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A RETROSPECTIVE ANALYSIS OF FREQUENCY OF ALK GENE REARRANGEMENTS IN SAUDI LUNG PATIENTS

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Reporting of ALK testing

- Preanalytical
- Analytical
- Result
- Interpretation/Conclusion

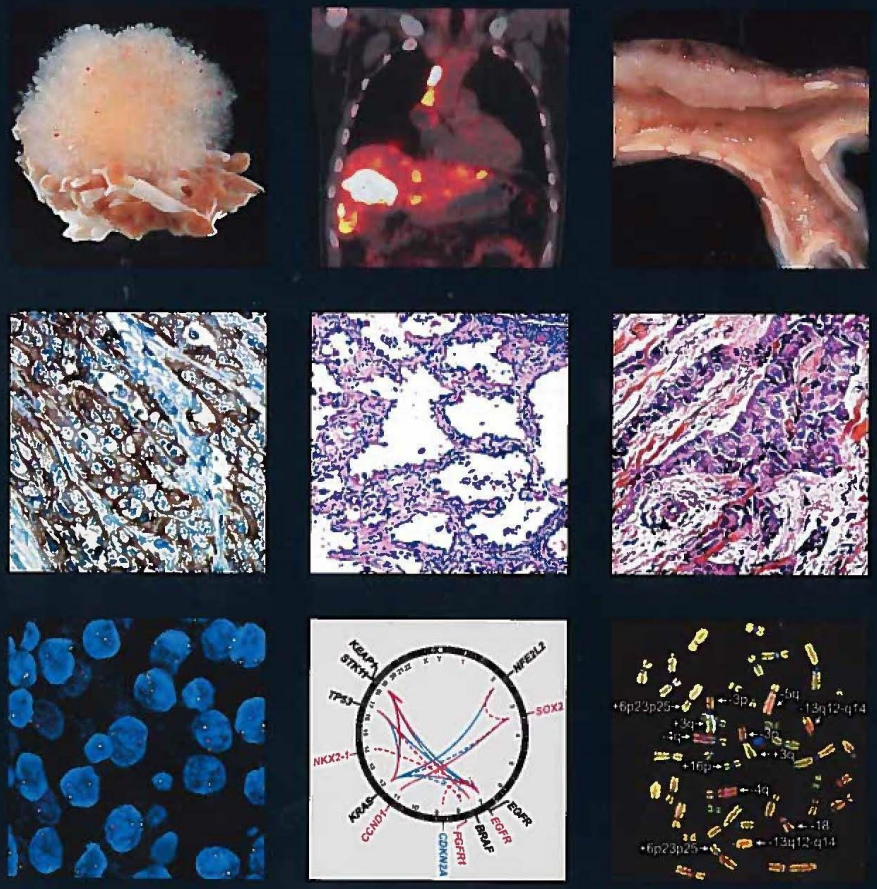
International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

William D. Travis, MD, Elisabeth Brambilla, MD, Masayuki Noguchi, MD, Andrew G. Nicholson, MD, Kim R. Geisinger, MD, Yasushi Yatabe, MD, David G. Beer, PhD, Charles A. Powell, MD, Gregory J. Riely, MD, Paul E. Van Schil, MD, Kavita Garg, MD, John H. M. Austin, MD, Hisao Asamura, MD, Valerie W. Rusch, MD, Fred R. Hirsch, MD, Giorgio Scagliotti, MD, Tetsuya Mitsudomi, MD, Rudolf M. Huber, MD, Yuichi Ishikawa, MD, James Jett, MD, Montserrat Sanchez-Cespedes, PhD, Jean-Paul Sculier, MD, Takashi Takahashi, MD, Masahiro Tsuboi, MD, Johan Vansteenkiste, MD, Ignacio Wistuba, MD, Pan-Chyr Yang, MD, Denise Aberle, MD, Christian Brambilla, MD, Douglas Flieder, MD, Wilbur Franklin, MD, Adi Gazdar, MD, Michael Gould, MD, MS, Philip Hasleton, MD, Douglas Henderson, MD, Bruce Johnson, MD, David Johnson, MD, Keith Kerr, MD, Keiko Kuriyama, MD, Jin Soo Lee, MD, Vincent A. Miller, MD, Iver Petersen, MD, PhD, Victor Roggli, MD, Rafael Rosell, MD, Nagahiro Saijo, MD, Erik Thunnissen, MD, Ming Tsao, MD, and David Yankelewitz, MD

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WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart

Edited by
William D. Travis, Elisabeth Brambilla, Allen P. Burke, Alexander Marx, Andrew G. Nicholson



WHO

IASLC/ATS/ERS Classification of Lung Adenocarcinoma in Resection Specimens

- **Preinvasive lesions**
  - **Atypical adenomatous hyperplasia**
  - **Adenocarcinoma in situ (≤ 3 cm formerly BAC)**
- **Minimally invasive adenocarcinoma (≤ 3 cm lepidic predominant tumor with ≤ 5 mm invasion)**
- **Invasive adenocarcinoma**

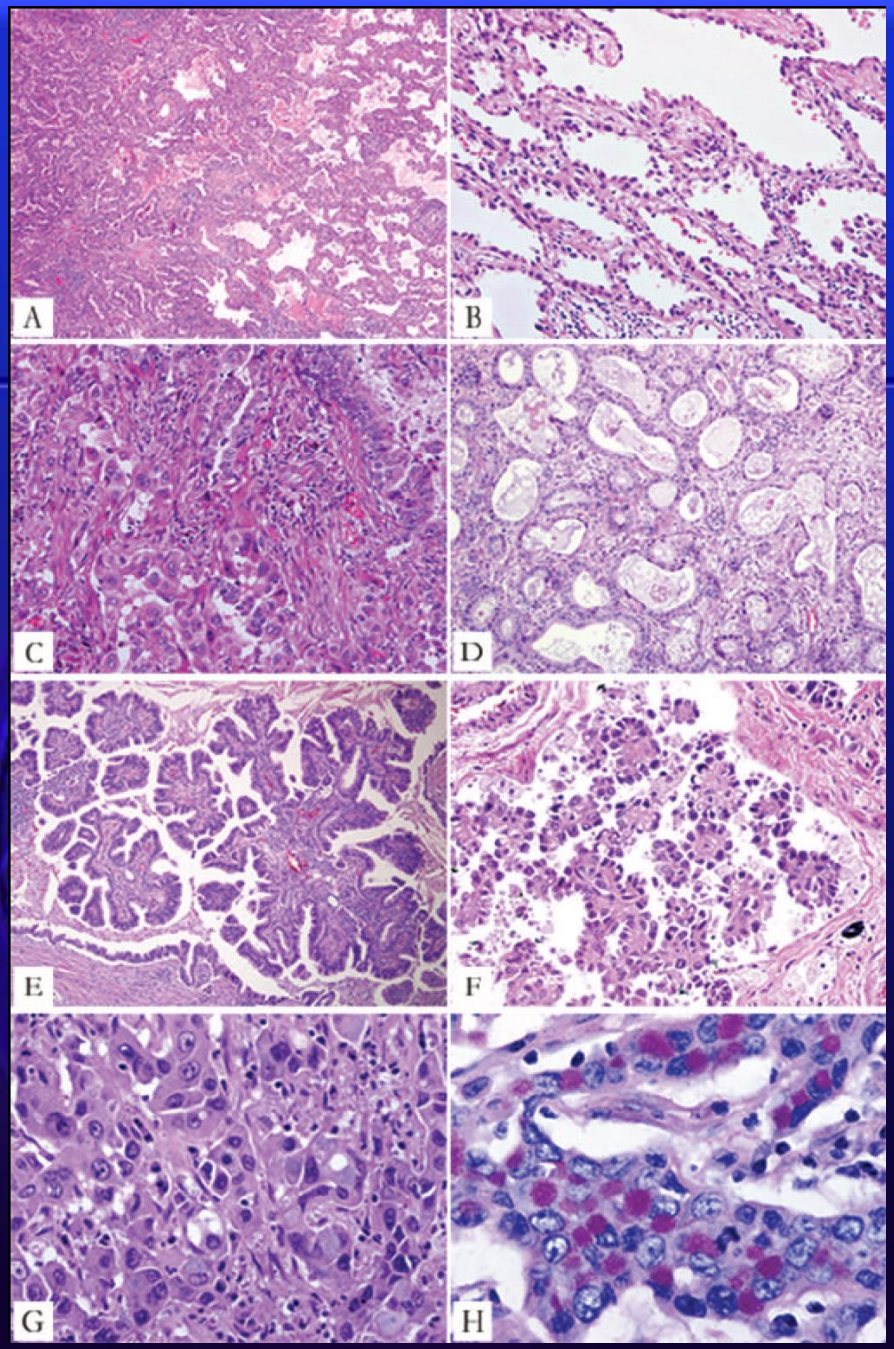
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Lepidic predominant pattern

Acinar adenocarcinoma

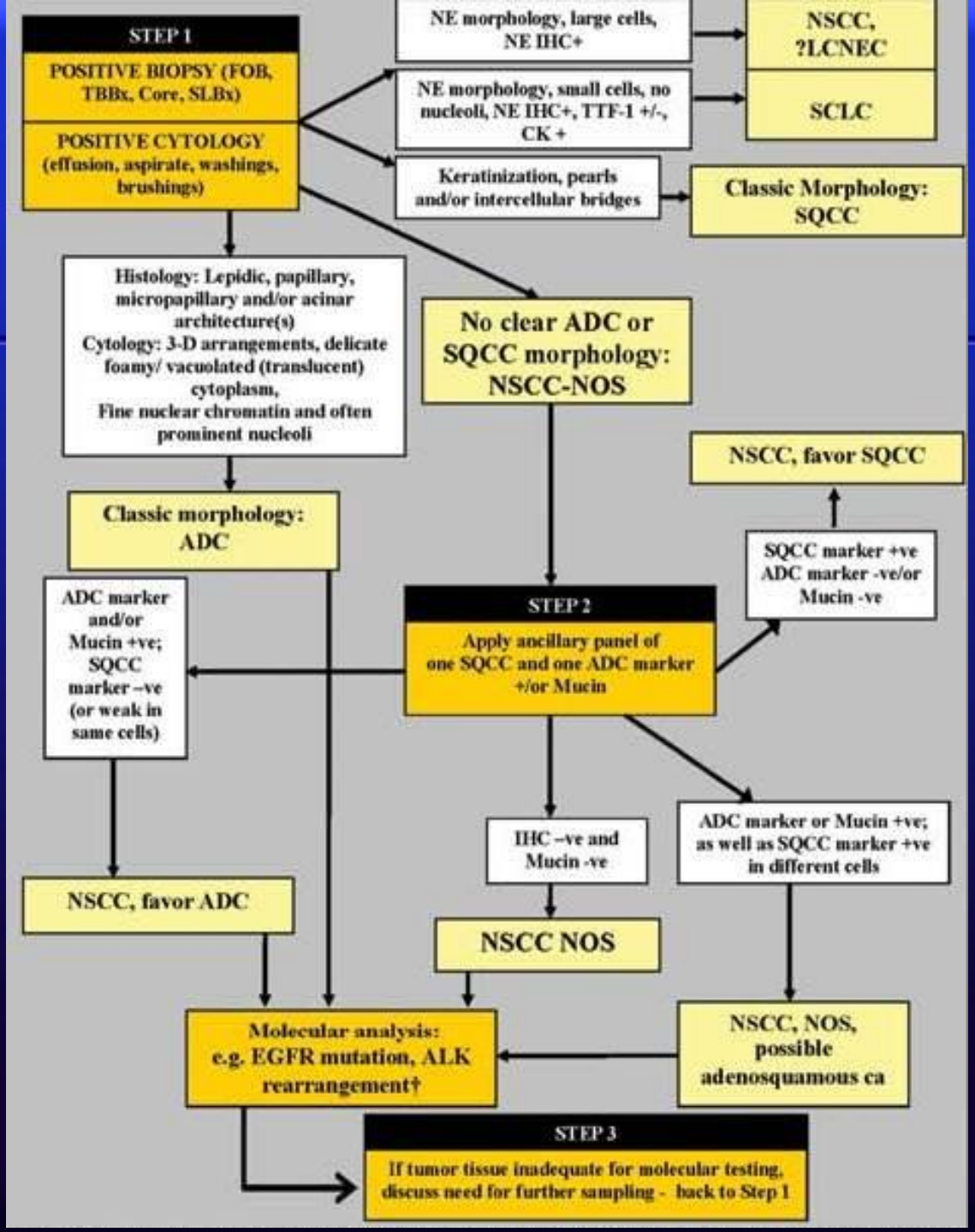
Papillary adenocarcinoma

Solid adenocarcinoma



Micropapillary adenocarcinoma

Journal of Thoracic Oncology, Vol. 6, Number2, February 2011


STEP 1
POSITIVE BIOPSY (FOB, TBBx, Core, SLBx)
POSITIVE CYTOLOGY (effusion, aspirate, washings, brushings)
NE morphology, large cells, NE IHC+
NSCC, ?LCNEC
NE morphology, small cells, no nucleoli, NE IHC+, TTF-1 +/-, CK +
SCLC
Keratinization, pearls and/or intercellular bridges
Classic Morphology: SQCC
Histology: Lepidic, papillary, micropapillary and/or acinar architecture(s)
Cytology: 3-D arrangements, delicate foamy/ vacuolated (translucent) cytoplasm,
Fine nuclear chromatin and often prominent nucleoli
No clear ADC or SQCC morphology: NSCC-NOS
NSCC, favor SQCC
Classic morphology: ADC
SQCC marker +ve ADC marker -ve/or Mucin -ve
ADC marker and/or Mucin +ve; SQCC marker –ve (or weak in same cells)
STEP 2
Apply ancillary panel of one SQCC and one ADC marker +/or Mucin
IHC –ve and Mucin -ve
ADC marker or Mucin +ve; as well as SQCC marker +ve in different cells
NSCC, favor ADC
NSCC NOS
Molecular analysis: e.g. EGFR mutation, ALK rearrangement†
NSCC, NOS, possible adenosquamous ca
STEP 3
If tumor tissue inadequate for molecular testing, discuss need for further sampling - back to Step 1

Lung Adenocarcinoma
Activating Oncogenes

Deletion and point Mutations

- **KRAS (30%)**
- **EGFR (15%)**

Gene Amplification
EGFR (6-9%)

Chromosomal rearrangement
EML4-ALK (5%)
ROS1 (2%)

EGFR, EML 4-ALK and KRAS are mutually exclusive

ALK-rearranged Adenocarcinoma

- 2-7% of adenocarcinomas
- Younger patients
- Never smoking
- Higher stage
- Solid tumor growth, frequent signal cells with abundant intracellular mucin

Similar to EGFR mutation positive patient except they are younger and male

Testing for EGFR mutations and AlK gene rearrangements is recommended in the NCCN NSCLC guidelines for adenocarcinoma patients.

NCCN Guidelines Version 2.20, 2013

Molecular Testing Guideline for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology.

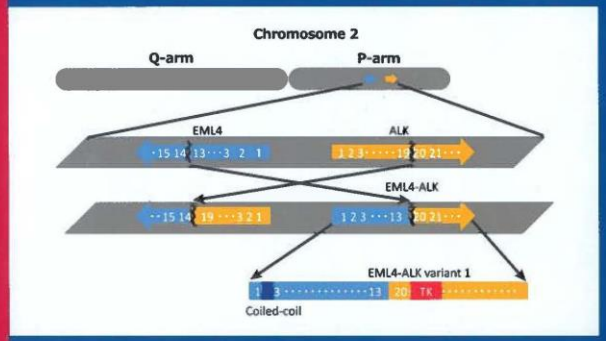
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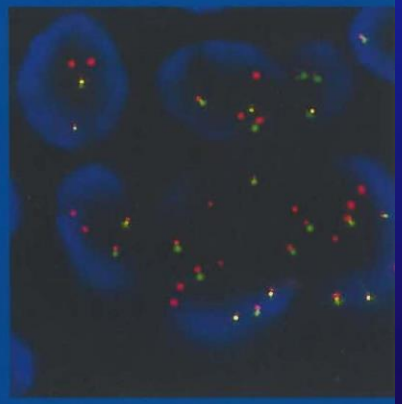
IASLC

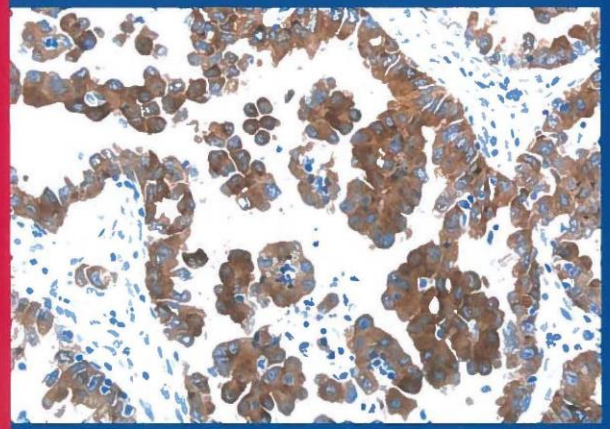
Conquering Thoracic Cancers Worldwide

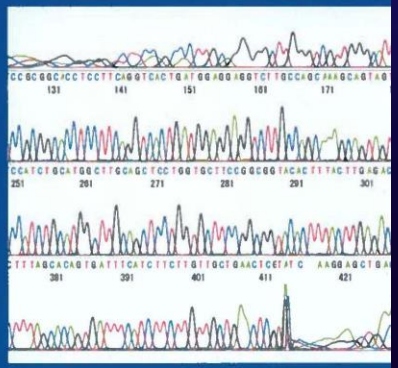
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IASLC ATLAS OF ALK TESTING IN LUNG CANCER









INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

How should ALK testing be performed?

What methods should be used for ALK testing?

- **Recommendation:** Laboratories should use an ALK FISH assay using dual labeled break-apart probes for selecting patients for ALK TKI therapy; ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for ALK FISH testing.
- **Recommendation:** RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.
- **Expert consensus opinion:** A pathologist should be involved in the selection of sections for ALK FISH testing, by assessing tumor architecture, cytology, and specimen quality.
- **Expert consensus opinion:** A pathologist should participate in the interpretation of ALK FISH slides, either by performing the analysis directly or by reviewing the interpretations of cytogeneticists or technologists with specialized training in solid tumor FISH analysis.
- **Expert consensus opinion:** Testing for secondary mutations in ALK associated with acquired resistance to ALK inhibitors is not currently required for clinical management.

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Lung Carcinoma

Distinction is critical between:

- **Adenocarcinoma**
- **Pure squamous cell carcinoma**
- **Pure small cell carcinoma**
- **Pure neuroendocrine carcinoma**

For EGFR and ALK testing

Lung Carcinoma

Lung carcinoma with mixed histology (adenosquamous, adeno/small cell) can have EGFR mutation or Alk rearrangement . Testing is required If possibility of adenocarcinoma component cannot be excluded.

Lung Cancer

It is important to retain sufficient tissue for molecular testing after establishing diagnosis of adenocarcnoma.

ALK Testing

Preanalytical

- Both tissue and cytology samples can be used
- Tumor assessment
- Percentage of tumor cells “tumor adequacy”
- Extent of necrosis, inflammation

Preanalytical Recommendations for Successful FISH

Parameter	Recommendation
Time of fixation	As short as possible, not exceeding 1 hr
Fixative	10% neutral buffered formalin
Time of fixation	6-48 hr
Preparation	Paraffin-embedded sections, cut at a thickness of 5 $\pm$ 1μm
Specimen Storage	Tissue blocks (ideal)
Storage time for blocks	Not relevant if in proper conditions
Storage conditions for blocks	Protected from light, heat, and humidity
Storage time for cut sections	4-6 weeks (ideal); older slides require customized protocol
Decalcification	EDTA, if necessary

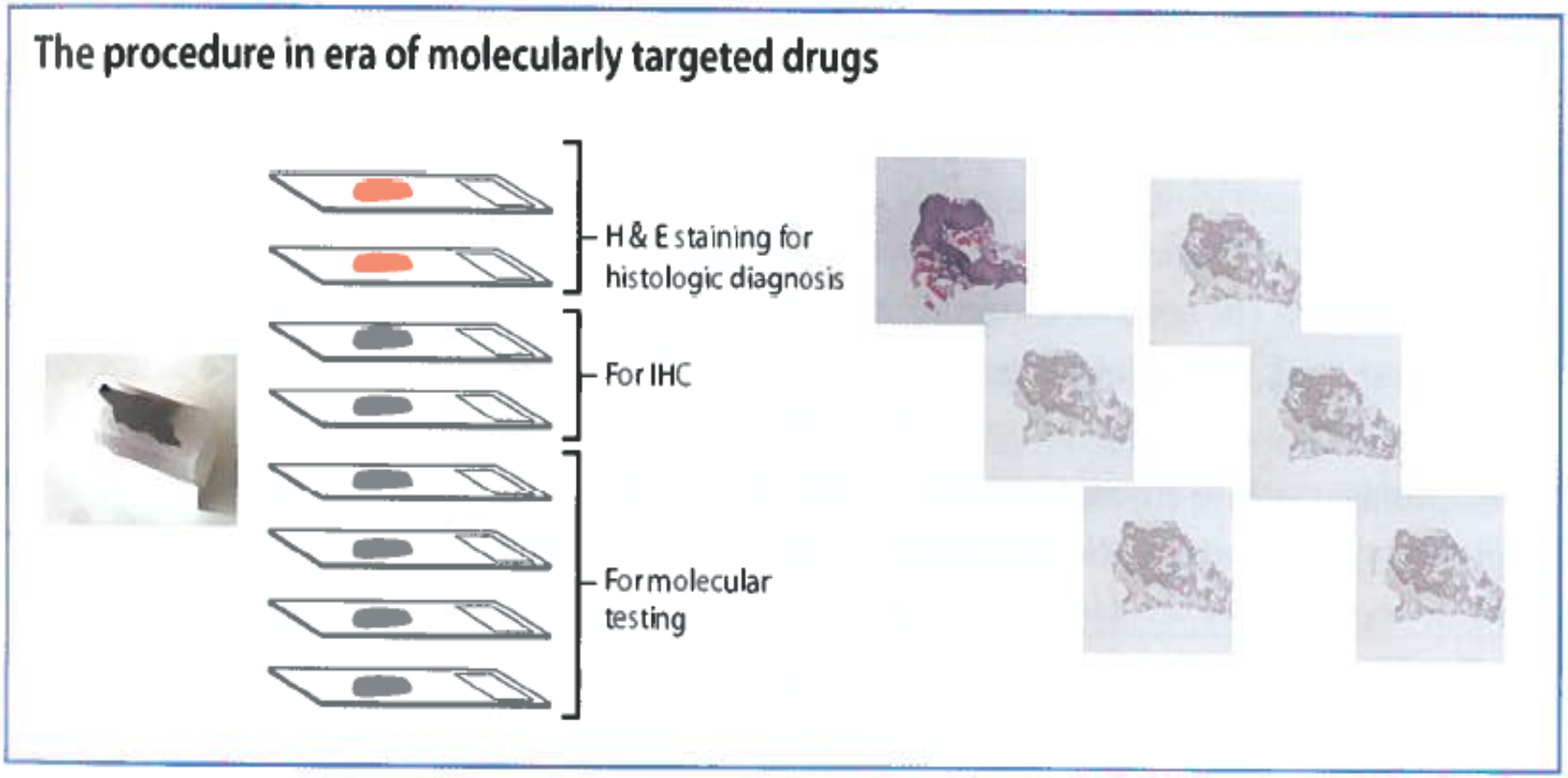


Figure 1. Preparation of tissue sections during diagnostic workup. Current routine practice involves making additional sections for IHC assay and/or molecular testing after the initial sectioning and hematoxylin and eosin (H & E) staining for histologic diagnosis. Multiple sequential sectioning may deplete the tumor volume each time block trimming is necessary. In the era of molecularly targeted therapies, the preparation of additional unstained sections for possible IHC analysis and/or molecular testing may significantly reduce the amount of tissue sample lost and improve turnaround time.

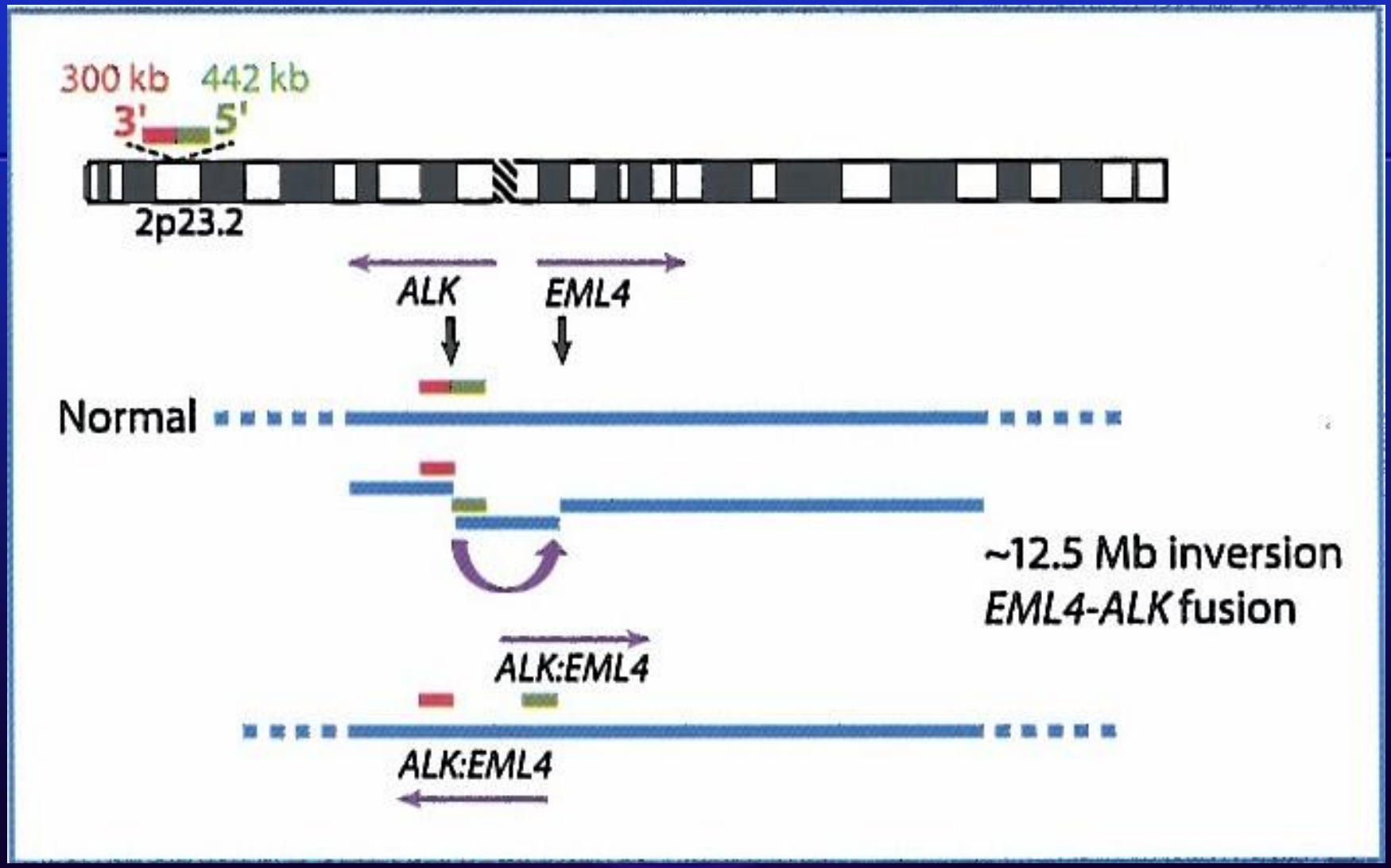
ALK Testing (Analytical)

- IHC
- FISH
- RT - PCR
- NGS
- CTCs

- **ALK FISH:** probe set (manufacturer, type) and threshold used to define a positive result

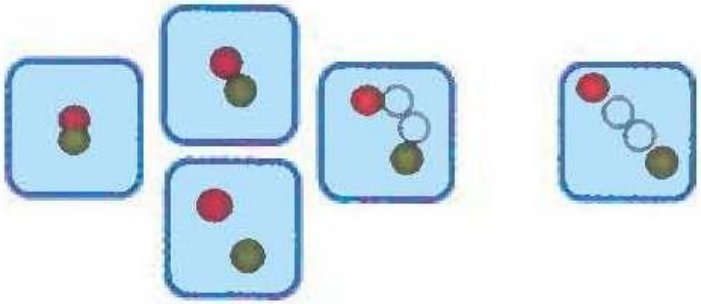
- **ALK IHC:** antibody type (source), antibody concentration, incubation time and temperature, and secondary signal enhancement system

- **ALK RT-PCR:** method used, primers, probes and their references, and assay sensitivity


300 kb
442 kb
3'
5'
2p23.2
ALK
EML4
Normal
~12.5 Mb inversion
EML4-ALK fusion
ALK:EML4
ALK:EML4

SIGNAL CLASSIFICATION

Patterns observed in native *ALK*



Red and green separated by <2 signal diameters

Classified as negative

Red and green separated by ≥2 signal diameters

Classified as positive

Patterns observed in split 3'-5' *ALK*



Red and green separated by ≥2 signal diameters

Classified as positive

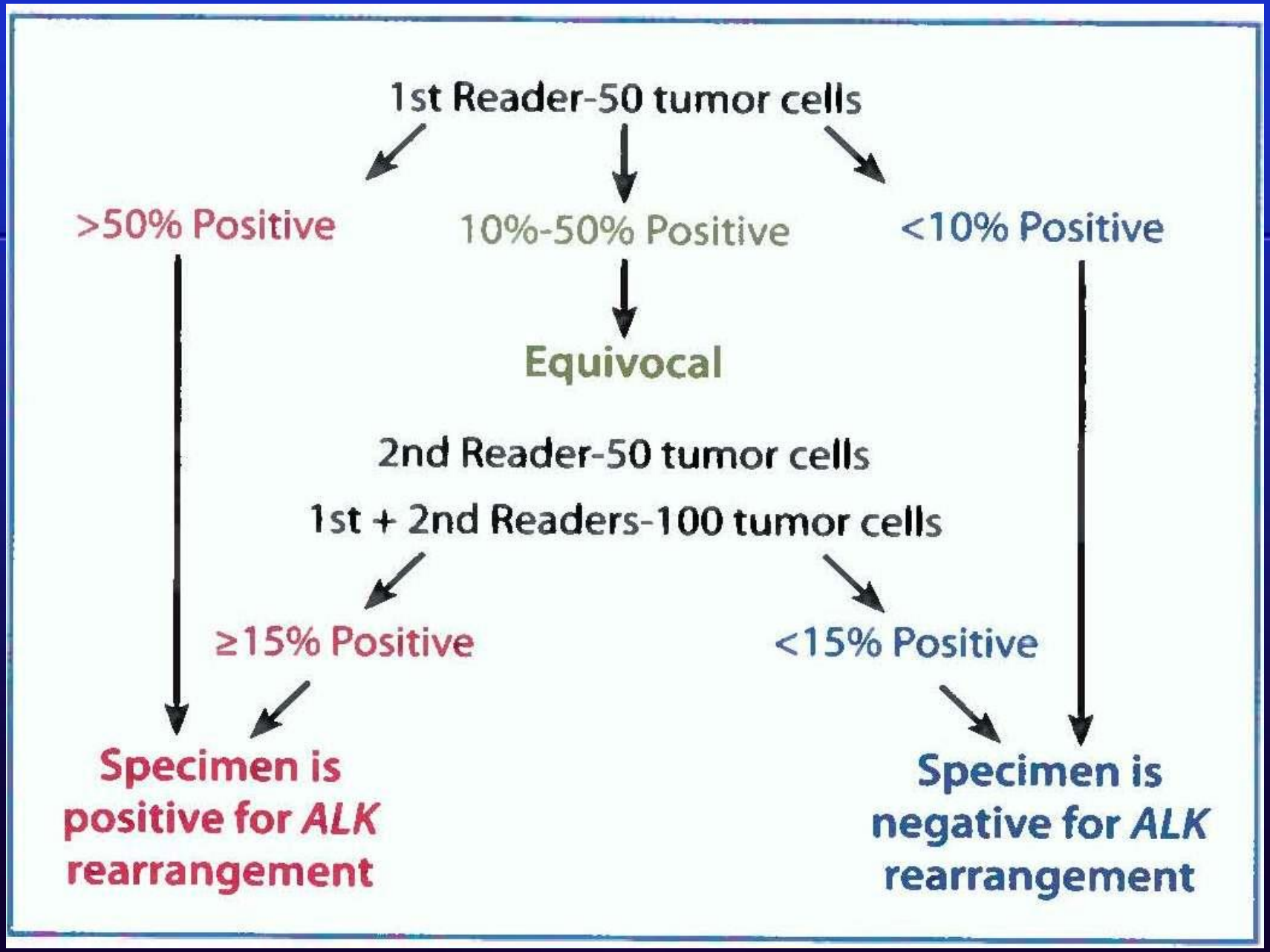
Red and green separated by <2 signal diameters

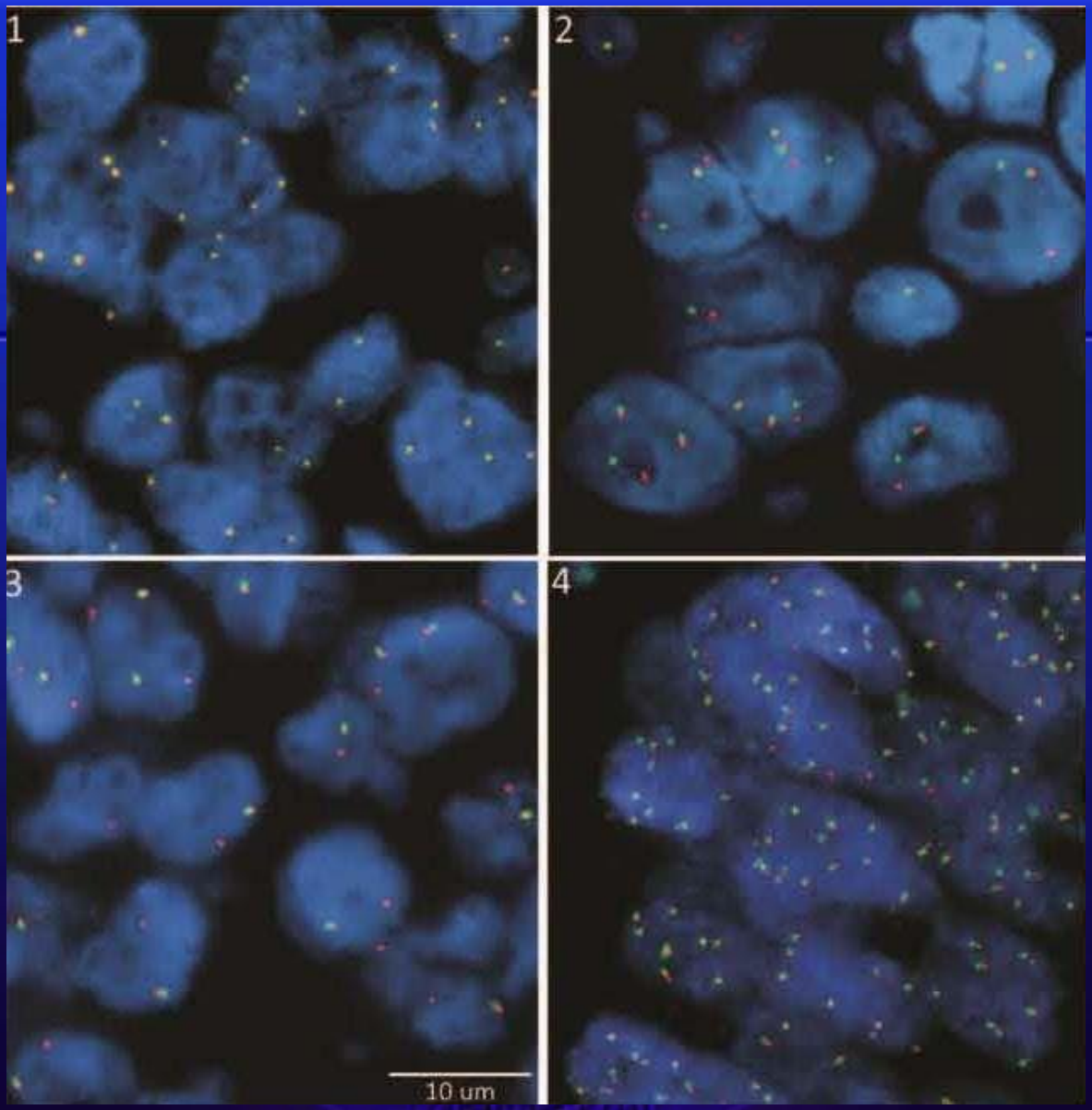
Classified as negative

SPECIMEN CLASSIFICATION

Nonrearranged tumors:
Rearrangement-positive cell rate
<15% of cells

Rearranged tumors:
Rearrangement-positive cell rate
≥15% of cells


1st Reader-50 tumor cells
>50% Positive
10%-50% Positive
<10% Positive
Equivocal
2nd Reader-50 tumor cells
1st + 2nd Readers-100 tumor cells
≥15% Positive
<15% Positive
Specimen is positive for ALK rearrangement
Specimen is negative for ALK rearrangement



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ALK Testing

KFSH&RC Experience:

Of 198 lung adenocarcinoma cases analyzed, 11 cases were positive for ALK rearrangement by FISH (5.5%).

ALK Positive Lung Adenocarcinoma

Age/Sex	Smoking History	Targeted Therapy	Response
36 M	Yes	Yes	Stable disease
64 M	Yes	No	Lobectomy
56 M	Yes	No	Died (Advanced stage on dx)
68 M	Not known	Yes	Yes
30 M	Not known	Yes	Yes
61 M	Not known	Yes	Yes
52 F	Not known	Yes	Started Rx
21 F	No	No	Lobectomy