKIs
Microarray results (Illumina I-Scan platform)

ERBB2 (HER2) amplification
# TMB – Tumor mutational burden

A rough measure of potential immunotherapy efficacy


## TMB Harmonization Project Overview

<table>
<thead>
<tr>
<th>Workflow</th>
<th>Phase 1: In silico analysis</th>
<th>Phase 2: Empirical analysis</th>
<th>Phase 3: Clinical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Publicly available TCGA data</td>
<td>Cells derived from human tumors</td>
<td>Clinical Samples</td>
</tr>
<tr>
<td>Goals</td>
<td>Identify sources of variability between TMB calculated using whole exome sequencing (WES) &amp; various targeted panels used in the clinic</td>
<td>Agree upon creation of a universal reference standard using WES Identify sources of variability after alignment of TMB scores from targeted panels to the reference standard</td>
<td>Propose standards for defining clinical application of TMB and inform clinical use</td>
</tr>
<tr>
<td>Timeframe</td>
<td>May 2018</td>
<td>Spring 2019</td>
<td>Summer 2019</td>
</tr>
</tbody>
</table>
The Future: Targeted or Genomic Approach?
Copy numbers, SNVs, InDels, structural variants, oh my

Tumor / Normal whole exome sequencing
Generated 152,708 candidate mutations
Per tumor (on average) by subtraction method
And 508 using [VarScan 2] pipeline…

IF EVERYTHING IS UNDER CONTROL, YOU ARE NOT MOVING FAST ENOUGH.

(Normally attributed: Mario Andretti)

"I DON'T KNOW WHAT THIS IS, BUT YOU SHOULD SEE HOW FAST IT'S GROWING!"

-Mario Andretti
Founded in 1824 in Charleston, the Medical University of South Carolina is the oldest medical school in the South. Today, MUSC continues the tradition of excellence in education, research, and patient care. MUSC educates and trains more than 3,000 students and residents, and has nearly 13,000 employees, including approximately 1,500 faculty members. As the largest non-federal employer in Charleston, the university and its affiliates have collective annual budgets in excess of $1.7 billion. MUSC operates a 750-bed medical center, which includes a nationally recognized Children’s Hospital, the Ashley River Tower (cardiovascular, digestive disease, and surgical oncology), Hollings Cancer Center (one of fewer than 70 National Cancer Institute designated centers), Level I Trauma Center, and Institute of Psychiatry. For more information on academic information or clinical services, visit www.musc.edu. For more information on hospital patient services, visit www.muschealth.org.