Optimizing Advanced NSCLC Testing, Treatment, and Management Virtual Summit

Session 1: Follow the Tissue: Testing Selection for Patients with Advanced NSCLC

May 21, 2021
11:00 am – 12:30 pm Eastern
Faculty

- Dana Herndon, RN, BSN
  Thoracic Oncology Nurse Navigator
  Cone Health Cancer Center
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  Thoracic Surgeon
  Virginia Cancer Specialists
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  Professor and Chair, Department of Pathology
  The University of Mississippi Medical Center
- Carolyn Presley, MD
  Assistant Professor and Associate Medical Director of the OncoGeriatrics Program
  Thoracic & Geriatric Oncology
  The Ohio State University Comprehensive Cancer Center

Molecular Process (Including Liquid)

TIMOTHY CRAIG ALLEN, MD, JD, FCAP, FASCP
THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER
Molecular Testing of NSCLC

Recommended testing targets include: **EGFR, ALK, ROS1, Met exon 14 skipping mutations, RET, and PD-L1**

**ALK** testing should be performed in the same NSCLC patient population as for **EGFR**: patients with advanced NSCLC and never-smokers with squamous subtype

Testing modalities for **ALK** include fluorescent in situ hybridization (FISH), immunohistochemistry (IHC), and Next-generation sequencing (NGS)

Testing must be clinically relevant, easy to interpret, have a short turnaround time, and be cost efficient

Testing considerations that impact the overall turnaround time

- Preanalytic (test ordering, specimen retrieval, slide/block review, transportation to the testing laboratory)
- Analytic (batching of tests, releasing reports)
- Postanalytic (report availability in the electronic medical record, notification of results to the treating physician)
- Reflex testing initiated by the pathologist at the time of biopsy diagnosis can increase testing rates and decrease turnaround time of molecular testing
Application of Testing Methods

- Most biomarker tests are performed as a series of single-gene evaluations, which need more tissue and potential limit the number of tests possible.

- While IHC is the usual diagnostic test for ALK, followed by FISH where results are indeterminate, NGS is more commonly being used as a testing panel.

- Laboratories are migrating away from the single gene test approach toward NGS assays incorporating gene panels able to detect a diverse set of alterations.

- The choice is driven by cost, urgency, clinical and laboratory focus, and time considerations; there is no “one size fits all” approach.

Plasma Genotyping (Liquid biopsy)

- Evolving; usefulness in clinical practice depends upon the number of circulating tumor molecules in the peripheral blood and tumor burden.

- Reasons to perform liquid biopsy include:
  - (1) inability to biopsy or rebiopsy due to the patient’s suboptimal clinical condition or unfavorable tumor site such as bone, central nervous system, or multiple small pulmonary nodules.
  - (2) sparing the patient the risk of complications of an invasive procedure.
  - (3) inadequacy of biopsy tissue for the performance of all necessary testing.
  - (4) lower cost of blood draw.
  - (5) shorter turnaround time.
  - (6) circulating markers are theoretically more likely to reflect systemic tumor burden, better depicting intratumoral heterogeneity that is missed with single-site biopsies.
Technical Considerations

- Blood collection: Ethylenediaminetetra-acetic acid (EDTA) tubes versus preservative tubes designed for cell-free DNA isolation
  - EDTA tubes: inexpensive, must be processed within 1-2 hours after collection, greater risk of release of normal genomic DNA, diluting the mutant species; therefore best for use with in-house laboratory testing
  - Preservative tubes: stabilize nucleated RBCs, preventing release of genomic DNA, inhibits cfDNA degradation, cfDNA stable up to 14 days, circulating tumor cells (CTCs) up to 7 days; therefore best for sending out to a laboratory
- No consensus but usually 20 ml of blood is suggested; both stable at room temperature; storage and transport guidelines must be strictly followed

Liquid Biopsy

- Tissue testing remains the gold standard for ALK testing; however, liquid biopsy is becoming more commonly employed
- Noninvasive, reliable, alternative approach for patients at the time of diagnosis for whom tumor biopsy is not feasible or with inadequate material for molecular analysis
- Can guide treatment strategies during the disease course, including evaluating recurrence
- Liquid biopsy has driven molecular testing from local pathology laboratories to high-throughput, centralized, often for-profit laboratories
Liquid Biopsy

- What does this mean for patient care?
- How is the pathologist’s role affected?
- What are the implications for integration of diagnostic information and appropriate therapy selection?
- How do we control quality?

Receiving the Reports & Treatment Decisions: It’s Complicated

CAROLYN J PRESLEY, MD, MHS
THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER
Lung cancer is responsible for 1 in 5 cancer deaths worldwide.
Optimizing Advanced NSCLC Testing, Treatment, and Management Virtual Summit
Session 1: Follow the Tissue: Testing Selection for Patients with Advanced NSCLC

**Histologic Subtypes of NSCLC: US**

- Decreasing Incidence of Squamous Cell Subtype Over Time
- Incidence of Histologic Types: Males and Females

85% of lung cancers are NSCLC.

**Molecular Subsets of Lung Cancer Defined by Driver Mutations**

<table>
<thead>
<tr>
<th>Driver Mutations in NSCLC, %</th>
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</thead>
<tbody>
<tr>
<td>AKT1</td>
</tr>
<tr>
<td>ALK</td>
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<tr>
<td>BRAF</td>
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<td>EGFR</td>
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<td>KRAS</td>
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<td>MEK1</td>
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<td>PIK3CA</td>
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<tr>
<td>RET</td>
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<tr>
<td>ROS1</td>
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</table>
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![EGFR mutations diagram](image)

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**Diagnostic Pathology**

*Analysis of the frequency of oncogenic driver mutations and correlation with clinicopathological characteristics in patients with lung adenocarcinoma from Northeastern Switzerland*

*Alexandra Grosse, Claudia Grosse, Markus Rechsteiner & Alex Soltermann*

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*bMCM Part of Springer Nature*
Optimizing Advanced NSCLC Testing, Treatment, and Management Virtual Summit

Session 1: Follow the Tissue: Testing Selection for Patients with Advanced NSCLC

It's easy to get lost in the cancer world
Optimizing Advanced NSCLC Testing, Treatment, and Management Virtual Summit
Session 1: Follow the Tissue: Testing Selection for Patients with Advanced NSCLC

**Stage IV Lung Cancer Treatments**

- Targeted Treatment
- Chemotherapy + Immunotherapy
- Immunotherapy alone

**Targeted Therapies Toolbox**

The James

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QuickSheet

<table>
<thead>
<tr>
<th></th>
<th>EGFR</th>
<th>ALK</th>
<th>ROS1</th>
<th>BRAF V600E</th>
<th>MET</th>
<th>NTRK</th>
<th>RET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred First-Line</td>
<td>Osimertinib</td>
<td>Alectinib&lt;br&gt;Brigatinib</td>
<td>Entrectinib</td>
<td>Dabrafenib&lt;br&gt;trametinib</td>
<td>Capmatinib&lt;br&gt;Tepotinib</td>
<td>Larotrectinib&lt;br&gt;Entrectinib</td>
<td>Selpercatinib&lt;br&gt;Pralsetinib</td>
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<tr>
<td>Alternative</td>
<td>Afatinib&lt;br&gt;Gefitinib&lt;br&gt;Dacomitinib&lt;br&gt;Erlotinib</td>
<td>Ceritinib</td>
<td>Crizotinib&lt;br&gt;Ceritinib</td>
<td>Vemurafenib</td>
<td>Crizotinib</td>
<td>Cabozantinib&lt;br&gt;Vandetanib</td>
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<tr>
<td>2nd line+</td>
<td></td>
<td>Crizotinib</td>
<td>Lorlatinib&lt;br&gt;Entrectinib</td>
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<td>Clinical trial</td>
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</tbody>
</table>
Best response: AMG510 at 960mg

<table>
<thead>
<tr>
<th>Efficacy outcomes</th>
<th>NSCLC, evaluable patients receiving 960mg N = 13</th>
<th>CRC, evaluable patients receiving 960mg N = 12</th>
<th>Other tumor types, evaluable patients receiving 960mg N = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best overall response</td>
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<tr>
<td>Partial response – No. (%)</td>
<td>7 (54)</td>
<td>1 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stable disease – No. (%)</td>
<td>6 (46)</td>
<td>10 (83)</td>
<td>0 (0)</td>
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<tr>
<td>Progressive disease – No. (%)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>1 (100)</td>
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<tr>
<td>Objective response rate – %</td>
<td>54%</td>
<td>8%</td>
<td>N/A</td>
</tr>
<tr>
<td>Disease control rate a – %</td>
<td>100%</td>
<td>92%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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OSU 19016: glutaminase inhibition + osimertinib in EGFR positive NSCLC after PD on osimertinib

- Cycle 1 Day 16
  - Phase I
    - NSCLC EGFR activating mutation positive, T790M mutation negative
    - OSU 19016: glutaminase inhibition
    - AZD9291: 80mg orally daily and CB-839 HCl orally BID
  - Cycle 1 Day 28
    - Cycle 2 and onward (28-day cycles): AZD9291: 80mg orally daily and CB-839 HCl orally BID

- Cycle 1 Day 15
  - Cycle 2 (onward) Day 1
    - AZD9291: 80mg orally daily and CB-839 HCl orally BID
    - Osimertinib
    - Oxometabolites
    - cfDNA

- Cycle 2 (onward) Day 1
  - AZD9291: 80mg orally daily and CB-839 HCl orally BID
  - Osimertinib
  - Oxometabolites
  - cfDNA

- Assess response with CT imaging every 2 months

- Cycle 1 Baseline
  - Oxometabolites
  - cfDNA

- Disease progression:
  - Blood sample
  - Oxometabolites
  - cfDNA
It's easy to get lost in the cancer world

Heterogeneity
Personalizing Care for Older Adults

Tip: Toxicity ↔ Comorbidities

- Lower extremity swelling
- Diarrhea and dehydration
- Rash
- Cardiac
Tip: Brain Mets versus no Brain Mets

Tip: Talk to your pharmacist!

- Drug-drug interactions
- BEERS Criteria
- Polypharmacy
Conclusions

- Test for at least 7 genes
- Brain versus no brain involvement
- Comorbidities
- Pharmacy involvement

Acknowledgements

- Oncogeriatics Program at OSUCCC
- Thoracic Oncology Center at OSUCCC
Biomarker Testing Methodology and Recommendations

May 21, 2021
12:45 – 2:15 pm Eastern

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Review of IHC

TIMOTHY CRAIG ALLEN, MD, JD, FCAP, FASCP
THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Immunohistochemistry (IHC)

- Many IHC antibodies are dependent upon fixation; IHC on formalin fixed paraffin embedded tissue is the most practical
- Careful control of preanalytical, analytical, and postanalytical variables is critical for successful IHC results
- The use of IHC for determination of pulmonary carcinoma biomarkers is a well-established and powerful technique
- IHC is readily available in pathology laboratories, is relatively easy to perform and assess, can provide clinically meaningful results quickly, and is relatively inexpensive
Preanalytic Variables

- Starts the moment the tissue is removed from the patient
- Variables include fixation delay, inappropriate fixation time, and issues of paraffin embedding
- Cold ischemia time (time from tissue removal until placement in formalin) should be less than 1 hour
- Fixation should be in an adequate amount, 10 times the specimen volume
- Fixation time should be 6-24 hours for biopsies, 24-72 hours for resection specimens
- Unstained sections not used within a few days should be stored at 2 to 8 degrees to preserve antigenicity

Analytic Variables

- Laboratory’s responsibility
- Development of adequate antibodies, antigen retrieval, type and concentration of the antibody, incubation time, incubation temperature, signal enhancement, epitope retrieval buffers
- Validation of the IHC test requires a minimum of 10 samples, which may be a practical difficulty for some laboratories as it may take a long time to acquire 10 positive samples for initial setup of the IHC
Postanalytic Variables

- Starts with the glass slide’s microscopic evaluation
- Standardization of positive and negative controls
- Subjectivity of staining intensity assessment can be reduced using uniform intensity scoring
- Identification of IHC staining artifact, including nonspecific background changes, crush artifacts, edge artifacts, and artifacts due to poor fixation and necrosis

ALK IHC

- ALK testing was originally performed via FISH assay; however, IHC is now accepted as an appropriate assay, with FISH used in cases of indeterminate IHC results
- ALK-specific preanalytic variables: D5F3 and 5A4 antibodies show equal sensitivity; however, the ALK1 antibody is less accurate and should not be used
- ALK-specific postanalytic variables: ALK protein is not expressed in normal mature lung tissue, so strong IHC amplification can be used as a marker of tumor ALK positivity; however, artifacts can cause strong false-positive staining
ALK IHC

ALK-specific postanalytic variables (cont): Positive ALK IHC shows strong granular cytoplasmic staining; however, granular staining can occur in alveolar macrophages nerve and ganglion cells, including within tumors, glandular epithelium, extracellular mucin, and areas of necrosis.

- Especially in mucin-containing cells such as signet ring tumor cells require careful evaluation for ALK staining; and signet ring cell morphology of ALK-rearranged adenocarcinomas is frequent.

- A thin membranous positive pattern on ALK IHC may be masked by an intracellular mucin vacuole, making it difficult to detect their ALK positivity.

ALK IHC

- ALK IHC may be used for screening with confirmatory FISH testing for some indeterminate (weak positive) cases.

- Because ALK testing was originally performed via FISH only, some in the lung oncology community may be somewhat suspicious of IHC biomarkers.

- In fact, there have been a number of failed trials, likely due to the nature of the IHC biomarker; however, this should not be used as evidence against the use of IHC biomarkers today.

- It is important to understand the practice of IHC and how the particular chemistry used in any assay may influence the test outcome.

- ALK IHC can be used to the patient’s advantage; today, some ALK IHC protocols do not require FISH confirmation.
ALK IHC

What about NGS replacing IHC and FISH?

NGS-based testing is fast emerging as a one-stop solution in lung cancer diagnostics; however, ALK IHC remains available, affordable, and sensitive, so NGS cannot be considered today as a complete replacement of ALK IHC.

Perhaps NGS will replace FISH as the confirmatory test for cases of indeterminate ALK IHC test results.
Faculty

Zahra Maleki, MD, FCAP, MIAC
Associate Professor of Pathology
Johns Hopkins Hospital
@ZMaleki_cyto

Pathology Evaluation

Goal of Pathologic evaluation:
A) to make an accurate diagnosis using 2015 WHO classification
B) to preserve the tissue for molecular studies, especially in cases of advanced-stage disease

The 2015 World Health Organization Classification of Lung Tumors
Impact of Genetic, Clinical and Radiologic Advances
Since the 2004 Classification


On Behalf of the WHO Panel
Molecular Study Selection

- **Molecular diagnostic studies in Non-Small Cell Lung Cancer:**
  - A) Gene alterations with impact on therapy
  - B) Avoidance of therapies with no/minimal clinical benefit

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### NCN Guidelines Version 4.2021

**Non-Small Cell Lung Cancer**

**NCCN Evidence Blocks™**

**CLINICAL PRESENTATION**

- Advanced or metastatic disease

**HISTOLOGIC SUBTYPE**

- **Adenocarcinoma**
  - Large cell NSCLC (not otherwise specified (NOS))
  - Molecular testing, including:
    -EGFR mutation (category 1), ALK (category 1), ROS1, BRAF, NTRK1/2/3, MET exon 14 skipping, RET
    - Testing should be conducted as part of broad molecular profiling
    - PD-L1 testing (category 1)

- **Squamous cell carcinoma**

**BIOMARKER TESTING**

- Consider molecular testing, including:
  - EGFR mutation, ALK, ROS1, BRAF, NTRK1/2/3, MET exon 14 skipping, RET
  - Testing should be conducted as part of broad molecular profiling
  - PD-L1 testing (category 1)

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**See Principles of Pathologic Review (NSCLC)**

**See Principles of Molecular and Biomarker Analysis (NSCLC).**

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Session 2: Biomarker Testing Methodology and Recommendations

NCCN Guidelines Version 4.2021
Non-Small Cell Lung Cancer
NCCN Evidence Blocks

Targeted Therapy or Immunotherapy 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 clinically 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 Guidelines parameters is a clinical decision.

Sensitizing EGFR Mutation Positive
- First-line therapy
  - Atezolizumab
  - Erlotinib
  - Dacomitinib
  - Gefitinib
  - Osimertinib
  - Erlotinib + bevacizumab (non-squamous)
  - Subsequent therapy
  - Osimertinib

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

RET Rearrangement Positive
- First-line therapy
  - Cabozantinib
  - Crizotinib
  - Lorlatinib

ROS1 Rearrangement Positive
- First-line therapy
  - Ceritinib
  - Entrectinib

BRAF V600E Mutation Positive
- First-line therapy
  - Dabrafenib/triametrexate
- Subsequent therapy
  - Dabrafenib/triametrexate

PD-L1 ≥ 1% (in addition to above)
- First-line therapy
  - Atezolizumab
  - Camlilumab

See Evidence Blocks on NSCL-29A

* An FDA-approved biomarker is an appropriate substitute for bevacizumab.

** Continuation maintenance refers to the use of at least one of the agents given in first line, beyond 4.6 cycles, in the absence of disease progression.

References

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Testing Results

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ALK rearrangement positive
- NSCL-23

ROS1 rearrangement positive
- NSCL-26

BRAF V600E mutation positive
- NSCL-27

NTRK1/2/3 gene fusion positive
- NSCL-28

MET exon 14 skipping mutation positive
- NSCL-29

RET rearrangement positive
- NSCL-29

PD-L1 100% and negative for actionable molecular markers above
- NSCL-31

PD-L1 31%-49% and negative for actionable molecular markers above
- NSCL-32

PD-L1 ≤ 1% and negative for actionable molecular markers above
- NSCL-33

Molecular Subsets of Lung Cancer Defined by Driver Mutations

EGFR

Unknown

KRAS

NRAS

PIK3CA

MEK

HER2

BRAF

RET

ROS1

AKT2

Frequency of Driver Mutations in NSCLC, %

AKT2

ALK

BRAF

EGFR

HER2

KRAS

MEK

NRAS

PIK3CA

RET

ROS1


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Molecular Study Methods

- “Up-front” slide sectioning when the tissue is minimal
- **Next-generation sequencing (NGS):** a broad-based panel
- **RNA-based NGS:** to maximize detection of fusion events (especially in never smokers)
- **Real-time polymerase chain reaction (PCR):** specific targeted fusion
- **Fluorescence in situ hybridization (FISH):** to examine copy numbers, amplification, and structural alterations

---

Fluorescence in situ hybridization (FISH)

- A molecular cytogenic technique
- To locate and detect a specific DNA sequence on a chromosome using a probe
- A probe is a small DNA or RNA sequence with an attached fluorescent molecule
- Binding occurs between a probe and part of the DNA with high degree of complementarity
FISH Process

- Denature the chromosome
- Denature the probe
- Hybridization
- Fluorescence staining
- Detection in the dark

https://www.genome.gov/sites/default/files/ig/illustration/fluorescence_in_situ_hybridization_fish.jpg

FISH advantages and disadvantages

- **Advantages**
  - High sensitivity, specificity and rapid turnover
  - High efficiency of detection
  - 4–24 h turn around time
  - Analysis of 1000–2000 cells accomplished in 15–45 min

- **Disadvantages**
  - High cost of fluorescence microscope
  - Loss of signals with time in FISH slides


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**FISH Specimen**

- **FFPE** (formalin fixed paraffin embedded) tissue, cell blocks
- **Smears**: unstained, Papanicolaou stain, Romanowsky stain, cytospin
- **Tissue imprints**
- **Liquid based preparations**

**FISH Probes for NSCLC**

- ALK translocation in 2-7% of NSCLC, never smoker
- EML4/ALK, 4-5% of NSCLC
- BRAF mutations in 5% of NSCLC, V600E and non-V600E
- ROS1 with CD74/ROS1 FISH probe
- EGFR FISH probe
- ERBB2 (HER2)
- KRAS FISH probe, 15% to 25% of NSCLC, 97% affecting KRAS exon 2 and 3, smoker
- MET FISH, 4% of lung cancers
- NTRK1 rearrangement in 1-3% of NSCLC
- PD-L1 (CD274) FISH probe
- PIK3CA FISH probe, chromosome 3q26
- PTEN, 2-7% of NSCLC
- RET rearrangement in 1-2% of NSCLC
- ROS, 1% of NSCLC, never smoker
**Optimizing Advanced NSCLC Testing, Treatment, and Management Virtual Summit**

**Session 2:** Biomarker Testing Methodology and Recommendations

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**FISH in NSCLC**

- **ALK** rearrangements, 2p23
- **ROS1** rearrangements, 6q22
- **RET** rearrangements, 10q11.2
- **MET** amplification

---

**ALK (Anaplastic Lymphoma Kinase) Gene Rearrangements**

- **ALK** is a tyrosine kinase receptor that can be rearranged in NSCLC
- **ALK rearrangement** is associated with responsiveness to oral ALK TKIs.
- Echinoderm microtubule-associated protein-like 4 (EML4) is the most common fusion partner
- FDA-approved IHC (ALK [D5F3] CDx Assay) can be utilized as a stand-alone test, not requiring confirmation by FISH.
- FISH, NGS, Targeted real time PCR

---

ALK FISH pattern scoring system. A, Normal cells showing two ALK fused signals (ALK_F); (B) ALK short split pattern (ALK_S); (C) ALK long split pattern (ALK_L); (D) ALK far-away split pattern (ALK_FA); (E) ALK deleted split pattern (ALK_D). Cells positive for ALK rearrangement (ALK_R) are those showing short, long, far-away splits, and deleted patterns. © ALK polysomy. ALK (anaplastic lymphoma kinase) gene rearrangement. 10.1038/jtco.2015.44. doi: 10.1097/JTO.0000000000000444. PMID: 25514802.

**References:**

An inv(2) leading to EML4-ALK fusion, or other rearrangements disrupting ALK gene would result in constitutive kinase activity.

ALK FISH probe is a break-apart probe (5' in green and 3' in red).

Crizotinib is a tyrosine kinase inhibitor, targeting ALK positive NSCLC.

ROS1 is a tyrosine kinase receptor

- CD74, SLC34A2, CCDC6, and FIG, common fusion partners
- Oral ROS1 TKIs
- FISH, NGS, Targeted real time PCR
- IHC for ROS1 fusions has low specificity, needs to be confirmed

https://www.empiregenomics.com/img/ideograms/catalog_probes/ROS1-BA/ROS1-BA_GROR.png
**RET** (rearranged during transfection)
Gene Rearrangements

- RET is a receptor tyrosine kinase
- Dysregulation and inappropriate signaling through the RET kinase domain
- Common fusion partners are KIF5B, NCOA4, and CCDC6
- Associated with responsiveness to oral RET TKIs regardless of fusion partner
- NGS-based methodology has a high specificity, and RNA-based NGS is preferable to DNA-based NGS for fusion detection.
- Targeted real-time reverse transcriptase PCR assays, FISH may under detect

---

**MET** (mesenchymal-epithelial transition)

- Exon 14 (METex14) skipping variants
- MET is a receptor tyrosine kinase
- Loss of MET ex14 leads to dysregulation
- Responsiveness to oral MET TKIs
- NGS, RNA-based NGS demonstrating improvement in detection

https://www.empiregenomics.com/img/ideograms/catalog_probes/MET/MET_OR.png
**FISH test for MET amplification**

Abnormal Cells with MET Amplification

**BRAF (B-Raf proto-oncogene) point mutations**

- A serine/threonine kinase
- MAP/ERK signaling pathway
- Change in amino acid position V600E

Combined therapy with oral inhibitors of BRAF and MEK
Real-time PCR, Sanger sequencing (ideally paired with tumor enrichment), and NGS
**EGFR (Epidermal Growth Factor Receptor)**

A receptor tyrosine kinase on the surface of epithelial cells

- **Exon 19 deletions, p.L858R point mutation in exon 21**
- Responsive to oral EGFR tyrosine kinase inhibitor (TKI) therapy
  - stage IIB-IIIA or high risk stage IB-IIA NSCLC
- **EGFR p.T790M**: a mechanism of resistance to first- and second-generation EGFR TKI
- **EGFR ex20**: a diverse group of in-frame duplication or insertion mutations
- Lack of response to EGFR TKI therapy exceptions are:
  - p.A763_Y764insFQEA is associated with sensitivity to TKI therapy
  - p. A763_Y764insLQEA may be associated with sensitivity to TKI therapy
- For this reason, the specific sequence of EGFRex20 insertion mutations is important
- Testing Methodologies: Real-time PCR, Sanger sequencing (ideally paired with tumor enrichment), and NGS are the most commonly deployed methodologies for examining EGFR mutation status.

**KRAS (KRAS proto-oncogene) point mutations**

G-protein with intrinsic GTPase activity, activating mutations result in unregulated signaling through the MAP/ERK pathway.

- KRAS are most commonly seen at codon 12
- KRAS mutation is prognostic of poor survival
- reduced responsiveness to EGFR TKI therapy
- presence of a known activating mutation in KRAS identifies patients who are unlikely to benefit from further molecular testing.
**NTRK1/2/3 (neurotrophic tyrosine receptor kinase) gene fusions**

*NTRK1/2/3* are tyrosine kinases receptor
Rarely rearranged in NSCLC
no specific clinicopathologic features
Point mutations in *NTRK1/2/3* are generally non-activating and have not been investigated in association with targeted therapy
FISH, IHC, PCR, and NGS
DNA-based NGS may under-detect *NTRK1* and *NTRK3* fusions.

**PD-L1** (Programmed Death Ligand 1)

**PD-L1**: A co-regulatory molecule, expressed on tumor cells, inhibiting T-cell-mediated cell death.

**PD-1**: a negative regulator, binds to ligands including PD-L1 (CD274) or PD-L2 (CD273)

PD-L1 is a suppressor for T cell activity
Immune checkpoint inhibitors block PD-L1 and PD-1 interaction and enhance antitumor effects of T cells.
IHC for PD-L1 is used to detect effectiveness of first line anti-PD-1 and PD-L1 therapy.

https://doi.org/10.1016/j.str.2017.06.011
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Session 2: Biomarker Testing Methodology and Recommendations

**PD-L1 (Programmed Death Ligand 1) (2)**

- PD-L1 IHC
- It is **FDA approved**
- PD-L1 IHC interpretation is based on membranous expression of tumor cells
- utilization of pembrolizumab in patients with NSCLC
- The guide-line is based on Tumor proportion score (TPS)
- TPS: is the percentage of viable cells with partial or complete membranous expression of PD-L1, regardless of its intensity

https://www.spandidos-publications.com/article_images/ol/18/1/ol-18-01-0161-g02.jpg

**PD-L1 (Programmed Death Ligand 1) (3)**

The definition of positive or negative testing for PD-L1 varies for each antibody and platform deployed.

In NSCLC patients harboring tumors with an oncogenic driver and PD-L1 expression, targeted therapy for the oncogenic driver should be considered first.
PD-L1 and the NCCN guidelines

- PD-L1 guidelines are based on
  - <1%
  - 1%–49%
  - Equal or >50%


CAP New Recommendations

- A minimal **first panel** of genes: *EGFR, ALK, and ROS1*.
- A **second expanded** panel of genes in NSCLC patients: *BRAF, MET, RET, ERBB2 (HER2), and KRAS*, if adequate material is available
- Pathologists and laboratories **should not use** EGFR copy number analysis (i.e., FISH or CISH) to select patients for EGFR-targeted tyrosine kinase inhibitor therapy.
Guideline for non-Small Cell Lung Cancer

- **Adenocarcinoma, NSCLC (NOS), Large cell carcinoma**
  - EGFR mutation (category 1), ALK (category 1), ROS1, BRAF, NTRK1/2/3, MET ex14 skipping, RET
  - PD-L1 testing (category 1)

- **Squamous cell carcinoma**
  - EGFR mutation, ALK, ROS1, BRAF, NTRK1/2/3, MET exon 14 skipping, RET
  - PD-L1 testing (category 1)

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CAP 2018

- **Any Cytology Sample With Adequate Cellularity and Preservation** May Be Tested.—The original recommendation preferred cell blocks over smears.
- Analytic methods must be able to detect mutation in a sample with 20% or more malignant cell content.
- It is not appropriate to use IHC for EGFR mutation testing.
- **ROS1** testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics.
- (1) offer a comprehensive cancer panel that includes all of the genes in the first 2 categories (EGFR, ALK, ROS1, BRAF, MET, ERBB2 [HER2], KRAS, RET) for all appropriate patients, or
  (2) offer targeted testing for the genes in the **must-test category (EGFR, ALK, ROS1)** for all appropriate patients and offer as a second test an expanded panel containing the second-category genes (BRAF, MET, ERBB2 [HER2], and RET) for patients who are suitable candidates for clinical trials, possibly after performing a single-gene KRAS test to exclude patients with KRAS-mutant cancers from expanded panel testing.
Strong Recommendation: Physicians must use **EGFR and ALK** molecular testing for lung adenocarcinoma patients at the time of diagnosis for patients presenting with advanced stage disease or at progression in patients who originally presented with lower stage disease but were not previously tested.

Recommendation: Pathologists may utilize either **cell blocks or other cytologic preparations** as suitable specimens for lung cancer biomarker molecular testing.

Pathologists and laboratories should not use EGFR copy number analysis (i.e., FISH or CISH) to select patients for EGFR-targeted tyrosine kinase inhibitor therapy.

Strong Recommendation: Laboratories should not use total **EGFR** expression by IHC testing to select patients for EGFR-targeted tyrosine kinase inhibitor therapy.

**RET, BRAF, ERBB2 (HER2), KRAS, MET**, molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include RET as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative.
CAP Recommendation

- **ROS1** IHC may be used as a screening test in advanced stage lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.
- Recommendation: Immunohistochemistry (IHC) is an equivalent alternative to FISH for **ALK** testing.
- Sensitizing **EGFR** mutations and have progressed after treatment with an EGFR targeted TKI **EGFR T790M** mutational testing when selecting patients for third-generation EGFR-targeted therapy. Recommendation: Laboratories testing for EGFR T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting EGFR T790M mutations in as little as 5% of viable cells. No Recommendation: There is currently insufficient evidence to support a recommendation for or against routine testing for ALK mutational status for lung adenocarcinoma patients with sensitizing ALK mutations who have progressed after treatment with an ALK-targeted tyrosine kinase inhibitor.

The role of testing for circulating, cell-free DNA for lung cancer patients

- There is currently insufficient evidence to support the use of circulating cell-free plasma DNA (cfDNA) molecular methods for the diagnosis of primary lung adenocarcinoma. Recommendation: In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA (cfDNA) assay to identify **EGFR mutations**.
- Expert Consensus Opinion: Physicians may use cell-free plasma DNA (cfDNA) methods to identify **EGFR T790M mutations** in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted tyrosine kinase inhibitors; testing of the tumor sample is recommended if the plasma result is negative.
**CAP Recommendation**

- Smoking status, ethnicity, and histology—are associated with the presence of an **EGFR** mutation
- Smoking status and histology—have been associated with the presence of an **ALK** rearrangement
- *These factors should not be considered in selecting patients for testing.*

**CAP 2018, emerging markers**

- Mitogen-activated protein kinase kinase 1 (MEK1/MAP2K1)
- Fibroblast growth factor receptor 1–4 (FGFR 1–4)
- Neurotrophic tyrosine kinase, receptor, type 1–3 (NTRK1-3)
- Neuregulin 1 (NRG1)
- Ras-like without CAAX 1 (RIT1)
- Neurofibromin 1 (NF1)
- Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) AKT serine/threonine kinase 1 (AKT1)
- NRAS proto-oncogene, GTPase (NRAS)
- Mechanistic target of rapamycin (MTOR)
- Tuberous sclerosis 1 (TSC1)
- Tuberous sclerosis 2 (TSC2)
- KIT proto-oncogene receptor tyrosine kinase (KIT)
- Platelet-derived growth factor receptor alpha (PDGFRA)
Thank you

@ZMaleki_cyto

Review of Molecular Single Assay

MOHAMED K. MOHAMED, MD, PHD
CONE HEALTH CANCER CENTER
Testing Methodologies

Appropriate possible testing methodologies are indicated below for each analyte separately; however, several methodologies are generally considered for use:
- Next-generation sequencing (NGS) is used in clinical laboratories. Not all types of alterations are detected by individual NGS assays and it is important to be familiar with the types of alterations identifiable in individual assays or combination(s) of assays.
- It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach, most typically performed by NGS. For patients who, in broad panel testing don’t have identifiable driver oncogenes (especially in never smokers), consider RNA-based NGS if not already performed, to maximize detection of fusion events.
- Real-time polymerase chain reaction (PCR) can be used in a highly targeted fashion (specific mutations targeted). When this technology is deployed, only those specific alterations that are targeted by the assay are assessed.
- Sanger sequencing requires the greatest degree of tumor enrichment. Unmodified Sanger sequencing is not appropriate for detection of mutations in tumor samples with less than 25% to 30% tumor after enrichment and is not appropriate for assays in which identification of subclonal events (eg, resistance mutations) is important. If Sanger sequencing is utilized, tumor enrichment methodologies are nearly always recommended.
- Other methodologies may be utilized, including multiplex approaches not listed above (ie, SNAPSHOT, MassARRAY).
- Fluorescence in situ hybridization (FISH) analysis is utilized for many assays examining copy number, amplification, and structural alterations such as gene rearrangements.
- IHC is specifically utilized for some specific analytes, and can be a useful surrogate or screening assay for others.

Plasma Cell Free Circulating Tumor DNA Testing

- Plasma Cell-Free/Circulating Tumor DNA Testing:
  - Cell-free circulating tumor DNA testing should not be used in lieu of a histologic tissue diagnosis.
  - Some laboratories offer testing for molecular alterations examining nucleic acids in peripheral circulation, most commonly in processed plasma (sometimes referred to as "liquid biopsy").
  - Studies have demonstrated cell-free tumor DNA testing to generally have very high specificity, but significantly compromised sensitivity, with up to 30% false-negative rate.
  - Standards for analytical performance characteristics of cell-free tumor DNA have not been established, and in contrast to tissue-based testing, no guidelines exist regarding the recommended performance characteristics of this type of testing.
  - Cell-free tumor DNA testing can identify alterations that are unrelated to a lesion of interest, for example, clonal hematopoiesis of indeterminate potential (CHIP).
  - The use of cell-free circulating tumor DNA testing can be considered in specific clinical circumstances, most notably:
    - If a patient is medically unfit for invasive tissue sampling
    - In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified (see NSCLC-18 for oncogenic drivers with available targeted therapy options).
Advanced NSCLC Treatment Opportunities and Optimizing Patient Care

May 21, 2021
2:30 – 4:00 pm Eastern

Faculty

- Mohamed Mohamed, MD, PhD
  Division Director Medical Oncology,
  Director of Thoracic Oncology
  Hematologist/Medical Oncologist
  Cone Health Cancer Center
- Kimberly Rohan, ANP-BC, AOCN
  Edward Hematology Oncology Group
- Julia Kathleen Rotow
  Associate Professor of Pathology
  Johns Hopkins University School of Medicine
- Dana Herndon, RN, BSN
  Thoracic Oncology Nurse Navigator
  Cone Health Cancer Center
- Zahra Maleki, MD
  Associate Professor of Pathology
  Johns Hopkins University School of Medicine
NCCN Guidelines

MOHAMED MOHAMED
CONE HEALTH CANCER CENTER

NCCN Guidelines Version 4.2021
Non-Small Cell Lung Cancer

CLINICAL PRESENTATION

- Advanced or metastatic disease
  - Establish histologic subtype* with adequate tissue
  - Consider genetic testing
  - Smoking cessation
  - Integrate palliative care

HISTOLOGIC SUBTYPE

- Adenocarcinoma
- Large cell
- NSCLC not specified (NOS)

Biomarker Testing

- Molecular testing, including:
  - EGFR mutation (category 1)
  - ALK (category 1)
  - ROS1
  - BRAF
  - METex14 skipping
  - RET
  - Testing should be conducted as part of broad molecular profiling
  - PD-L1 testing (category 1)

- Consider molecular testing, including:
  - EGFR mutation
  - ALK
  - ROS1
  - BRAF
  - RVK1222
  - MET exon 14 skipping
  - RET
  - Testing should be conducted as part of broad molecular profiling
  - PD-L1 testing (category 1)

* See Principles of Pathologic Review (NSCL-5)

††† The NCCN NSCLC Lung Cancer Panel strongly advises broader molecular testing with the goal of identifying more driver mutations for which effective drugs might become available, to improve care of patients maximizing the availability of clinical trials. Broad molecular profiling is a component of the improvement of care of patients with NSCLC. See: Emerging Biomarkers to

††††† See Principles of Molecular and Biomarker Analysis (NSCL-11)

Note: All recommendations are category 2 unless otherwise indicated.
32 years old never smoker white female recently diagnosed with stage IV non-small cell lung cancer, poorly differentiated adenocarcinoma with signet ring features presented with bilateral pulmonary nodules and masses in addition to mediastinal lymphadenopathy as well as axillary and upper abdominal lymphadenopathy.

Alectinib vs. Crizotinib PFS

NSCLC

Improved PFS with alectinib

Hazard ratio for disease progression or death, 0.47 (95% CI, 0.34–0.65)
P < 0.001 by log-rank test

Peters et al. NEJM 2017
April 2020 Treatment Paradigm for Molecular Biomarker–Positive Advanced NSCLC

1. **Advanced NSCLC (molecular biomarker positive)**
   - EGFR mutation positive
     - Osimertinib (preferred), crizotinib, afatinib, gefitinib, or dacomitinib
   - ALK positive
     - Alectinib (preferred), brigatinib, ceritinib, or crizotinib
   - ROS1 positive
     - Crizotinib or entrectinib
   - BRAF V600E positive
     - Dabrafenib/trametinib
   - NTRK positive
     - Entrectinib or larotrectinib
   - EGFR T790M mutation positive
     - Osimertinib
   - EGFR T790M mutation negative or previous osimertinib
     - Alectinib, brigatinib, ceritinib, or lorlatinib
   - BRAF V600E mutation positive
     - Dabrafenib/trametinib
   - PD-L1 positive
     - Pembrolizumab
   - PD-L1 negative
     - Pembrolizumab
   - Other biomarkers
     - Treatment options for adenocarcinoma or squamous cell carcinoma without actionable biomarker

Follow treatment options for adenocarcinoma or squamous cell carcinoma without actionable biomarker.

*Afatinib, dacomitinib, erlotinib, gefitinib, and osimertinib approved for EGFR exon19del, exon 21 L858R; afatinib for EGFR G719X, S768I, L861Q.
†Or as second line after CT.

**First line**
- Osimertinib
- Alectinib (preferred), brigatinib, ceritinib, or crizotinib
- Crizotinib or entrectinib
- Dabrafenib/trametinib
- Entrectinib or larotrectinib

**Second line and beyond**
- Osimertinib
- Alectinib, brigatinib, ceritinib, or lorlatinib
- Dabrafenib/trametinib
- Pembrolizumab
- Pembrolizumab

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**Slide credit:** clinicaloptions.com
Lorlatinib has Broad Activity Against ALK Resistance Mutations

- Secondary mutations in the ALK kinase domain can induce resistance to first- and second-generation ALK TKIs\(^1\).
- Lorlatinib has broad-spectrum potency against most known ALK resistance mutations, including ALK G1202R\(^1,2\).

**Table adapted from Gainor 2016.**

*Slide credit: clinicaloptions.com*

**Phase III CROWN: Firstline Lorlatinib has Superior PFS to Crizotinib**

**Cumulative CNS Progression as First Event**

- HR for CNS PD w/o previous non-CNS PD or death: 0.06 (95% CI: 0.02-0.18)
- 12-mo cumulative incidence: 3.3% (95% CI: 2.46%-4.47%)
- 12-mo cumulative incidence: 2.8% (95% CI: 1.0%-8.1%)

**Patients Alive Without Disease Progression (%)**

- Median PFS, Mo (95% CI): 12 - Mo PFS, % (95% CI)
- Lorlatinib: 9.3 (7.6-11.1) Crizotinib: 39 (30-48)

**Patients at Risk, n**

- Lorlatinib: 149, Crizotinib: 147

**HR for PD or death: 0.28 (95% CI: 0.19-0.41); P<001**

Lorlatinib Toxicity Poses Unique Challenges

<table>
<thead>
<tr>
<th>AEs, %</th>
<th>Lorlatinib (n = 149)</th>
<th>Crizotinib (n = 142)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Any</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>19</td>
<td>25</td>
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<tr>
<td>Edema</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>Increased weight</td>
<td>7</td>
<td>14</td>
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<tr>
<td>Peripheral neuropathy</td>
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<td>7</td>
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<tr>
<td>Cognitive effects</td>
<td>13</td>
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<td>Diarrhea</td>
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<td>Fatigue</td>
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</tr>
<tr>
<td>Hypertension</td>
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<td>7</td>
</tr>
<tr>
<td>Vision disorder</td>
<td>17</td>
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<tr>
<td>Increased ALT</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Mood effects</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Increased AST</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>


Patient Case 5: Newly Diagnosed ROS1-Positive Advanced NSCLC

- 56-yr-old male nonsmoker presents with abnormal chest x-ray
- CT/PET shows large LUL mass with extensive metastatic lymphadenopathy
- Cervical lymph node biopsy reveals stage IV adenocarcinoma
- Brain MRI shows 3 small lesions in frontal lobe
- NGS biomarker testing with tissue shows:
  - **ROS1 rearrangement positive**; negative for **EGFR, ALK, BRAF, MET, RET, NTRK**
  - PD-L1 expression 60%
**Crizotinib: First Agent Approved in ROS1-Positive NSCLC**

**PROFILE 1001: ORR in ROS1+ (N = 50)**

- ORR: 72%
- Median PFS: 19.2 mo

*Later determined to not be a ROS1 mutation.

A, FISH positive, negative for ROS1 rearrangement, positive for ALK rearrangement.

M, FISH positive, positive for MET amplification.

**ROS1-Rearranged NSCLC (N = 53)**

- Deaths, n (%): 26 (49.1)
- Median OS, mo (95% CI): 51.4 (29.3-NR)

- 1-yr OS rate: 79%
- 4-yr OS rate: 51%

**Entrectinib in ROS1-Positive NSCLC: Efficacy**

**Change in Tumor Size (N = 161)**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total (N = 161)</th>
<th>CNS Disease at Baseline (n = 56)</th>
<th>No CNS Disease at Baseline (n = 105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, n (%) (95% CI)</td>
<td>108 (67.1) (59.3-74.3)</td>
<td>35 (62.5) (48.6-75.1)</td>
<td>73 (69.5) (59.8-78.1)</td>
</tr>
<tr>
<td>Median PFS, mo (95% CI)</td>
<td>15.7 (11.0-21.1)</td>
<td>11.8 (6.4-15.7)</td>
<td>19.0 (12.0-29.6)</td>
</tr>
</tbody>
</table>

Investigator determined CNS status.

Data cutoff: May 1, 2019. Median follow-up: 15.8 mo. Both treatment-naive (37.3%) and previously treated (62.7%) patients included in analysis.
Approach to Selecting ROS-Targeted TKI Therapy for ROS1+ NSCLC

<table>
<thead>
<tr>
<th></th>
<th>Crizotinib¹</th>
<th>Entrectinib²</th>
<th>Lorlatinib³</th>
<th>Repotrectinib⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 52)</td>
<td>(N = 161)</td>
<td>(N = 69)</td>
<td>(N = 33)</td>
</tr>
<tr>
<td>Median PFS, mo</td>
<td>19.2</td>
<td>15.7 (19.0 without CNS mets)</td>
<td>21.0 (crizotinib naive)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Intracranial ORR, %</td>
<td>26 (ALK+)²</td>
<td>79.2*</td>
<td>64†</td>
<td>100‡</td>
</tr>
<tr>
<td>Efficacy in pretreated disease?</td>
<td>--</td>
<td>Yes⁶</td>
<td>Yes (35%³)</td>
<td>Yes (39%⁴)</td>
</tr>
<tr>
<td>Safety considerations</td>
<td>Visual impairment, peripheral edema, GI</td>
<td>Weight gain, dizziness, dysgeusia</td>
<td>Peripheral neuropathy, cognitive AEs</td>
<td>Dizziness, dyspnea, neuropathy</td>
</tr>
</tbody>
</table>

* n = 19; DoR: 12.9 mo. ¹ n = 7 crizotinib naive; intracranial ORR in 12 crizotinib-pretreated patients: 50%. ² n = 6. ³ Patients with pretreated disease included in overall analysis. ⁴ ORR for 40 crizotinib-pretreated patients. ⁵ ORR for 3 patients treated with second-line repotrectinib 80 mg; for 160 mg: 55%.

A New Frontline Option for ALK+ NSCLC?

- Multiple FDA-approved options exist for newly diagnosed patients with ALK+ NSCLC
  - Crizotinib (approved in 2011 but not recommended)
  - Ceritinib (2017)
  - Alectinib (2017; preferred second-generation TKI)
  - Brigatinib (2020)
  - Lorlatinib?
    - Approval expanded to frontline setting in March 2021
Treatment Failure – Next Steps: ALK Rearrangement Positive Patients

- Biopsy and send for Next Gen Sequencing
- Asymptomatic Patients: consider oligometastatic lesion and consider local therapy such as SBRT or surgery. Continue Alectinib
- Symptomatic Patients:
  - Brain: SRS for limited lesions and continue therapy
  - Systemic
    - Limited: SBRT/surgery and continue therapy
    - Multiple lesions: Change therapy to Lorlatinib
    - Clinical Trial