The Role of Pathology in the Era of Targeted Therapy

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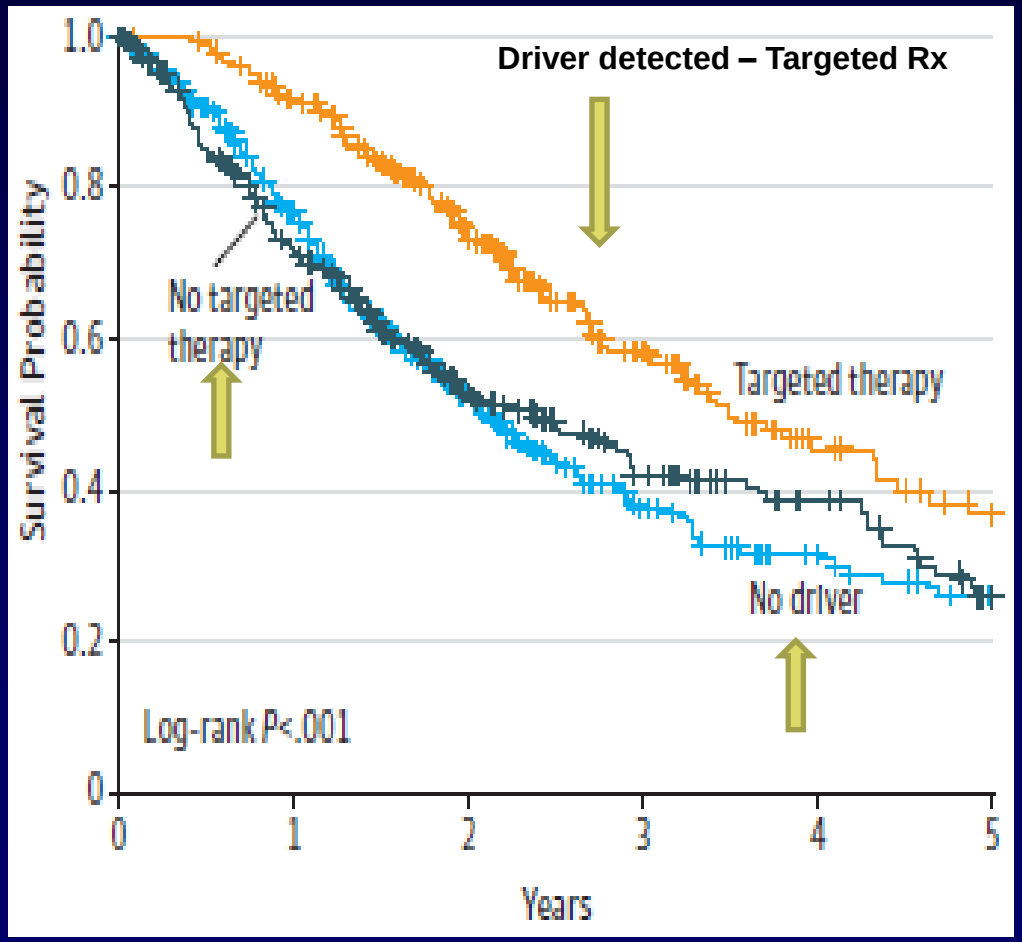
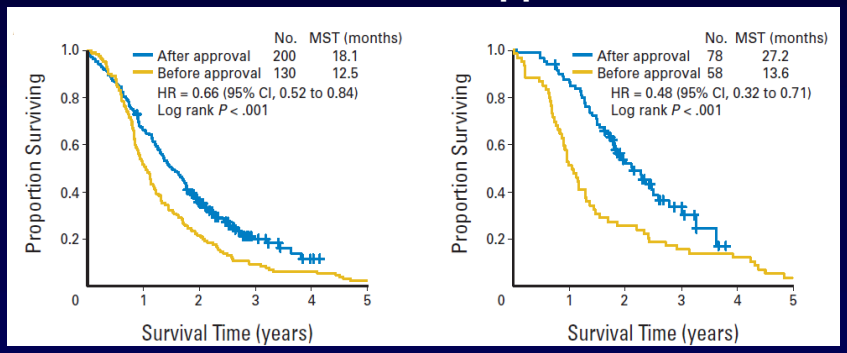
It Is Worthwhile Finding an Actionable Genetic Alteration in Lung Cancer

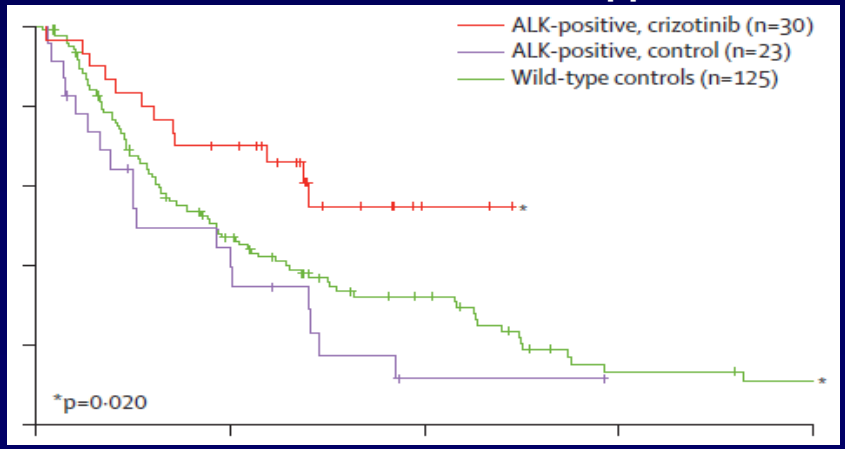
Using Multiplexed Assays of Oncogenic Drivers in Lung Cancers to Select Targeted Drugs[1]



Comparison of Survival for Patients With Lung Adenocarcinoma in Japan Before and After Gefetinib Approval[2]

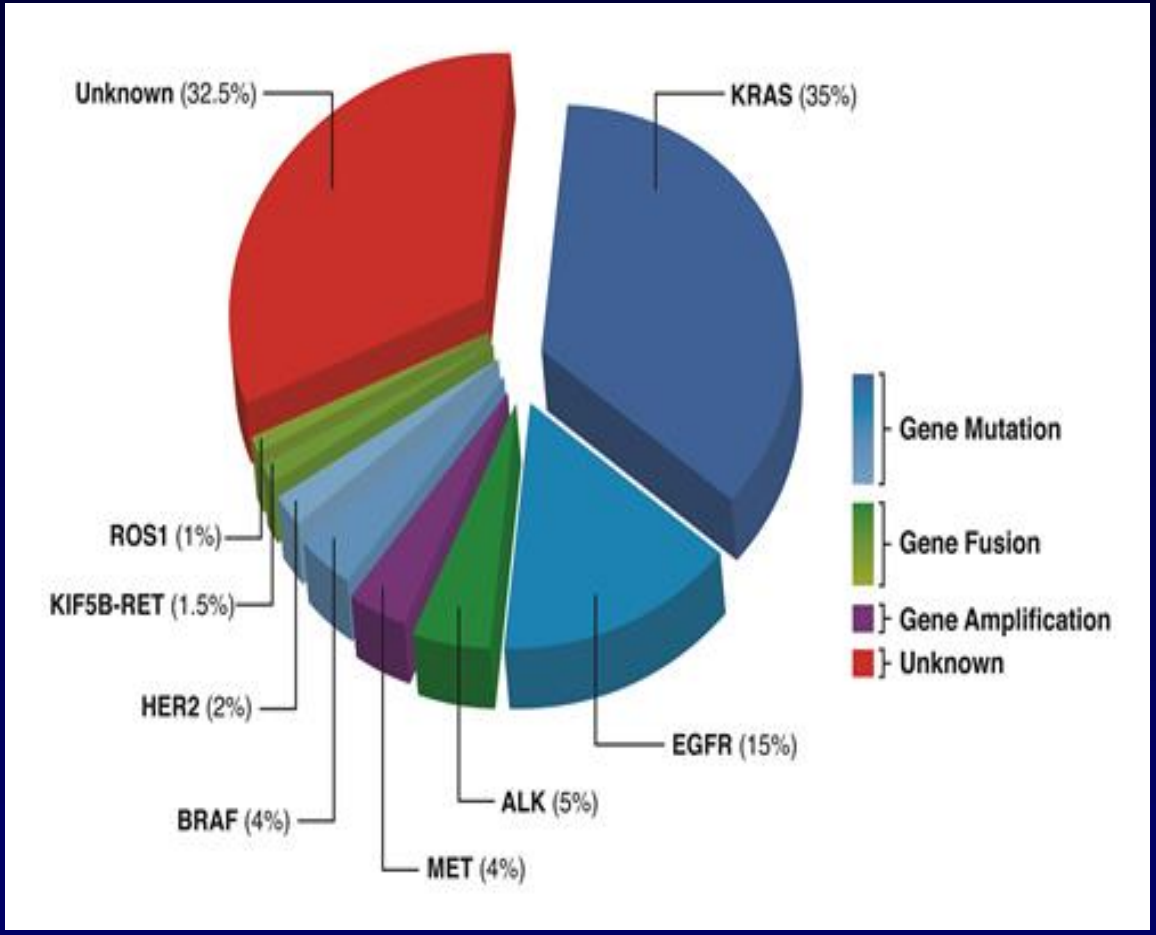


Comparison of Survival for Patients With Lung Adenocarcinoma in Second Line Before and After Crizotinib Approval[3]



1. Kris MG, et al. *JAMA.* 2014;311(19):1998-2006. 2. Takano T, et al. *J Clin Oncol.* 2008;26(34):5589-5595. 3. Shaw AT, et al. *Lancet Oncol.* 2011;12(11):1004-1012.

Oncogene “Drivers” in Adenocarcinoma



NTRK1 fusion

MPRIP-NTRK1 and *CD74-NTRK1*

3.3% of ‘onco-negative’ adenocarcinomas

Trk inhibitors exist

Vaishnavi A, et al. *Nat Med.* 2013;19(11):1469-1472.

***CD74-NRG1* fusion**

Search in ‘onco-negative’ adenocarcinomas

ERBB3 and PI3K-AKT pathway activation

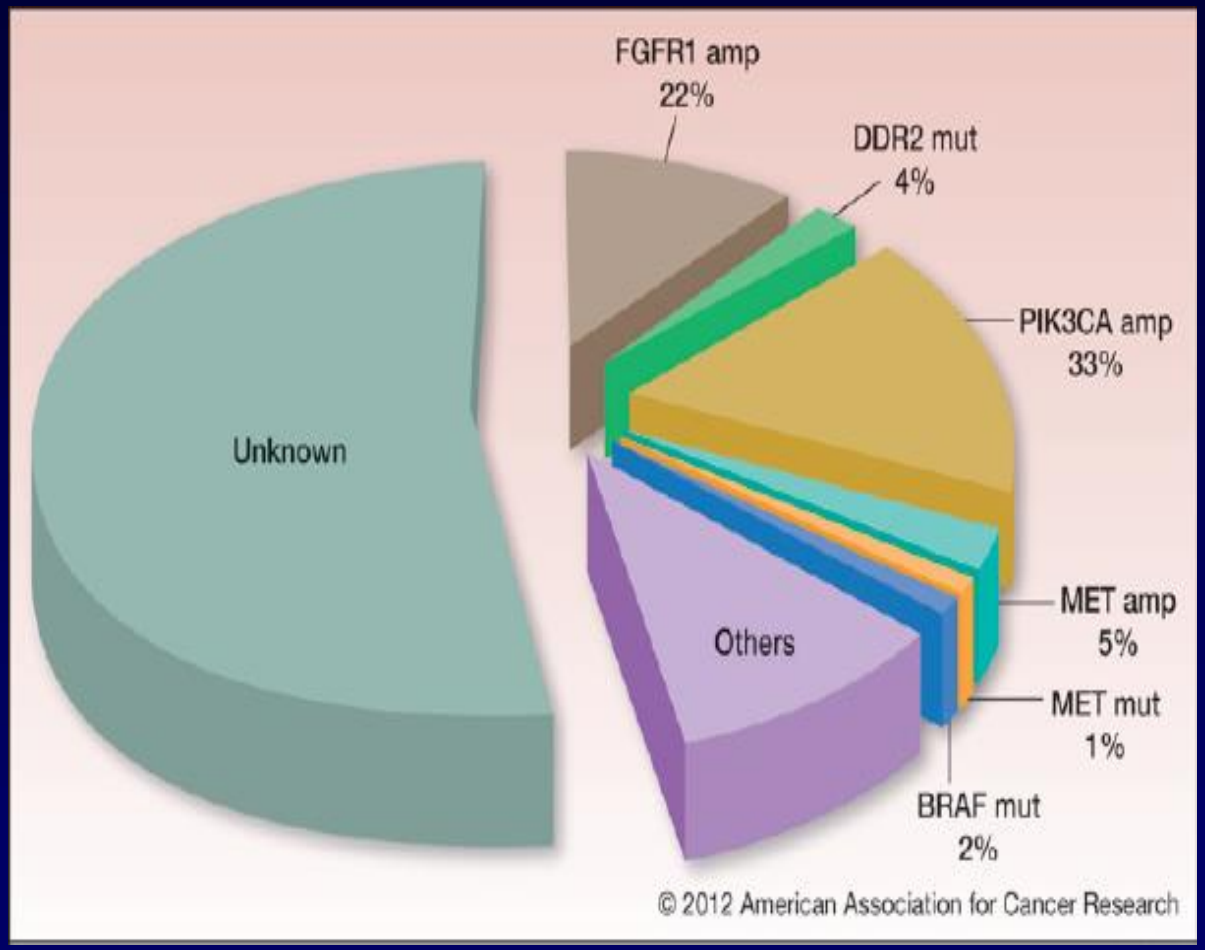
Mucinous adenocarcinomas

Potential therapeutic target

Fernandez-Cuesta L, et al. *Cancer Discov.* 2014;4(4):415-422.

Kerr KM. *J Clin Pathol.* 2013;66(10):832-838.

Squamous Cell Carcinoma of the Lung:
Molecular Subtypes and Therapeutic Opportunities



EGFR
- TKI vs MoAb
- Mutations – rarity (vIII – 8%)
- Targeting the receptor

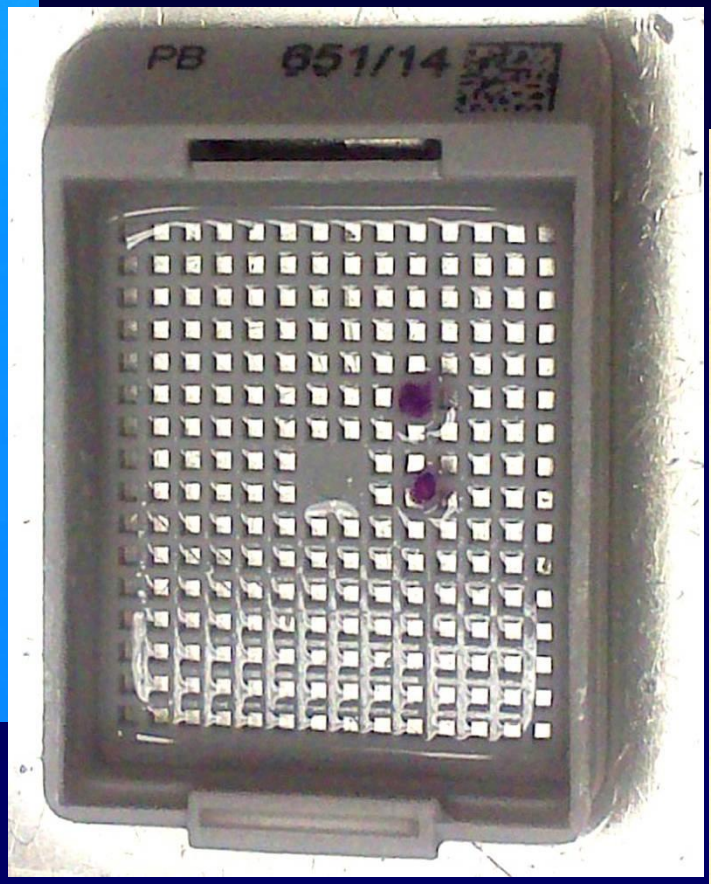
IGFR1
- Figitumumab
- Some effect in squamous
- Toxicity

MoAB, monoclonal antibody; TKI, tyrosine kinase inhibitor

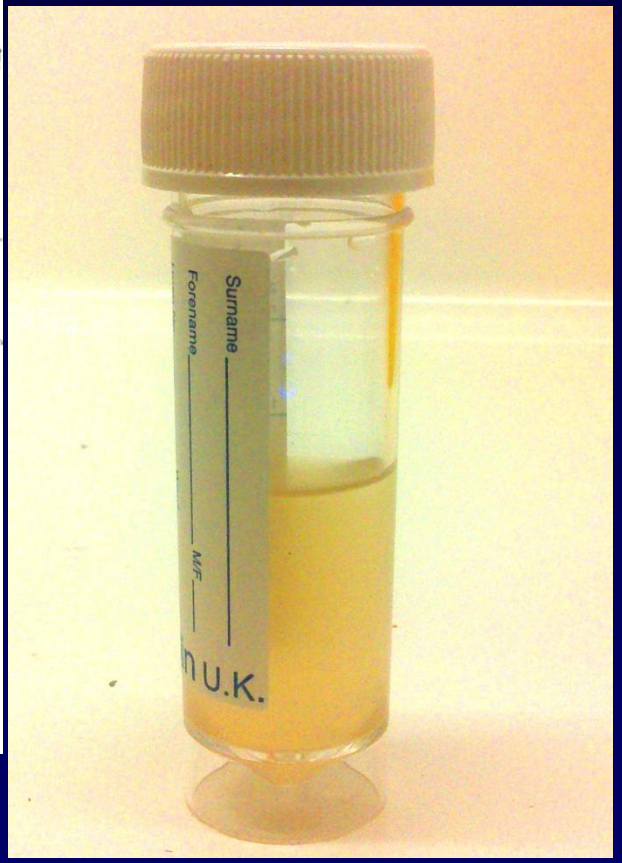
Perez-Moreno P, et al. *Clin Cancer Res.* 2012;18(9):2443-2451.

Most Lung Cancer Samples Are Small Biopsies or Cytology-Type Samples

Surgically resected tumour

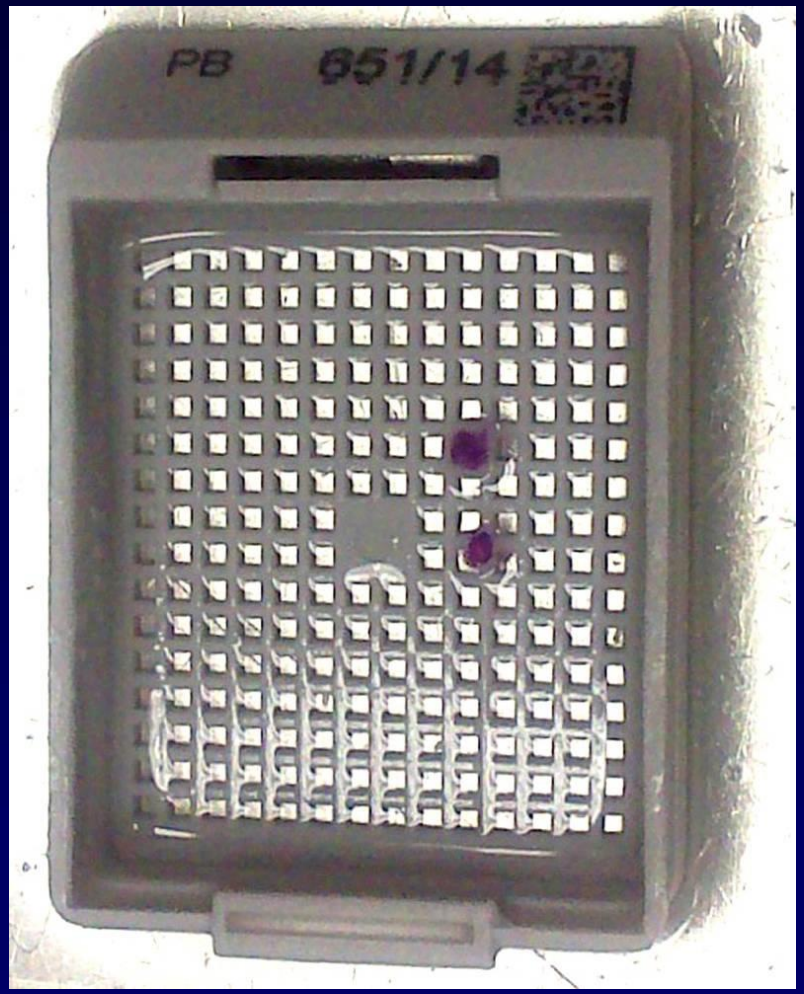


Small biopsy samples



Cytology samples

Is There Enough Material for These Studies?

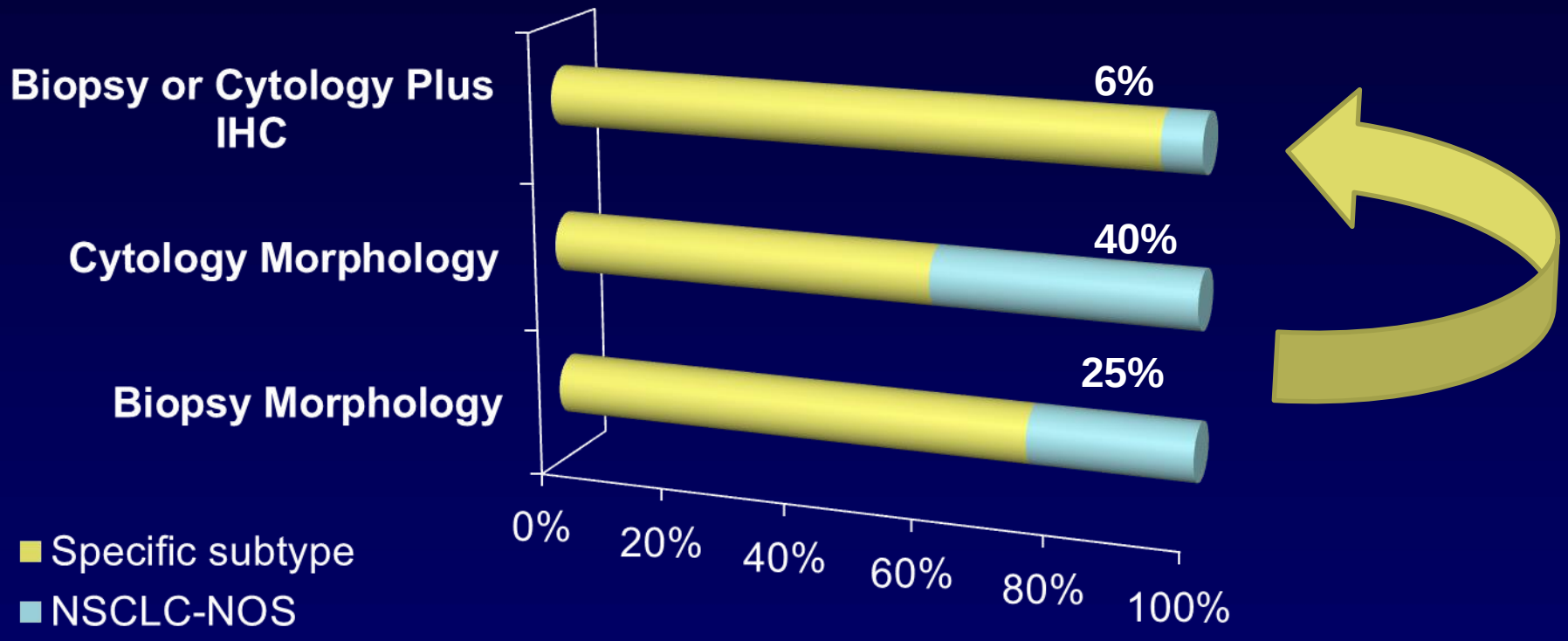


Two biopsy fragments <1 mm

- Morphologic diagnosis
- Immunohistochemistry (IHC)
- Molecular testing
- Conserve tissue
- Don’t waste

On average, only 20% of this tissue is tumour

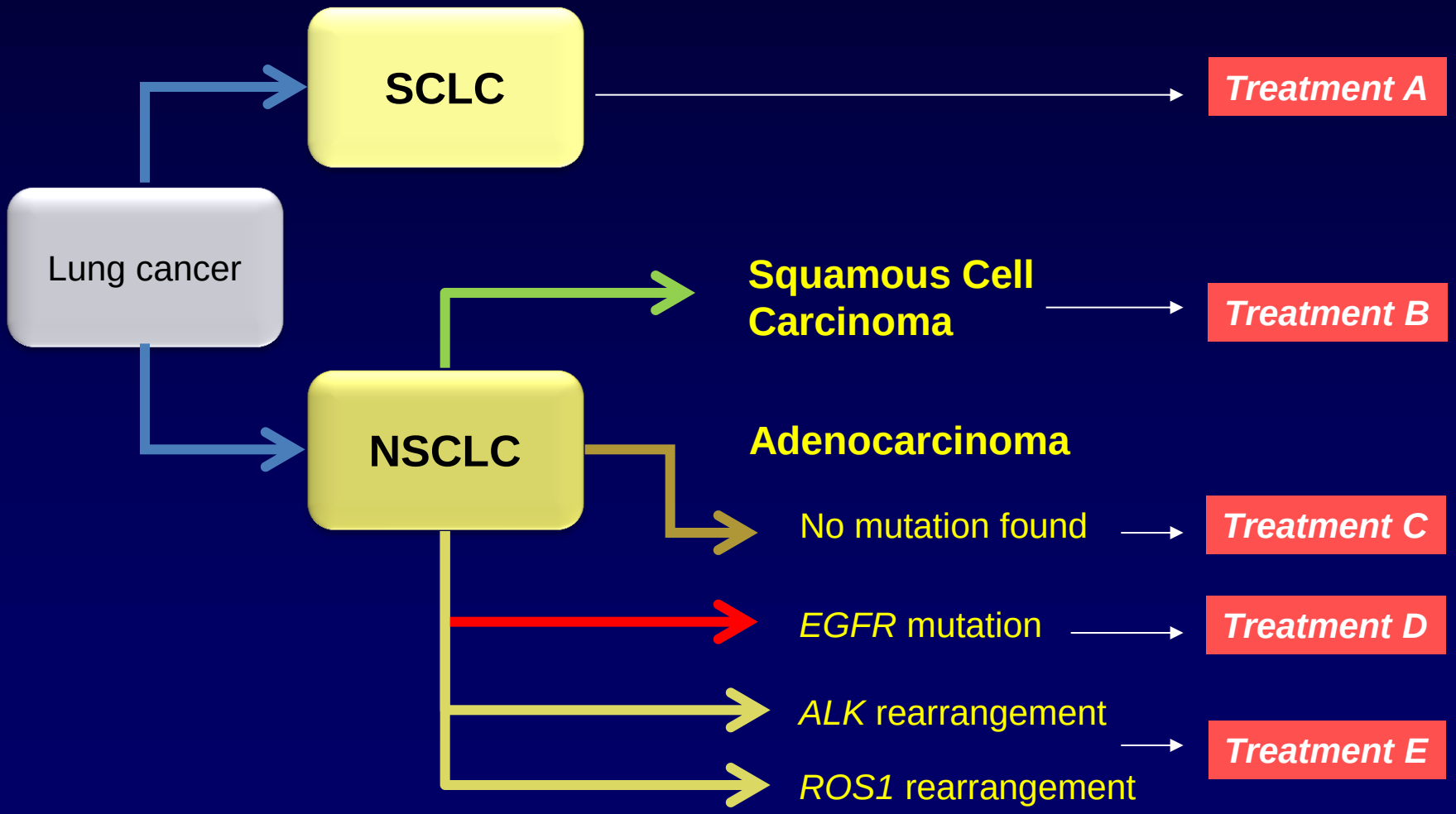
Immunohistochemical (IHC) Subtyping of NSCLC



- **Predictive IHC has ‘levelled the playing field’**
- **Better diagnosis possible on poorer specimens**

NSCLC-NOS, non-small cell lung cancer not otherwise specified

Tumour Histology, Genotype and Treatment of Lung Cancer



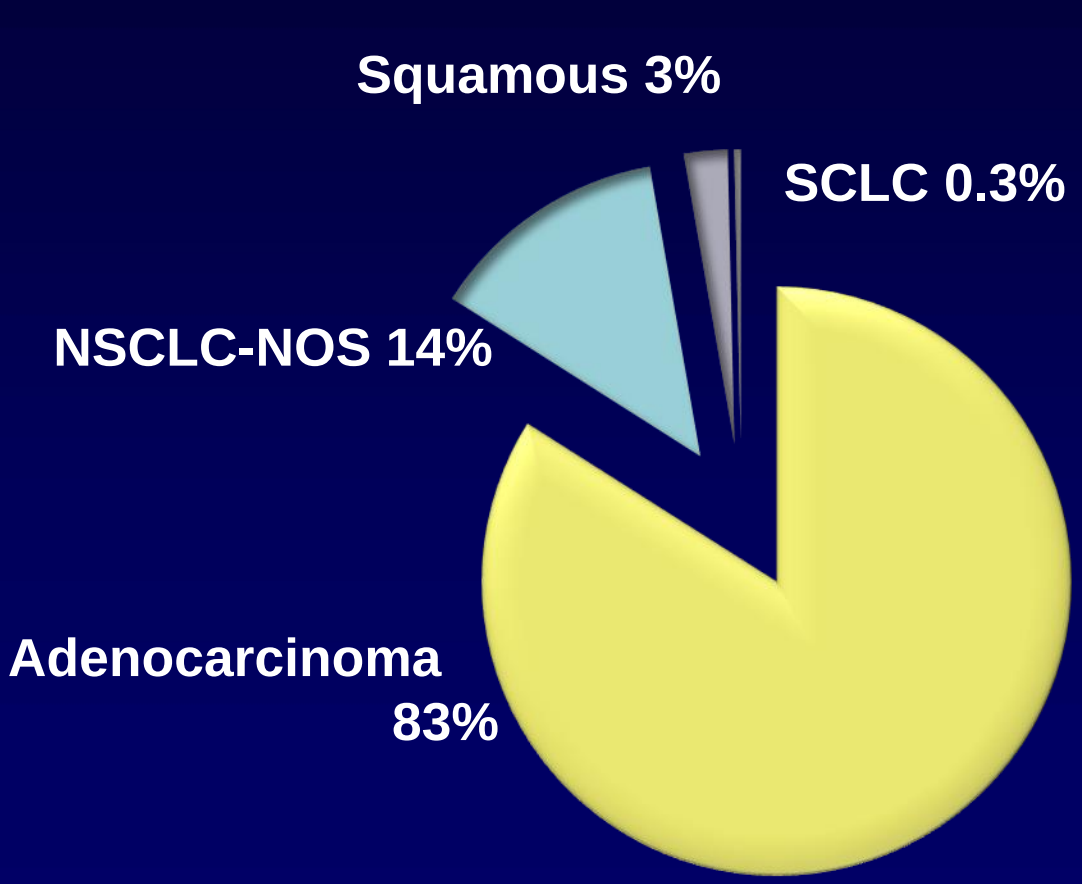
SCLC, small cell lung cancer

Whom Should We Be Testing for *EGFR* Mutation and *ALK* Rearrangement?

- ***All nonsquamous tumours in patients with advanced/recurrent disease should be tested for EGFR mutation and ALK rearrangement***
- *Selected squamous tumours (from patients with minimal or remote smoking history) should strongly be considered for testing*

2nd ESMO Consensus Conference on Lung Cancer: Pathology and Molecular Biomarkers for Non-Small-Cell Lung Cancer. Kerr KM, et al. *Ann Oncol.* 2014;25(9):1681-1690.

Which Tumours Do We Test for *EGFR* Mutation and *ALK* Arrangement?



Squamous and adenoca cases include ‘probable’ cases on IHC

What Do We Use for the Test?

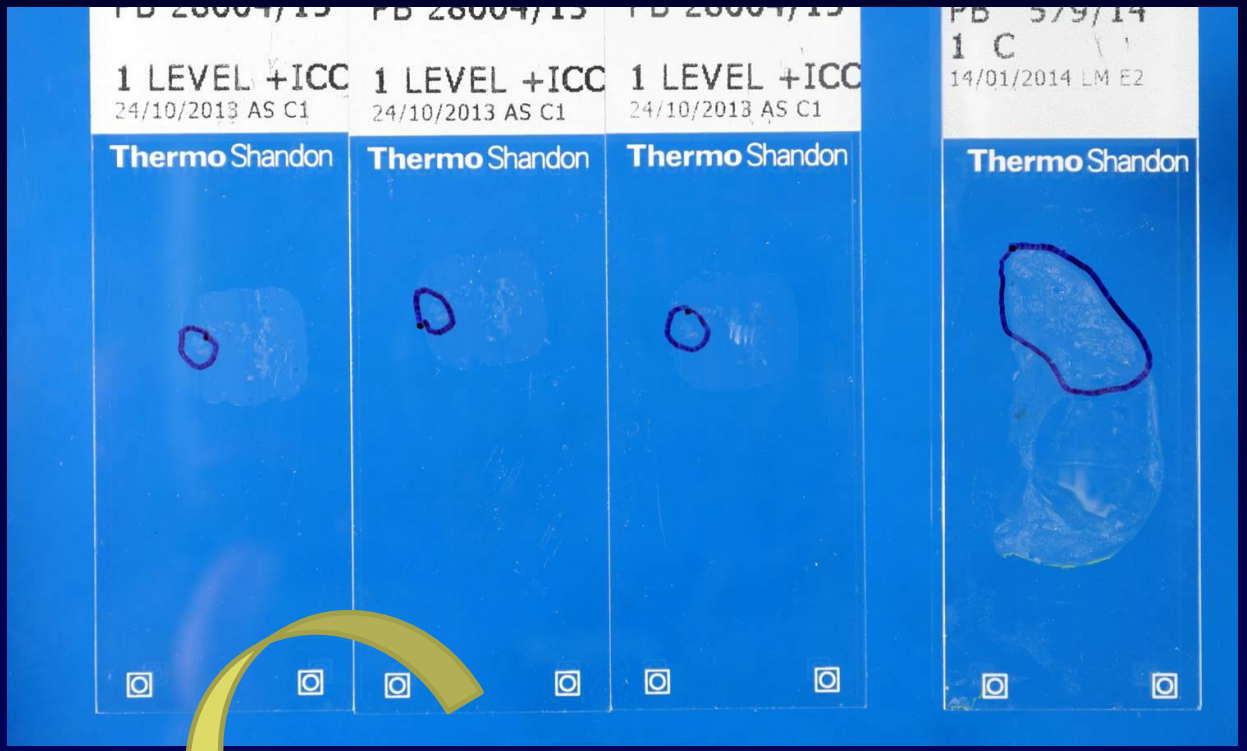
- Whatever is available – we need tumour cells!!!
- Tissue or cytology cell block sections
  - Maximise tumour cells &…….
  - Minimise nontumour cells in material submitted for DNA extraction
    - >10%, >50%.....
    - >100 cells?
  - For FISH – at least 50 assessable cells for *ALK*
  - For IHC?

FISH, fluorescence *in situ* hybridization

Pathologic Assessment for Molecular Testing

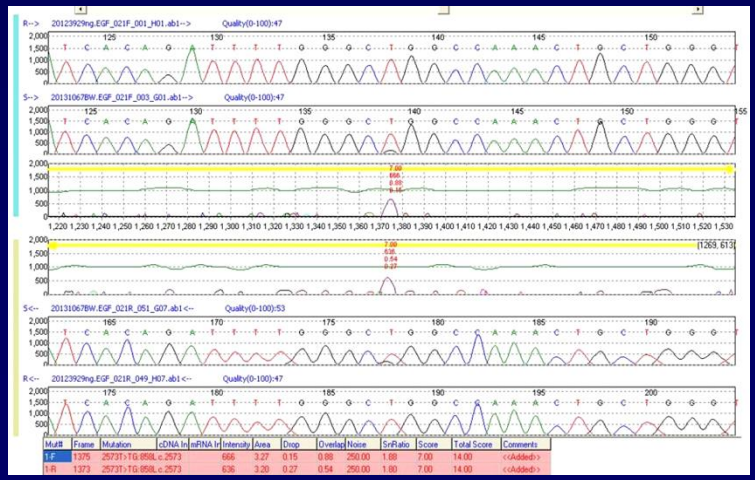
- Tumour present?
- Prepared appropriately?
- Is there is enough tumour?
- The molecular lab knows what it is getting?

- Integration of the molecular results
  - Are they meaningful?
  - Are they reliable?



Tissue from sections: a standard source of tumour DNA

EGFR c.2573T>G; p.Leu858Arg
(exon21 L858R)



EGFR Mutation Testing Methodology

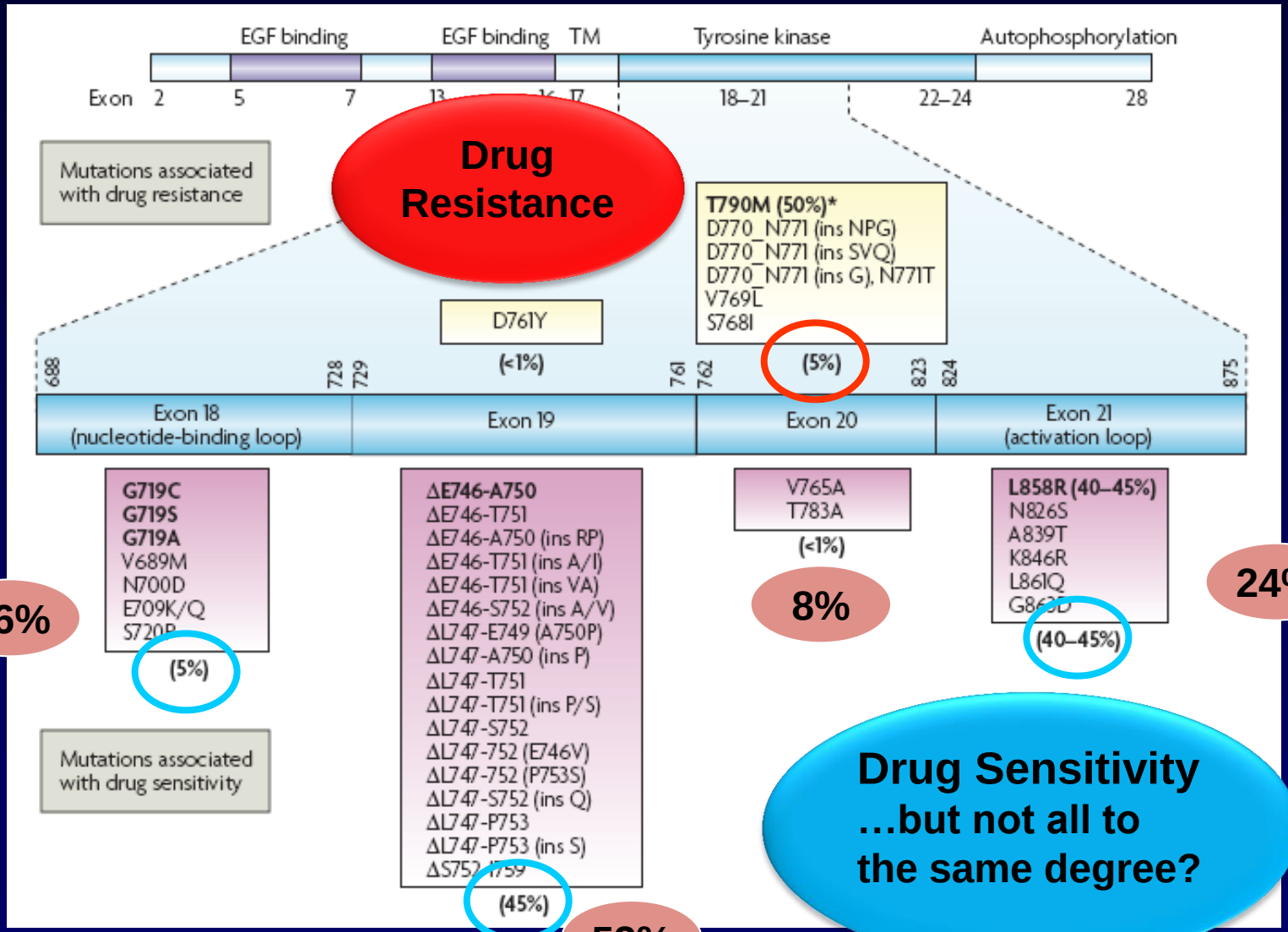
Increasing Sensitivity

Technique	Limit of Detection, % Mutant DNA	Mutations Identified
Direct sequencing	10 - 20	Known and new
TaqMan PCR	10	Known only
Loop-hybrid mobility shift assay	10	Known only
Pyrosequencing	5	Known and new
PCR-SSCP	5	Known and new
dHPLC (WAVE surveyor)	3-5	Known and new
Cycleave PCR	5	Known only
PCR-RFLP and length analysis	5	Known only
MALDI-TOF MS-based genotyping	5	Known only
High resolution melting (HRM)	3-5	Known and new
Scorpion ARMS	1	Known only
PNA-LNA PCR clamp	1	Known only
Single molecule sequencing	*0.1*	*Known and new*
Mutant-enriched sequencing	*0.1*	*Known only*
SMAP	*0.1*	*Known only*

ARMS, amplification refractory mutation system; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; PCR, polymerase chain reaction; PNA-LNA, peptide nucleic acid-locked nucleic acid; RFLP, restriction fragment length polymorphisms; SMAP, smart amplification process; SSCP, single-strand conformation polymorphism

Pao W, Ladanyi M. *Clin Cancer Res*. 2007;13(17):4954-4955.

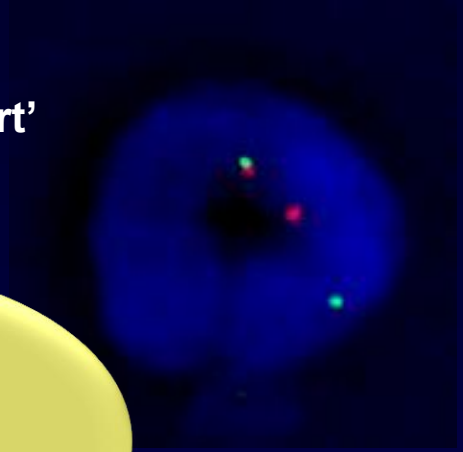
All *EGFR* Mutations Are Not Equal



Sharma SV, et al. *Nat Rev Cancer.* 2007;7(3):169-181.

ALK: Which Test?

The 'break apart' FISH test



Change in DNA Sequence *ALK* Translocation

Transcription

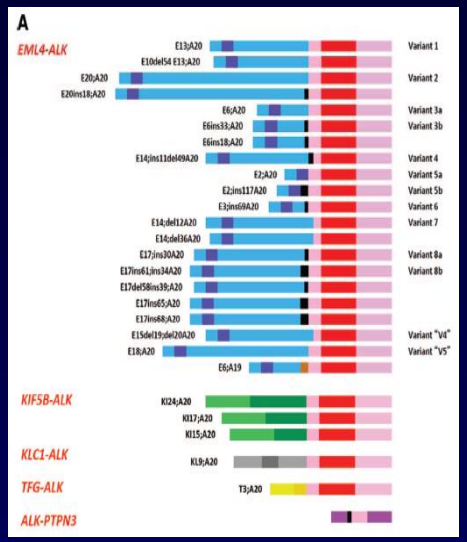
ALK Fusion Gene mRNA Transcript

Translation

ALK PROTEIN

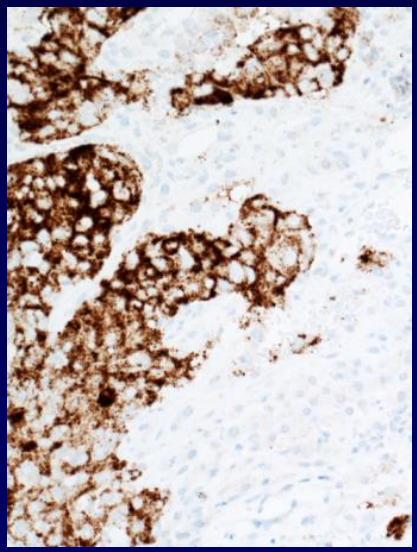
Biologic Activity
Oncogenesis
Drug target

Multiplex PCR

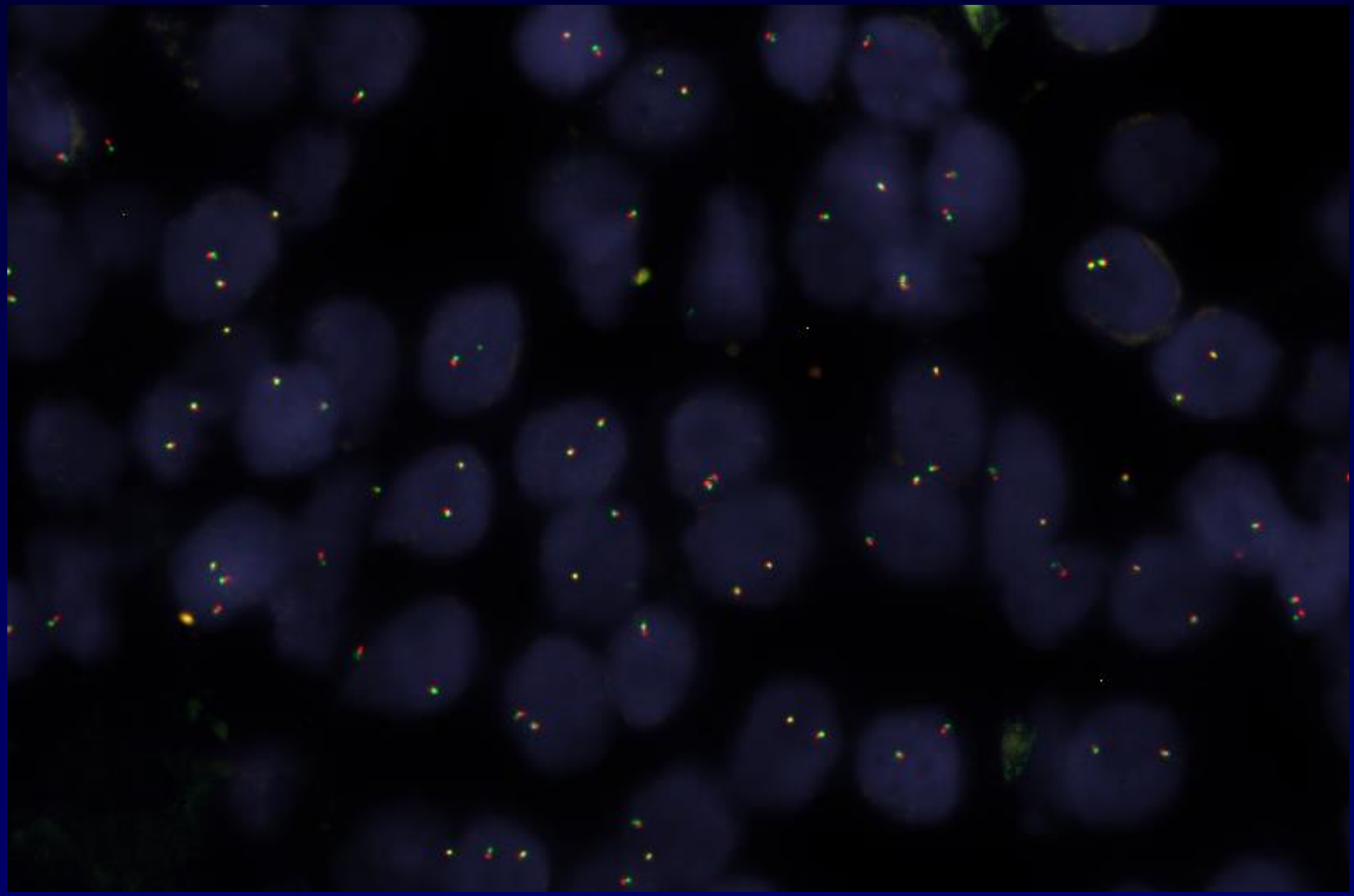


Ou SH, et al. *Oncologist.* 2012; 17(11):1351-1375.

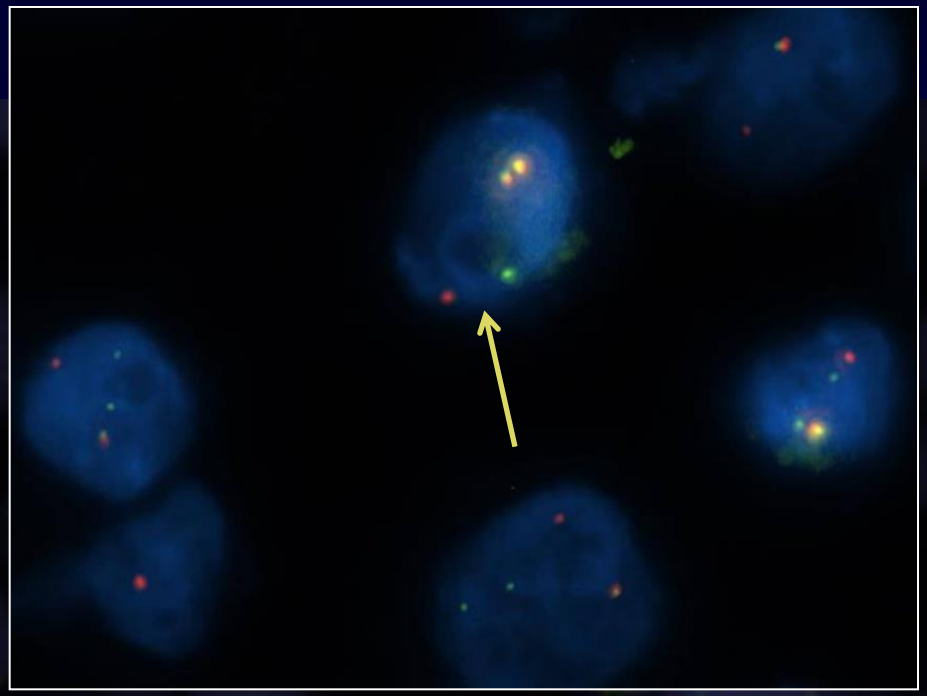
IHC



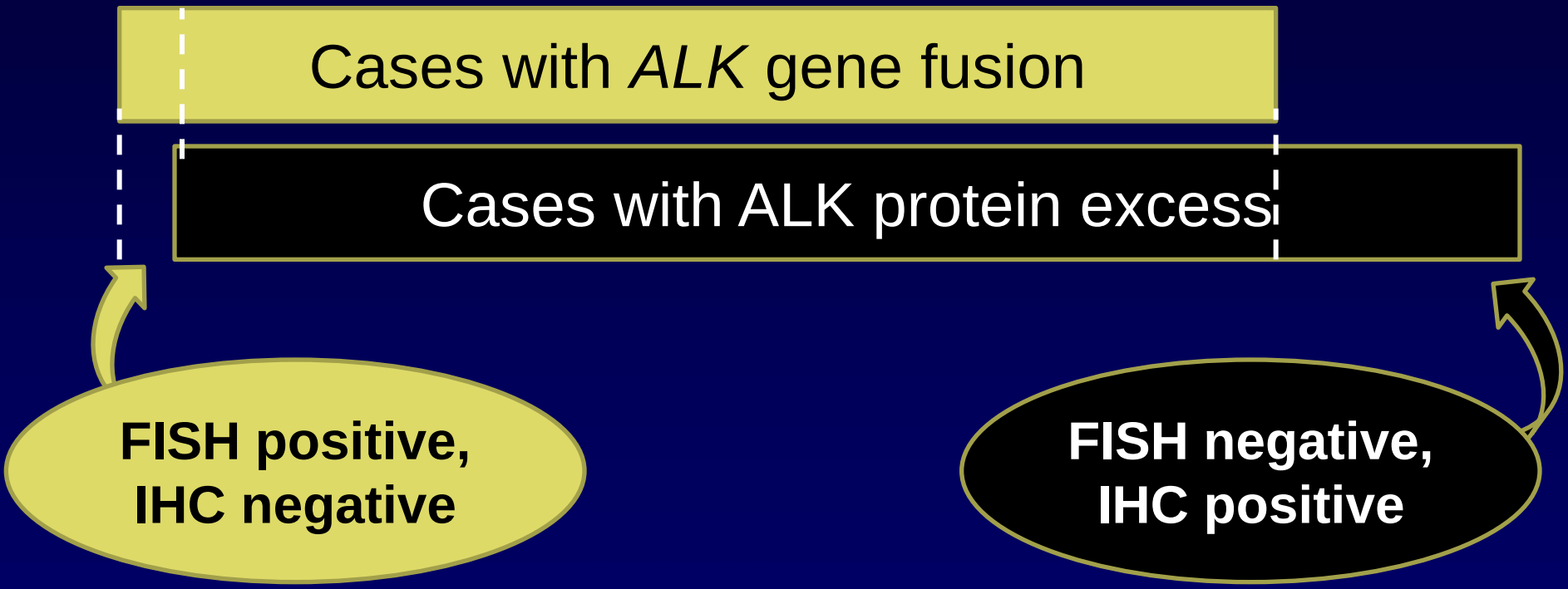
ALK Negative



ALK Positive



The Protein Does the Job
The Protein Is the Target of the Drug



Who Orders the Test? Reflex vs Bespoke Testing

Reflex—pathologist driven

- **Fast**
- **Becomes ‘routine’**
- **Ready for tumour board decision**
- ***Potential for waste***
  - ***Time***
  - ***Tissue***
  - ***Money***

Bespoke—to order from oncologist

- **Only when needed**
- **Preserves tissue**
- **Time not wasted**
- ***Slower turnaround***
- ***Could be illogical; cases may be missed***

Do We Always Succeed?

- **Diagnostic IHC—rarely insufficient**
  - **Occasionally it just doesn't work!**
- ***EGFR* mutation**
- ***ALK* rearrangement**
  - **IHC screening**
  - **Confirmation by FISH**

EBUS Samples for EGFR Mutation?

Reference	% EBUS INSUFF for *EGFR* Mutation Test	Comment
Garcia-Olive et al	28%	12% for core biopsy
Schuurbiers et al	23%	
Esterbrook et al	12%	Cell block based
Navani et al	10%	
Rekhtman et al	2%	

INSUFFICIENT FOR TEST

EBUS, endobronchial ultrasound

Garcia-Olivé I, et al. *Eur Respir J.* 2010;35(2):391-395. Schuurbiers OC, et al. *J Thorac Oncol.* 2010;5(10):1664-1667. Esterbrook G, et al. *Lung Cancer.* 2013;80(1):30-34. Navani N, et al. *Am J Respir Crit Care Med.* 2012;185(12):1316-1322. Rekhtman N, et al. *J Thorac Oncol.* 2011;6(3):451-458.

ALK Testing 'Success'??

- In Aberdeen
  - About 4% of cases insufficient for *ALK* IHC
  - About 10% cases insufficient for *ALK* FISH
    - 50-60 assessable cells
    - 4 high power fields to assess

- Up to 20% of samples may be 'insufficient' for *ALK* FISH testing

Lantuéjoul S, et al. In IASLC ALK Atlas.

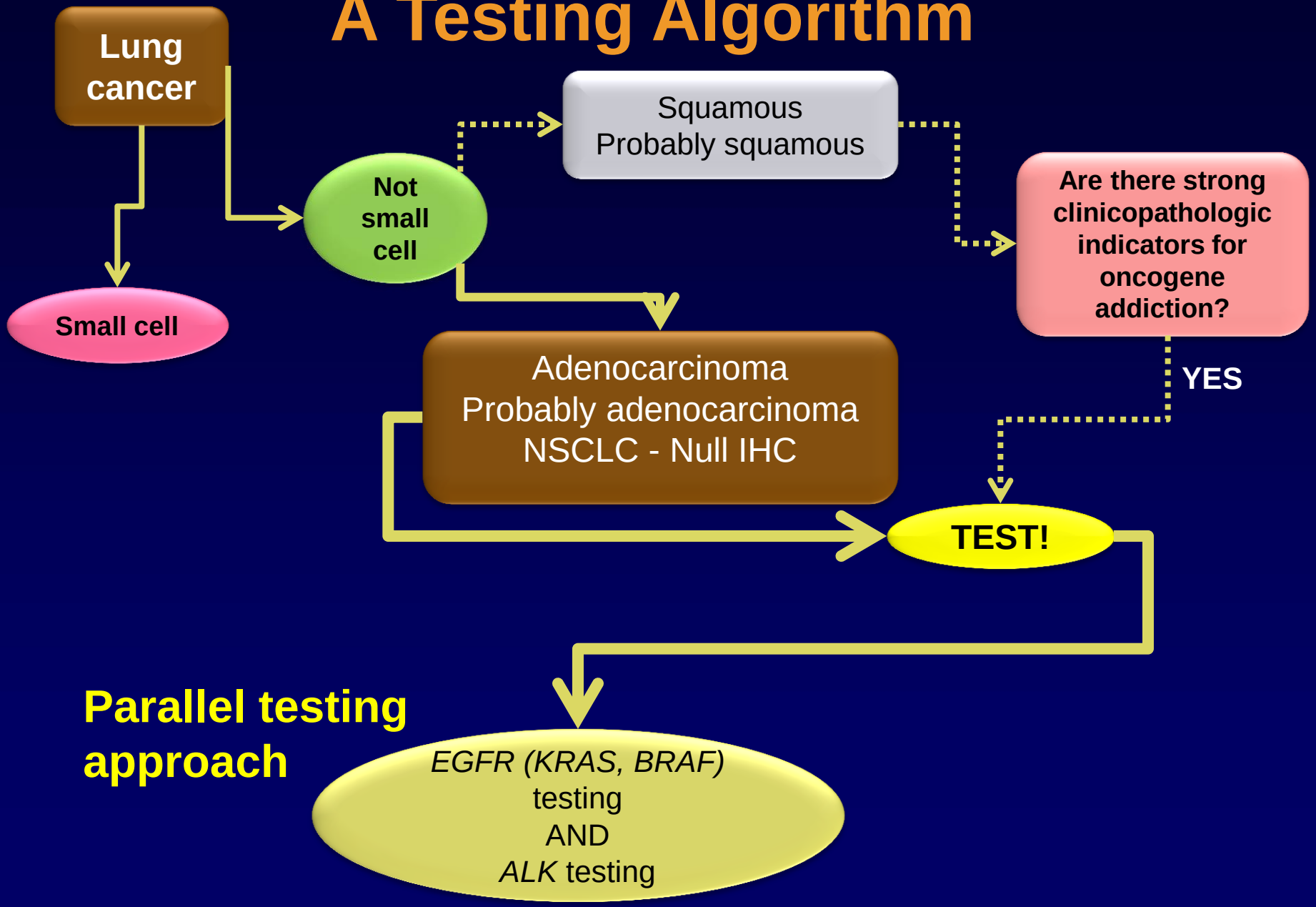
- Cytology samples less often assessable (69%) versus biospy (89%) for *ALK* FISH

Vidal J, et al. *J Thorac Oncol.* 2014:9(12):1816-1820.

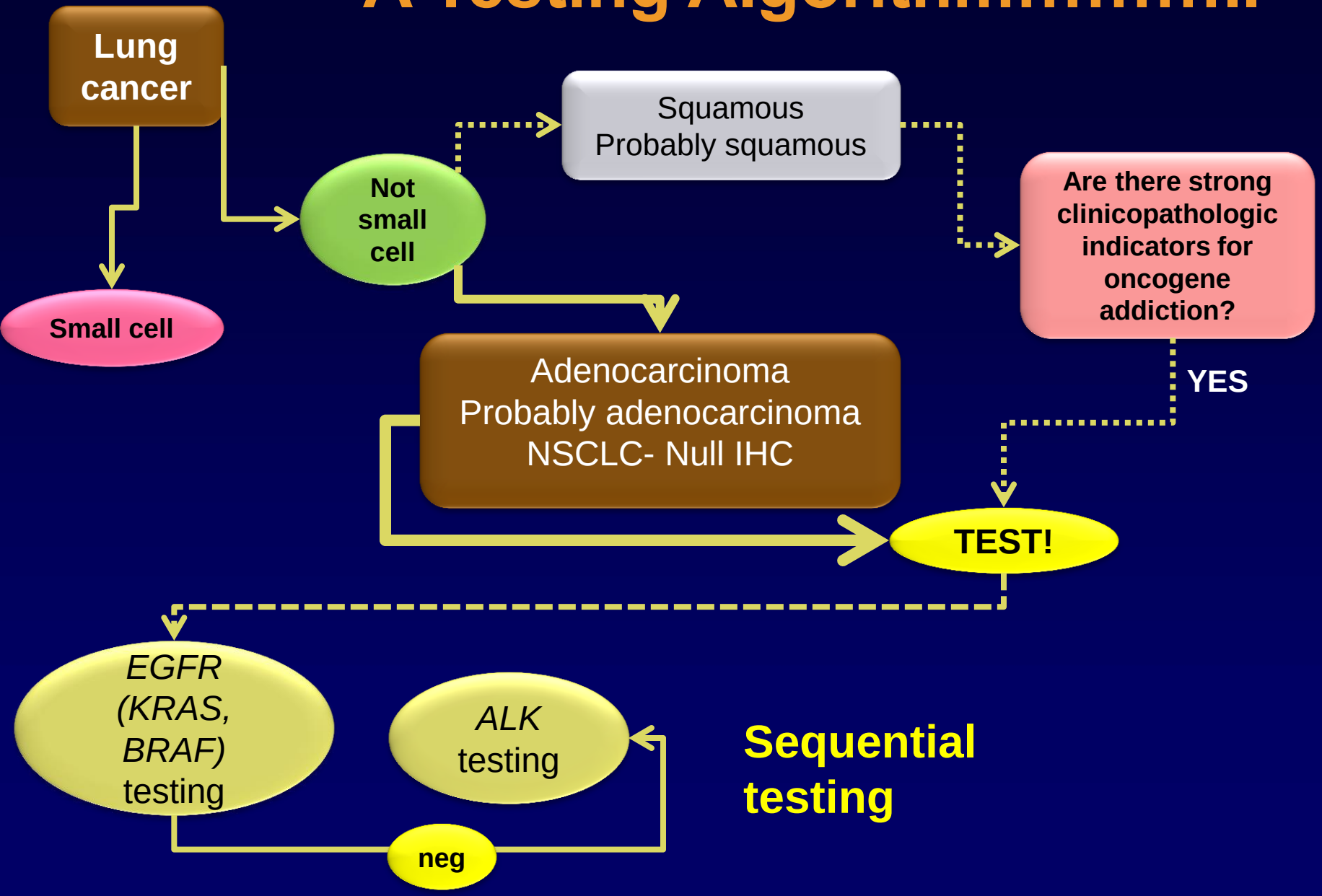
- Cytology samples suitable for *ALK* IHC

Savic S, et al. *J Thorac Oncol*. 2013:8(8):1004-1011.

A Testing Algorithm



A Testing Algorithm……..



More Complex, Ambitious Testing

- More markers to be tested
- Sequential testing increases risk
  - 30% failure
  - 15% fails in EURTAC trial

Buettner R, et al. *J Clin Oncol.* 2013;31(15):1858-1865.
Benlloch S, et al. *J Clin Oncol.* 2012;30(Suppl): Abstract 10596.

- Trials (Battle, MSKCC SCC)
  - 13% to 17% incomplete test sets

Tam AL, et al. *J Thorac Oncol.* 2013;8(4):436-442.
Paik PK, et al. *J Clin Oncol.* 2012;30(Suppl): Abstract 7505.

MSKCC, Memorial Sloan Kettering Cancer Center; SCC, squamous cell carcinoma

NGS for Molecular Testing

- Quoted amounts of DNA required rather variable
  - Technology dependant
  - Size of panel
- Mutation > fusion gene > gene copy number
- Fragmentation of DNA
- Bioinformatic analysis

- 80% samples – Complete panel of mutations
- 95% samples – *EGFR, KRAS, BRAF, HER2* mutations
- 'Minimum 2000 cells' - 5 x 10 um thick sections

Meyerson M, et al. *Nat Rev Genet.* 2010;11(10):685-696.

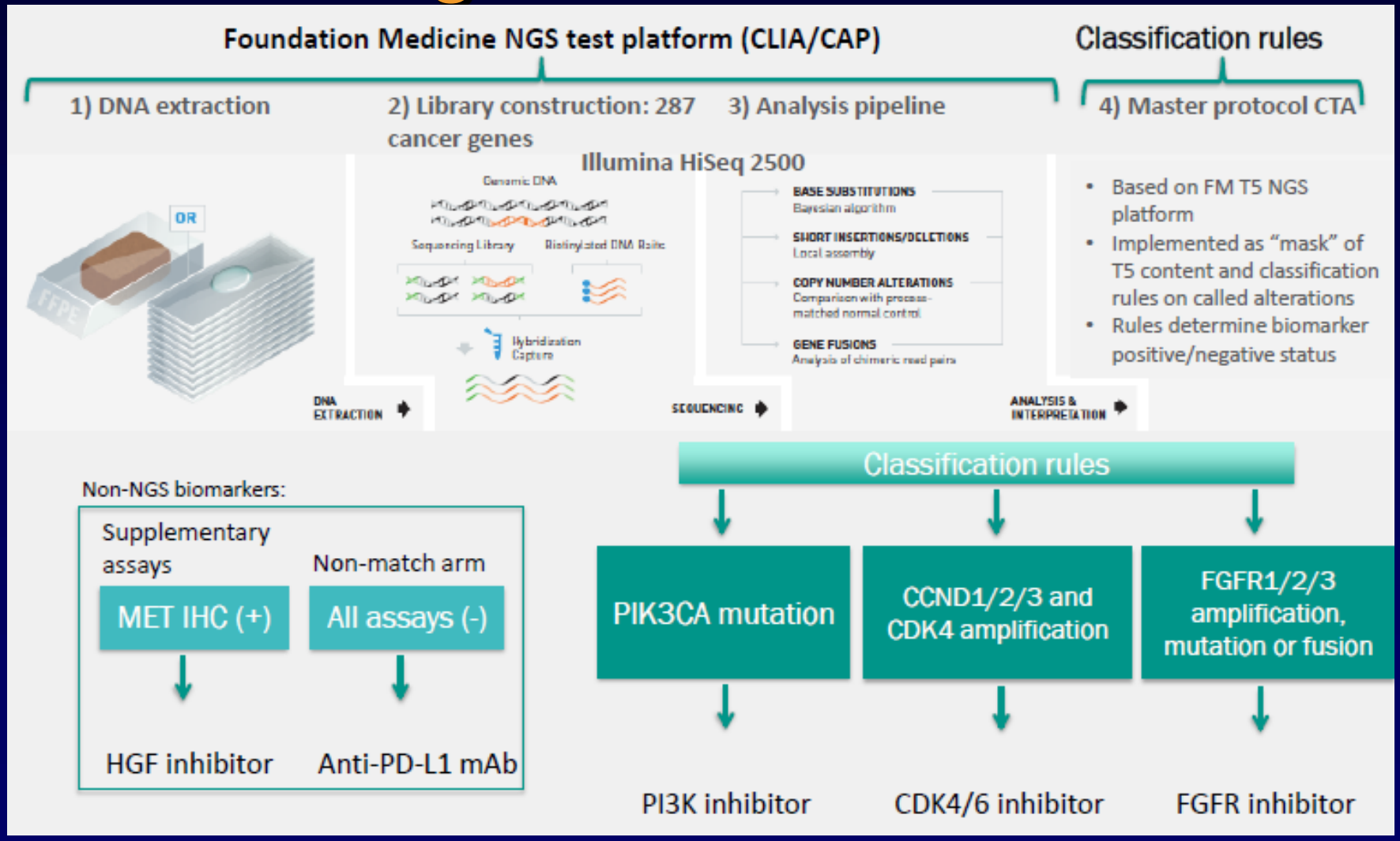
- Much still to define

NGS, next generation sequencing

Next Generation Sequencing: Different Approaches

- **Whole Genome Sequencing (WGS):** Determines the complete DNA sequence of an organism's genome at a single time
- **Whole Exome Sequencing (WES):** Selectively sequences only the coding areas of the genome
- **"Fully Informative" Sequencing:** Sequences a defined subset of genes of interest in their entirety
- **Targeted Sequencing (Hot Spot):** Sequences only the hot spots of a subset of genes of interest

Lung Cancer Master Protocol: Lung MAP Trial in SCC



National Institutes of Health. Available at: https://clinicaltrials.gov/ct2/show/NCT02154490. Accessed 12 December 2014.

Testing Plasma Samples

- Free plasma DNA ……or CTCs
- Referred to in some guidelines
- Needs sensitive methodology
- Primary analysis
  - cfDNA 58% sensitivity, 86% specificity
- Monitoring role?
  - 84% sensitivity (*EGFR*) using CTCs
  - Relapse on treatment
- High research priority
- Not currently recommended

Couraud S, et al, *Clin Cancer Res.* 2014;20(17):4613-4624.

Marchetti A, et al. *PLoS One.* 2014;9(8):e103883.

CTC, circulating tumour cells

Gridelli C, et al. *Clin Lung Cancer.* 2014;15(3):173-181. Kerr KM, et al, *Ann Oncol.* 2014;25(9):1681-1690.

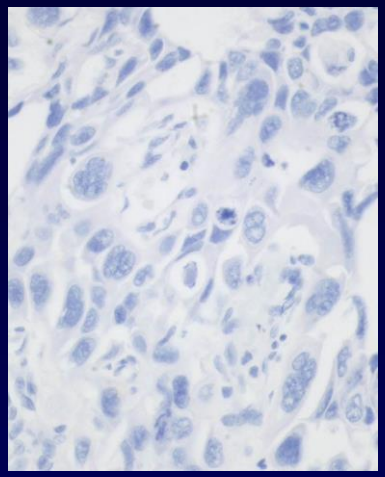
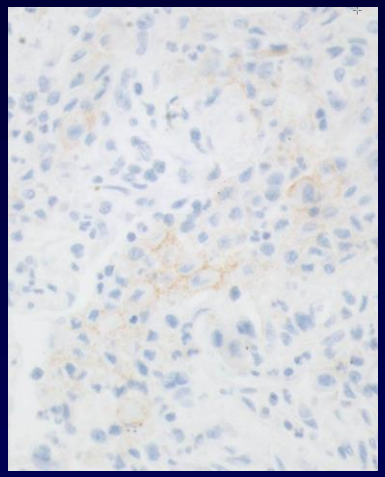
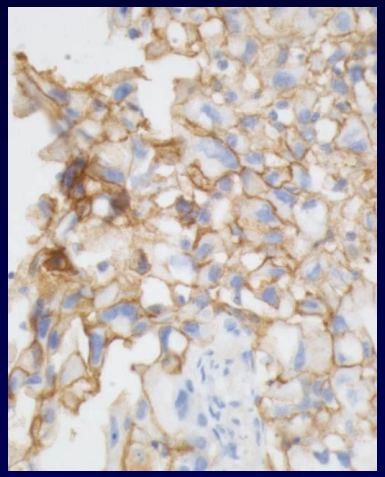
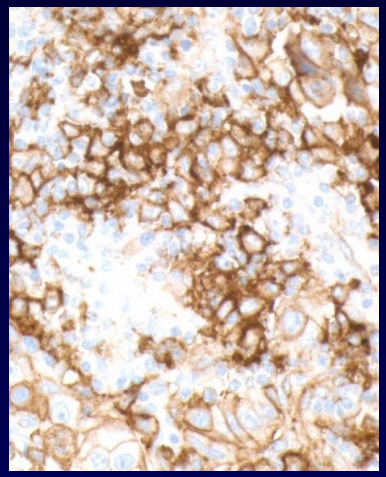
Gefitinib Treatment in *EGFR*-Mutated Caucasian NSCLC: Circulating-Free Tumour DNA as a Surrogate for Determination of *EGFR* Status

- Mutation status concordance between tumor and matched plasma was 94%, sensitivity 66% and specificity of 100% (n = 652)
- Reproducibility also high: Mutation concordance of 97% for 224 matched plasma specimens
- *Post hoc* analysis of the efficacy of first-line gefitinib revealed that PFS was similar for those with *EGFR* mutation–positive tissue (9.7 months) vs both mutation-positive tissue and plasma (10.2 months)
- Conclusions: Although these results are encouraging and suggest that plasma is a suitable substitute for mutation analysis regardless of mutation subtype, tumor tissue should be considered the preferred sample type when available

26 Sept 2014, CHMP of EMA gave positive opinion to include a label in gefitinib SmPc for the use of circulating tumour DNA (ctDNA) obtained from a blood sample, to be used for the assessment of *EGFR* mutation status in those patients where a tumour sample is not an option.

Douillard JY, et al. *J Thorac Oncol.* 2014;9(9):1345-1353.

Biomarkers for Immunotherapy?

PD-L1 Negative **PD-L1 Positive (predictive of response)**

Less response More response

1% **5%** **10%** **50% cell positive**

?

Several therapeutics
Several companion diagnostics……

Intensity of staining?
Immune cell staining?

Gandhi L, et al. *AACR Meeting Abstracts.* 2014;2014: Abstract CT105.

The Role of Pathology in the Era of Targeted Therapy

- Pathologic diagnosis
- Pathologic assessment
- Tissue handling
- Adapt to range of markers required
- Multiple test modalities