Molecular testing of lung carcinoma

Los Angeles Society Of Pathologists
January 25, 2014

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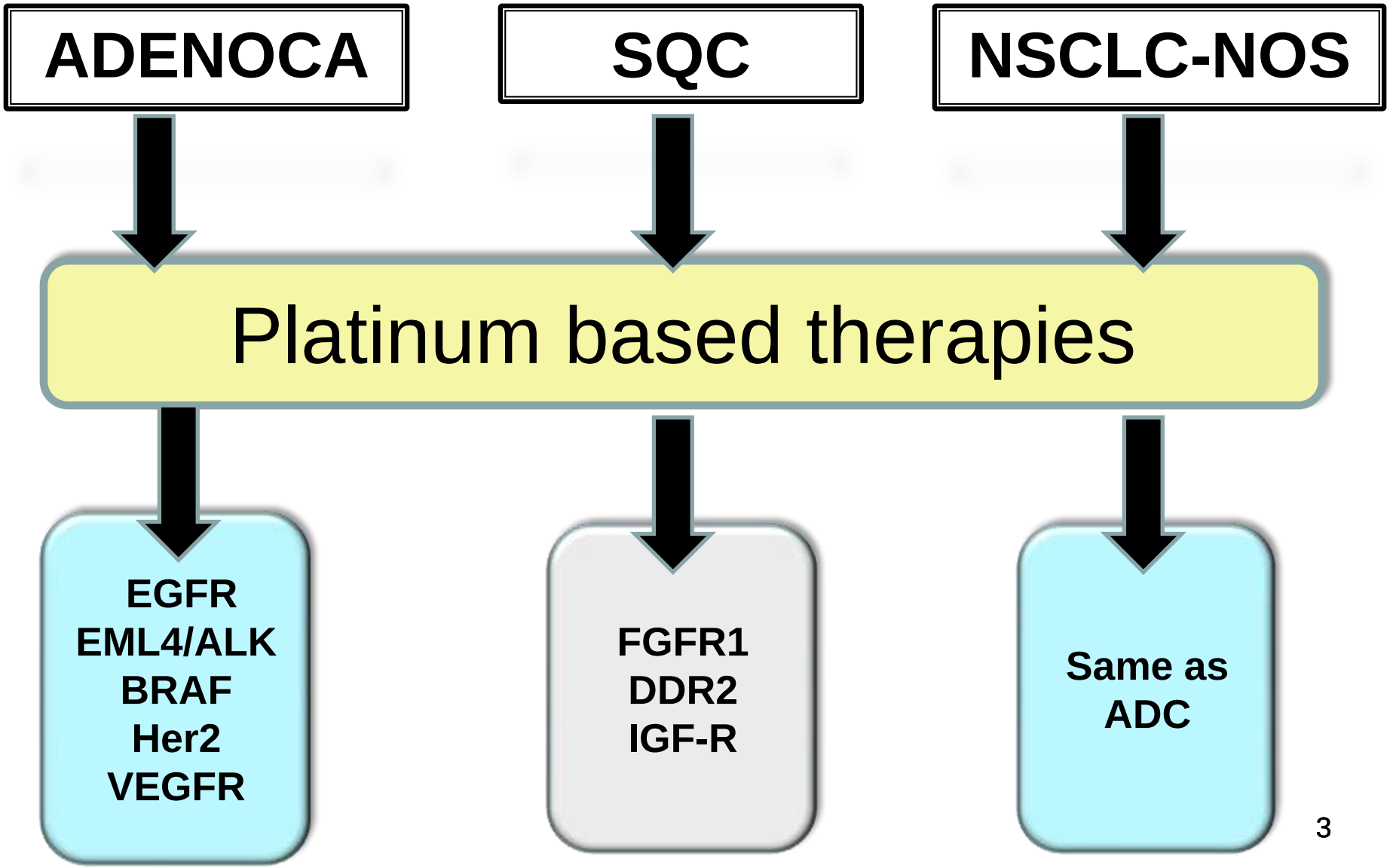
University of Pittsburgh Medical Center

OUTLINE

- Clinical testing for predictors of therapy response
  - *EGFR*, *ALK*, other
- Role of surgical pathologists in targeted therapies

Treatment of advanced NSCLC

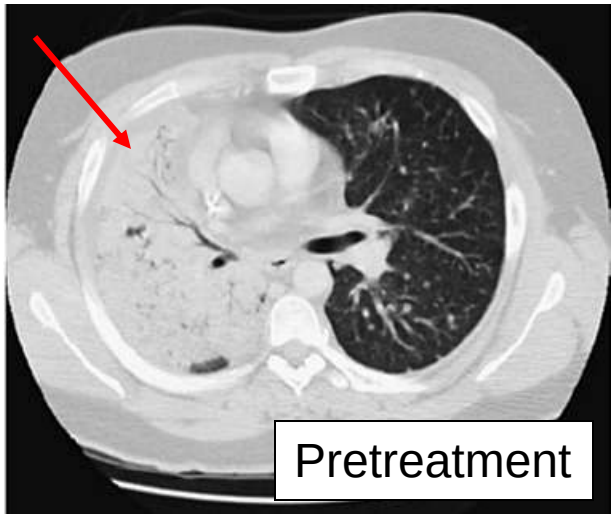


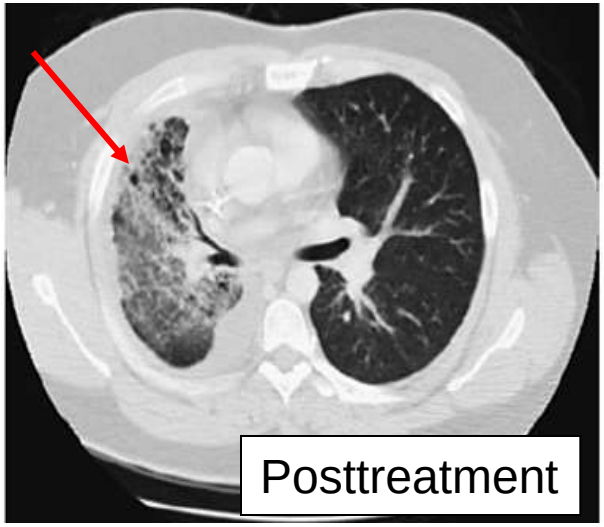
Diagnosis of lung carcinoma

- Before 2005.
  - Small cell carcinoma vs. non-small cell carcinoma

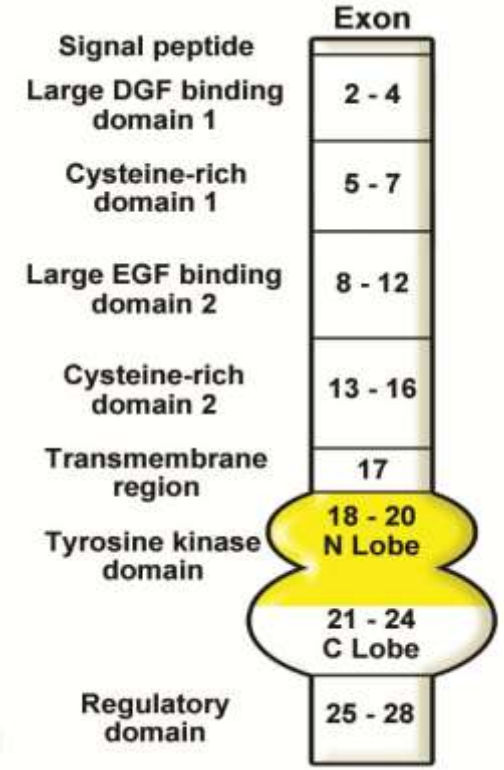
- From 2005- present
  - Small cell carcinoma vs. non-squamous cell carcinoma

EGFR mutations and *EGFR*- TKI responders



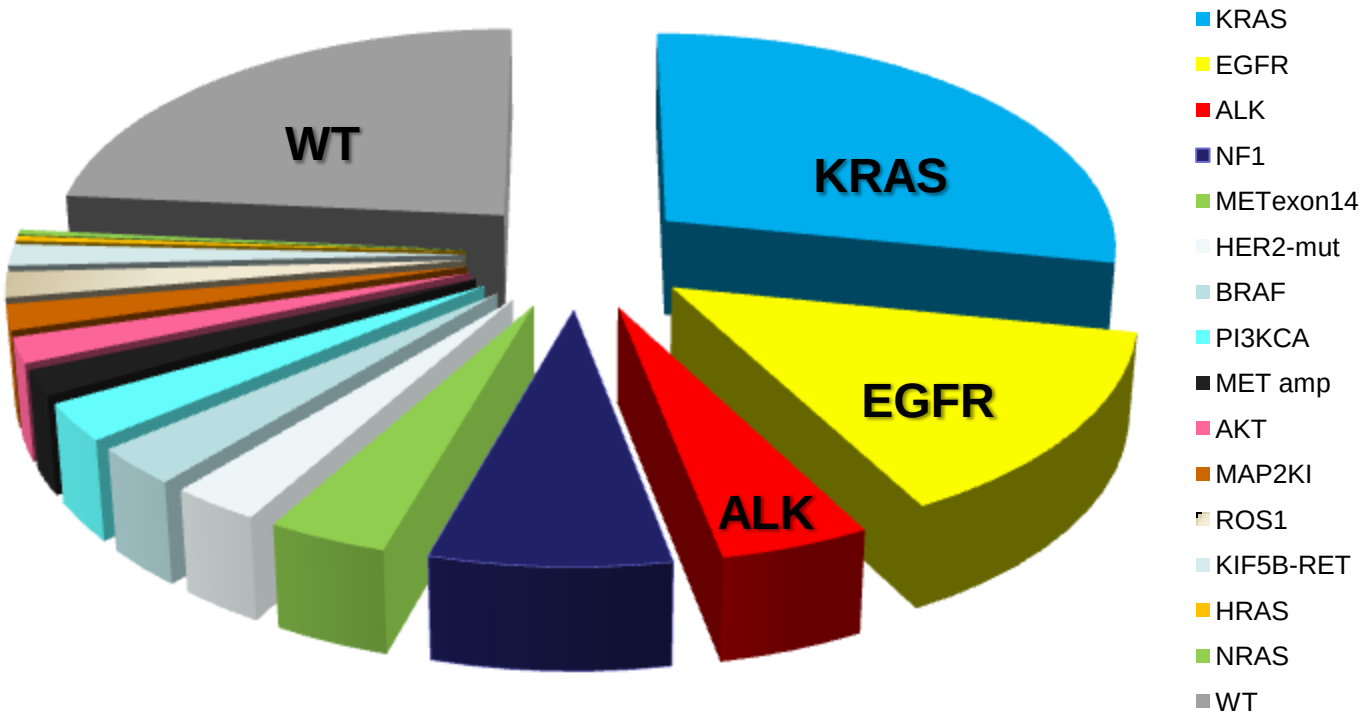


- Women; never smokers
- Adenocarcinoma
- Non-squamous carcinoma



N Engl J Med 2004; 350:2129-39

Genetic alterations in lung adenocarcinoma



COMMON QUESTIONS

- What assay to choose?
- What type of sample to send for molecular analysis?
- What histologic subtype of NSCLC should be tested?
- What is the future of molecular testing?

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

See related Guest Editorial on page 413.

SPECIAL ARTICLE

Molecular Testing Guideline for Selection of Lung Cancer Patients 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

Neal I. Lindeman,* Philip T. Cagle,† Mary Beth Beasley,‡ Dhananjay Arun Chitale,§ Sanja Dacic,¶ Giuseppe Giaccone,‖ Robert Brian Jenkins,** David J. Kwiatkowski,†† Juan-Sebastian Saldivar,‡‡ Jeremy Squire,§§ Erik Thunnissen,¶¶ and Marc Ladanyi‖‖

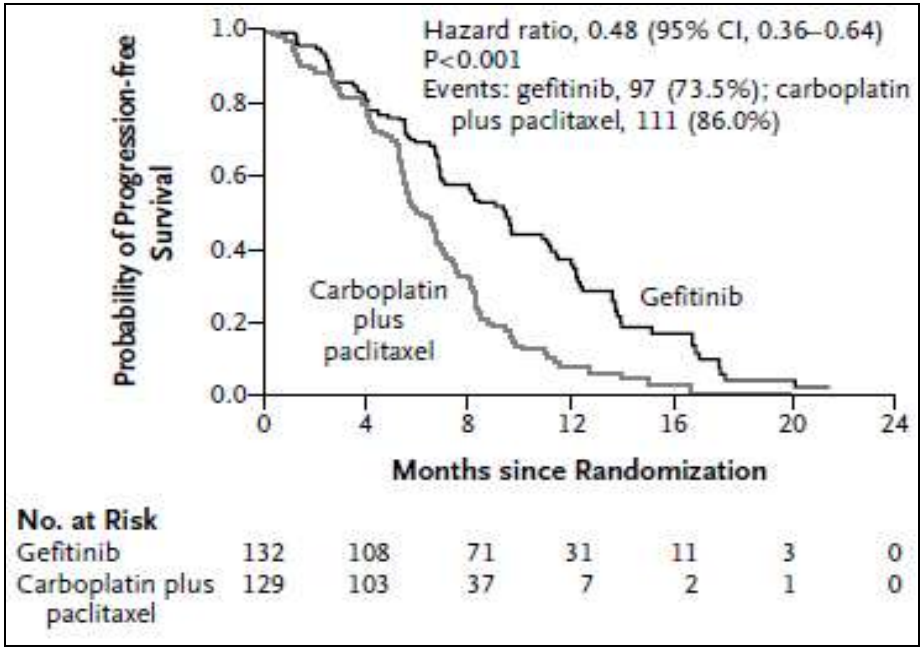
Journal of Thoracic Oncology

INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

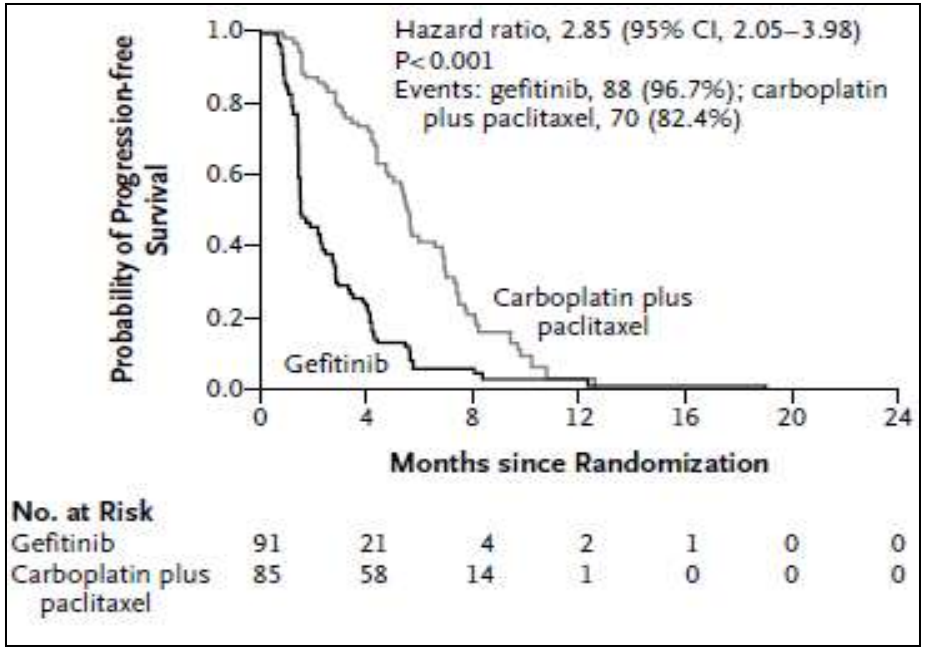
ARCHIVES of Pathology & Laboratory Medicine

Iressa Pan-Asian Study (IPASS)

***EGFR*-Mutation-Positive**

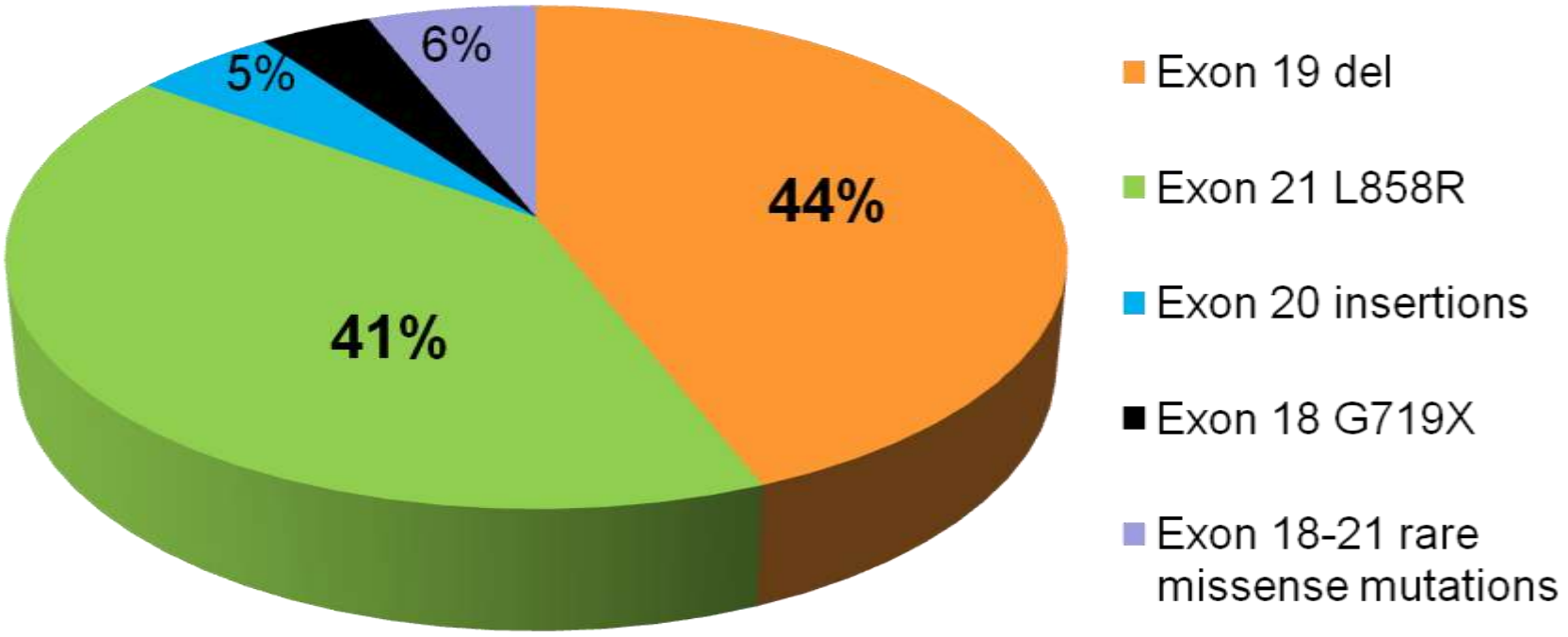


***EGFR*-Mutation-Negative**



Mok TS et al. NEJM 2009;361:947-957

Frequency of *EGFR* mutations



Modified from Shigematsu et al. Int J Cancer 2006;118:257-262.

CAP/IASLC/AMP recommendation

- Multiple test platforms are acceptable for *EGFR* mutation testing
- A mutation method must be at least as sensitive as Sanger sequencing
- EGFR mutation analysis should capture all mutations that individually account for 1% or more of mutant case
- Assay for the *T790M* should have sensitivity in the 1-5% range

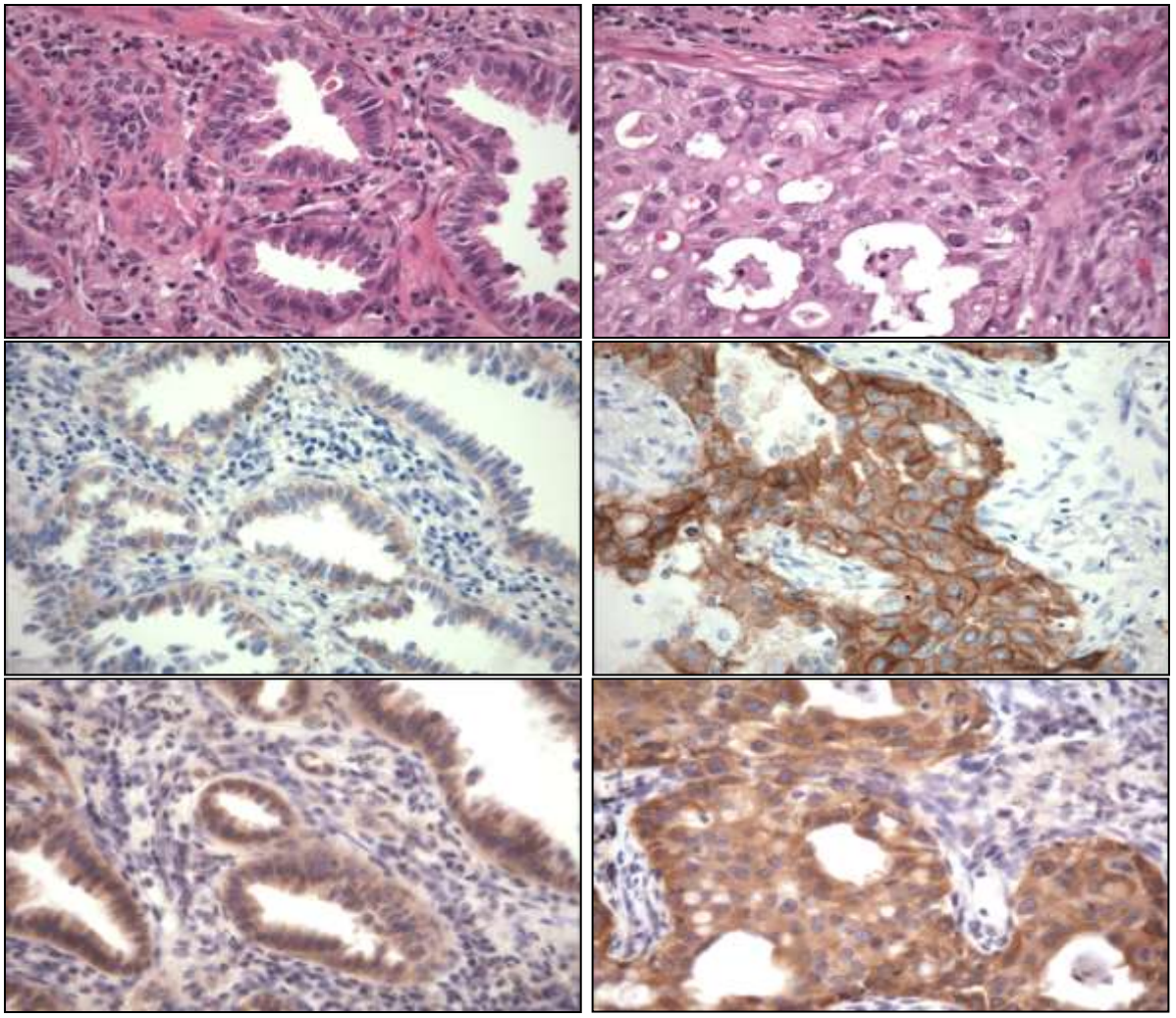
DETECTION OF EGFR ABNORMALITIES

- Immunohistochemistry
- FISH/CISH
- Detection of mutations
  - DNA sequencing or other mutation detection techniques

EGFR IHC

- IHC for total EGFR
  - Not acceptable
- IHC for phosphorylated EGFR
  - Limited experience, unreliable
- IHC for mutant forms of EGFR

EGFR MUTATION SPECIFIC ANTIBODIES



Clin Cancer Res 2009;15(9):3023-3028

Courtesy of Lucian Chirieac, MD, Brigham and Women's Hospital

EGFR exon 19 and L858R mutation specific antibodies

Antibody	Sensitivity (%)	Specificity (%)
Exon 19		
15 bp	100	98.8
<15 bp	74.2	98.8
Exon 21	95.2	98.8

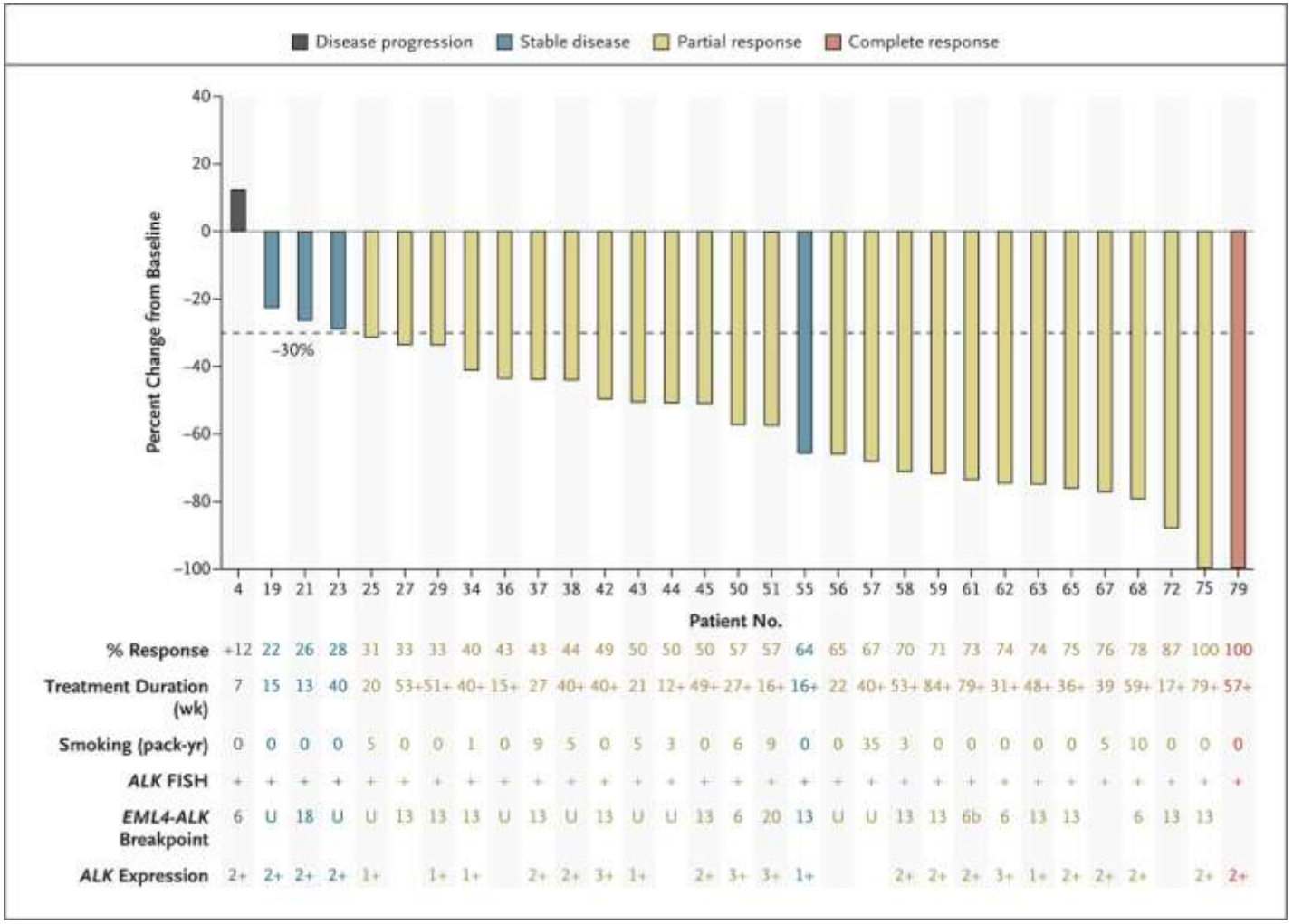
Brevet et al. JMD 2010; 12:169-176.

CAP/IASLC/AMP recommendation

- EGFR IHC is NOT recommended test for *EGFR* TKI treatment selection

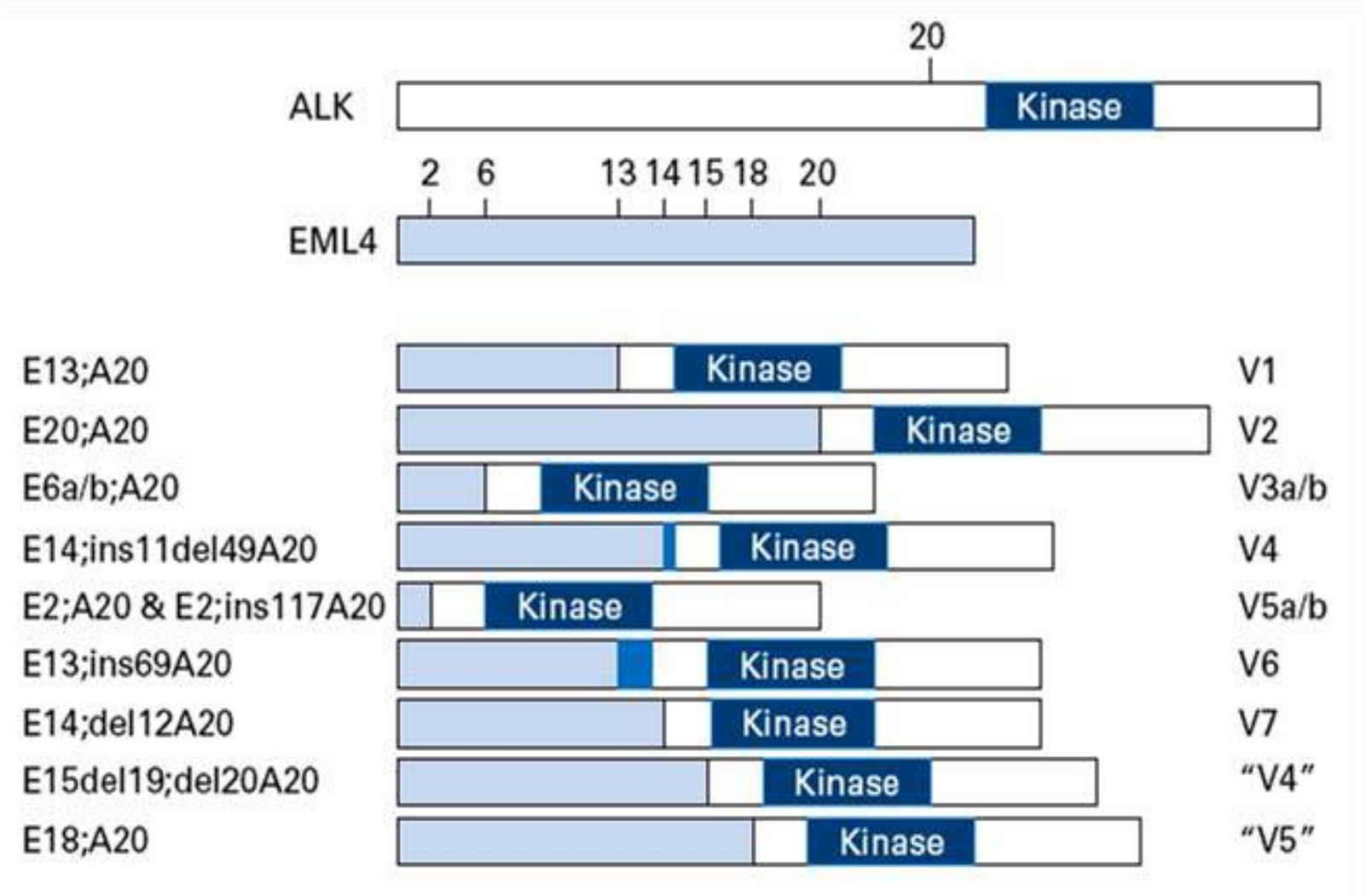
- Mutant *EGFR* allele-specific IHC is too insensitive to be used as a stand alone assay for *EGFR*-TKI treatment selection

Response to Crizotinib in *ALK*-Positive Tumors



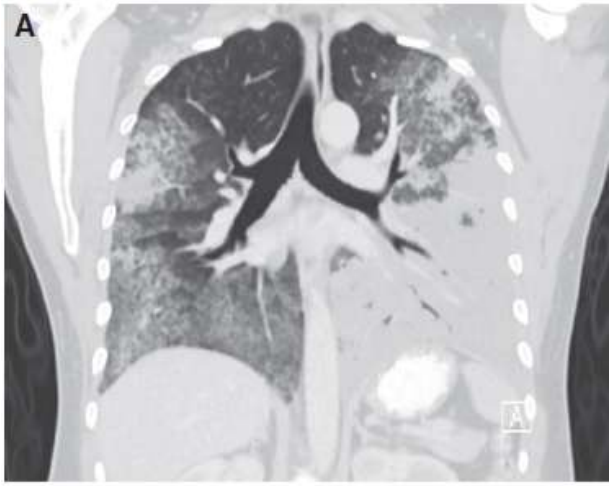
Kwak EL et al. N Engl J Med 2010;363:1693-1703.

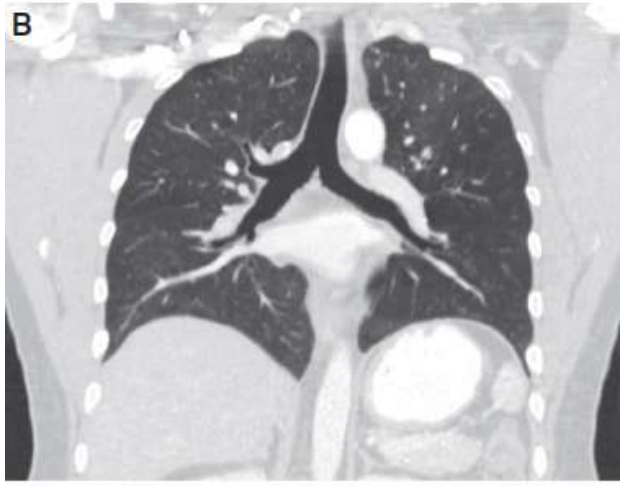
ALK and fusion products in NSCLC

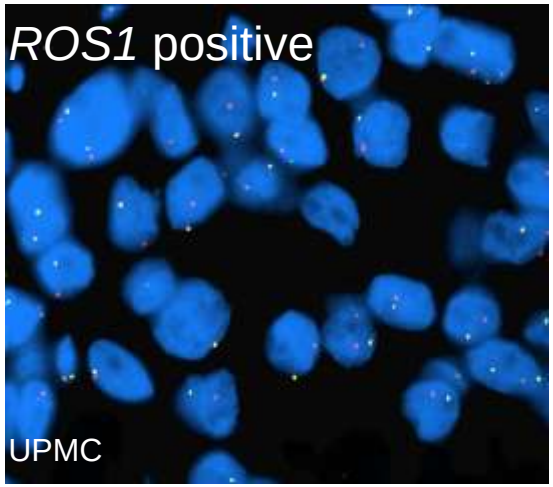


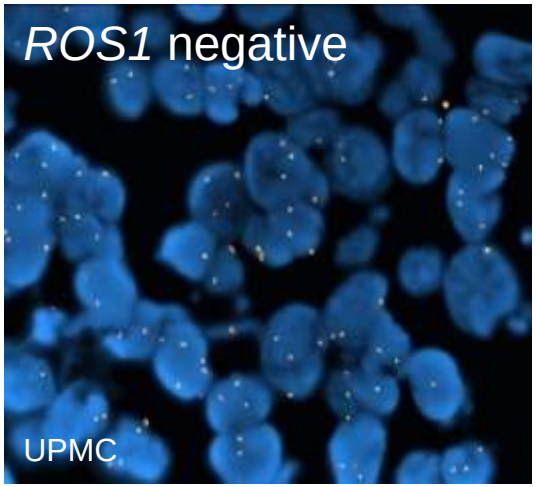
JCO 2009;27 (26):4232-35.

ROS1 rearrangements









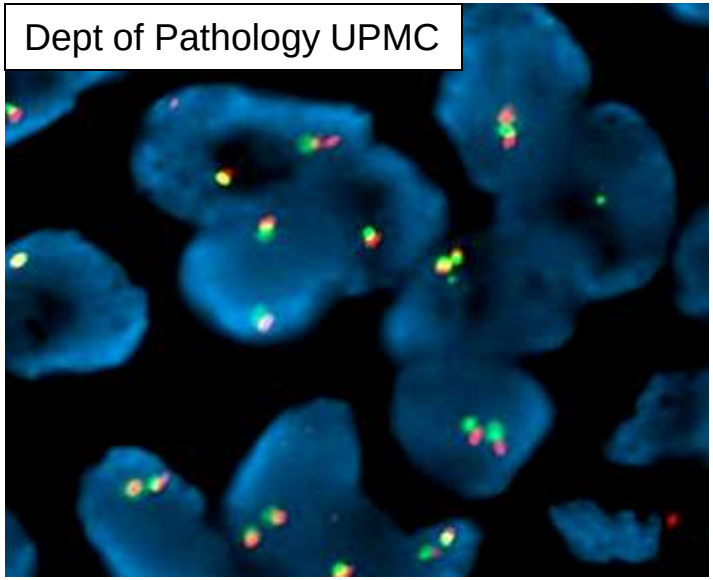
- receptor tyrosine kinase of the insulin receptor family
- 6q22
- 2% lung ADC
- Young, never smokers
- Respond to crizotinib

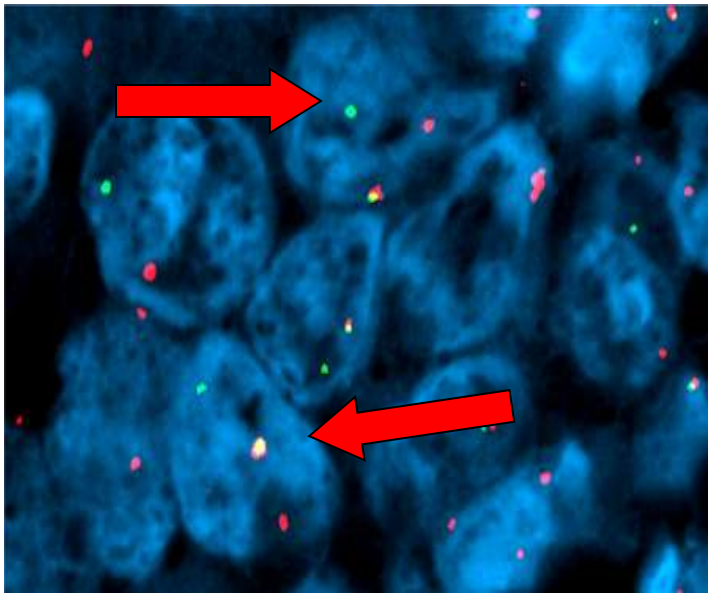
Bergethon et al., JCO 2012; 30(8): 863-70, 2012; Takeuchi et al., Nat Med 2012; 18(3): 378-81.

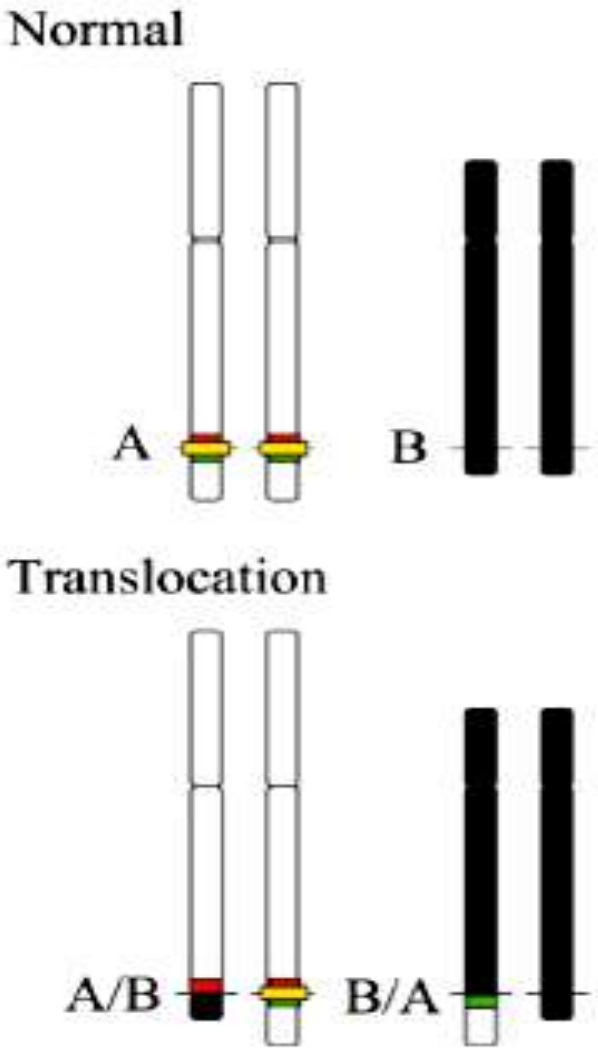
METHODS OF DETECTION

- Classical cytogenetics
- FISH
- Immunohistochemistry
- RT-PCR

ALK- FISH







Diagn Mol Pathol 2004; 13(4):197-206

ALK-IHC

CLONE	PROVIDER	SPECIFICITY (%)	SENSITIVITY (%)
D5F3 Rabbit monoclonal	Ventana Medical System, Inc. Cell Signaling Technology, Danvers, MA	75-99	91-100
5A4 Rabbit monoclonal	Novocastra, New Castle, UK	87.5-98	100
ALK1 M7195 Mouse monclonal	Dako, Carpinteria, CA	91-99	64-100

CCR 2010; JCP 2010; JTO 2011, 2012, 2013; Ann Oncol 2012, Mod Pathol 2013, Plos One 2013

IHC ASSAY IMPLEMENTATION CHALLENGES

- Tissue quality and quantity
- Antibody clone
- IHC protocol (antigen retrieval method, detection system)
- Interpretation criteria

ALK IHC agreement with FISH

	Agreement Between IHC and FISH	
Agreement Rate	n/N (%)	95% CI
Overall Percent Agreement	92/98 (93.9)	87.3, 97.2
Positive Percent Agreement	39/43 (90.7)	78.4, 96.3
Negative Percent Agreement	53/55 (96.4)	87.7, 99.0

WCLC , Sydney 2013

IASLC

Conquering Thoracic Cancers Worldwide

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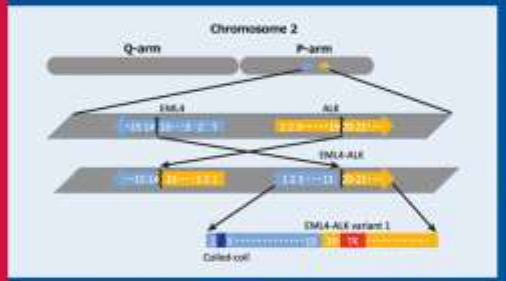
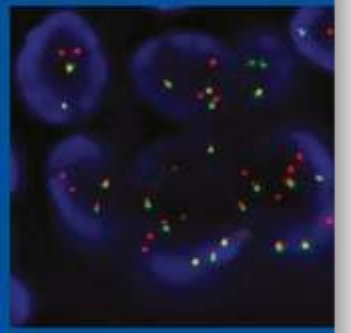
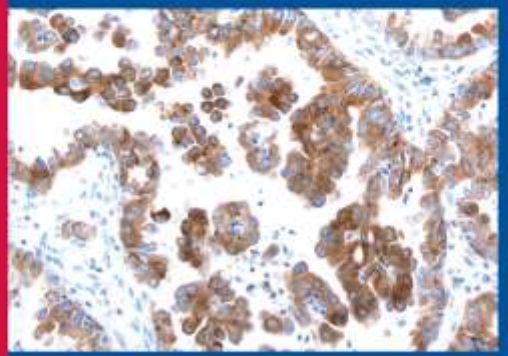
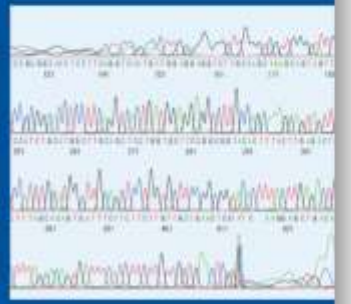
IASLC ATLAS OF ALK TESTING IN LUNG CANCER









INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

CAP/IASLC/AMP recommendation

- A commercial break-apart FISH assay developed by Abbott Molecular is recommended
- DAKO ALK1 antibody is not reliable for ALK rearrangement screening
- RT-PCR is not currently recommended as a first-line diagnostic method for *ALK* fusion status

WHAT TYPE OF TISSUE SAMPLE SHOULD BE TESTED?

SAMPLE FOR *EGFR/ALK* TESTING

- Sample processing
- Primary vs. metastatic tumor
- Selecting a block for analysis
- Multiple primary lesions

SAMPLE PROCESSING

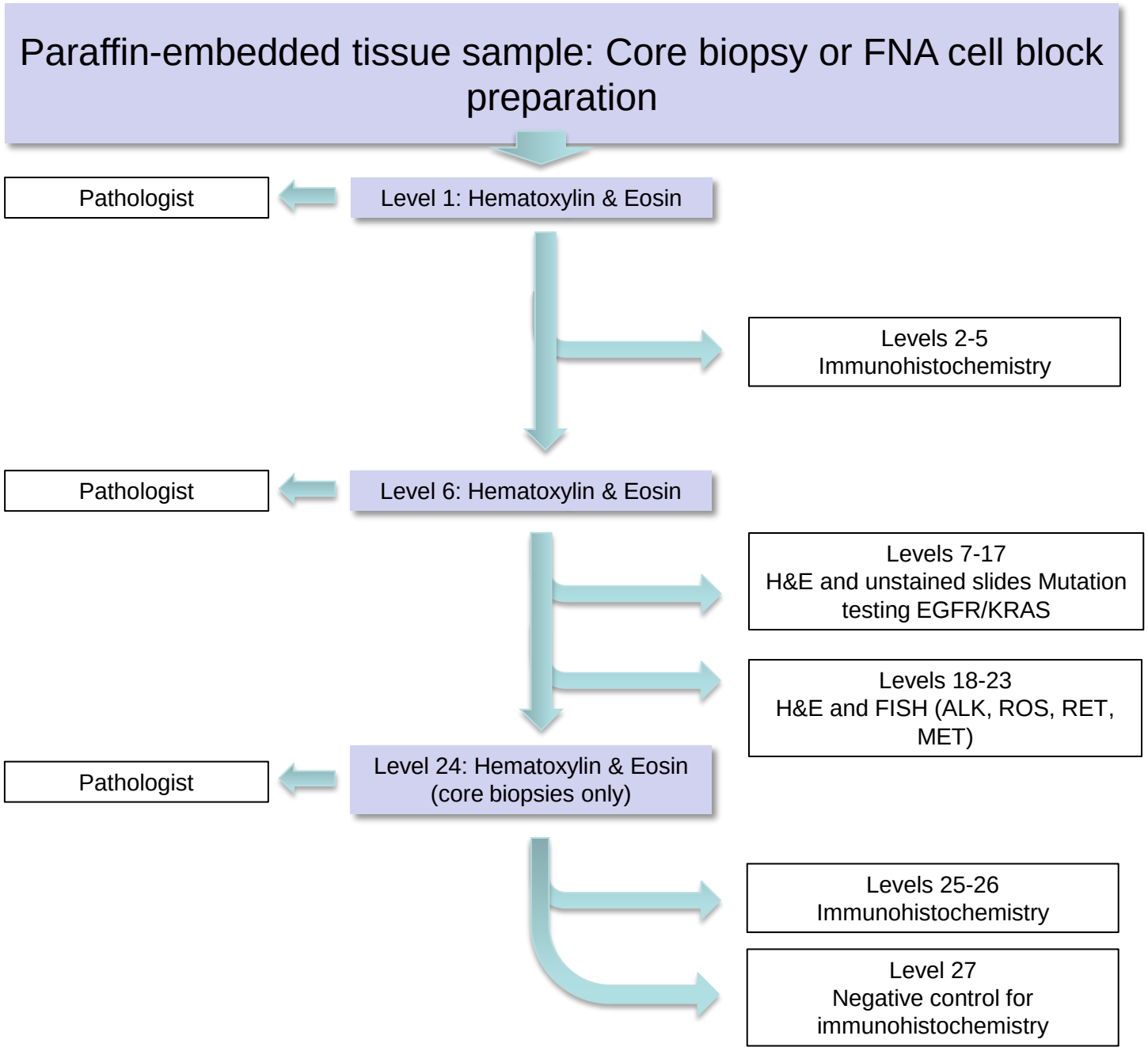
- **ACCEPTABLE fixatives**
  - 10% neutral-buffered formalin (NBF)
  - Alcohol (70% ethanol)

- **UNACCEPTABLE fixatives**
  - Heavy metal fixatives (e.g. Zenker's, B5, AZF, B plus)
  - Acidic solutions (Bouin's solution, bone decalcifying solutions)

SAMPLE PROCESSING
CAP/IASLC/AMP recommendation

- Specimen should be fixed in 10% NBF for no less than 6 hours and no more than 48 hours before processing

- Cell block is recommended for cytology specimens


Paraffin-embedded tissue sample: Core biopsy or FNA cell block preparation
Pathologist
Level 1: Hematoxylin & Eosin
Levels 2-5
Immunohistochemistry
Pathologist
Level 6: Hematoxylin & Eosin
Levels 7-17
H&E and unstained slides Mutation testing EGFR/KRAS
Levels 18-23
H&E and FISH (ALK, ROS, RET, MET)
Pathologist
Level 24: Hematoxylin & Eosin (core biopsies only)
Levels 25-26
Immunohistochemistry
Level 27
Negative control for immunohistochemistry

WHAT TUMOR SAMPLE TO TEST

PRIMARY TUMOR OR METASTASES ?

Heterogeneity of *EGFR* mutations

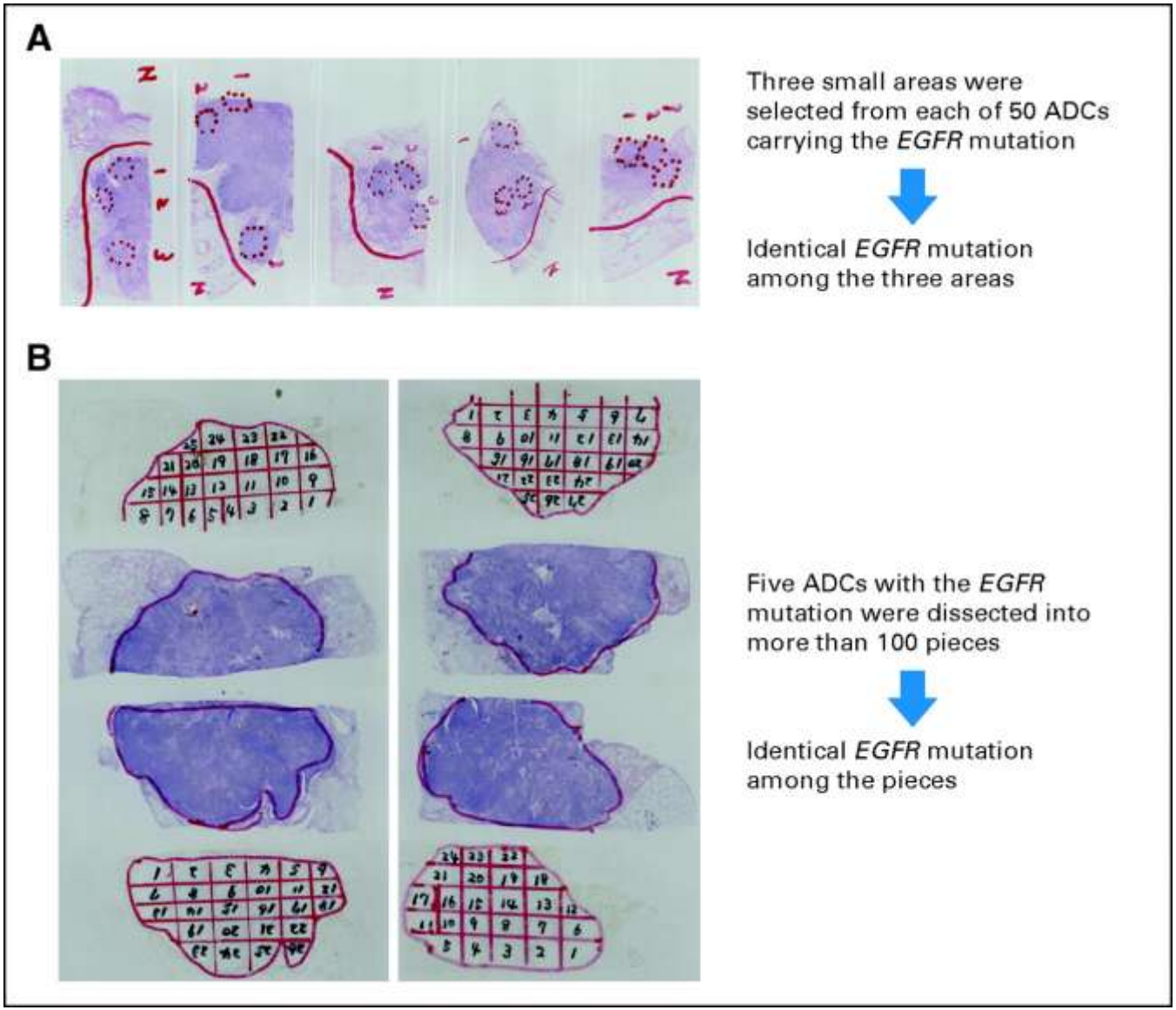
- Intratumor heterogeneous population of both *EGFR* mutated and non-mutated cancer cells, resulting in a reduced response to gefitinib (*Taniguchi K. et al Cancer Sci 2008; 99:929; Nakano H. Lung Cancer 2008; 60:136)*

- Discrepancy in *EGFR* mutations between primary tumors and metastatic lymph nodes, suggesting tumor heterogeneity at the molecular level during the process of metastasis (*Park S. JTO 2009;4:809; Schmid K. CCR 2009; 15:4554; Chang YL. Ann Surg Oncol 2011; 18:543*)

- Discrepancy in EGFR mutations between primary and recurrent NSCLC

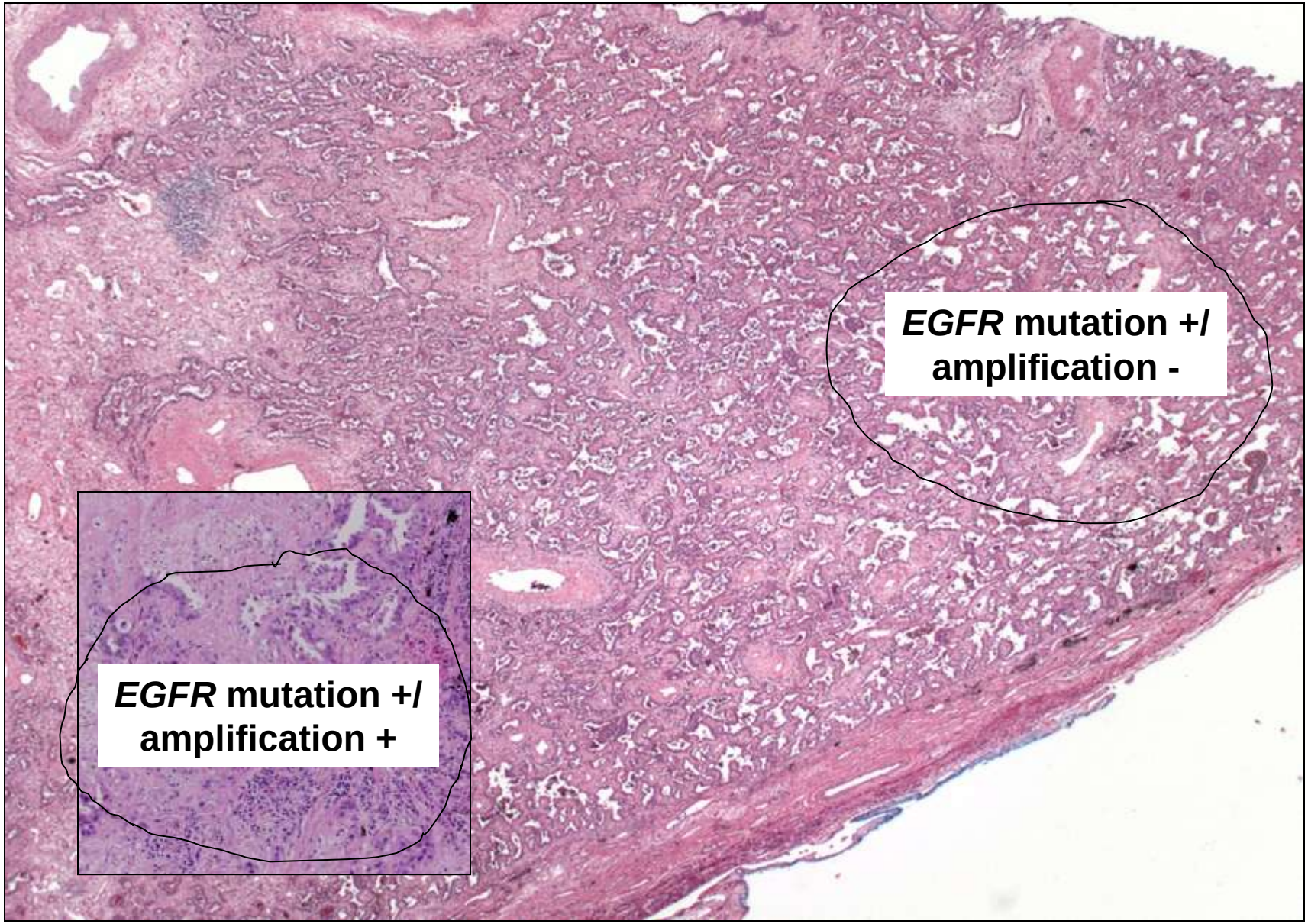
- Role of chemotherapy (Bai H. JCO 2012 ;30:3077)

The distribution of the *EGFR* mutations

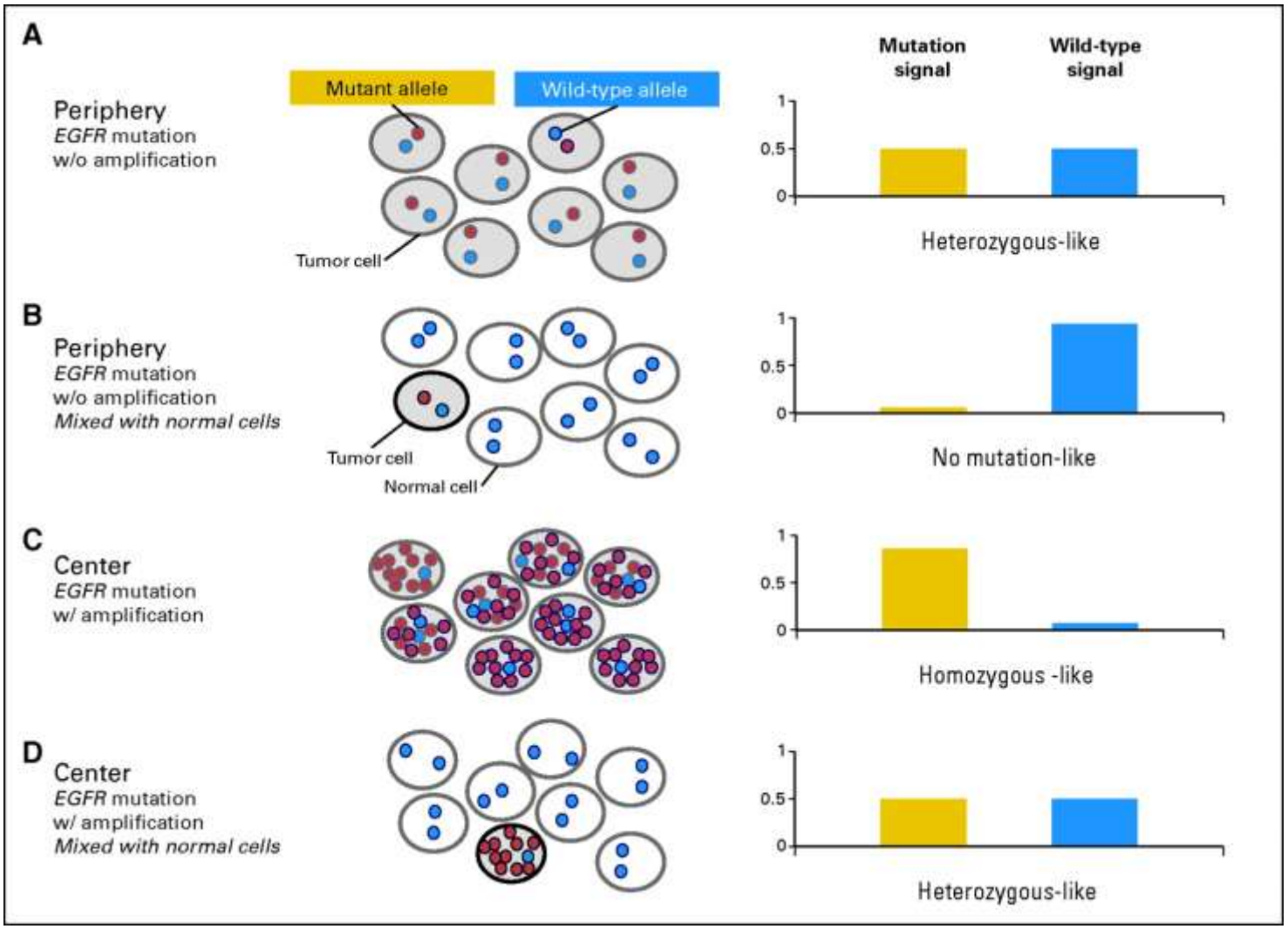


Yatabe Y et al. JCO 2011;29:2972-2977

EGFR mutation and amplification

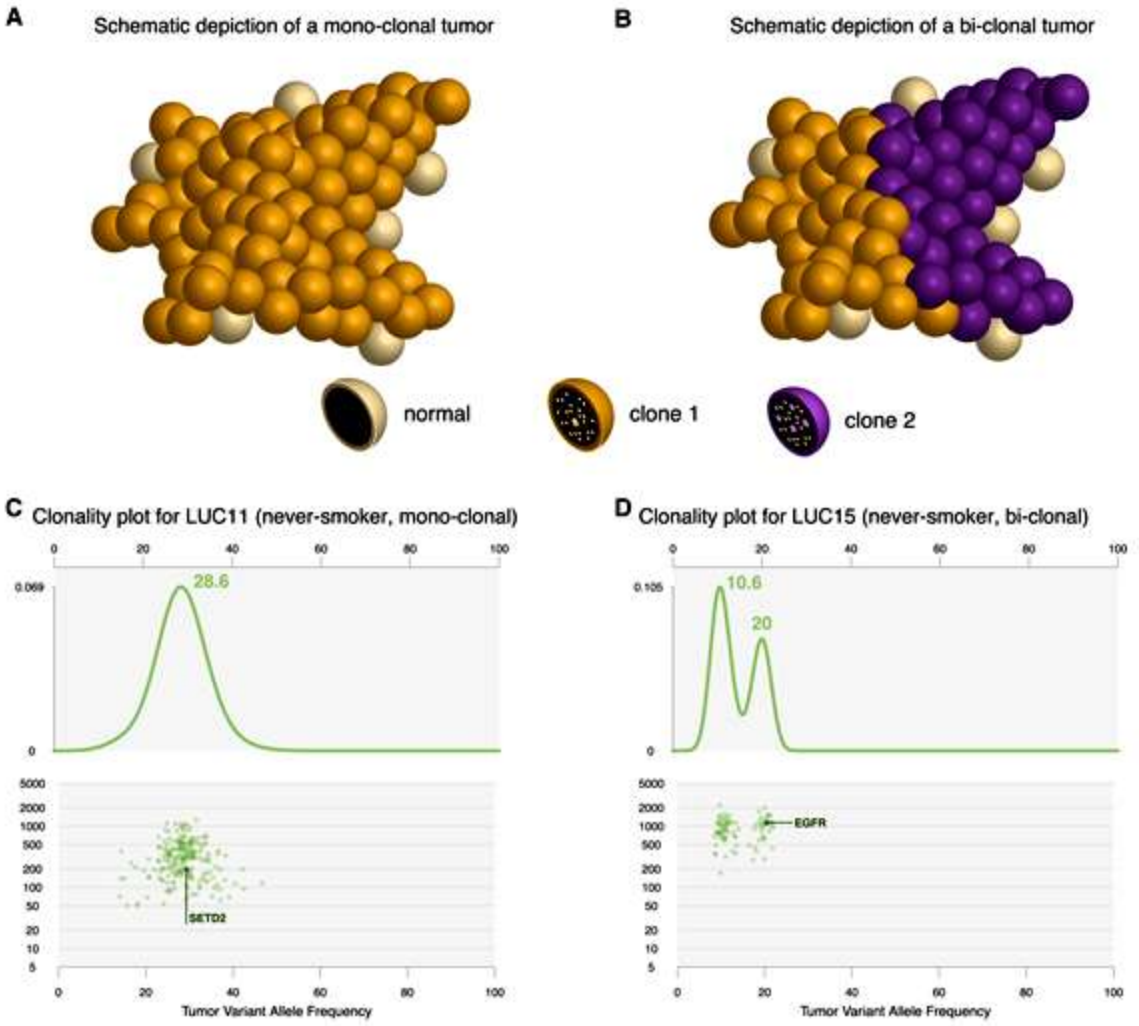


Pseudoheterogeneity of *EGFR* mutations in lung adenocarcinoma



Yatabe Y et al. JCO 2011;29:2972-2977

Tumor heterogeneity revisited by deep sequencing

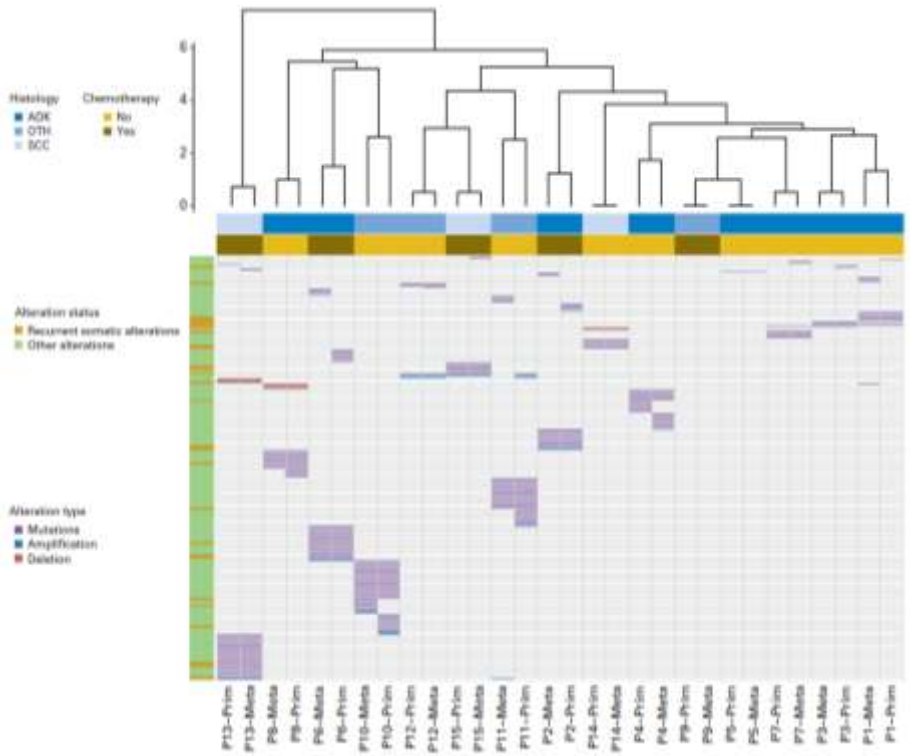


Cell 2012; 150:1121.

HIGH CONCORDANCE BETWEEN PRIMARY TUMORS AND METASTASES

Table 4. Concordance Between Primary Tumor and Matched Metastasis for Recurrent Somatic Alterations and Likely Passenger Alterations

Alterations	No. of Evaluated Alterations	Shared	Unshared	Concordance Rate (%)
Mutations				
Recurrent	28	26	2	93
Passenger	144	88	56	61
Large structural alterations				
Recurrent	5	5	0	100
Passenger	15	7	8	40
Global				
Recurrent	33	31	2	94
Passenger	159	95	64	63



Vignot S. JCO 2013; 31(17); 2167

PRIMARY TUMOR VS. METASTASES CAP/IASLC/AMP recommendation

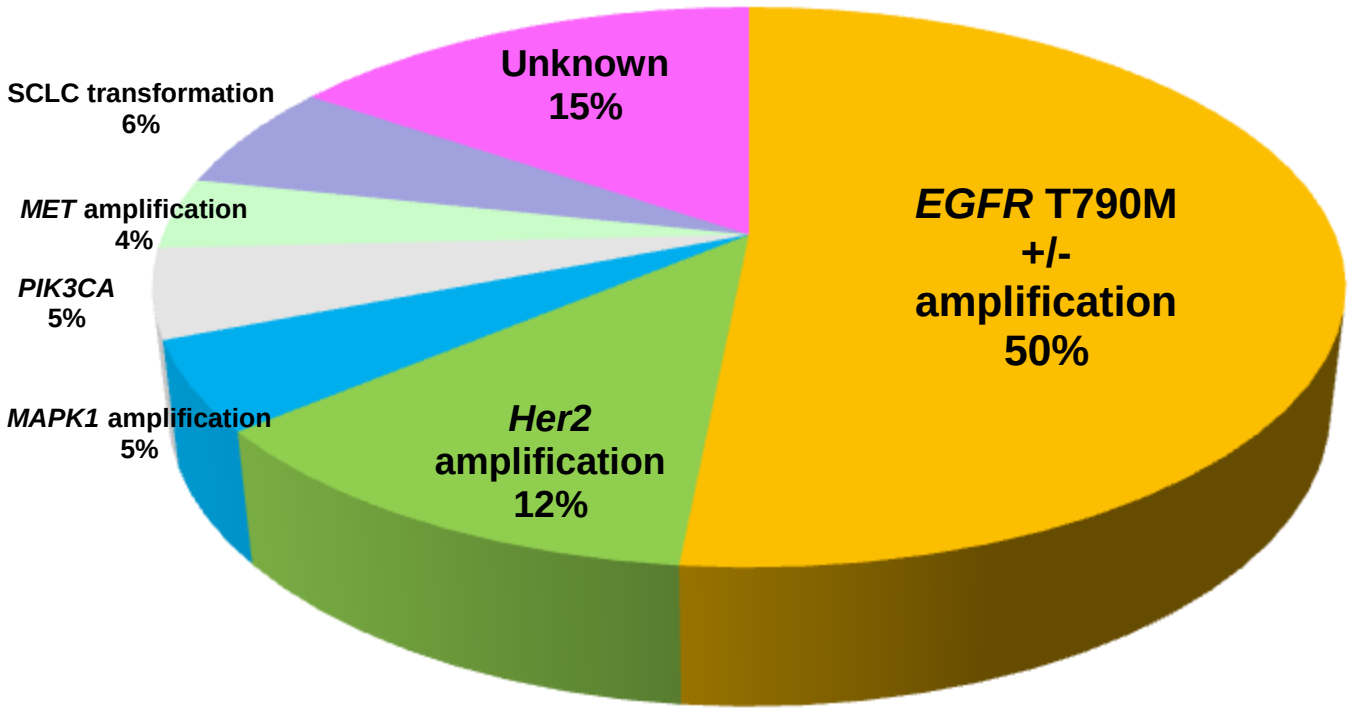
- The choice of primary vs. metastatic tumor should be based on the sample qualities (tumor content and preservation)

MULTIPLE PRIMARY LESIONS
CAP/IASLC/AMP recommendation

- Both separate primary tumors should be tested if tissue available

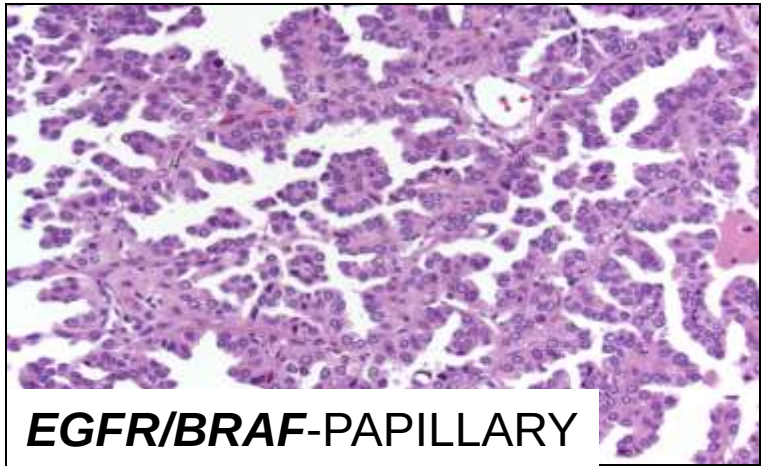
- Testing of multiple areas of a single primary tumor is not recommended

EGFR inhibitor acquired resistance

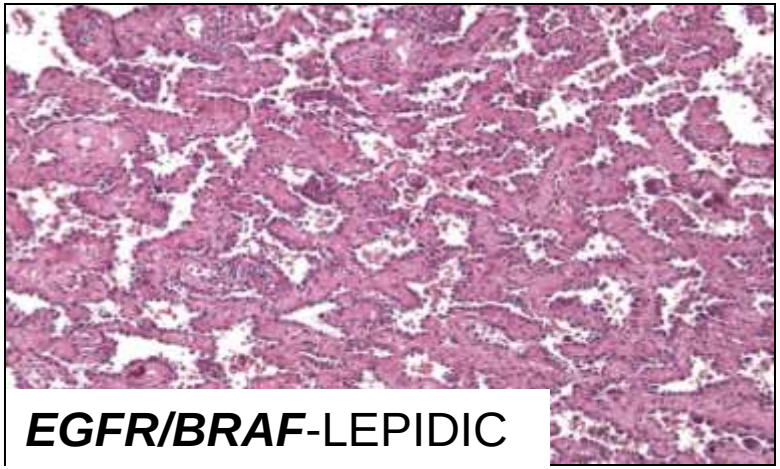


CAN HISTOLOGIC SUBTYPE OF LUNG ADENOCARCINOMA PREDICT MUTATION PROFILE?

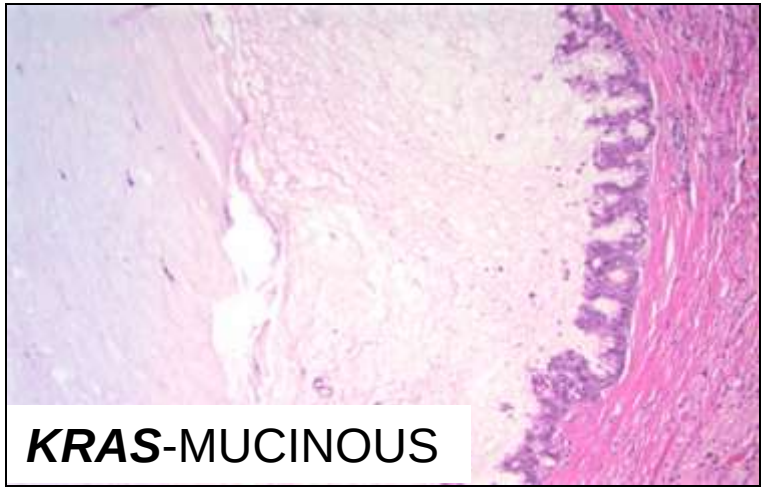
MUTATION and MORPHOLOGY



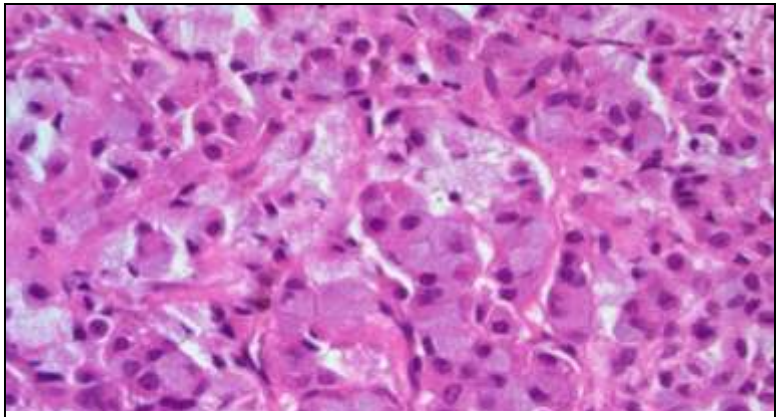
EGFR/BRAF-PAPILLARY



EGFR/BRAF-LEPIDIC

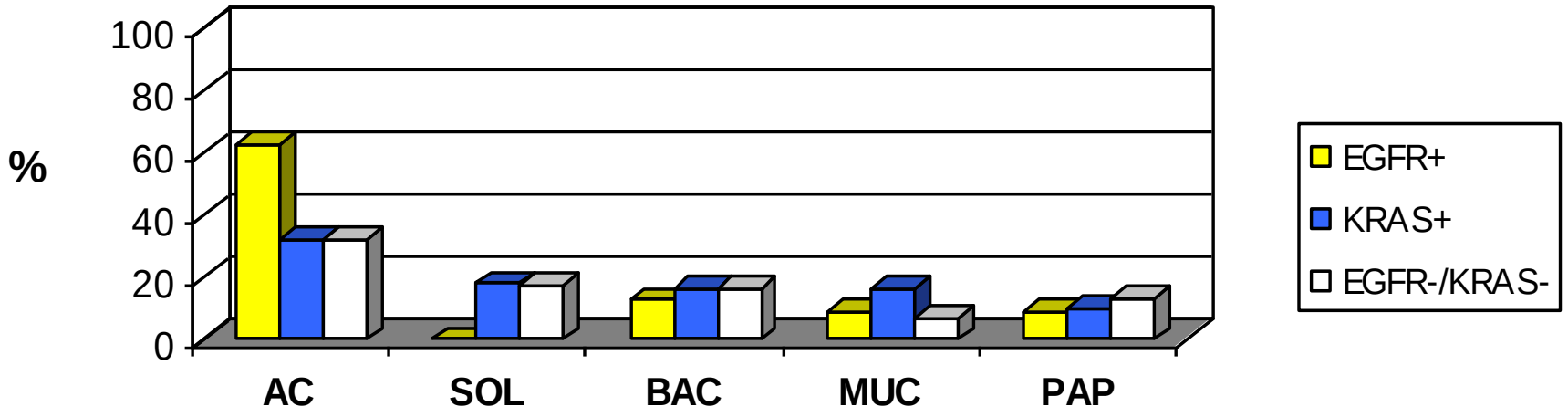


KRAS-MUCINOUS



ALK-SOLID WITH SIGNET RING

PRIMARY HISTOLOGIC PATTERNS IN MIXED SUBTYPE ADENOCARCINOMAS AND MUTATION TYPE

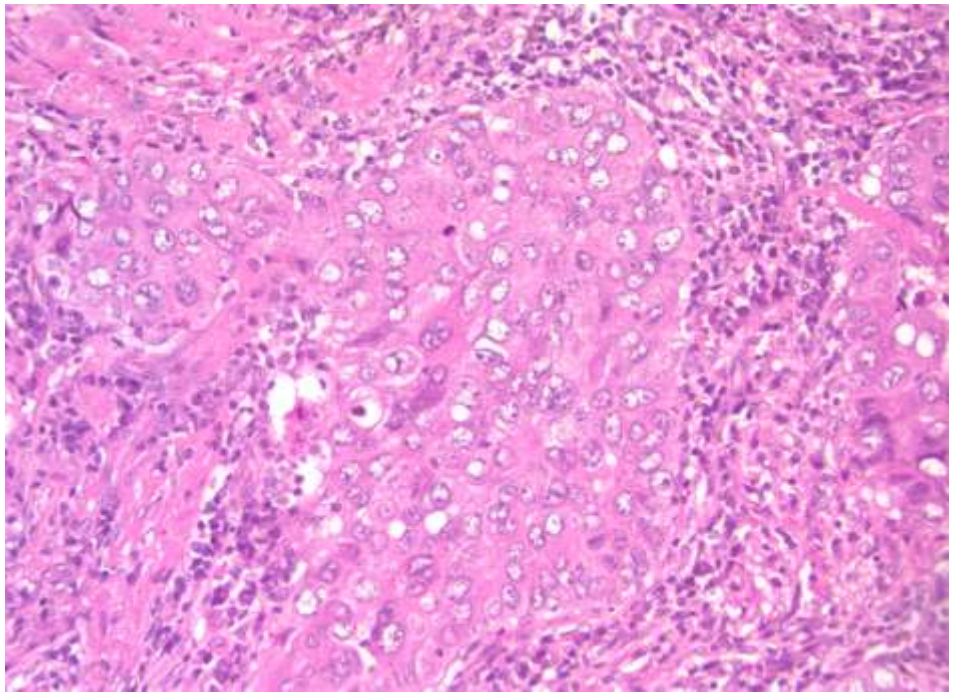


AC- acinar; SOL-solid; BAC-bronchioloalveolar; MUC-mucinous; PAP-papillary

Dacic S. et al. Mod Pathol 2010;23(2):159-68.

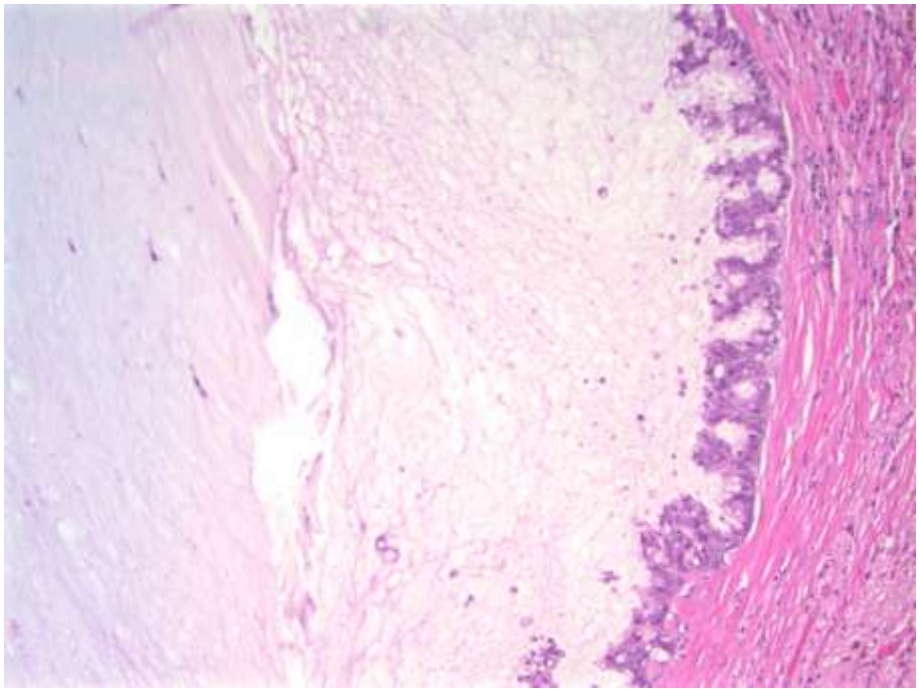
MORPHOLOGIC PREDICTORS OF MUTATIONAL PROFILE

***EGFR* + predictor**



Absence of solid growth pattern
OR 0.024; 95% CI <0.001-0.825
P=0.0388

***KRAS* + predictor**

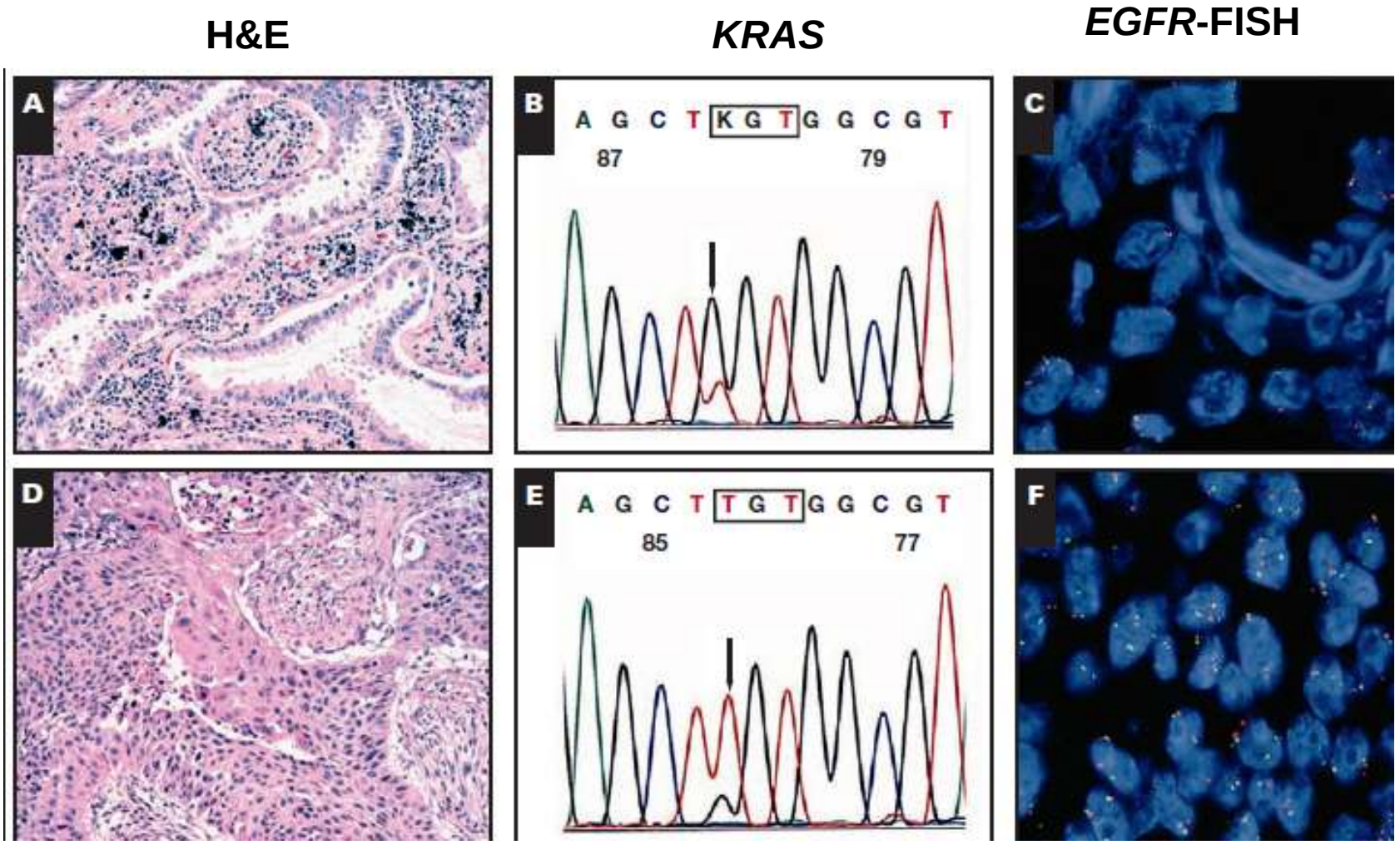


Mucinous growth pattern
OR 3.938; 95% CI 1.574-9.852
P=0.001

Dacic S. et al. Mod Pathol 2010;23(2):159-68.

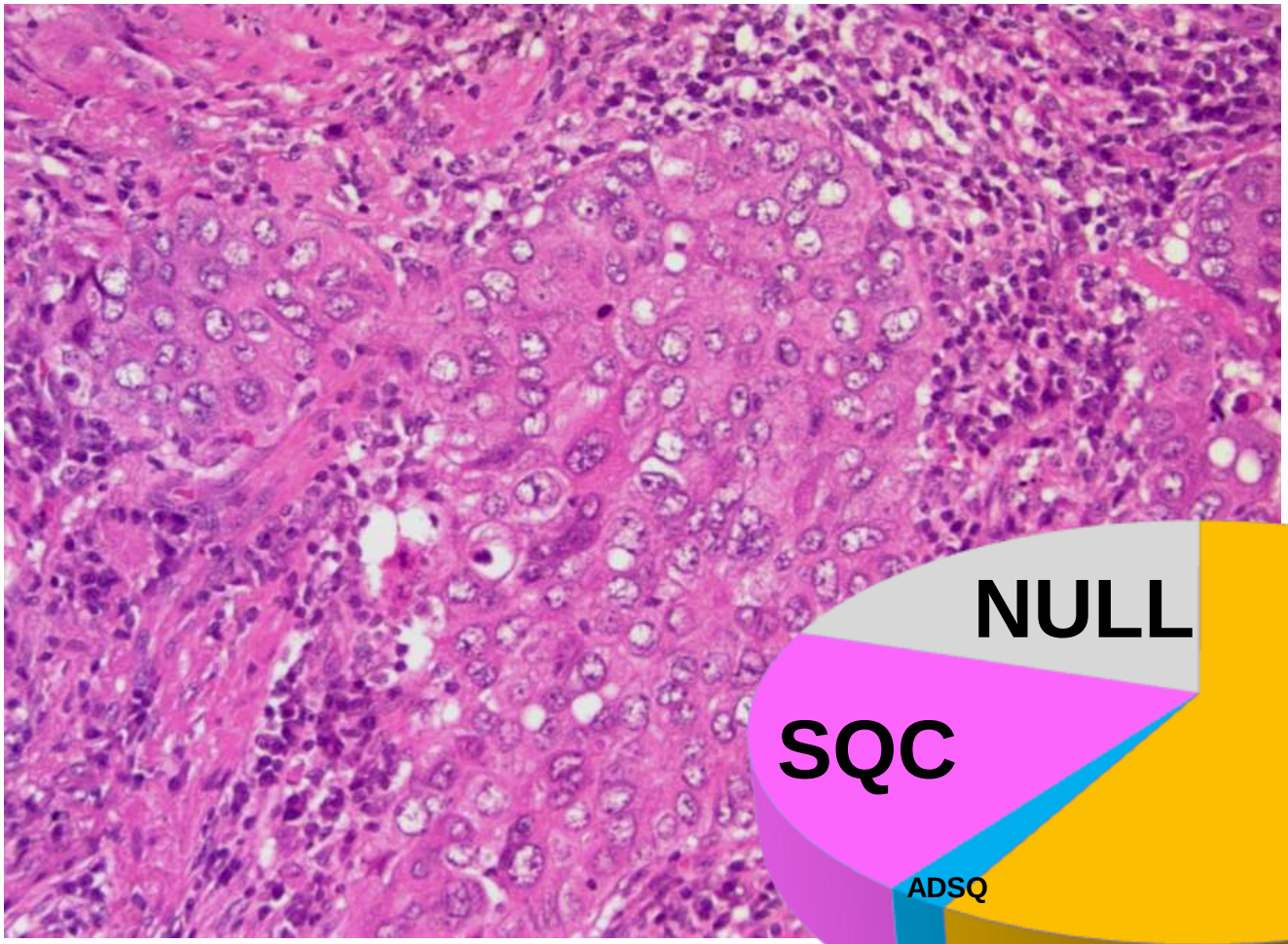
WHAT ABOUT OTHER NSCLC WITH GLANDULAR DIFFERENTIATION?

KRAS/EGFR in adenosquamous carcinoma and sarcomatoid carcinomas

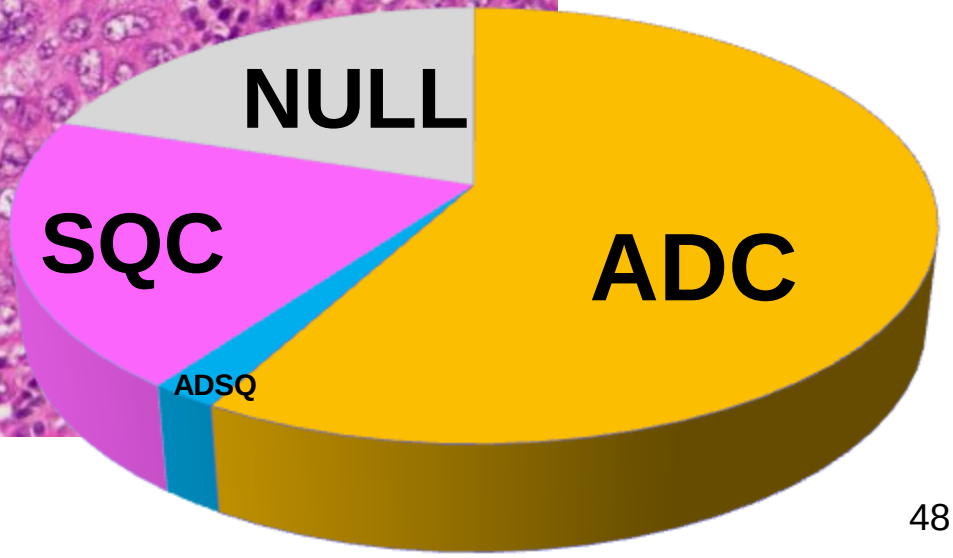


Tochigi et al. AJCP 2011

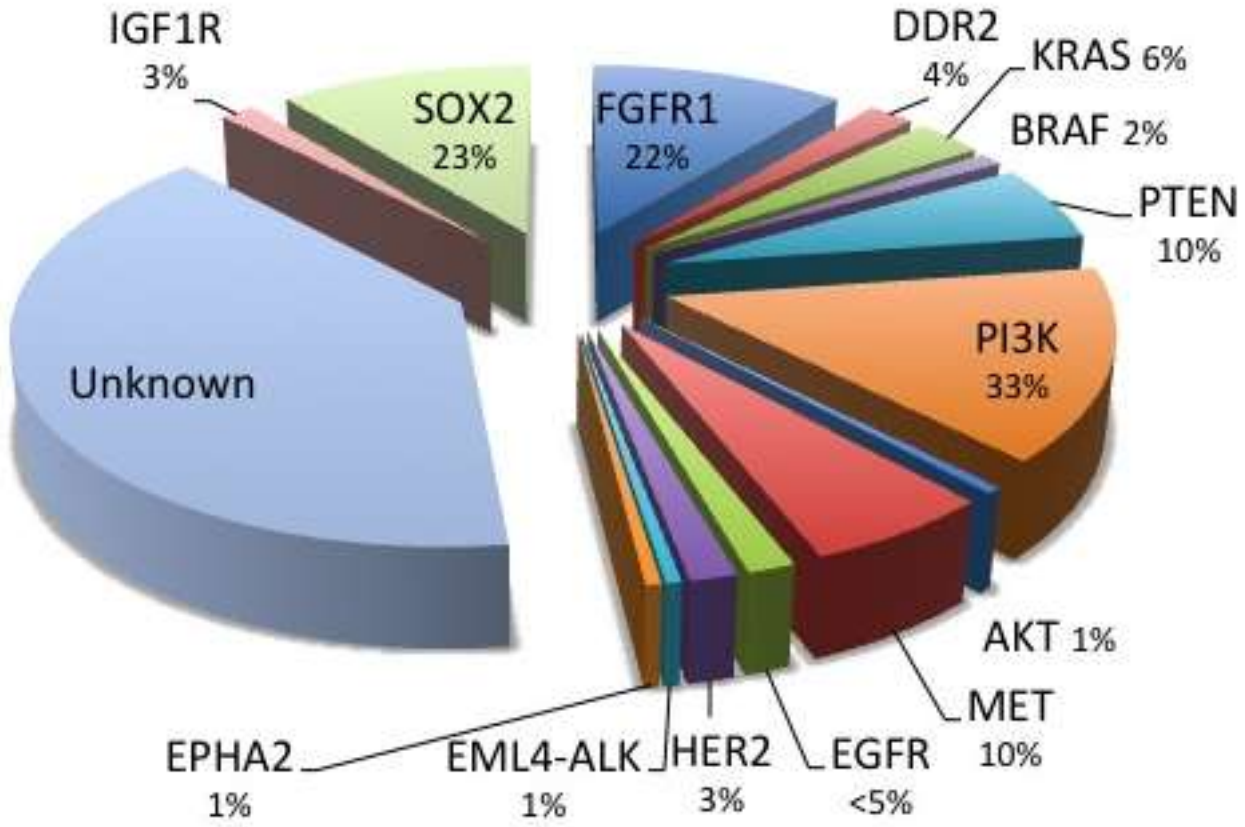
LARGE CELL CARCINOMA



- TTF-1
- P40
- Mucin

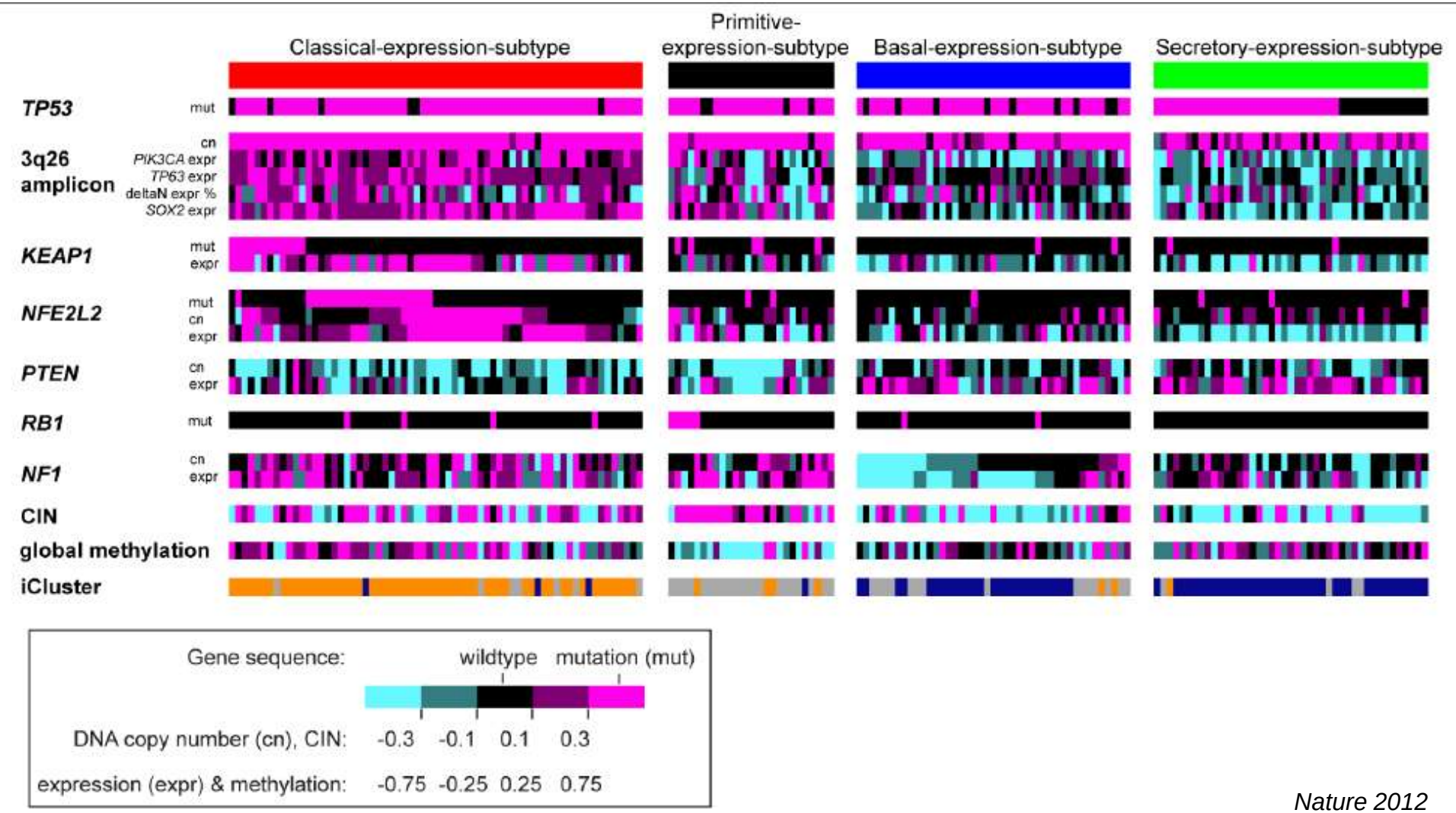


Genetic Alterations in SQC (mutations, amplifications)



Courtesy of Dr. Elisabeth Brambilla

Gene expression subtypes of SQC



Nature 2012

HISTOLOGY AND GENOTYPIC ANALYSIS CAP/IASLC/AMP recommendation

- All NSCLC that contain an adenocarcinoma component, regardless of histologic grade

- Not recommended for pure squamous cell carcinoma, small cell carcinoma or large cell neuroendocrine carcinoma

RESEARCH ARTICLE

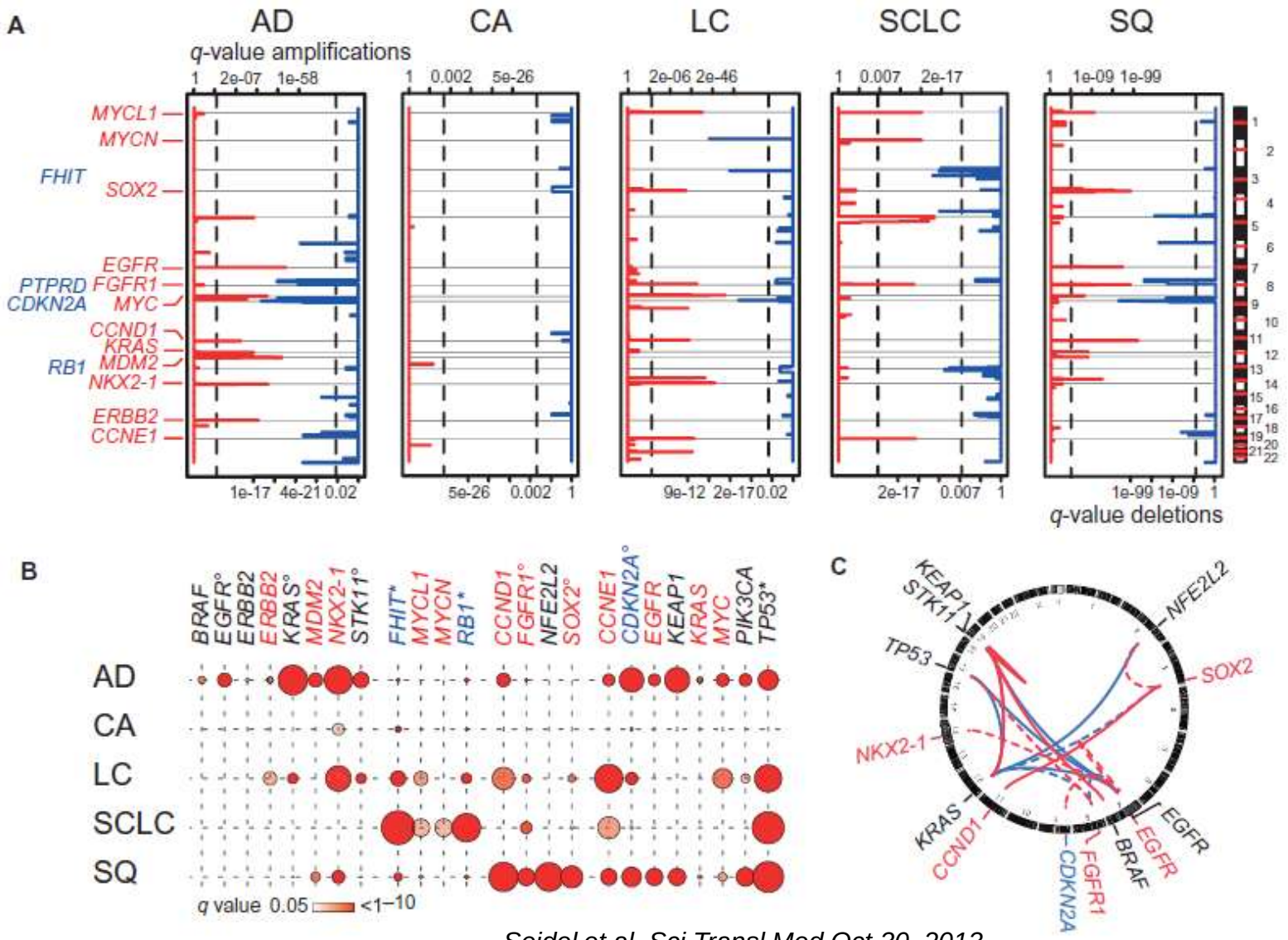
CANCER

A Genomics-Based Classification of Human Lung Tumors

The Clinical Lung Cancer Genome Project (CLCGP) and Network Genomic Medicine (NGM)*†

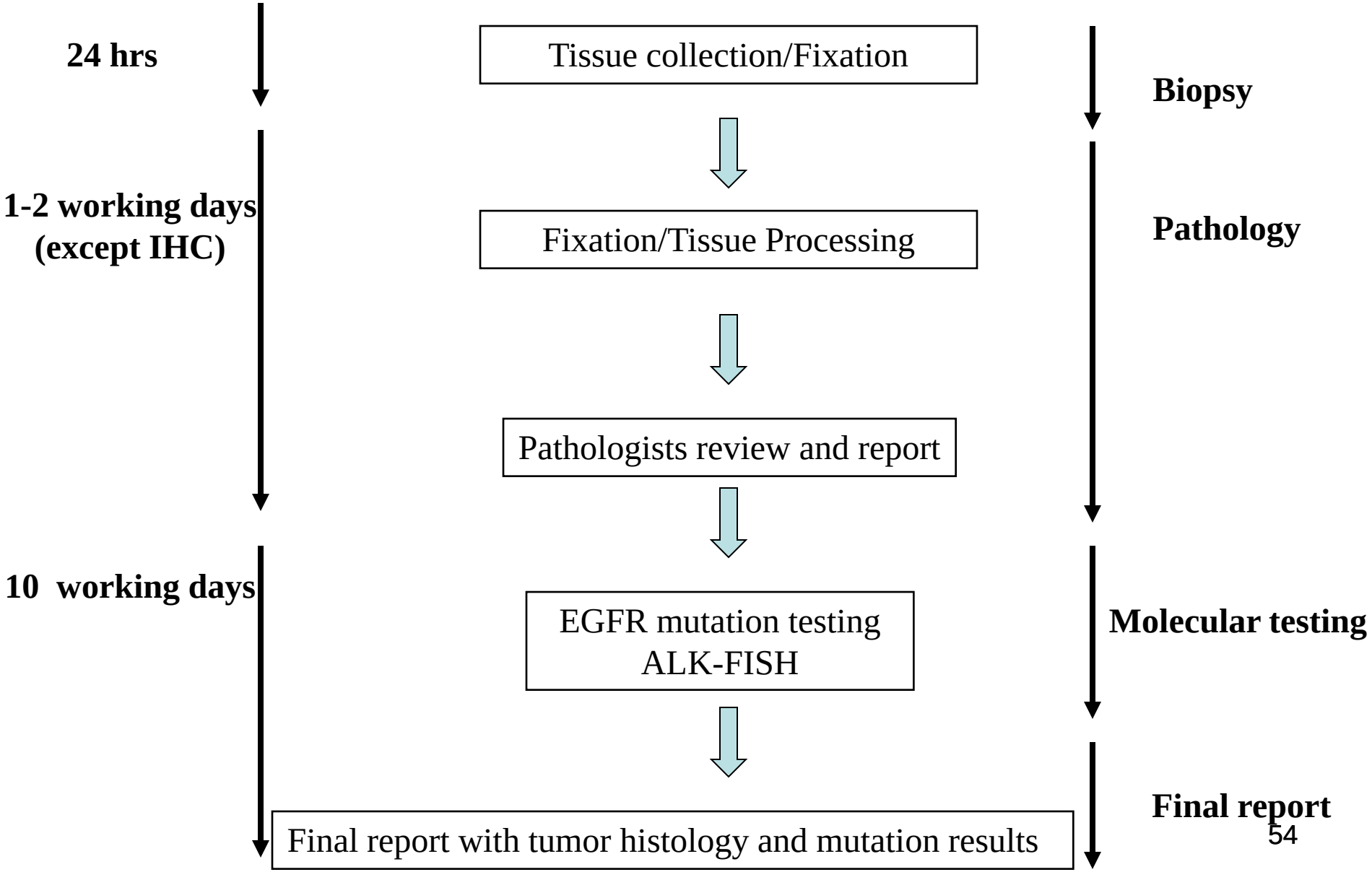
Seidel et al. Sci Transl Med Oct 30, 2013

Genomic alterations and histology



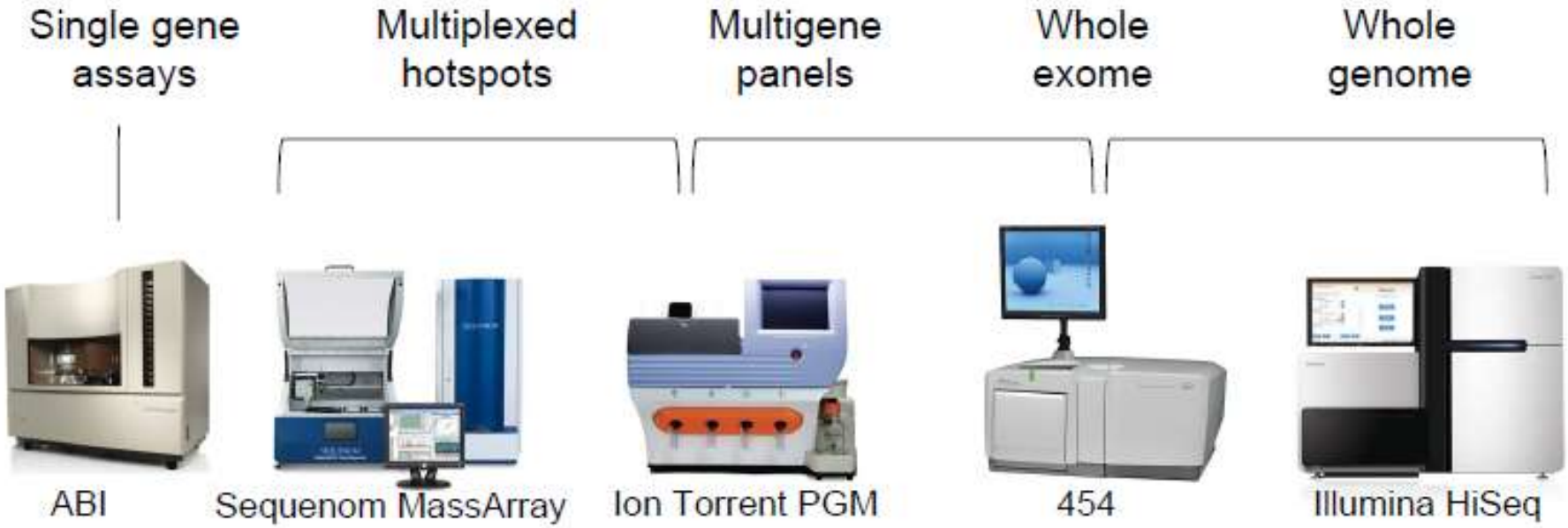
Seidel et al. Sci Transl Med Oct 30, 2013

Routine work up of lung adenocarcinomas



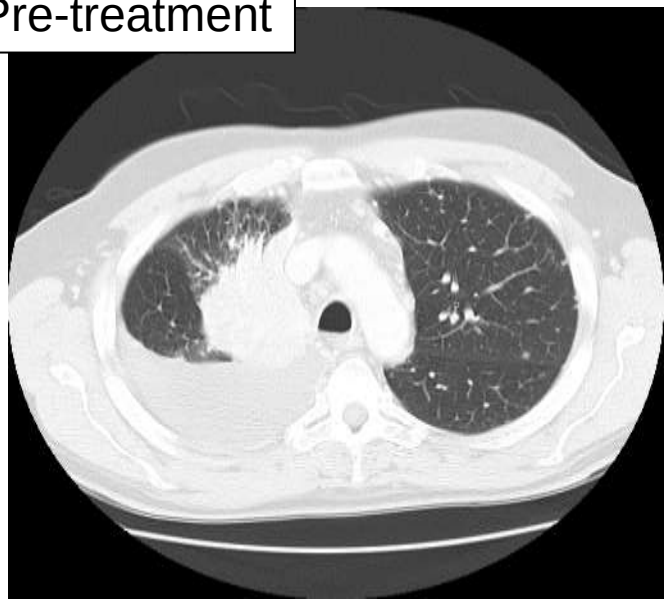
WHAT IS NEXT?

Next Generation Sequencing

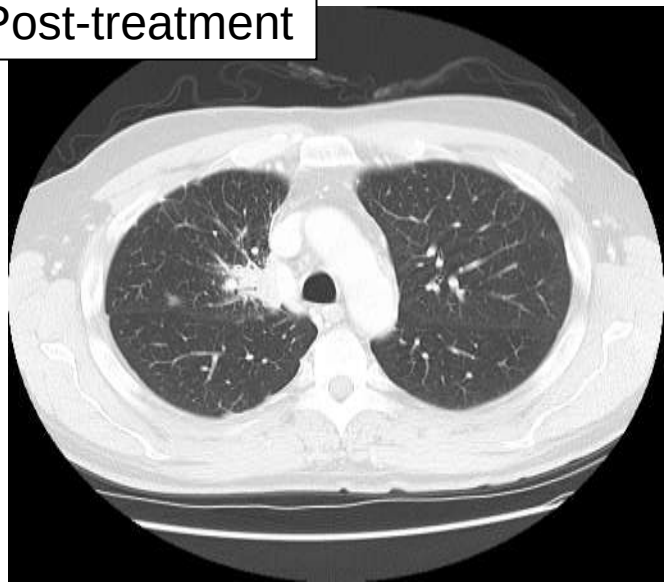


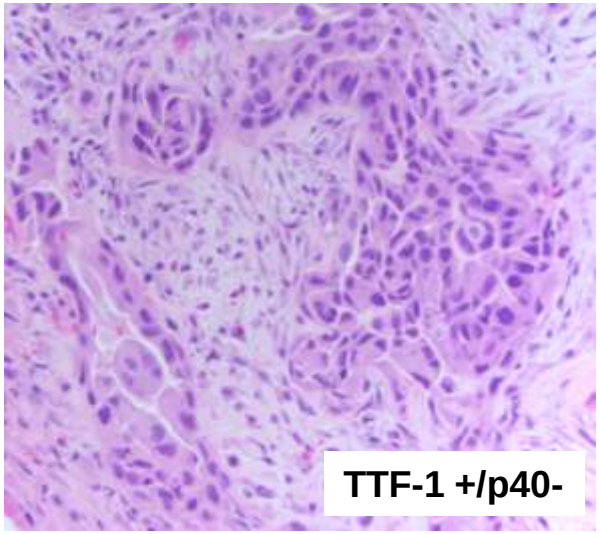
A RECENT CASE –SUCCESS STORY

Pre-treatment

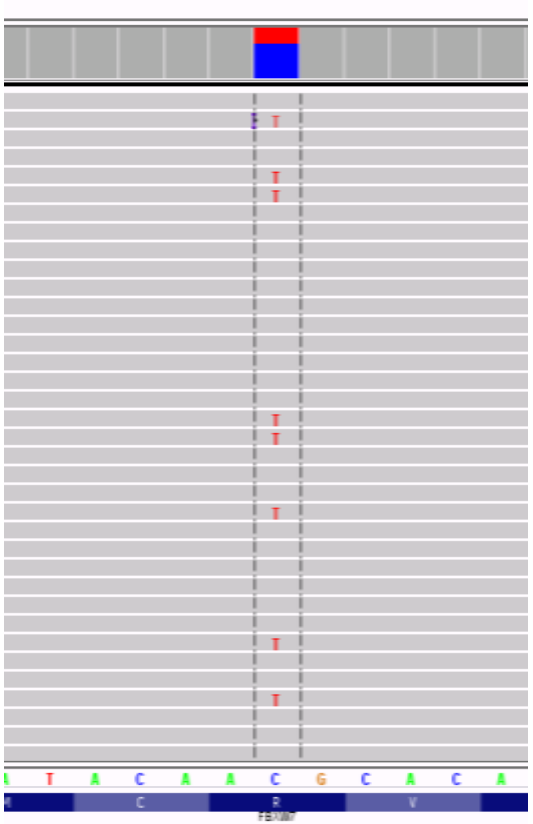


Post-treatment

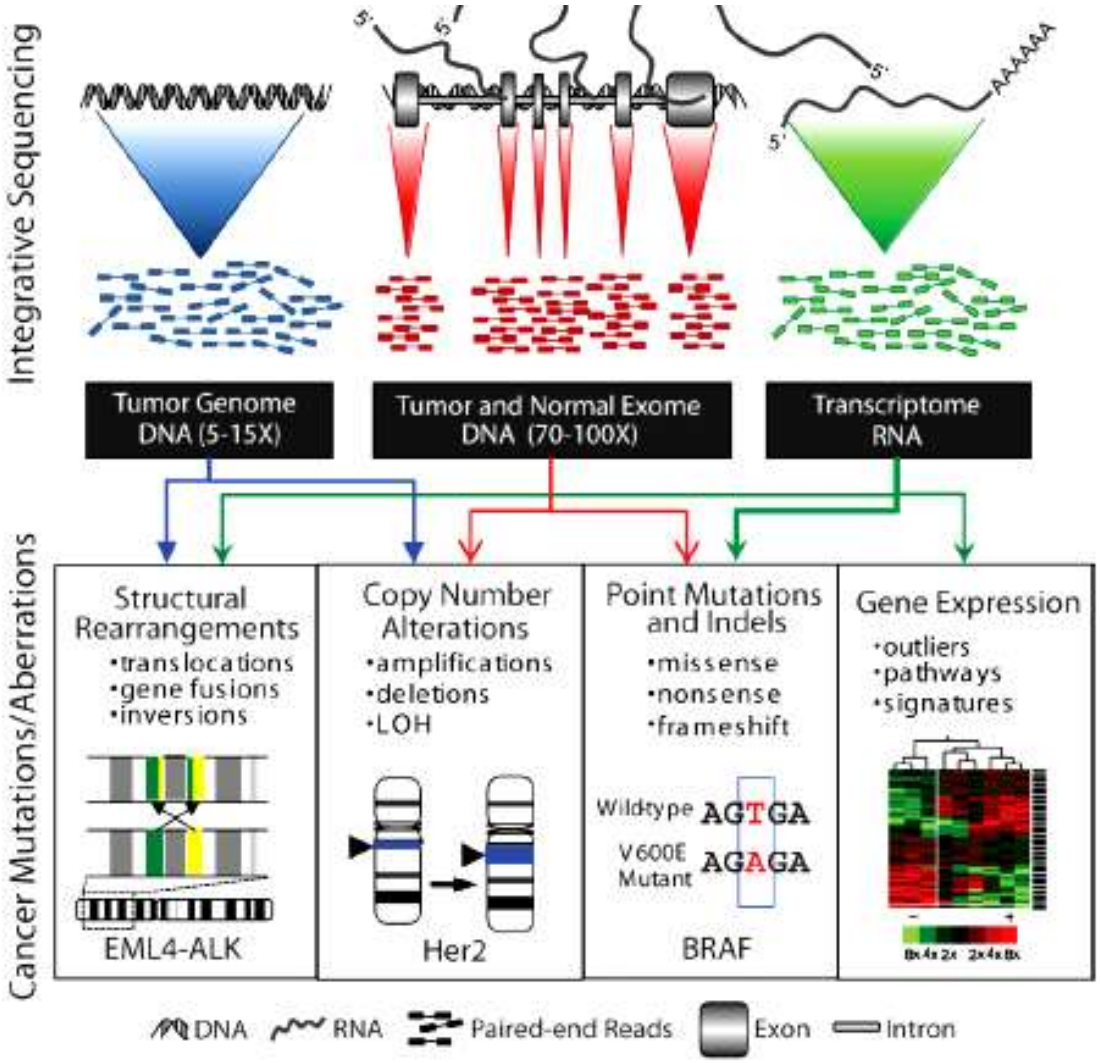


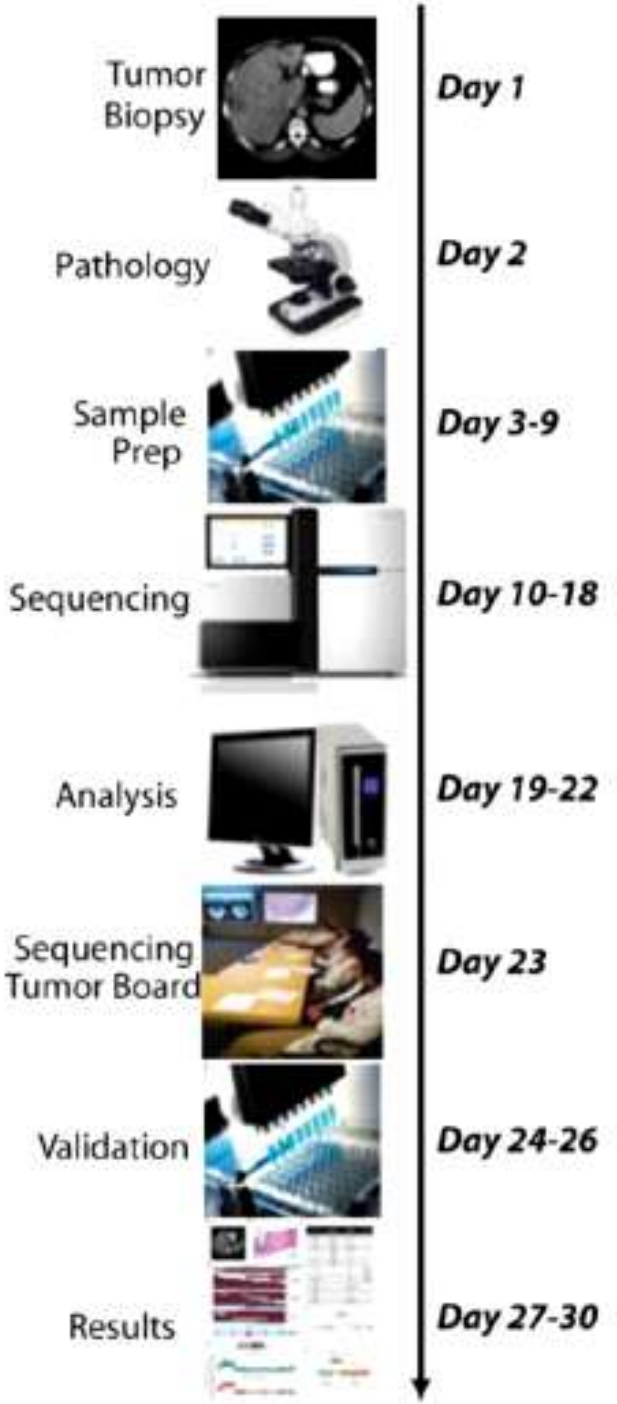


FBXW7 p.R465H
Point Mutation



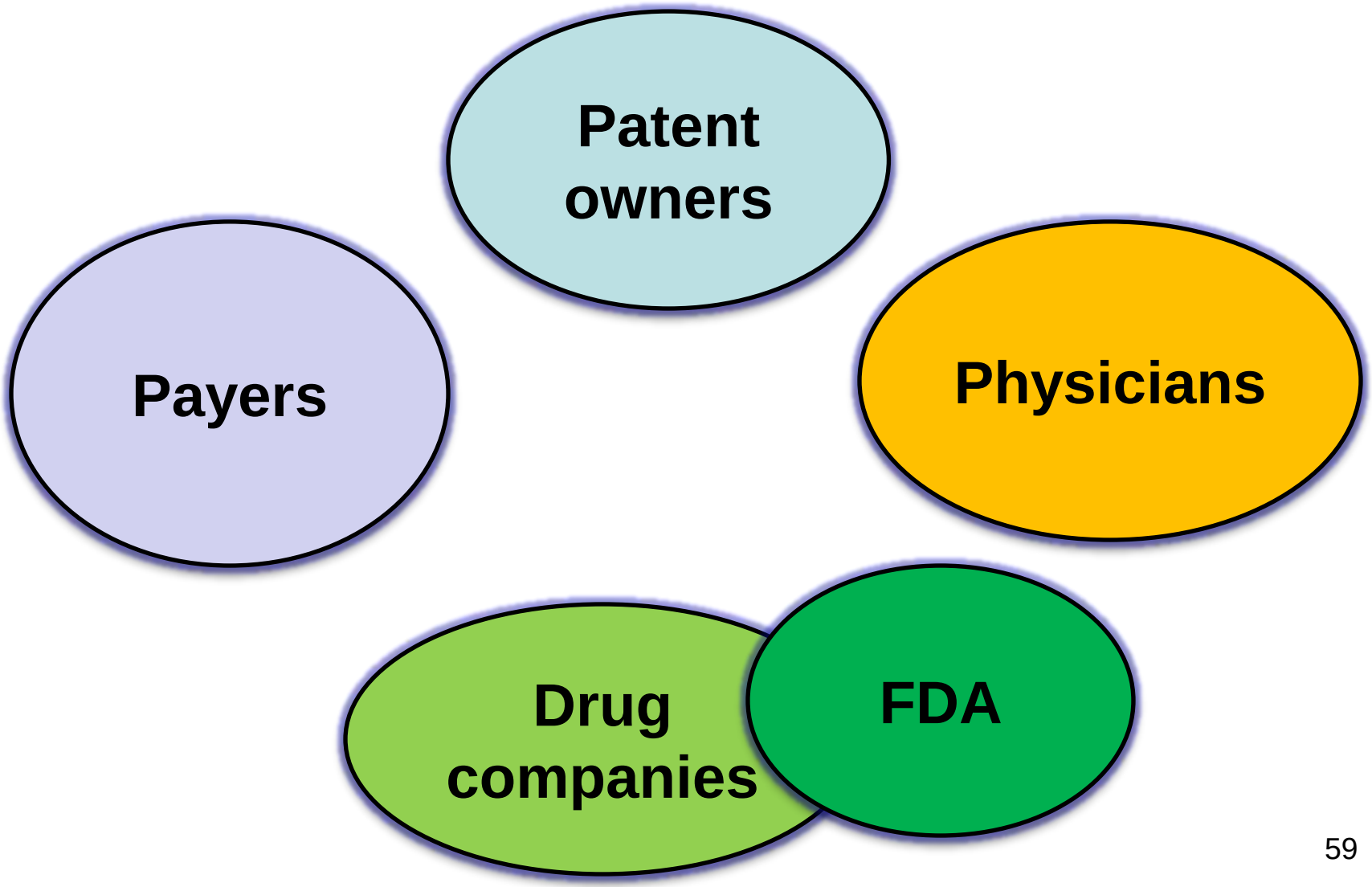
HOW MUCH IS ENOUGH ?





Roychowdhury et al. Sci Transl Med 2011

Obstacles in molecular testing



What does a surgeon/oncologist expect from a pathologist?

- Close interactions, good communication, respect
- Accuracy ⟷ fast response
- Integration of molecular profiling and diagnostic work up of NSCLC
- Team approach!

SUMMARY

- Molecular testing for predictors of targeted therapy response in lung adenocarcinoma must include *EGFR* mutation analysis for exons 19-21 and *ALK*-FISH

- Testing for other molecular biomarkers in NSCLC is not currently indicated for clinical management

- Pathologist must make every effort to spare the tissue for molecular testing after histologic diagnostic evaluation


Thank you