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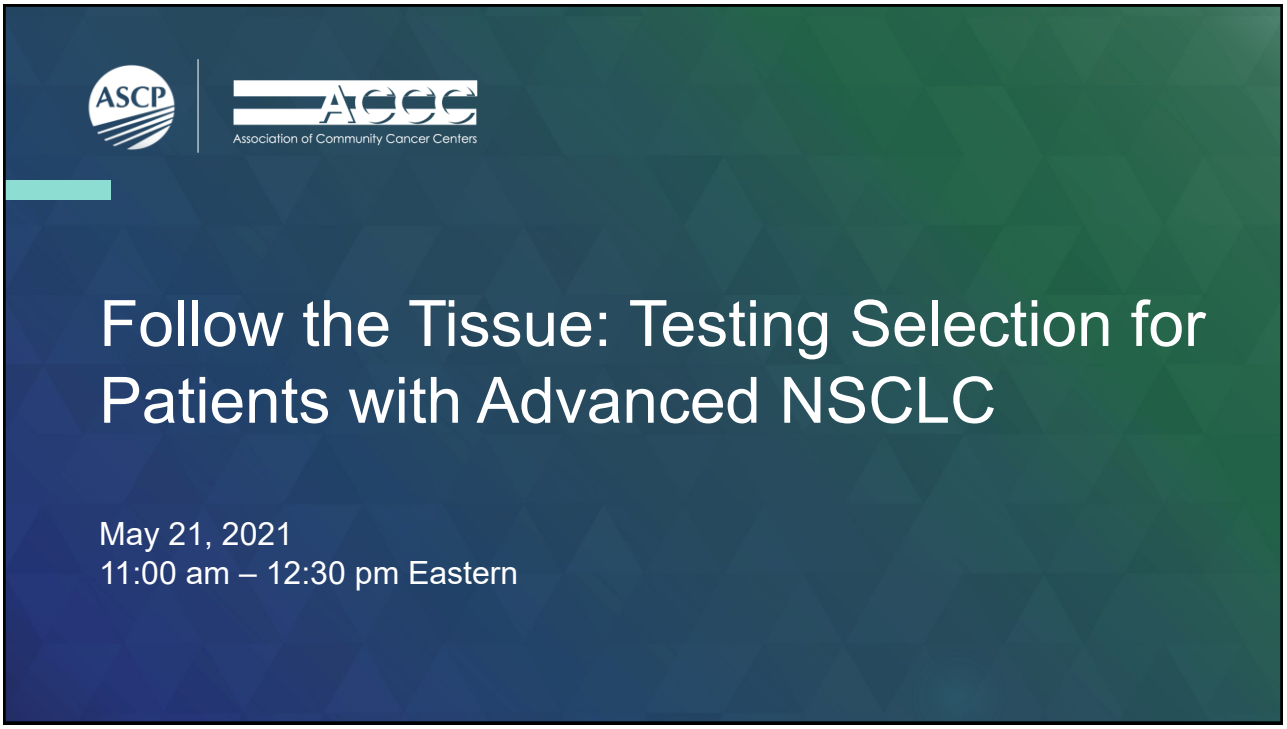

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ASCP

Association of Community Cancer Centers

Molecular Process (Including Liquid)

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THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

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Molecular Testing of NSCLC

5

- 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

5

Testing considerations that impact the overall turnaround time

6

- 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

6

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Application of Testing Methods

7

- 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

7

Plasma Genotyping (Liquid biopsy)

8

- 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

8

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Technical Considerations

9

- 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

9

Liquid Biopsy

10

- Tissue testing remains the gold standard for *ALK* testing; however, liquid biopsy is becoming more commonly employed
- Noninvasive, reliable, alterative 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

10

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Liquid Biopsy

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- 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?

11

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Receiving the Reports & Treatment Decisions: It's Complicated

CAROLYN J PRESLEY, MD, MHS
THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER

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Lung cancer is responsible for 1 in 5 cancer deaths worldwide

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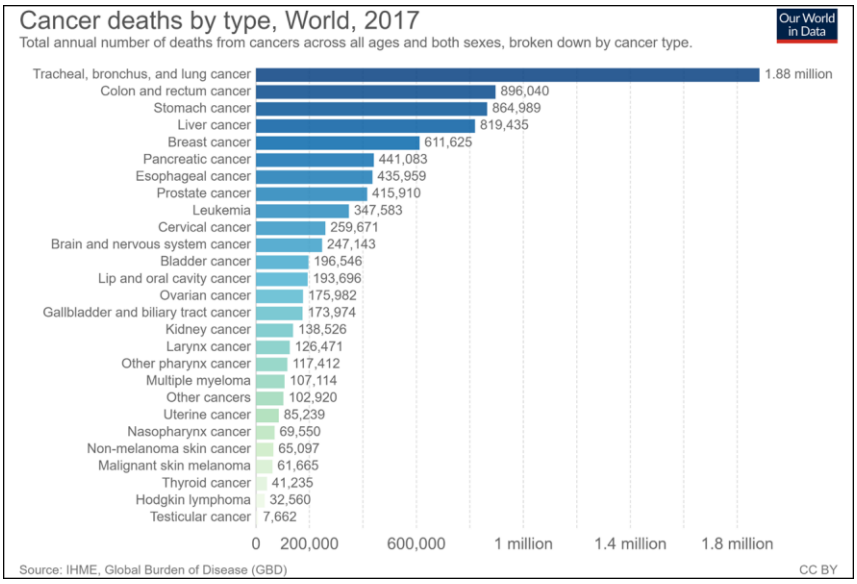

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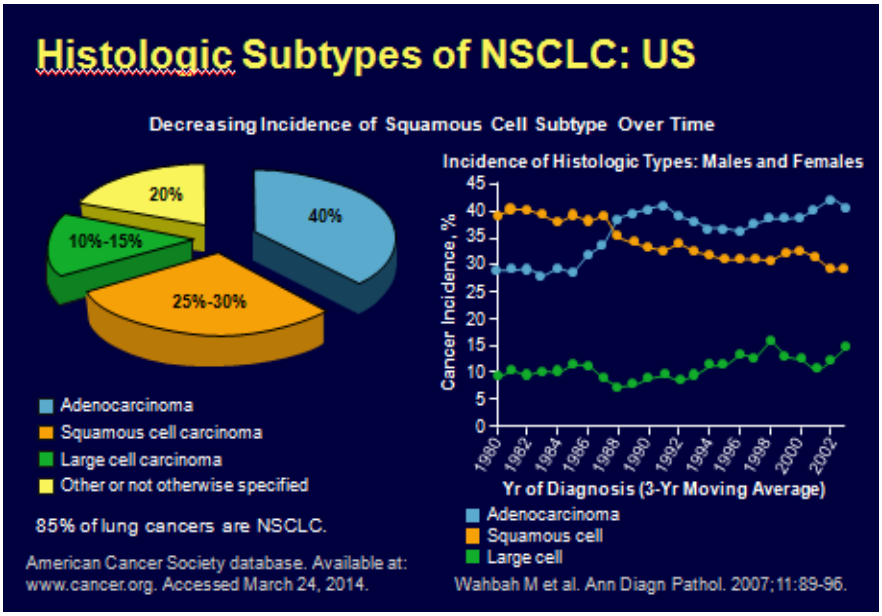

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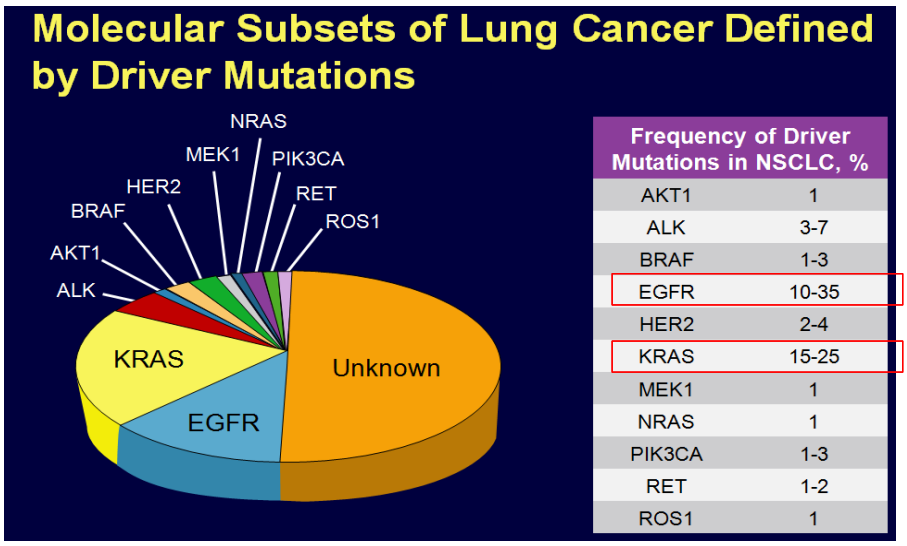

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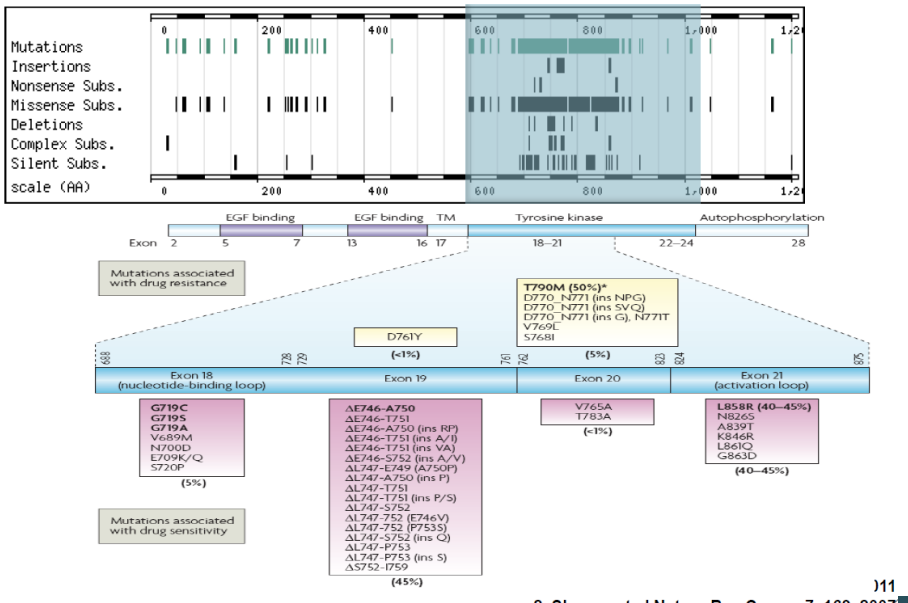
EGFR mutations



2. Sharma et al Nature Rev Cancer 7, 169, 2007

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BMC Part of Springer Nature

Diagnostic Pathology

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Research | Open Access | Published: 11 February 2019

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

Diagnostic Pathology **14**, Article number: 18 (2019) | Cite this article

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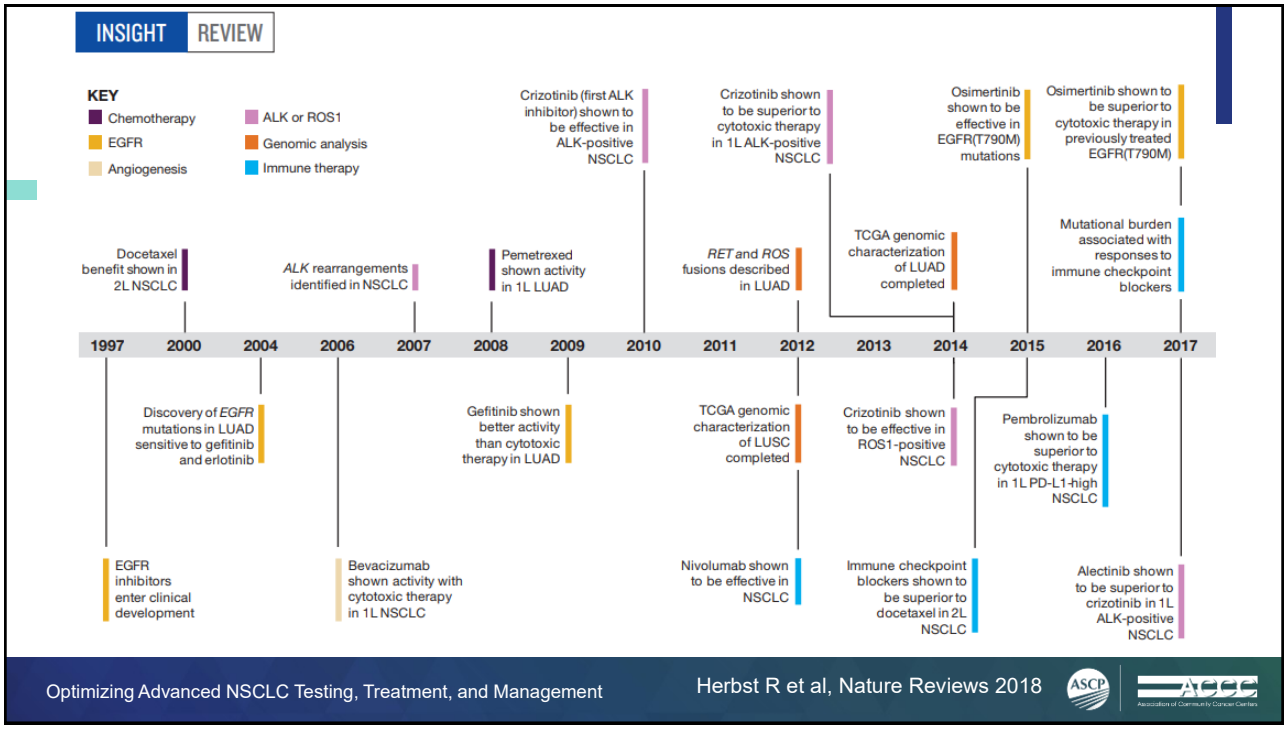

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Stage IV Lung Cancer Treatments

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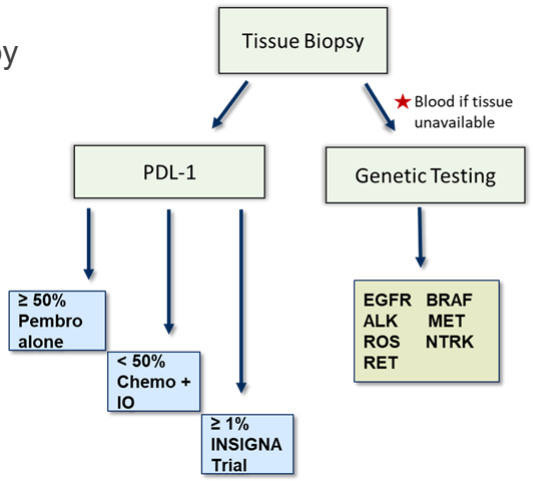
- Targeted Treatment
- Chemotherapy + Immunotherapy
- Immunotherapy along



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Targeted Therapies

Toolbox

The James

The Ohio State University
Comprehensive Cancer Center

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QuickSheet

	EGFR	ALK	ROS1	BRAFV600E	MET	NTRK	RET
Preferred First-Line	Osimertinib	Alectinib[1] Brigatinib Lorlatinib	Entrectinib	Dabrafenib + trametinib	Capmatinib[2] Tepotinib[3]	Larotrectinib[4] Entrectinib	Selpercatinib[5] Pralsetinib[6]
Alternative	Afatinib Gefitinib Dacomitinib Erlotinib +ramu or bev	Ceritinib	Crizotinib Ceritinib	Vemurafenib	Crizotinib		Cabozantinib Vandetanib
2nd line+		Crizotinib	Lorlatinib Entrectinib				
Clinical trial	Clinical trial	Clinical trial	Clinical trial	Clinical trial	Clinical trial	Clinical trial	Clinical trial
Reference	FLAURA NEJM 2018	ALEX[1] NEJM 2017	ALKA, STARTRK-1 STARTRK-2 Lancet Oncology	BRF113928 Lancet Oncology 2017	GEOMETRY-01[2] NEJM 2020 Paik NEJM 2020[3]	Drilon NEJM 2018[4]	Drilon NEJM 2020[5] ARROW[6] JCO 2020

23

ESMO Congress 2019, Barcelona, Spain, 27 SEPTEMBER – 1 OCTOBER 2019

Phase 1 Study of AMG 510 (Sotorasib), a Novel KRAS^{G12C} Inhibitor, in Advanced Solid Tumors With *KRAS G12C* Mutation

Ramaswamy Govindan, MD;[1] Marwan G Fakih, MD;[2] Timothy J Price, MBBS, DHlthSci, FRACP;[3] Gerald S Falchook, MD;[4] Jayesh Desai, MBBS, FRACP;[5] James C Kuo, MBBS, FRACP;[6] John H Strickler, MD;[7] John C Krauss, MD;[8] Bob T Li, MD;[9] Crystal S Denlinger, MD;[10] Greg Durm, MD;[11] Jude Ngang, PharmD;[12] Haby Henary, MD;[12] Gataree Ngarmchamnanrith, MD;[12] June Kim, PhD;[12] Phuong Khanh Morrow, MD;[12] David S Hong, MD[13]

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Best response: AMG510 at 960mg

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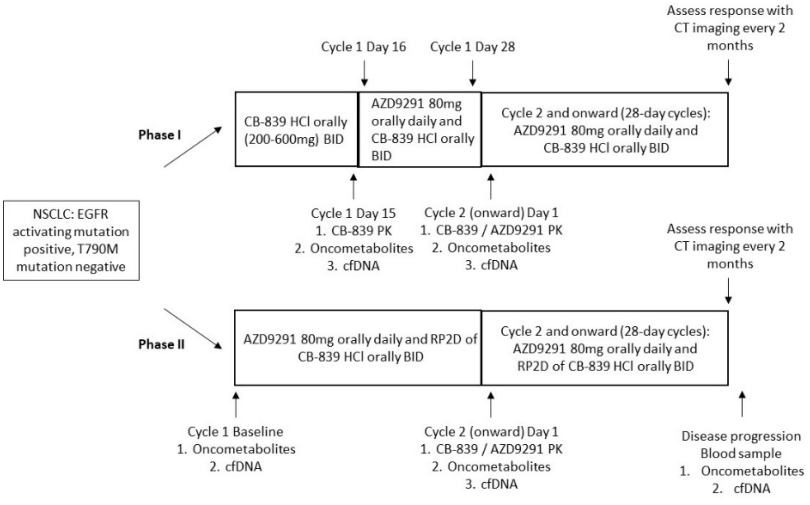
Efficacy outcomes	NSCLC, evaluable patients receiving 960mg N = 13	CRC, evaluable patients receiving 960mg N = 12	Other tumor types, evaluable patients receiving 960mg N = 1
Best overall response			
Partial response – No. (%)	7 (54)	1 (8)	0 (0)
Stable disease – No. (%)	6 (46)	10 (83)	0 (0)
Progressive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

[a]PR or SD at week 6; [b]the tumor type of this patient was recorded as small cell lung cancer ("other tumor types" category) by the data cutoff, and the participating site updated the tumor type to NSCLC after cutoff. Evaluable patients: patients who had been followed up for at least 6 weeks as of the data cutoff; NSCLC: non-small cell lung cancer; CRC: colorectal cancer; SCLC: small cell lung cancer; PR: partial response; SD: stable disease.

25

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

26



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It's easy to get lost in the cancer world

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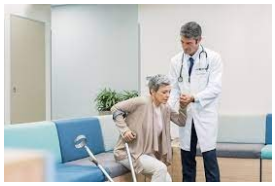
Heterogeneity







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Personalizing Care for Older Adults

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Tip: Toxicity ⟷ Comorbidities

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- Lower extremity swelling
- Diarrhea and deyhydration
- Rash
- Cardiac

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Tip: Brain Mets versus no Brain Mets

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Tip: Talk to your pharmacist!

- Drug-drug interactions
- BEERS Criteria
- Polypharmacy

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Conclusions

33

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

33

Acknowledgements

34

- Oncogeriatrics Program at OSUCCC
- Thoracic Oncology Center at OSUCCC

National Institute on Aging

NIH National Cancer Institute

The James
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Comprehensive Cancer Center

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Biomarker Testing Methodology and Recommendations

May 21, 2021
12:45 – 2:15 pm Eastern

1

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Optimizing Advanced NSCLC Testing, Treatment, and Management

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3

Review of IHC

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THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

3

Immunohistochemistry (IHC)

4

- 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

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Preanalytic Variables

5

- 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

5

Analytic Variables

6

- 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

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Postanalytic Variables

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- 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

7

ALK IHC

8

- 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: D5F3and 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

8

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ALK IHC

9

- 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 patten on ALK IHC may be masked by an intracellular mucin vacuole, making it difficult to detect their ALK positivity

9

ALK IHC

10

- 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

10

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ALK IHC

11

- 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

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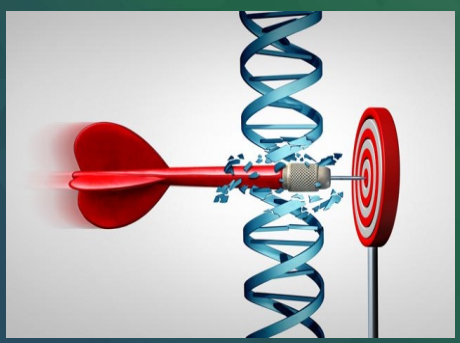

Review of Cytogentics

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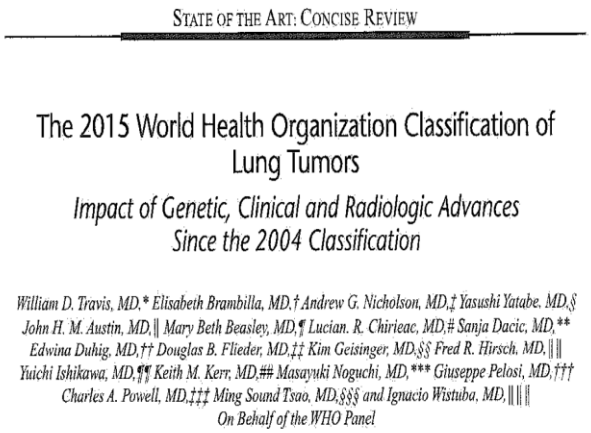
  Associate Professor of Pathology
  Johns Hopkins Hospital
  @ZMaleki_cyto

13

Pathology Evaluation

14

- **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



STATE OF THE ART: CONCISE REVIEW

The 2015 World Health Organization Classification of Lung Tumors

Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification

William D. Travis, MD, Elisabeth Brambilla, MD,† Andrew G. Nicholson, MD,‡ Yasushi Yatabe, MD,§ John H. M. Austin, MD,‖ Mary Beth Beasley, MD,¶ Lucian. R. Chirieac, MD,# Sanja Dacic, MD,** Edwina Duhig, MD,†† Douglas B. Flieder, MD,‡‡ Kim Geisinger, MD,§§ Fred R. Hirsch, MD,‖‖ Yuichi Ishikawa, MD,¶¶ Keith M. Kerr, MD,## Masayuki Noguchi, MD,*** Giuseppe Pelosi, MD,††† Charles A. Powell, MD,‡‡‡ Ming Sound Tsao, MD,§§§ and Ignacio Wistuba, MD,‖‖‖ On Behalf of the WHO Panel*

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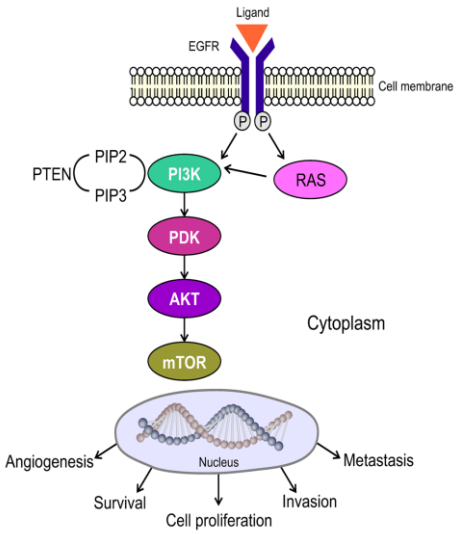
Molecular Study Selection

- **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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National Comprehensive Cancer Network®

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

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CLINICAL PRESENTATION	HISTOLOGIC SUBTYPE[a]	BIOMARKER TESTING[ll]	
Advanced or metastatic disease → • Establish histologic subtype[a] with adequate tissue for molecular testing (consider rebiopsy[kk] if appropriate) • Smoking cessation counseling • Integrate palliative care[c] (See NCCN Guidelines for Palliative Care)	• Adenocarcinoma • Large cell • NSCLC not otherwise specified (NOS)	• Molecular testing, including: ▸ *EGFR* mutation (category 1), *ALK* (category 1), *ROS1*, *BRAF*, *NTRK1/2/3*, *METex14* skipping, *RET* ▸ Testing should be conducted as part of broad molecular profiling[mm] • PD-L1 testing (category 1)	See Testing Results (NSCL-19)
	Squamous cell carcinoma	• Consider molecular testing, including:[nn] ▸ *EGFR* mutation, *ALK*, *ROS1*, *BRAF*, *NTRK1/2/3*, *MET* exon 14 skipping, *RET* ▸ Testing should be conducted as part of broad molecular profiling[mm] • PD-L1 testing (category 1)	See Testing Results (NSCL-19)

[a] See Principles of Pathologic Review (NSCL-A).
[c] Temel JS, et al. N Engl J Med 2010;363:733-742.
[kk] If there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *ROS1*, *BRAF*, *NTRK1/2/3*, *MET*, and *RET*, repeat biopsy and/or plasma testing should be done. If these are not feasible, treatment is guided by available results and, if unknown, these patients are treated as though they do not have driver oncogenes.
[ll] See Principles of Molecular and Biomarker Analysis (NSCL-H).
[mm] The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC. See Emerging Biomarkers to Identify Patients for Therapies (NSCL-I).
[nn] Lam VK, et al. Clin Lung Cancer 2019;20:30-36.e3; Sands JM, et al. Lung Cancer 2020;140:35-41.

Note: For more information regarding the categories and definitions used for the NCCN Evidence Blocks™, see page EB-1.
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.

NSCL-18

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Non-Small Cell Lung Cancer
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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
  - Afatinib[1]
  - Erlotinib[2]
  - Dacomitinib[3]
  - Gefitinib[4,5]
  - Osimertinib[6]
  - Erlotinib + ramucirumab[7]
  - Erlotinib + bevacizumab* (nonsquamous)[8]
- Subsequent therapy
  - Osimertinib[9]

***ALK* Rearrangement Positive**
- First-line therapy
  - Alectinib[10,11]
  - Brigatinib[12]
  - Ceritinib[13]
  - Crizotinib[10,14]
  - Lorlatinib[15]
- Subsequent therapy
  - Alectinib[16,17]
  - Brigatinib[18]
  - Ceritinib[19]
  - Lorlatinib[20]

See Evidence Blocks on NSCL-29A

***ROS1* Rearrangement Positive**
- First-line therapy
  - Ceritinib[21]
  - Crizotinib[22]
  - Entrectinib[23]
- Subsequent therapy
  - Lorlatinib[24]
  - Entrectinib[23]

***BRAF* V600E Mutation Positive**
- First-line therapy
  - Dabrafenib/trametinib[25]
- Subsequent therapy
  - Dabrafenib/trametinib[26,27]

***NTRK1/2/3* Gene Fusion Positive**
- First-line/Subsequent therapy
  - Larotrectinib[28]
  - Entrectinib[29]

***MET* Exon 14 Skipping Mutation**
- First-line therapy/Subsequent therapy
  - Capmatinib[30]
  - Crizotinib[31]
  - Tepotinib[32]

***RET* Rearrangement Positive**
- First-line therapy/Subsequent therapy
  - Selpercatinib[33]
  - Pralsetinib[34]
  - Cabozantinib[35,36]
  - Vandetanib[37]

PD-L1 ≥1%
- First-line therapy**
  - Pembrolizumab[38-40]
  - (Carboplatin or cisplatin)/pemetrexed/pembrolizumab (nonsquamous)[41]
  - Carboplatin/paclitaxel/bevacizumab*/atezolizumab (nonsquamous)[42]
  - Carboplatin/(paclitaxel or albumin-bound paclitaxel)/pembrolizumab (squamous)[43]
  - Carboplatin/albumin-bound paclitaxel/atezolizumab (nonsquamous)[44]
  - Nivolumab/ipilimumab[45]
  - Nivolumab/ipilimumab/pemetrexed/(carboplatin or cisplatin) (nonsquamous)[46]
  - Nivolumab/ipilimumab/paclitaxel/carboplatin (squamous)[46]

PD-L1 ≥50% (in addition to above)
- First-line therapy**
  - Atezolizumab[47]
  - Cemiplimab-rwlc[48]

See Evidence Blocks on NSCL-31A

*An FDA-approved biosimilar 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

Note: For more information regarding the categories and definitions used for the NCCN Evidence Blocks™, see page EB-1.
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.

NSCL-J
1 OF 2

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

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TESTING RESULTS[kk,ll]

Sensitizing *EGFR* mutation positive	NSCL-20
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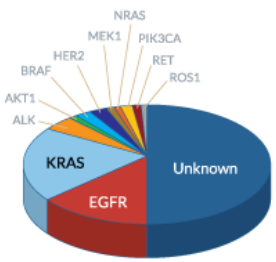
BRAF V600E mutation positive	NSCL-27
NTRK1/2/3 gene fusion positive	NSCL-28
METex14 skipping mutation positive	NSCL-29
RET rearrangement positive	NSCL-30
PD-L1 ≥50% and negative for actionable molecular markers above	NSCL-31
PD-L1 ≥1%–49% and negative for actionable molecular markers above	NSCL-32
PD-L1 <1% and negative for actionable molecular markers above	NSCL-33

Molecular Subsets of Lung Cancer Defined
by Driver Mutations



Frequency of Driver Mutations in NSCLC, %	
AKT1	1
ALK	3-7
BRAF	1-3
EGFR	10-35
HER2	2-4
KRAS	15-25
MEK1	1
NRAS	1
PIK3CA	1-3
RET	1-2
ROS1	1

www.mycancergenome.org

[kk] If there is insufficient tissue to allow testing for all of *EGFR, ALK, ROS1, BRAF, NTRK1/2/3, MET,* and *RET,* repeat biopsy and/or plasma testing should be done. If these are not feasible, treatment is guided by available results and, if unknown, these patients are treated as though they do not have driver oncogenes.
[ll] See Principles of Molecular and Biomarker Analysis (NSCL-H).

https://1dzi041pbrf98jfu01xrpf3k-wpengine.netdna-ssl.com/wp-content/uploads/2019/01/Lung-Cancer-Diagram.png

Note: For more information regarding the categories and definitions used for the NCCN Evidence Blocks™, see page EB-1.
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NSCL-19

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Molecular Study Methods

19

- "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

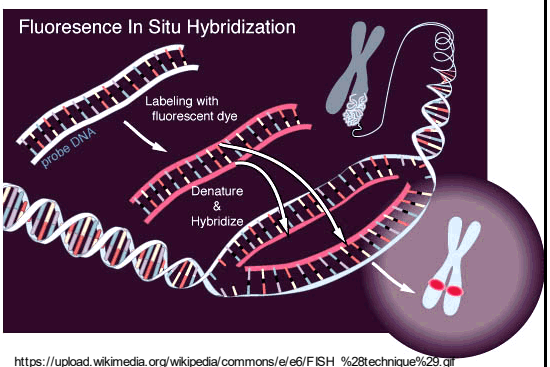
https://swisscheckup.com/wp-content/uploads/2013/07/ngsvssanger_small.jpg

19

Fluorescence in situ hybridization (FISH)

20

- A molecular cytogentic 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



https://upload.wikimedia.org/wikipedia/commons/e/e6/FISH_%28technique%29.gif

20

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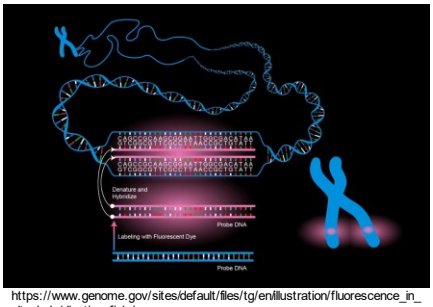
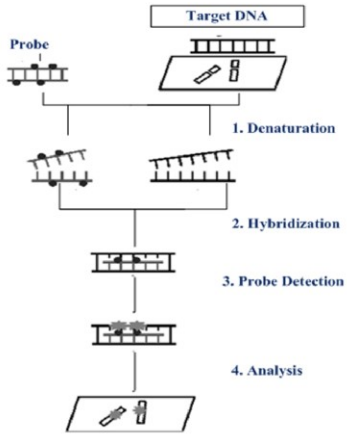
FISH Process

21

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



https://www.genome.gov/sites/default/files/tg/enIllustration/fluorescence_in_situ_hybridization_fish.jpg



Ratan Z, Zaman S, Mehta V, et al. (June 09, 2017) Application of Fluorescence In Situ Hybridization (FISH) Technique for the Detection of Genetic Aberration in Medical Science. Cureus 9(6): e1325. DOI 10.7759/cureus.1325

21

FISH advantages and disadvantages

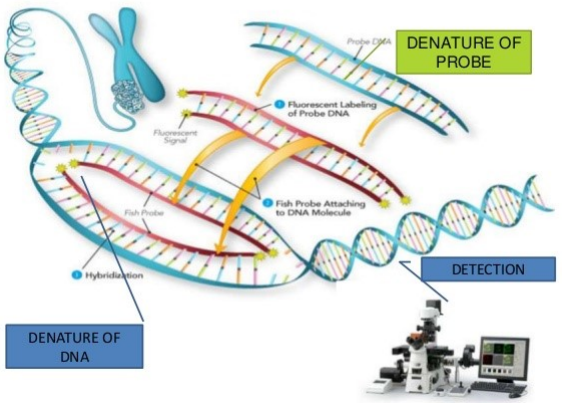
22

- ***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

Ryan Bishop, Applications of fluorescence *in situ* hybridization (FISH) in detecting genetic aberrations of medical significance, *Bioscience Horizons: The International Journal of Student Research*, Volume 3, Issue 1, March 2010, Pages 85–95, https://doi.org/10.1093/biohorizons/hzq009

FISH Procedure



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22

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FISH Specimen

23

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

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23

FISH Probes for NSCLC

24

- 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

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FISH in NSCLC

25

ALK rearrangements, 2p23

ROS1 rearrangements, 6q22

RET rearrangements, 10q11.2

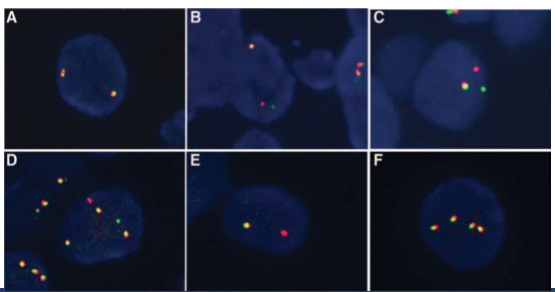
MET amplification

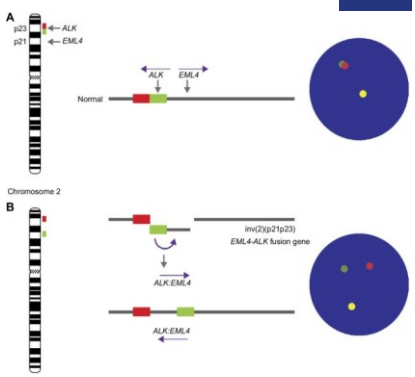
25

ALK (Anaplastic Lymphoma Kinase) Gene Rearrangements

26

- 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





https://www.researchgate.net/profile/Milena-Rondon-Lagos/publication/333481329/figure/fig3/AS:764250259730436@1559222869574/A LK-Break-Apart-FISH-Probe-for-detecting-formation-of-EML4-ALK-fusion_W640.jpg

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; (*F*) *ALK* polysomy. *ALK*, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization

Martin V, Bemasconi B, Merlo E, Balzarini P, Vermi W, Riva A, Chiaravalli AM, Fraitini M, Sahnane N, Facchetti F, Mazzucchelli L, Sessa F, Tibiletti MG. ALK testing in lung adenocarcinoma: technical aspects to improve FISH evaluation in daily practice. J Thorac Oncol. 2015 Apr;10(4):595-602. doi: 10.1097/JTO.0000000000004 44. PMID: 25514802.

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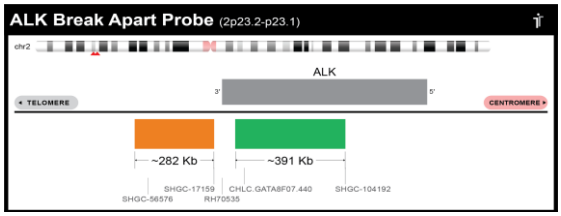
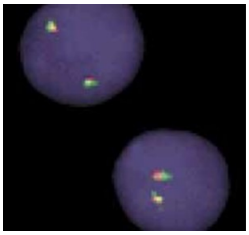
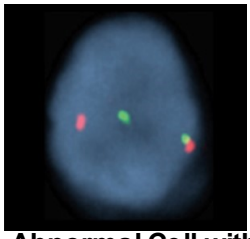
Anaplastic Lymphoma Kinase (ALK) Rearrangements Using a break-apart/ rearrangement probe set





Normal Cell



Abnormal Cell with *ALK* rearrangements

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

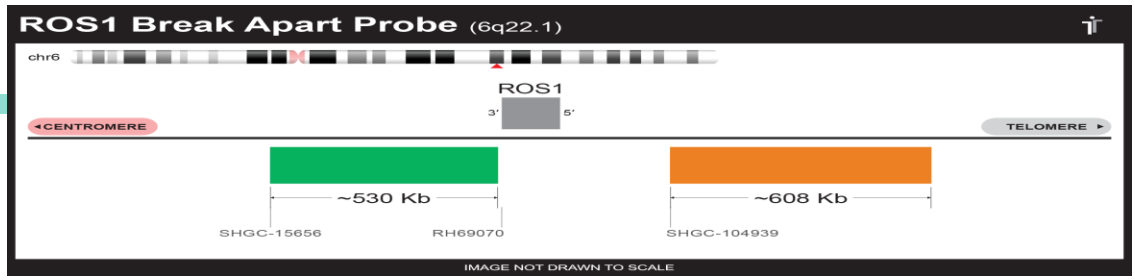
ALK FISH probe is a break-apart probe (5' in green and 3' in red).

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

27

ROS1 (ROS proto-oncogene 1)

28



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

28

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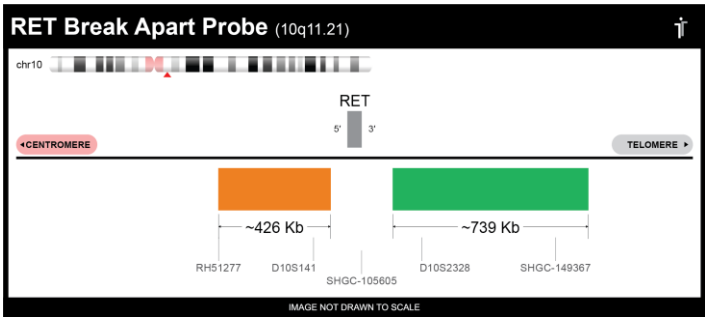
RET (rearranged during transfection) Gene Rearrangements

29

- 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

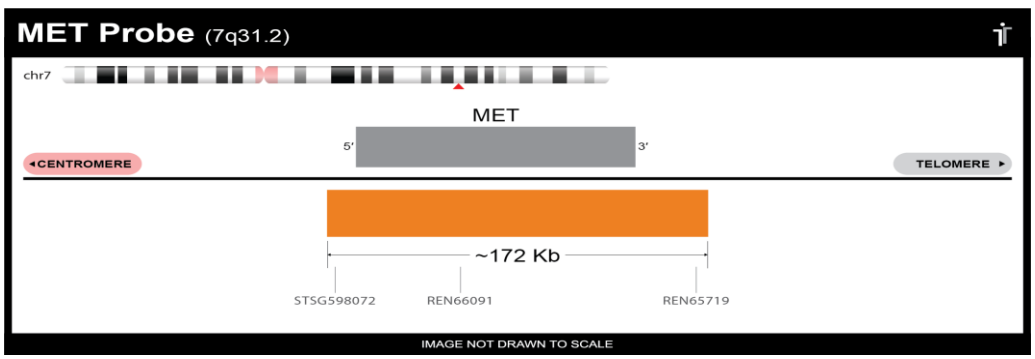


https://www.empiregenomics.com/img/deograms/catalog_probes/RET-BA/RET-BA_ORGR.png

29

MET (mesenchymal-epithelial transition)

30



- 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/deograms/catalog_probes/MET/MET_OR.png

30

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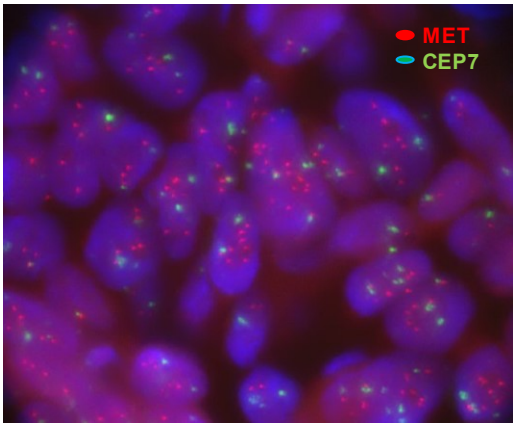
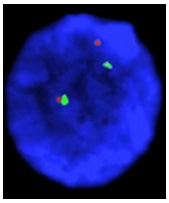
FISH test for MET amplification



Abnormal Cells with MET Amplification

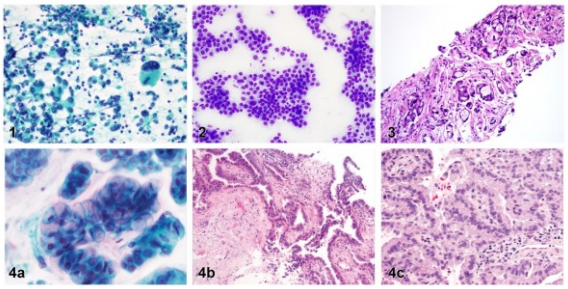


Normal Cell

31

BRAF (B-Raf proto-oncogene) point mutations

32



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

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EGFR (Epidermal Growth Factor Receptor)

33

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.

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KRAS (KRAS proto-oncogene) point mutations

34

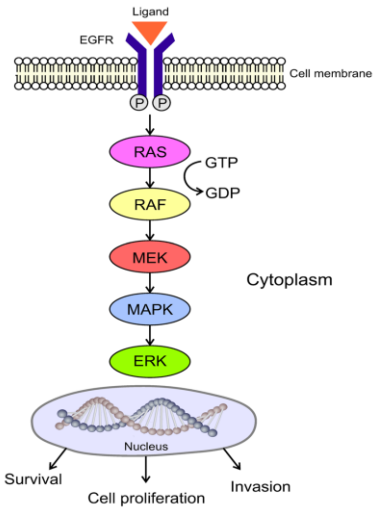
G-protein with intrinsic GTPase activity, activating mutations result in unregulated signaling through the MAP/ERK pathway.

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.



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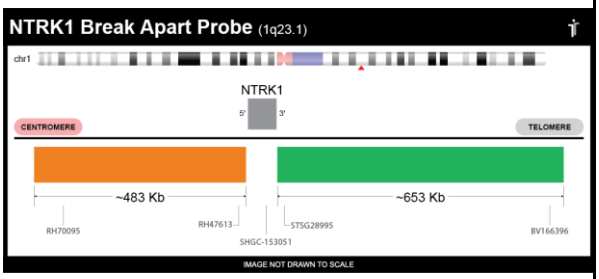
NTRK1/2/3 (neurotrophic tyrosine receptor kinase) gene fusions

35

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.



35

PD-L1(Programmed Death Ligand 1)

36

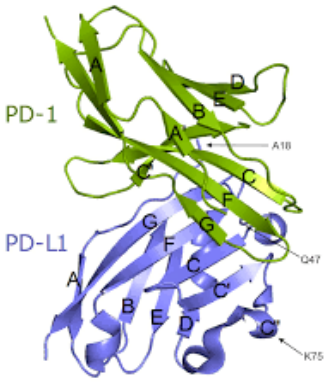
PD-L1 : A co-regulatory molecule, expressed on tumor cells, inhibiting T-cell-mediated cell death.

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

36

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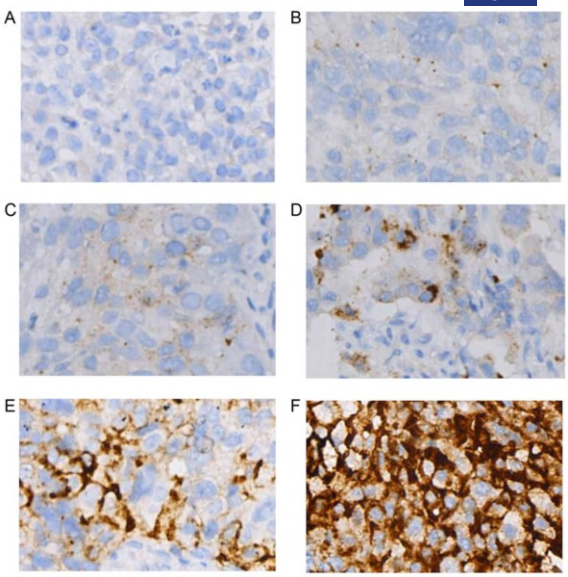
PD-L1(Programmed Death Ligand 1) (2)

37

- 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

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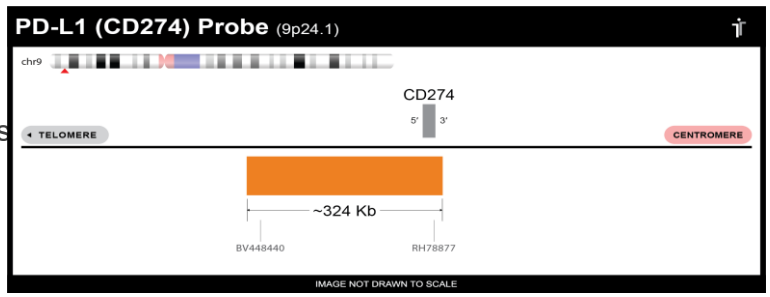
PD-L1(Programmed Death Ligand 1) (3)

38

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.



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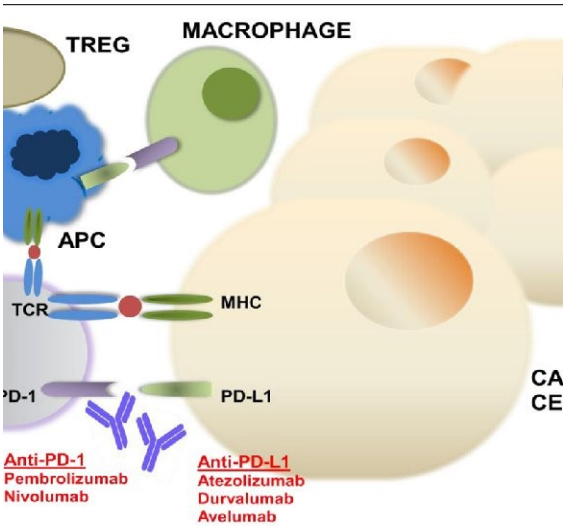
PD-L1 and the NCCN guidelines

39

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



https://www.researchgate.net/publication/322664614/figure/fig2/AS:631611628453915@1527599355219/Mechanism-of-action-of-PD-1-and-PD-L1-inhibitors-The-programmed-cell-death-1-PD-1_Q640.jpg

39

CAP New Recommendations

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

40

- 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.

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Guideline for non-Small Cell Lung Cancer

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- **Adenocarcinoma, NSCLC (NOS), Large cell carcinoma**

EGFR mutation (category 1), ALK (category 1), ROS1, BRAF, NTRK1/2/3, METex14 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)

41

CAP 2018

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- **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

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CAP Recommendation

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- 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.

CAP Recommendations

44

- 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.

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CAP Recommendation

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- **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.

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

46

- 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.

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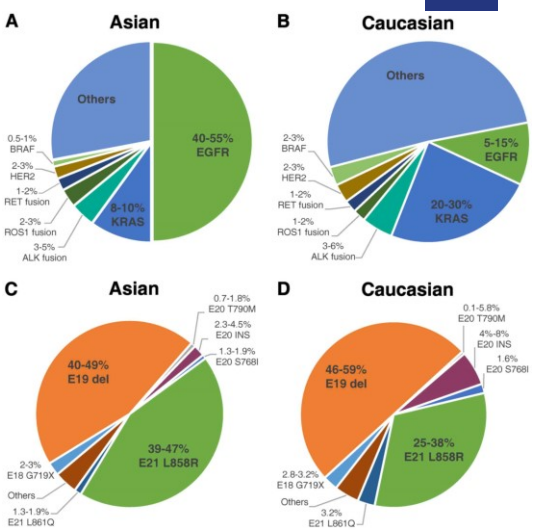
CAP Recommendation

- 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.***



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CAP 2018, emerging markers

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- 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)

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Thank you

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@ZMaleki_cyto

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50

Association of Community Cancer Centers

Review of Molecular Single Assay

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

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Testing Methodologies

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- Appropriate possible testing methodologies are indicated below for each analyte separately; however, several methodologies are generally considerations 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.

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Plasma Cell Free Circulating Tumor DNA Testing

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- 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 NSCL-18 for oncogenic drivers with available targeted therapy options).

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Advanced NSCLC Treatment Opportunities and Optimizing Patient Care

May 21, 2021
2:30 – 4:00 pm Eastern

1

Faculty

2

- Mohamed Mohamed, MD, PhD
  Division Director Medical Oncology,
  Director of Thoracic Oncology
  Hematologist/Medical Oncologist
  Cone Health Cancer Center
- Kimberly Rohan, ANP-BC, AOCN
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- 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

2

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NCCN Guidelines

MOHAMED MOHAMED
CONE HEALTH CANCER CENTER

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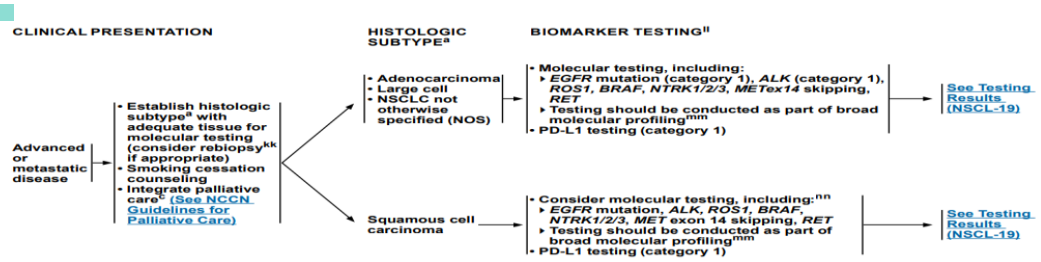

[a] See Principles of Pathologic Review (NSCL-A).
[c] Temel JS, et al. N Engl J Med 2010;363:733-742.
[kk] If there is insufficient tissue to allow testing for all of *EGFR, ALK, ROS1, BRAF, NTRK1/2/3, MET, and RET*, repeat biopsy and/or plasma testing should be done. If these are not feasible, treatment is guided by available results and, if unknown, these patients are treated as though they do not have driver oncogenes.
[ll] See Principles of Molecular and Biomarker Analysis (NSCL-H).

[mm] The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC. See Emerging Biomarkers to Identify Patients for Therapies (NSCL-J).
[nn] Lam VK, et al. Clin Lung Cancer 2019;20:30-36.e3; Sands JM, et al. Lung Cancer 2020;140:35-41.

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.

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NSCL-18

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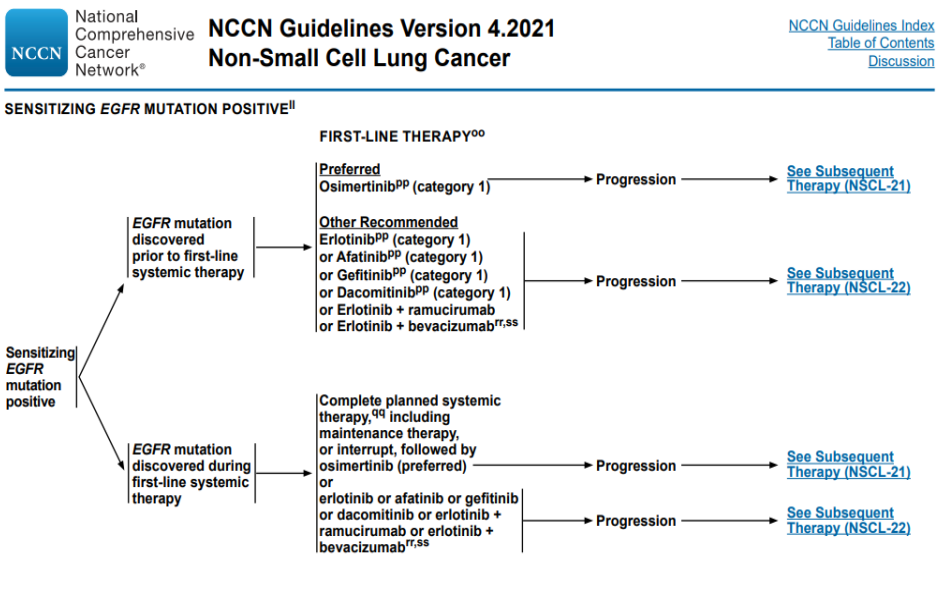

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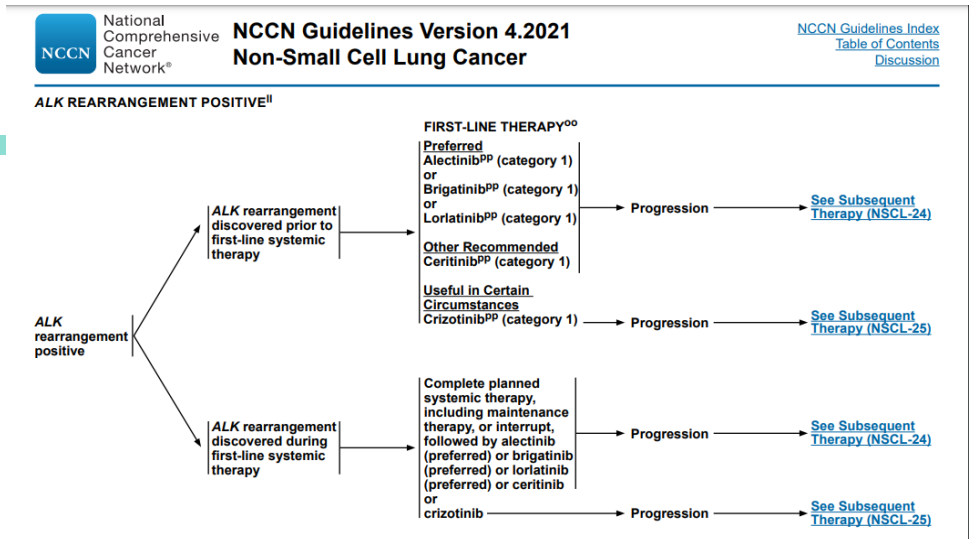

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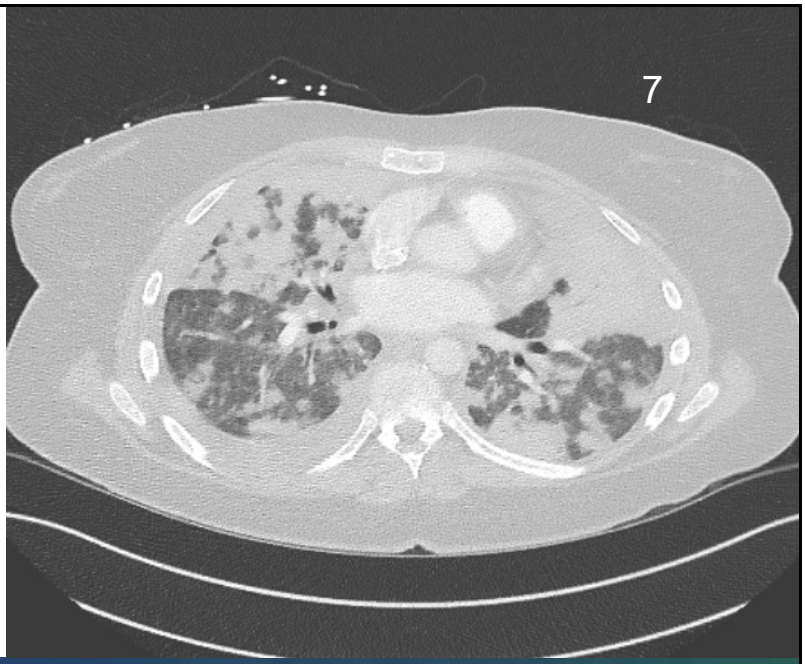
32 y ears old never smoker white female recently diagnosed with stage IV non-small cell lung cancer, poorly dif ferentiated adenocarcinoma with signet ring f eatures presented with bilateral pulmonary nodules and masses in addition to mediastinal ly mphadenopathy as well as axillary and upper abdominal ly mphadenopathy



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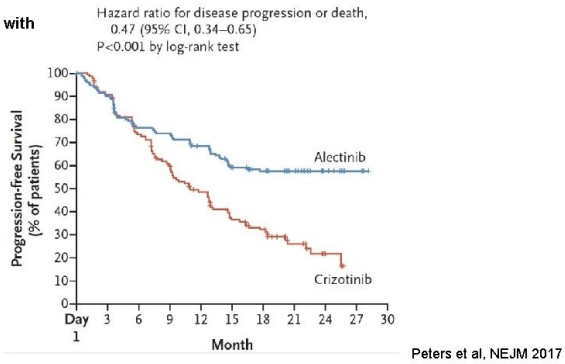
Alectinib vs. Crizotinib PFS

NSCLC

Improved PFS with alectinib



Peters et al, NEJM 2017

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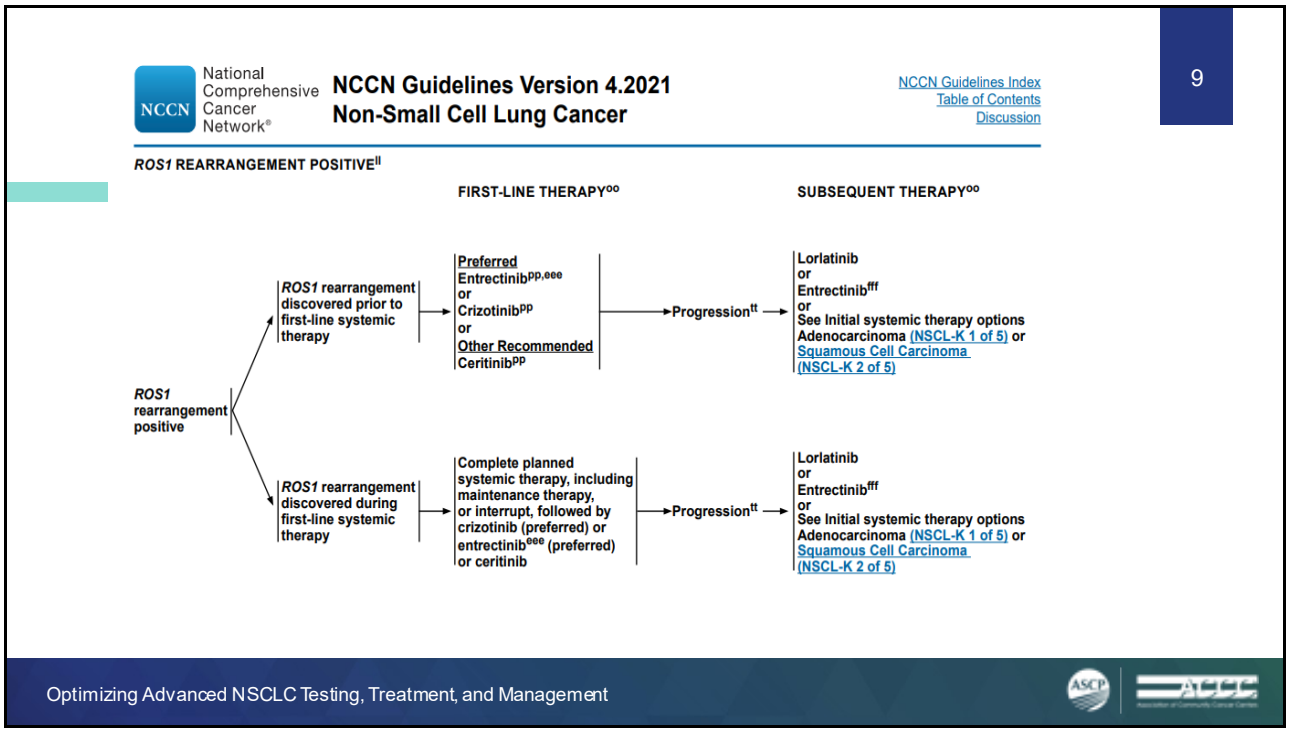

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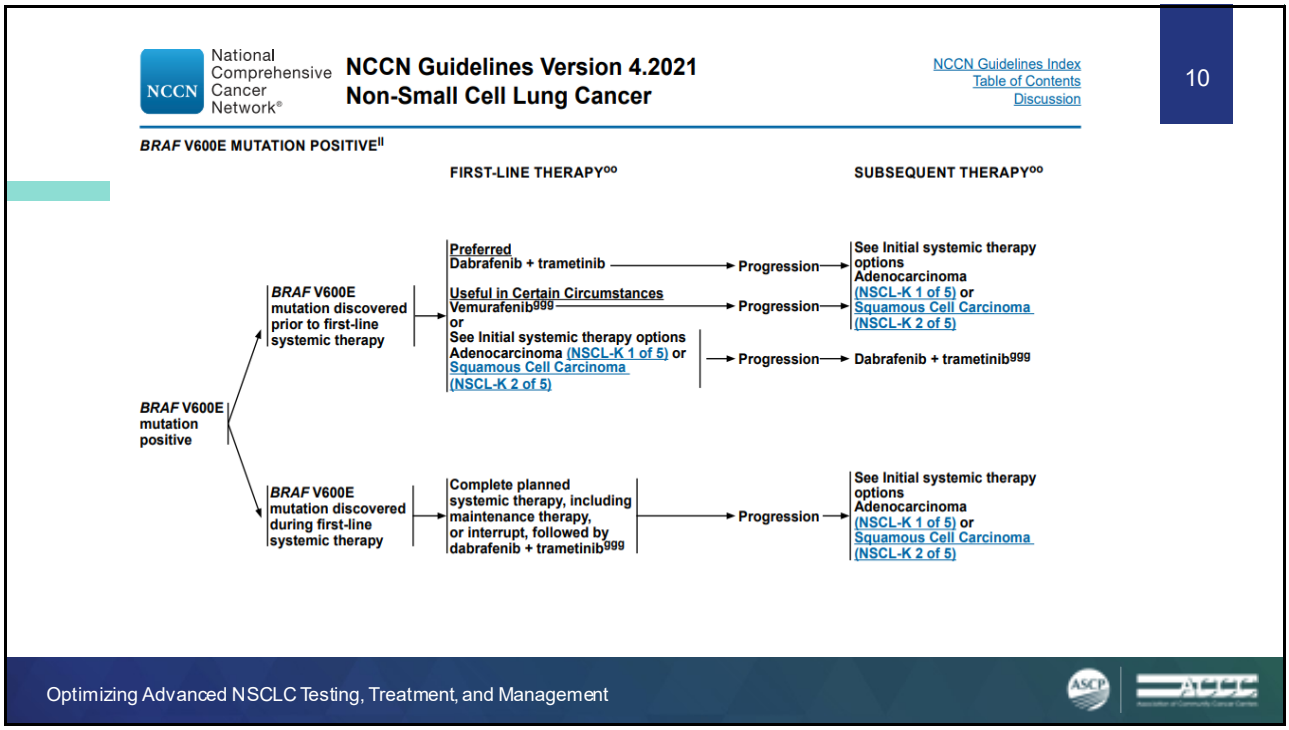

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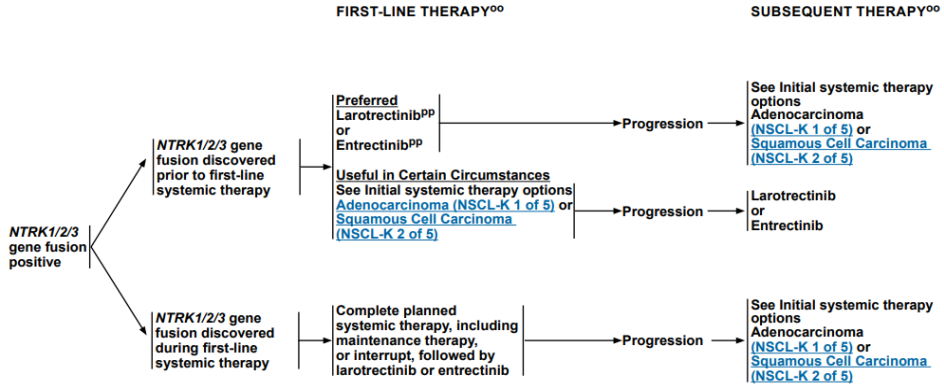
***NTRK* GENE FUSION POSITIVE**[ll]



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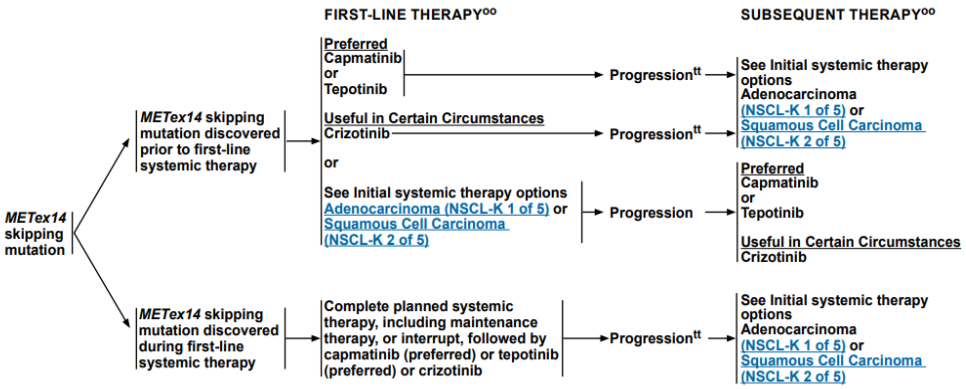
***METex14* SKIPPING MUTATION**[ll]



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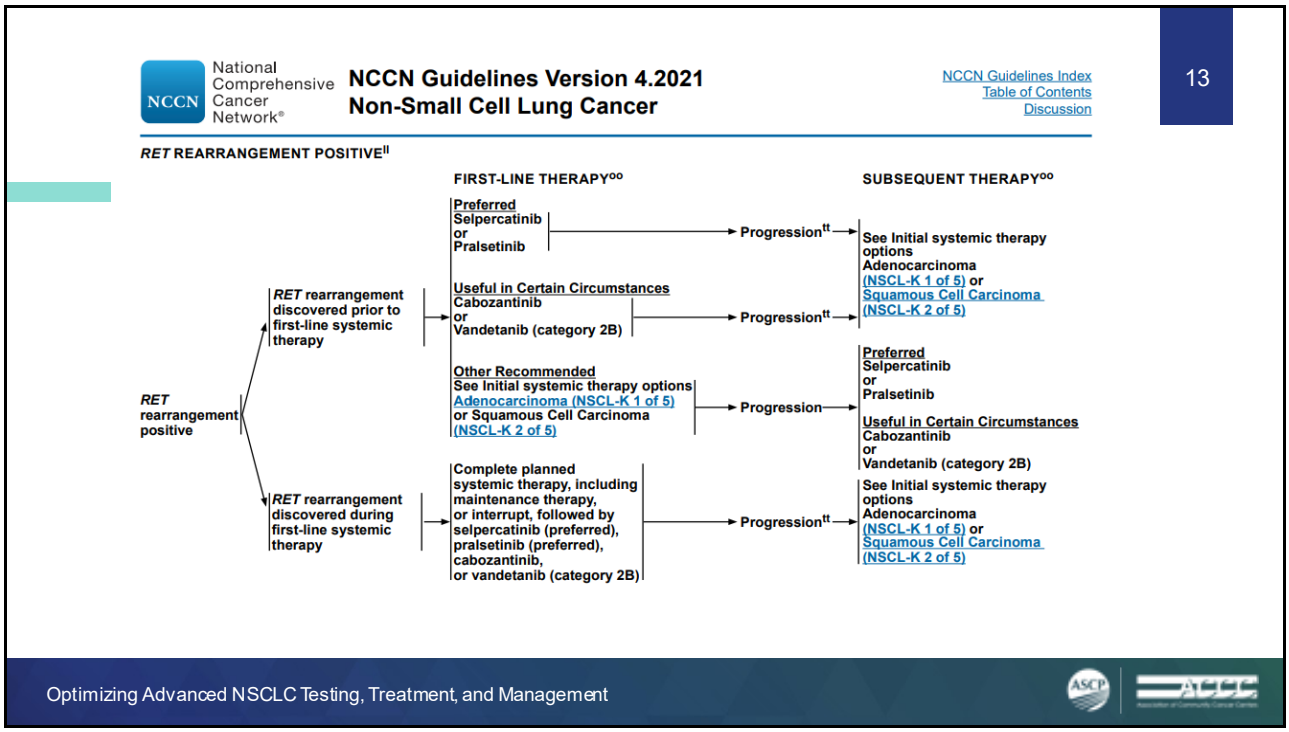

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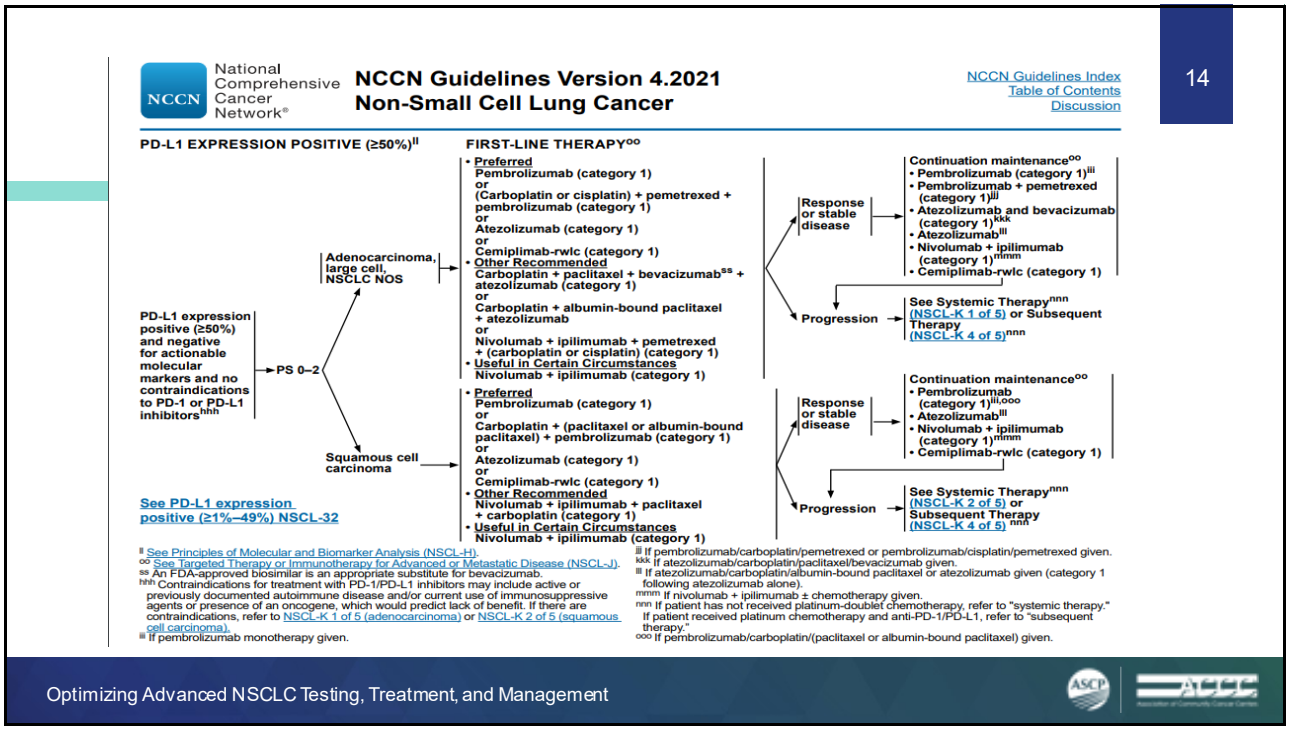

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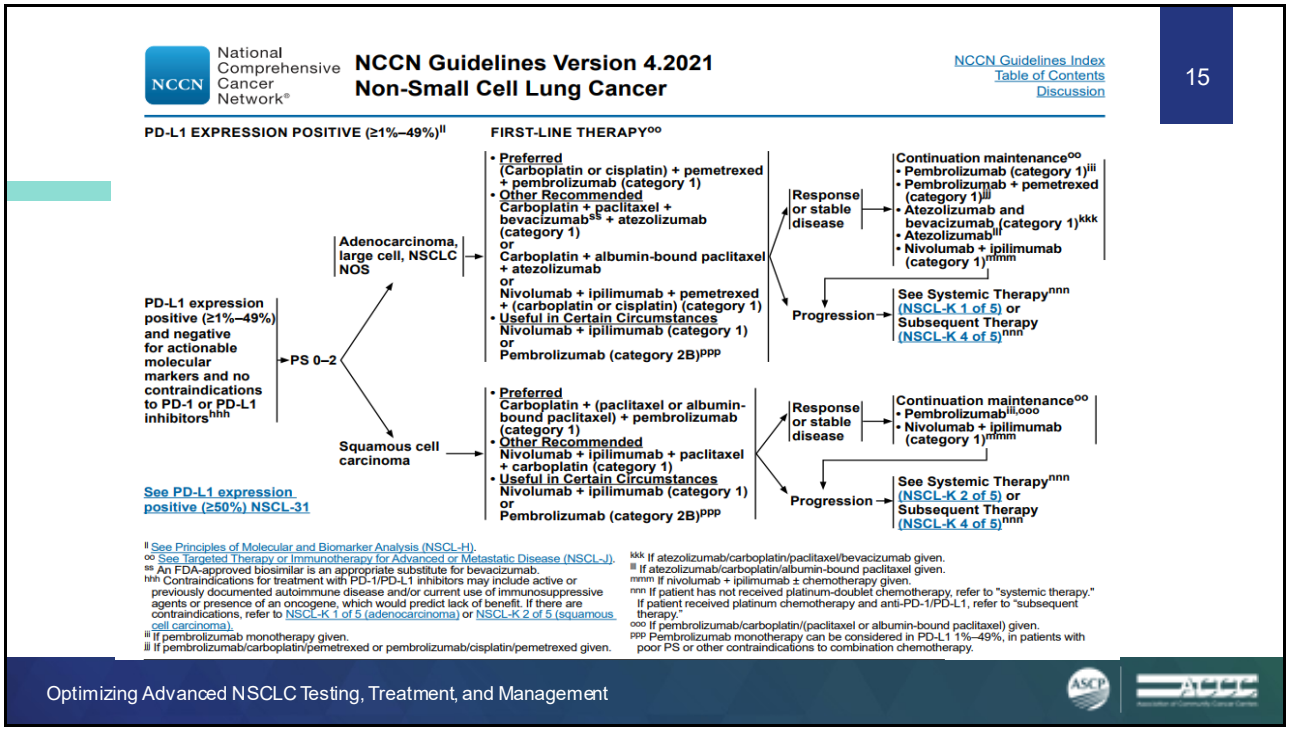

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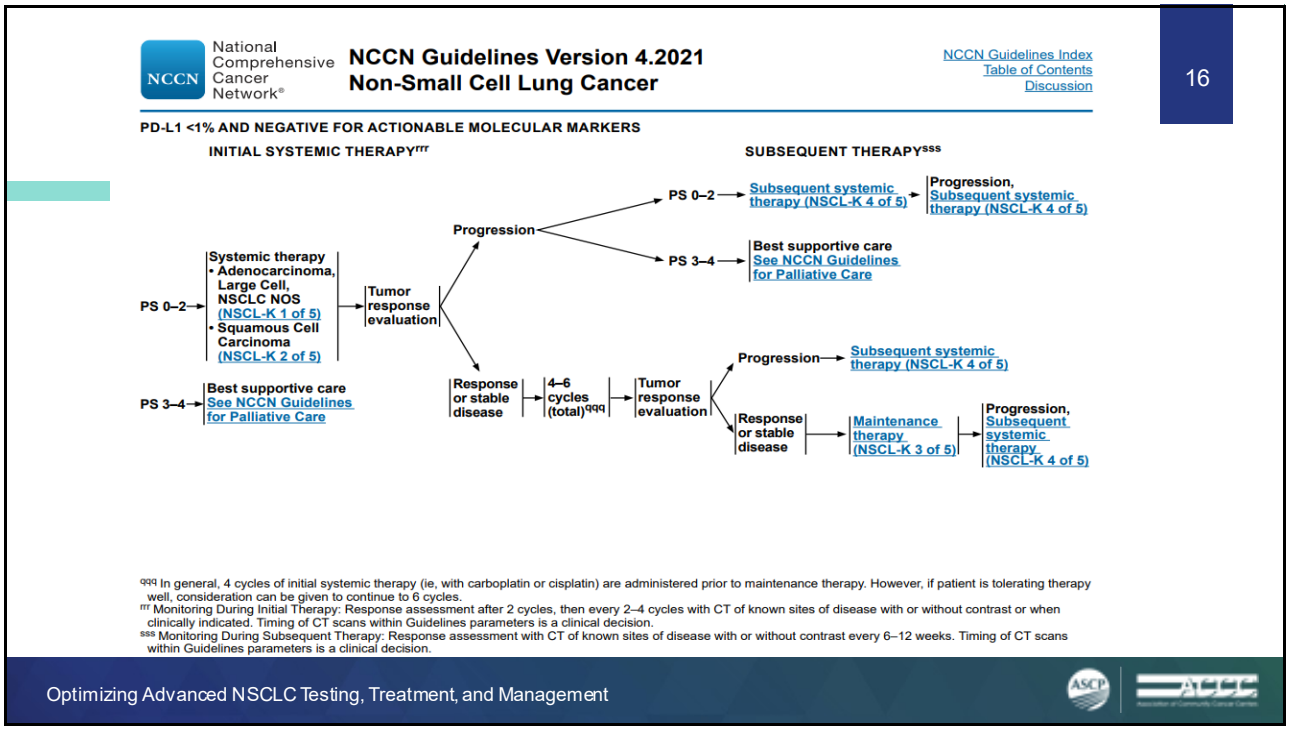

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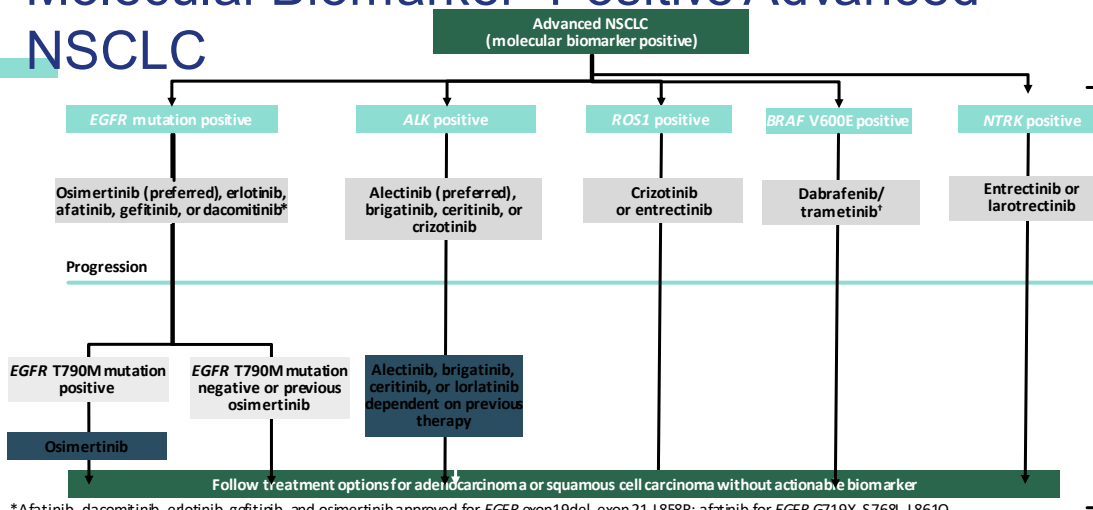
Treatment Failure: Next Steps

KIMBERLY ROHAN ANP-BC, AOCN
EDWARD HEMATOLOGY ONCOLOGY GROUP
NAPERVILLE, IL

17

April 2020 Treatment Paradigm for Molecular Biomarker–Positive Advanced NSCLC



*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.

Slide credit: clinicaloptions.com

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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]

Mutation Status	Cellular ALK Phosphorylation Mean IC_{50} (nM)				
	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
EML4-ALK	38.6	4.9	11.4	10.7	2.3
C1156Y	61.9	5.3	11.6	4.5	4.6
I1171N	130.1	8.2	397.7	26.1	49.0
I1171S	94.1	3.8	177.0	17.8	30.4
I1171T	51.4	1.7	33.6	6.1	11.5
F1174C	115.0	38.0	27.0	18.0	8.0
L1196M	339.0	9.3	117.6	26.5	34.0
L1198F	0.4	196.2	42.3	13.9	14.8
G1202R	381.6	124.4	706.6	129.5	49.9
G1202del	58.4	50.1	58.8	95.8	5.2
D1203N	116.3	35.3	27.9	34.6	11.1
E1210K	42.8	5.8	31.6	24.0	1.7
G1269A	117.0	0.4	25.0	ND	10.0

IC_{50} ≤50 nM IC_{50} >50 to <200 nM IC_{50} ≥200 nM

1. Gainor. Cancer Discov. 2016. 6:1118. 2. Johnson. J Med Chem. 2014;57:4720.

Table adapted from Gainor 2016.

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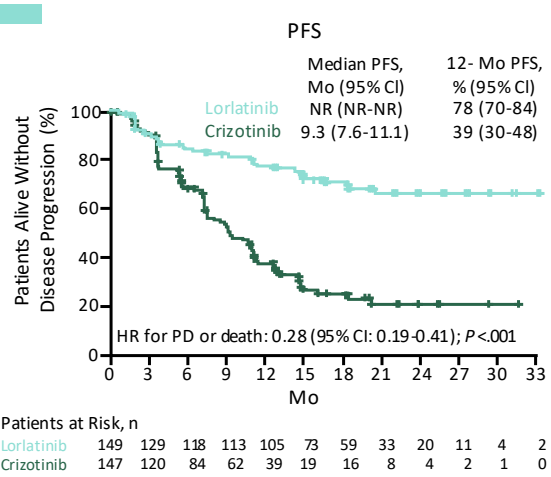
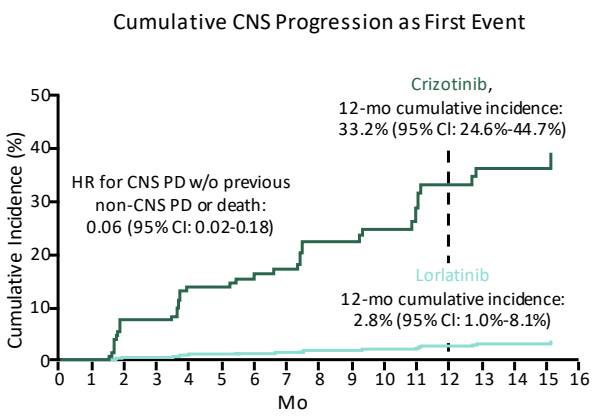
Phase III CROWN: Firstline Lorlatinib has Superior PFS to Crizotinib





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Lorlatinib Toxicity Poses Unique Challenges

AEs, %	Lorlatinib (n = 149)				Crizotinib (n = 142)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Any	4	19	58	14	6	32	47	8
Hypercholesterolemia	16	38	15	1	4	0	0	0
Hypertriglyceridemia	19	25	13	7	4	2	0	0
Edema	36	15	4	0	27	11	1	0
Increased weight	7	14	17	0	4	6	2	0
Peripheral neuropathy	24	7	2	0	13	1	1	0
Cognitive effects	13	6	2	0	5	1	0	0
Diarrhea	14	6	1	0	47	4	1	0
Anemia	11	6	3	0	2	3	3	0
Fatigue	17	1	1	0	18	12	3	0
Hypertension	1	7	10	0	0	2	0	0
Vision disorder	17	1	0	0	38	1	1	0
Increased ALT	15	0	3	0	18	11	4	1
Mood effects	9	5	1	0	3	2	0	0
Increased AST	12	0	2	0	21	3	4	0

Shaw. NEJM. 2020;383:2018.

Slide credit: clinicaloptions.com

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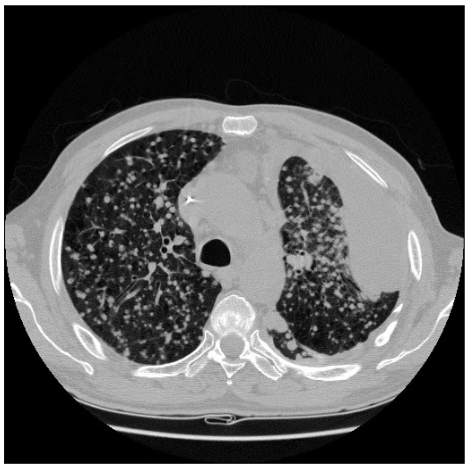
Patient Case 5: Newly Diagnosed *ROS1*-Positive Advanced NSCLC

- 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%



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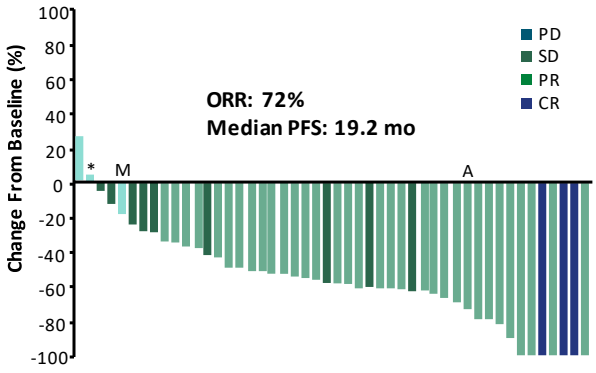
Crizotinib: First Agent Approved in *ROS1*-Positive NSCLC

Shaw. NEJM. 2014;371:1963. Shaw. Ann Oncol. 2019;30:1121.

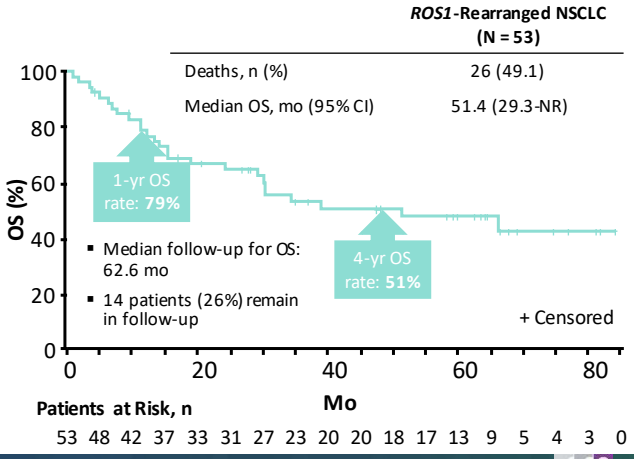
Slide credit: clinicaloptions.com

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



*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.

PROFILE 1001: OS in *ROS1+*



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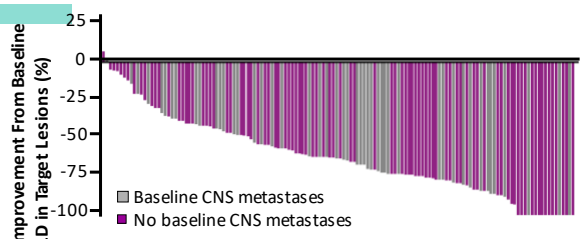
Entrectinib in *ROS1*-Positive NSCLC: Efficacy

Dziadziuszko. JCO. 2021;39:1253.

Slide credit: clinicaloptions.com

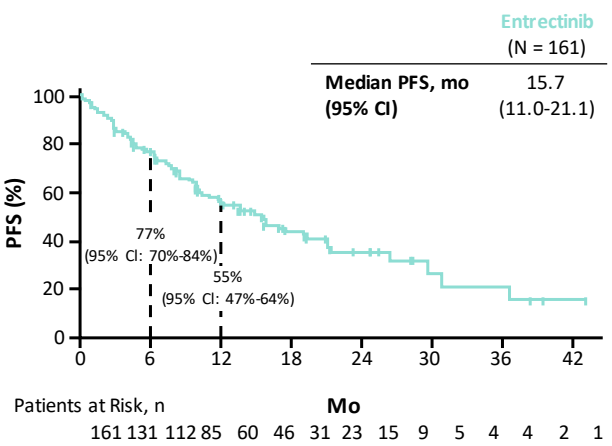
Change in Tumor Size (N = 161)



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

Investigator determined CNS status.

PFS



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.

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Approach to Selecting ROS-Targeted TKI Therapy for *ROS1*+ NSCLC

	Crizotinib[1] (N = 52)	Entrectinib2[2] (N = 161)	Lorlatinib[3] (N = 69)	Repotrectinib[4] (N = 33)
Median PFS, mo	19.2	15.7 (19.0 without CNS mets)	21.0 (crizotinib naive)	Not reported
Intracranial ORR, %	26 (*ALK*+)[5]	79.2*	64†	100‡
Efficacy in pretreated disease?	--	Yes§	Yes (35%‖)	Yes (39%¶)
Safety considerations	Visual impairment, peripheral edema, GI	Weight gain, dizziness, dysgeusia	Peripheral neuropathy, cognitive AEs	Dizziness, dyspnea, neuropathy

*n = 19; DoR: 12.9 mo. †n = 7 crizotinib naive; intracranial ORR in 12 crizotinib-pretreated patients: 50%. ‡n = 6. §Patients with pretreated disease induded in overall analysis. ‖ORR for 40 crizotinib-pretreated patients. ¶ORR for 3 patients treated with second-line repotrectinib 80 mg; for 160 mg: 55%.

1. Shaw. Ann Oncol. 2019;30:1121. 2. Dziadziuszko. JCO. 2021;39:1253. 3. Shaw. Lancet Oncol. 2019;20:1691. 4. Cho. ASCO 2019. Abstr 9011. 5. Peters. NEJM. 2017;377:829.

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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**

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Treatment Failure – Next Steps: ALK Rearrangement Positive Patients

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- 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

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