Biopsy and Mutation Detection Strategies

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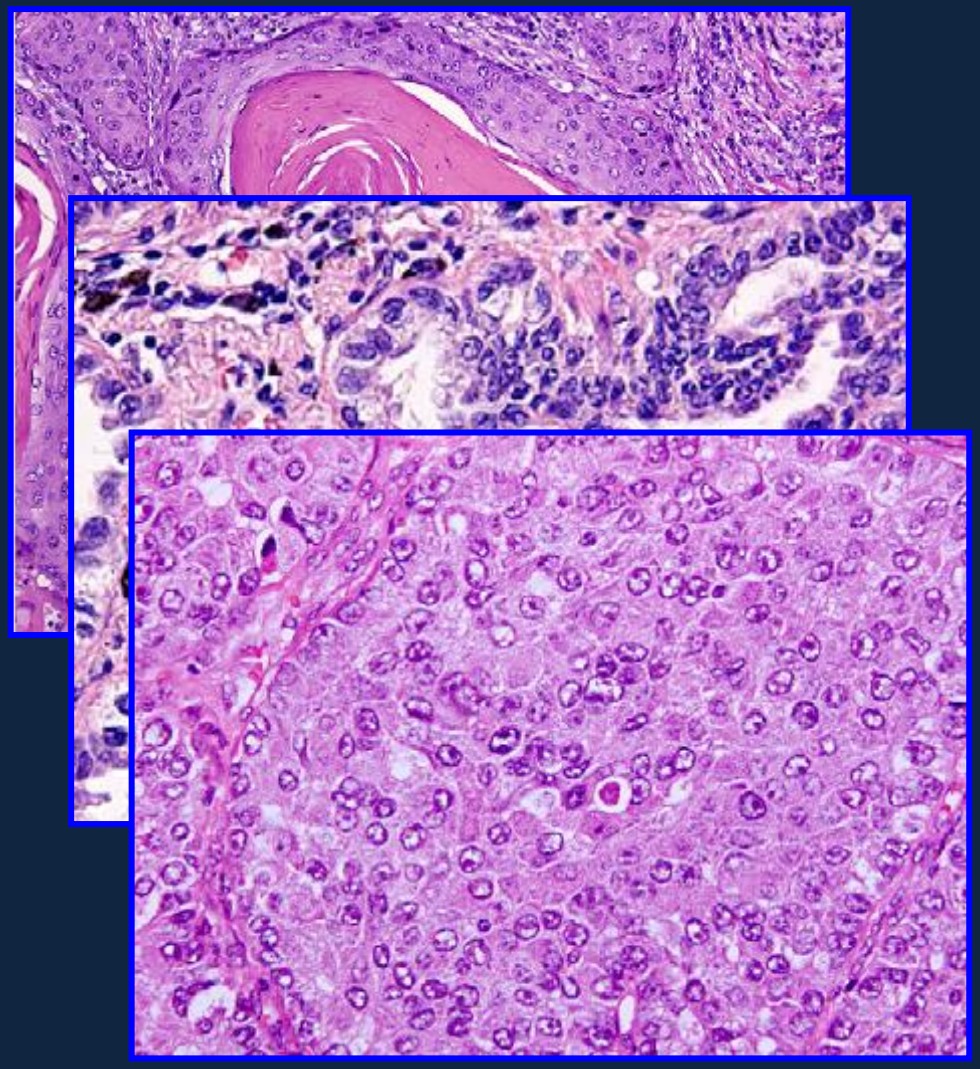
Objectives

- **Background**
- **Tissue acquisition & Pathology strategy**
- **Histological subtyping:**
  - **Immunohistochemistry (IHC)**
- **Predictive testing: Molecular analysis**
  - ***EGFR* mutation & *ALK* rearrangement**

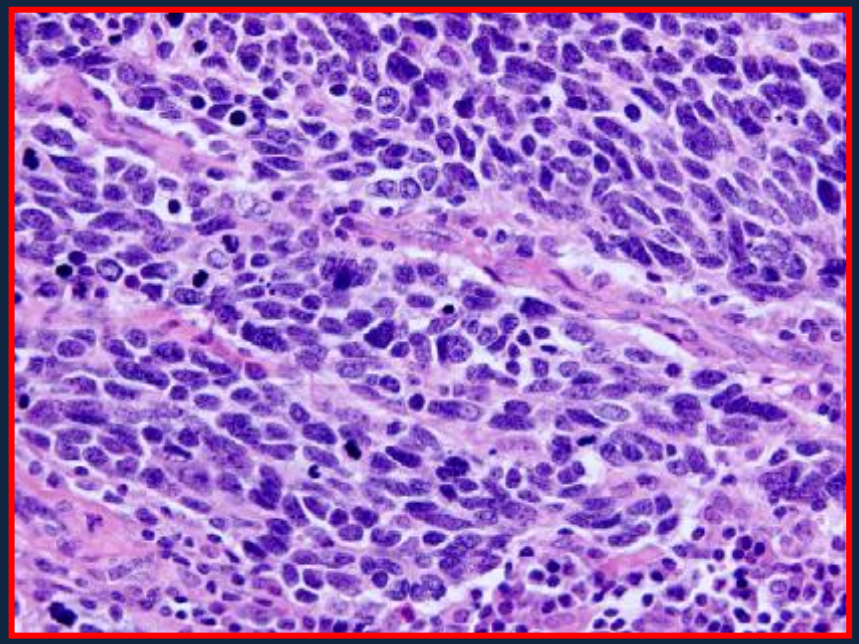
Background

Non-small cell lung cancer vs Small cell lung cancer

NSCLC

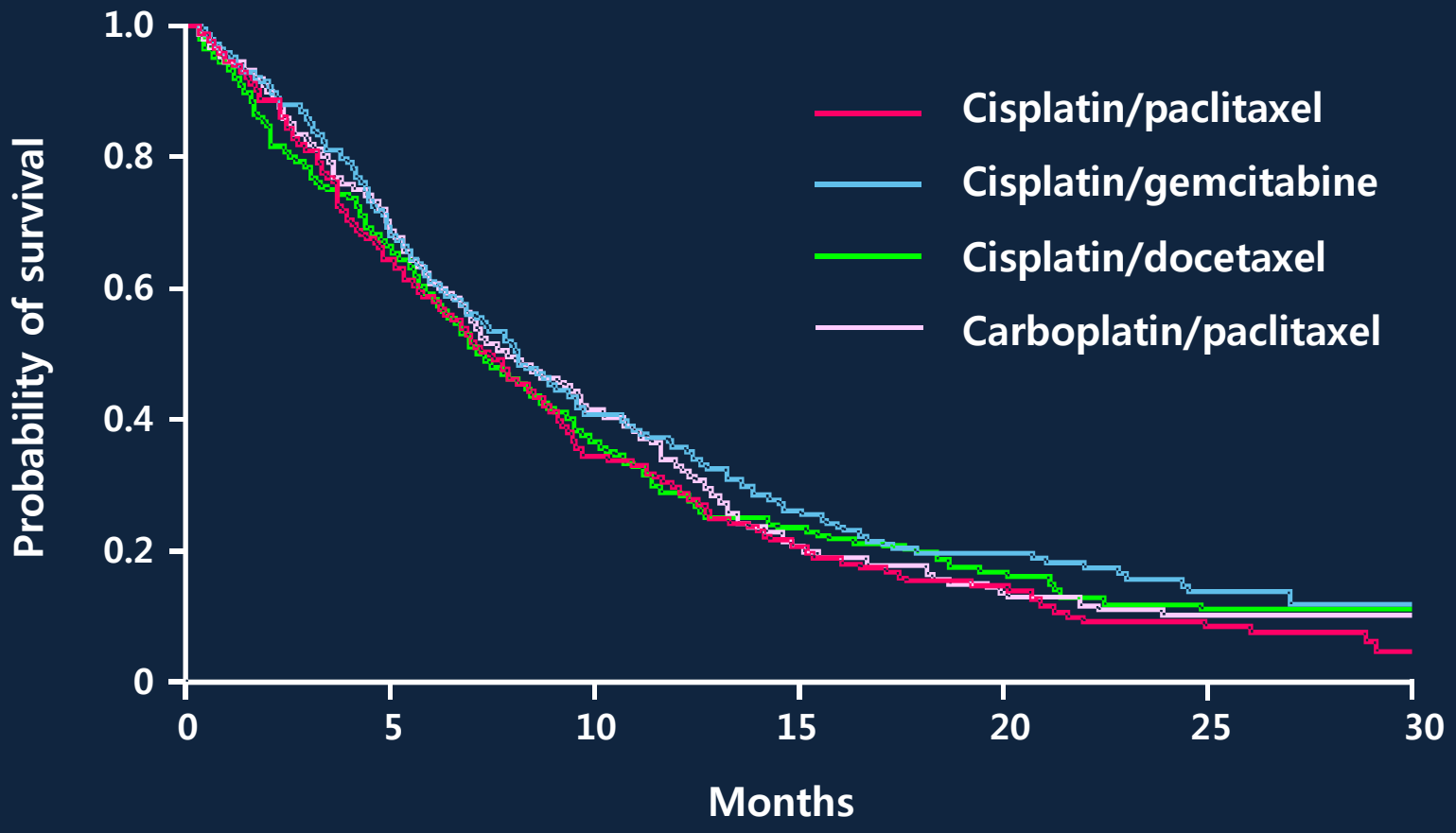


SCLC



First-line chemotherapy for advanced NSCLC

ECOG 1594: Platinum doublets

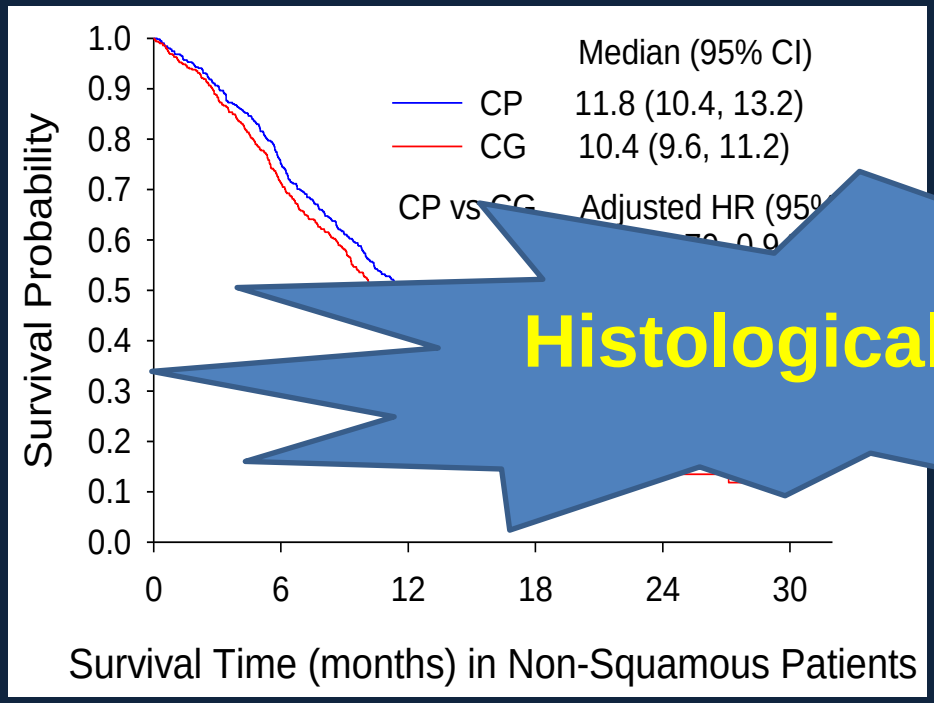


Schiller JH et al. N Engl J Med 2002;346:92-98.

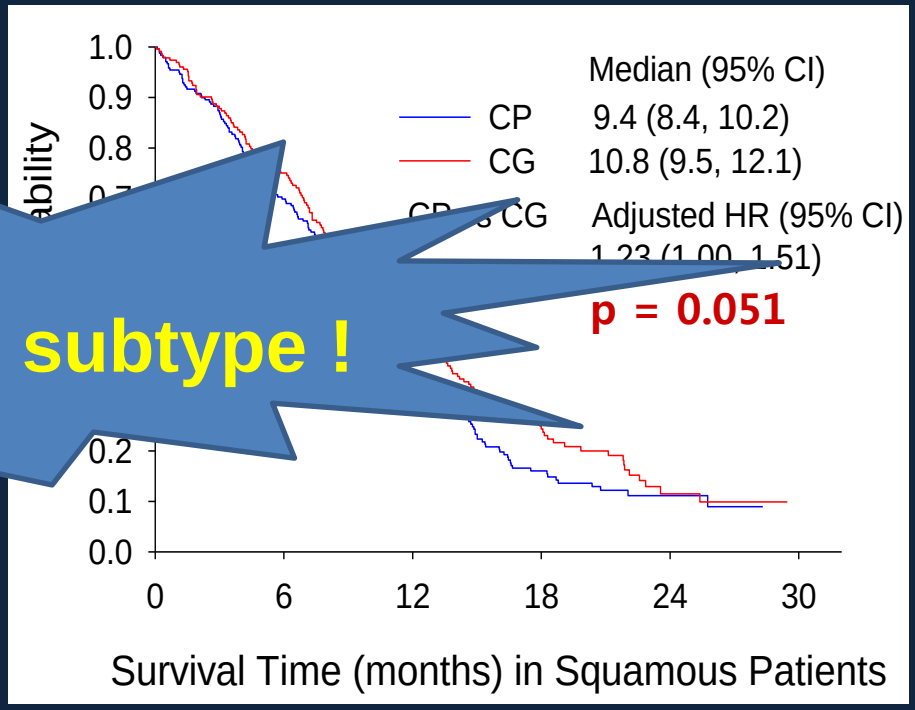
Cis/Pemetrexed vs Cis/Gemcitabine in 1st line NSCLC

JMDB

Non-Squamous



Squamous

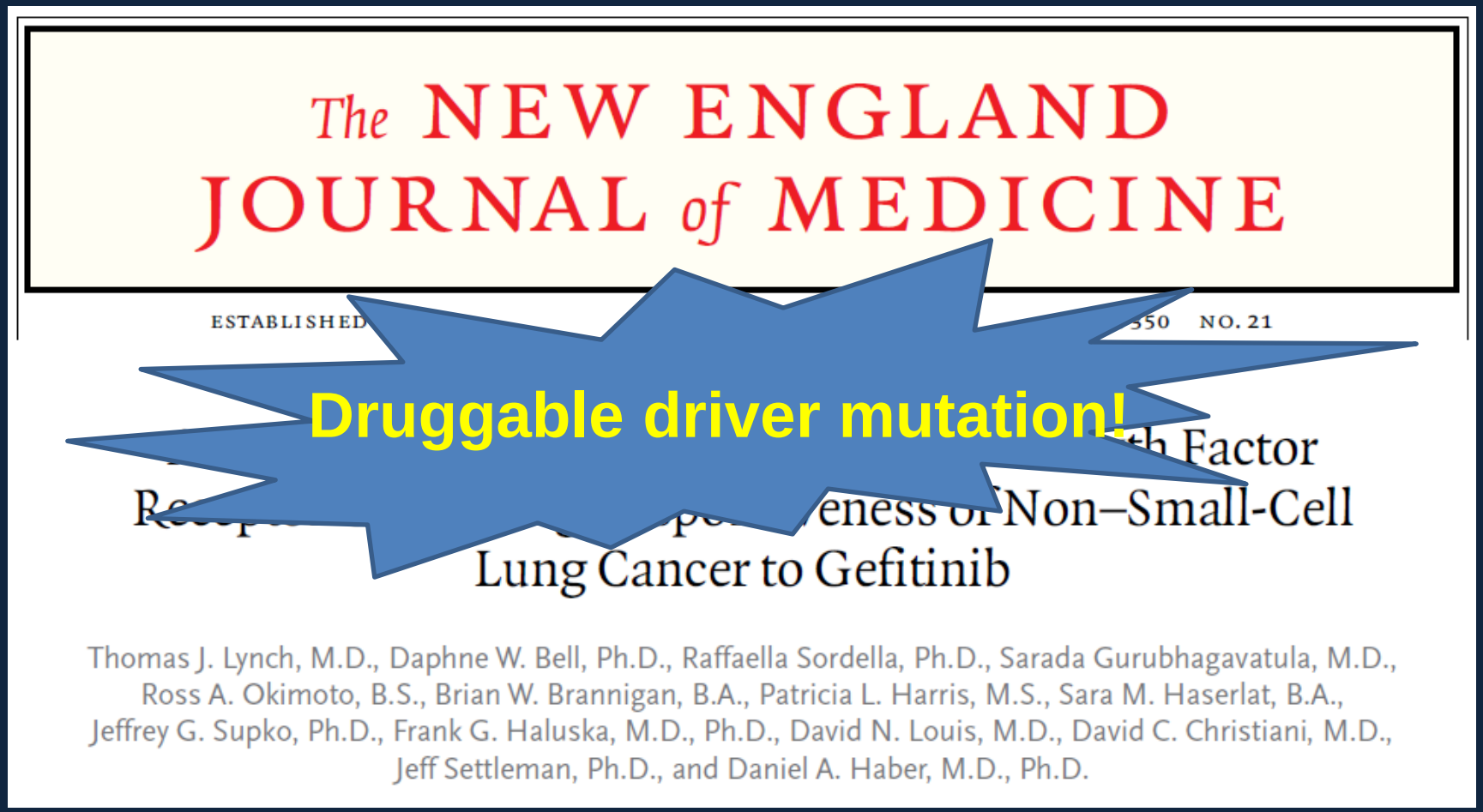


Histological subtype !

Scagliotti GV et al. J Clin Oncol 2008;26:3543-51.

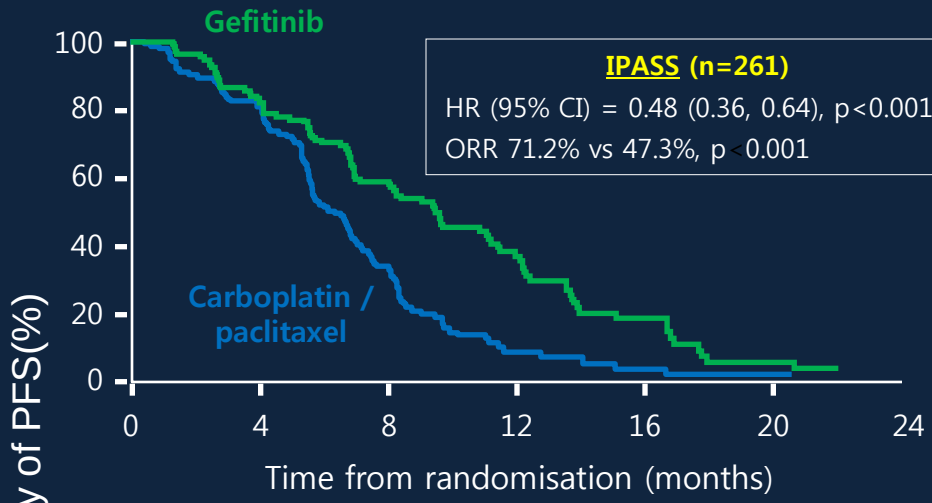
Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitor

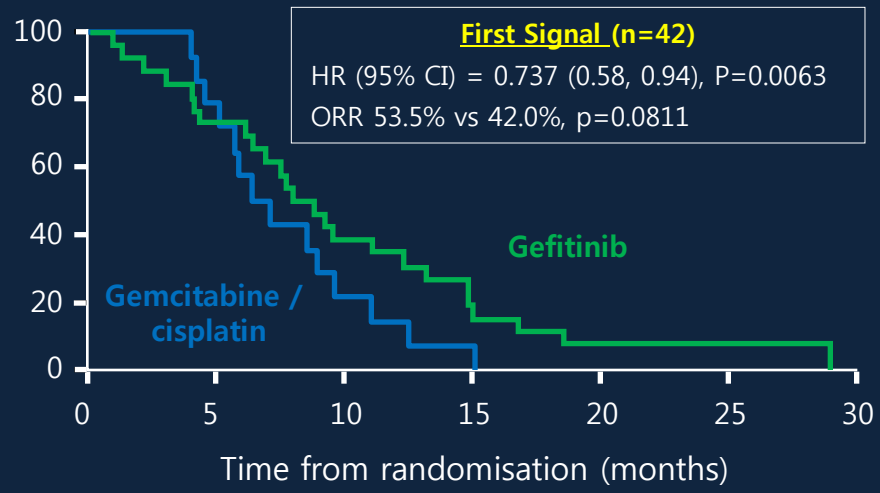
EGFR mutation

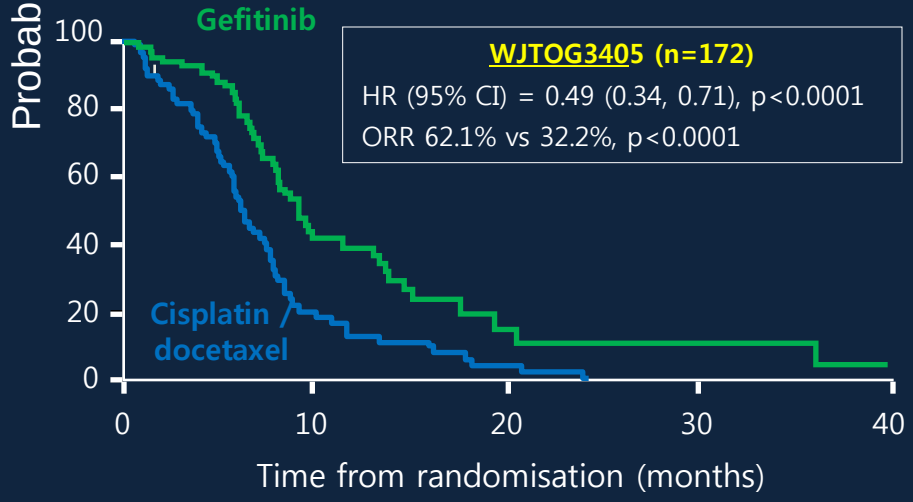


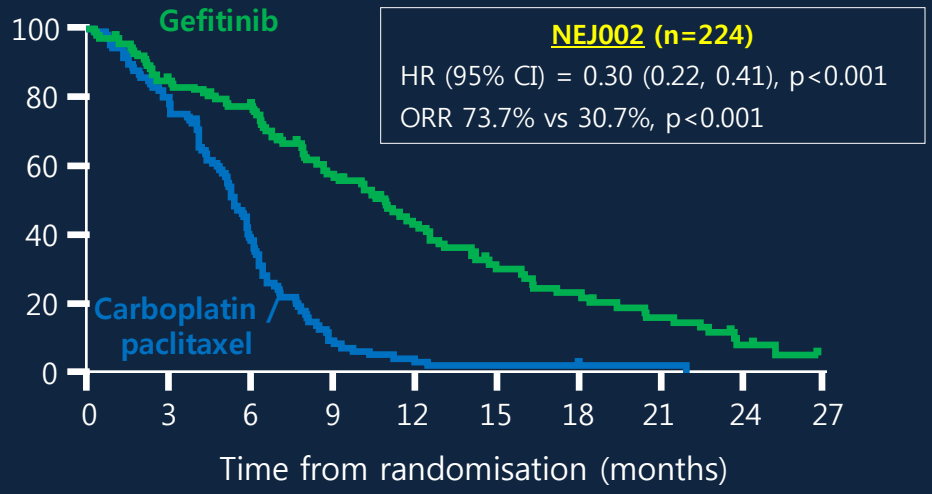
Lynch TJ et al. N Engl J Med 2004;350:2129-39.

PFS for Gefitinib vs Doublet chemotherapy in *EGFR* M+ patients from 4 Phase III first-line trials



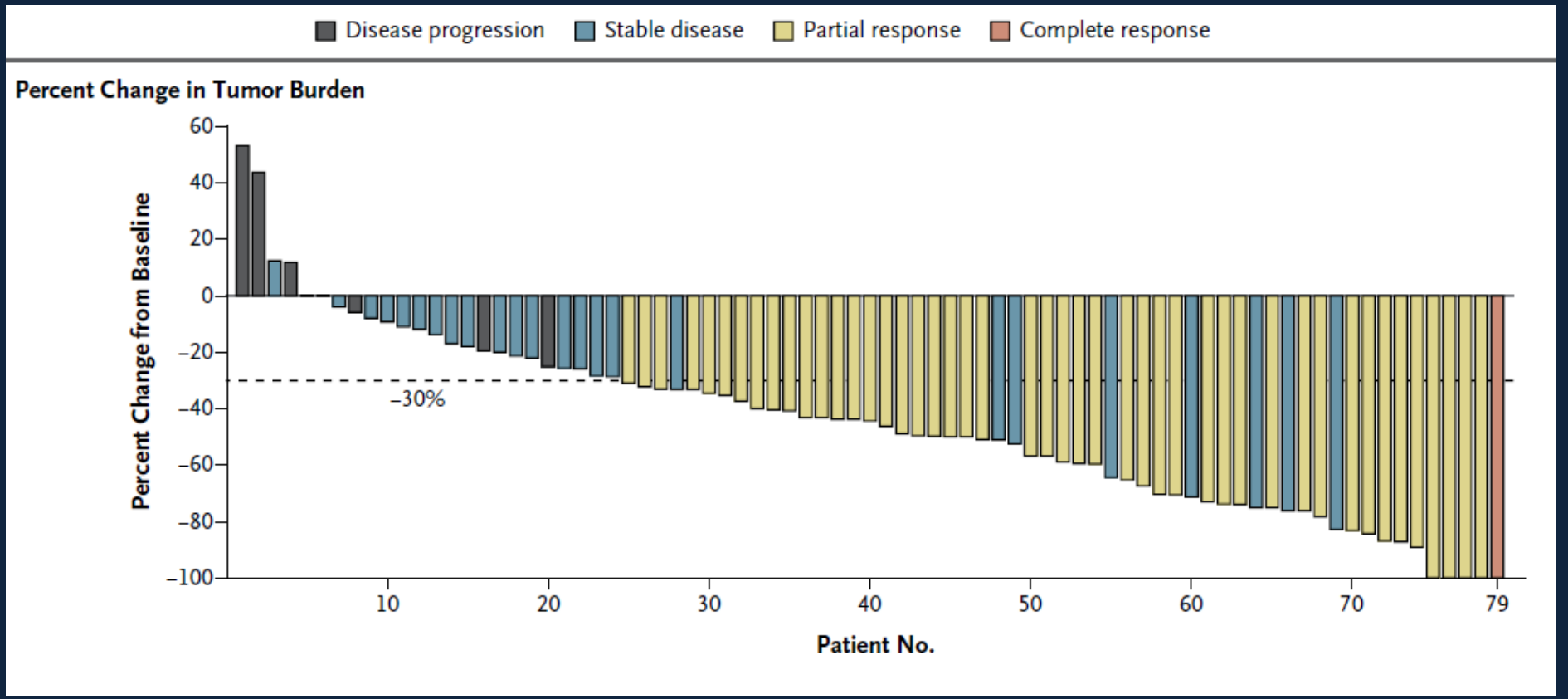






Mok et al 2009, Lee et al 2009, Mitsudomi et al 2009, Maemondo et al 2010

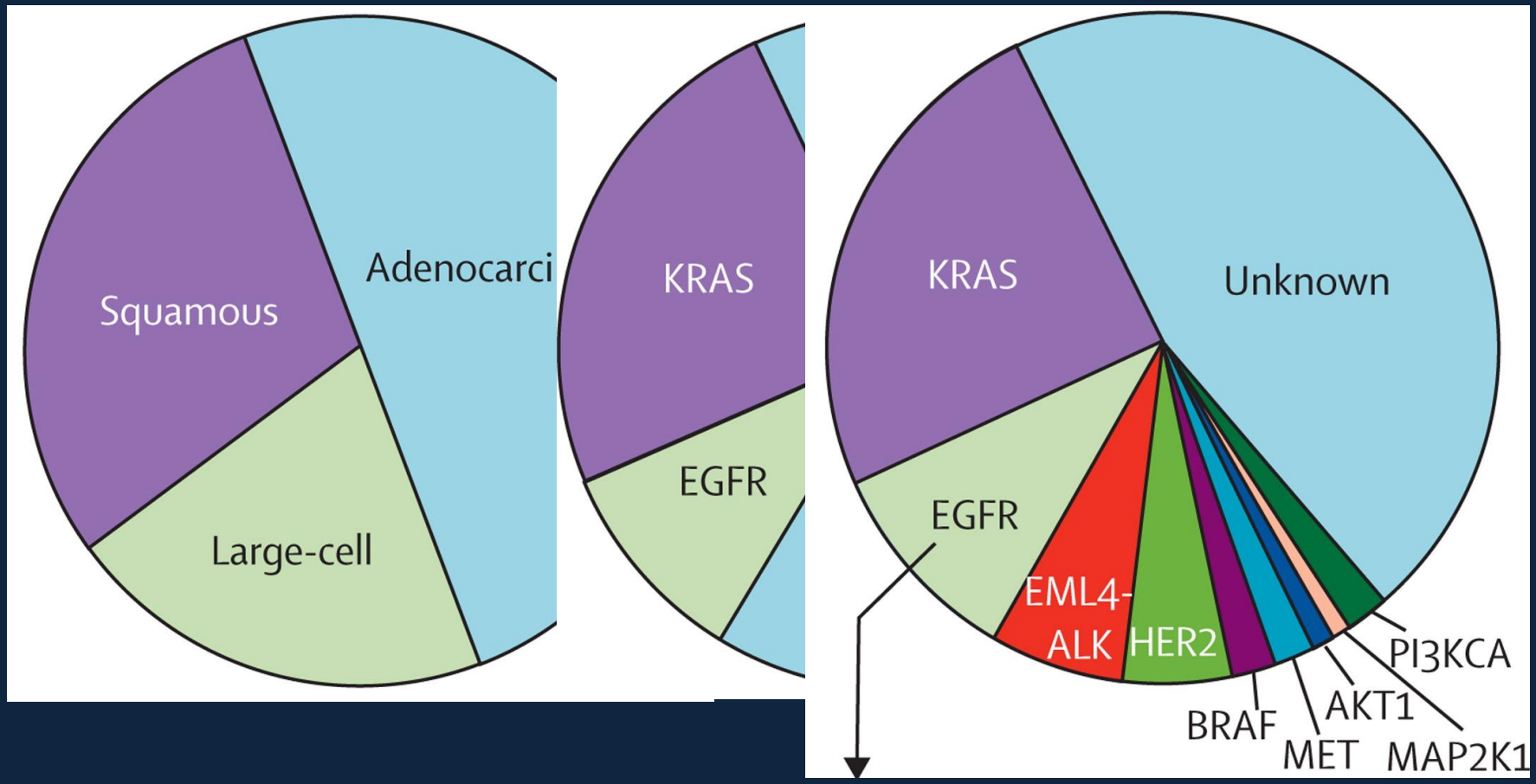
Crizotinib in *ALK* rearrangement of NSCLC



OR: 57%, SD: 33%

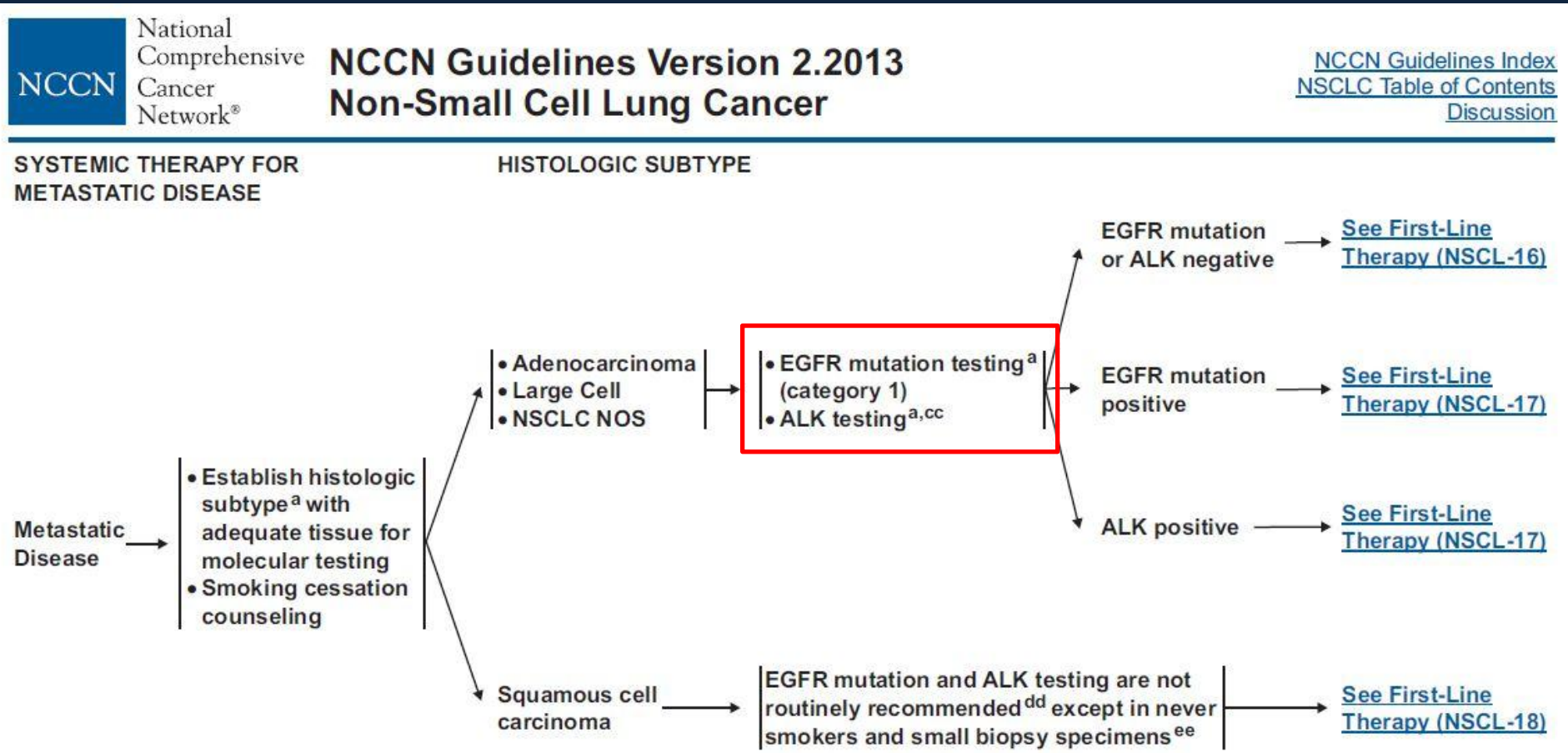
Kwak EL, et al. N Engl J Med 2010;363:1693-703.

Evolution of knowledge in NSCLC



Pao W et al. Lancet Oncol 2011;12:175-80.

NCCN guidelines



Tissue acquisition
&
Pathology strategy

Tissue acquisition

- **Correct sampling and processing of tissue!**

- **Methology**

  Exfoliation cytology: bronchial washing, BAL, bronchia brushing

  Aspiration cytology: TBNA, EBUS, EUS

  Biopsy: bronchial & transbronchial biopsy, transthoracic needle biopsy

- **Rapid on site examination (ROSE)**
- **Telepathology**

Pathology strategy

- **Accurate & relevant clinical information!**

- **Pathologist:**

  Determine priorities of the diagnostic approach

  Determine how specific a dianosis is required

  Plan the necessary investigations &

  anticipate the use of IHC and molecular tests

  Prevent unnecessary usage of tissue on test

  which are not required

- **Preserving scarce tissue for the most important test**

Approach to lung cancer work up for diagnosis

Cancer in the lung: primary vs metastases

Primary lung cancer: NSCLC vs SCLC

NSCLC: Adenocarcinoma vs Squamous cell ca.

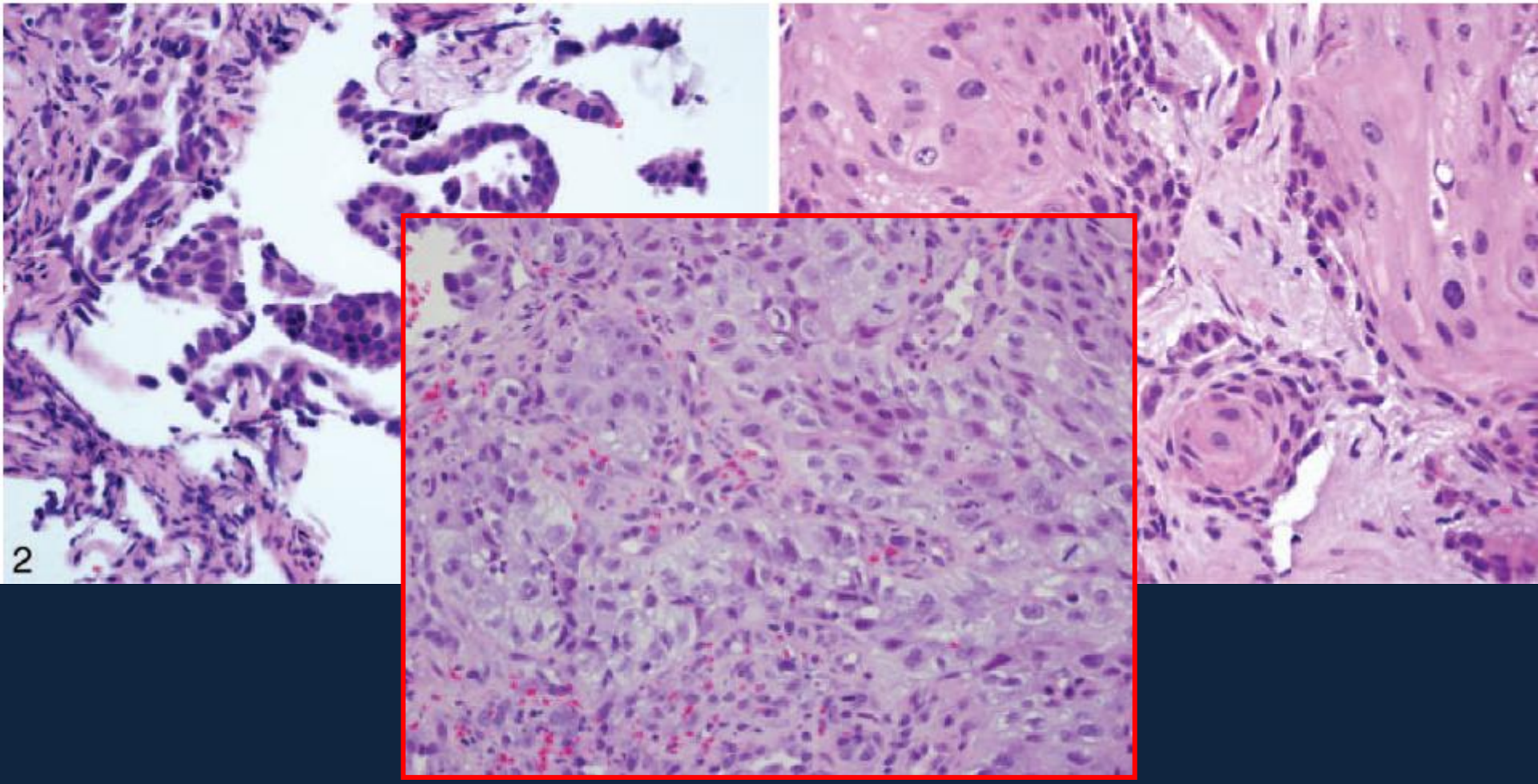
Molecular predictive testing: EGFR & ALK…

Histological subtyping

Non-small cell lung cancer: Pathology

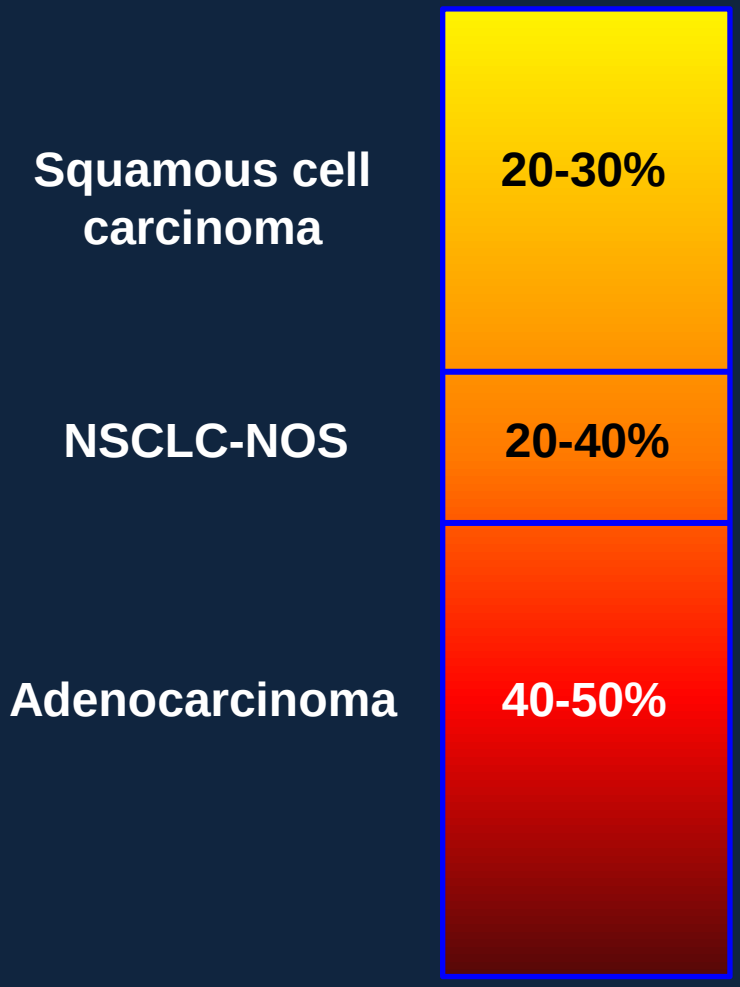
Adenocarcinoma

Squamous cell carcinoma

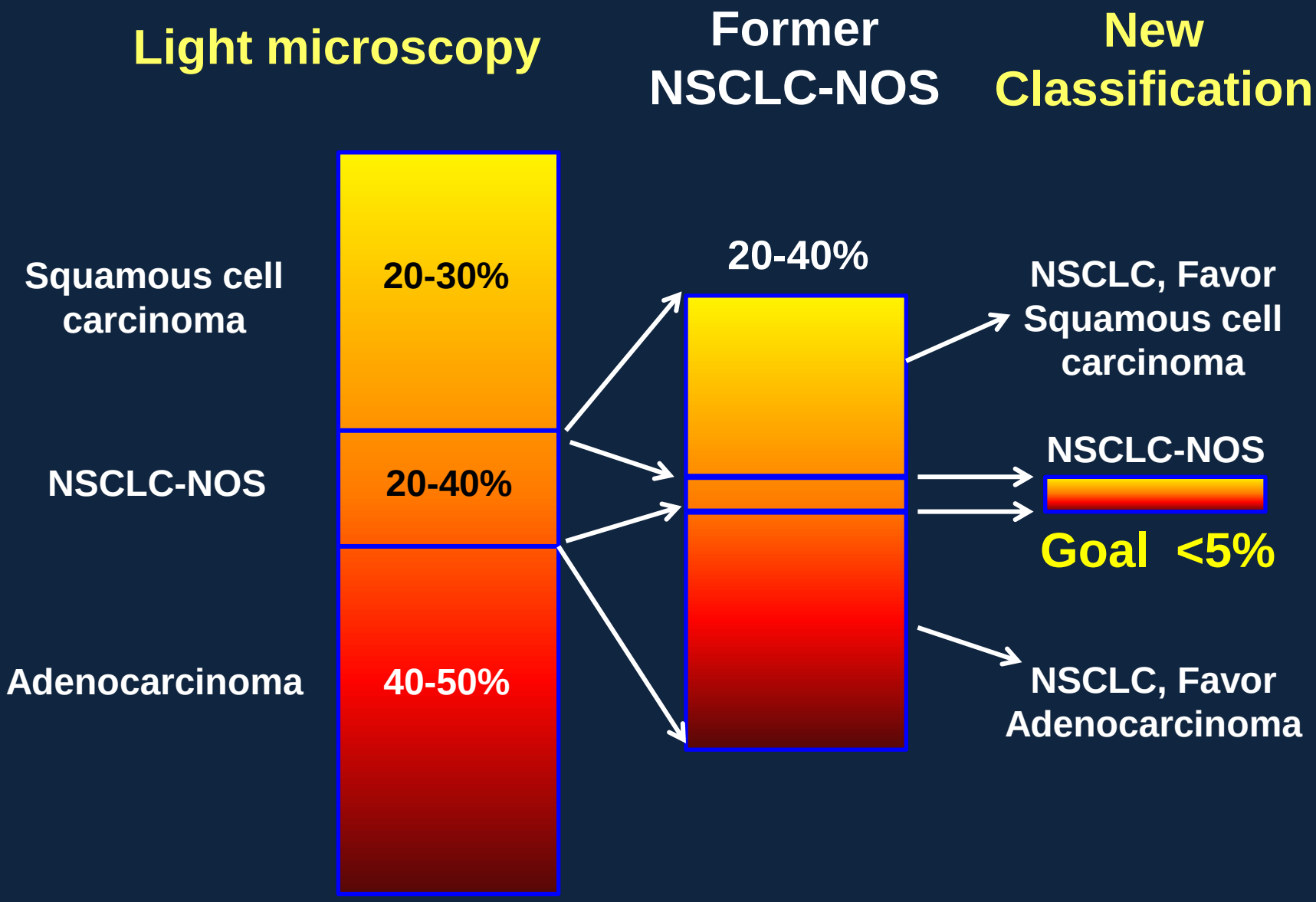


NSCLC-NOS

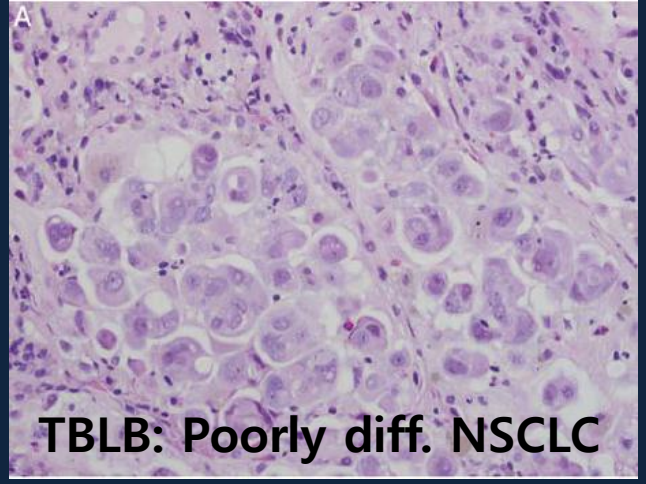
Light microscopy: Small biopsy and Cytology

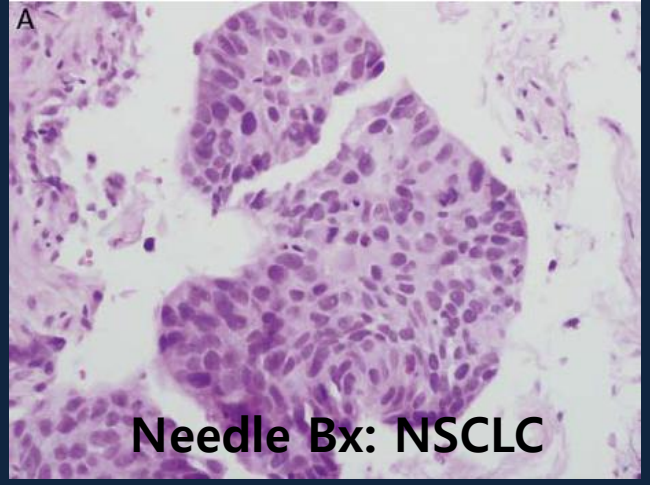


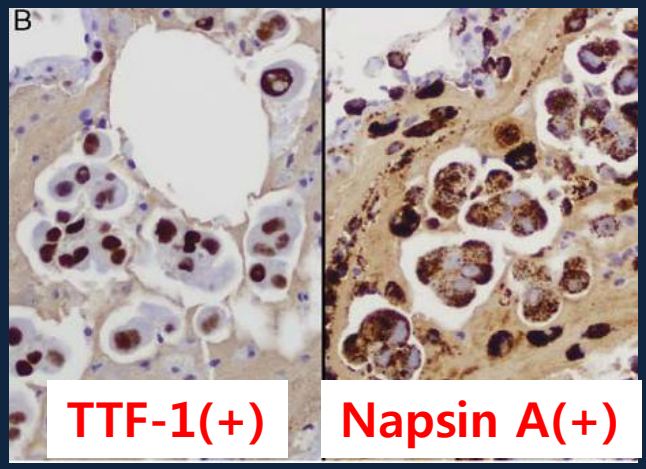
Travis WD et al. J Thorac Oncol 2011;6:244-85.

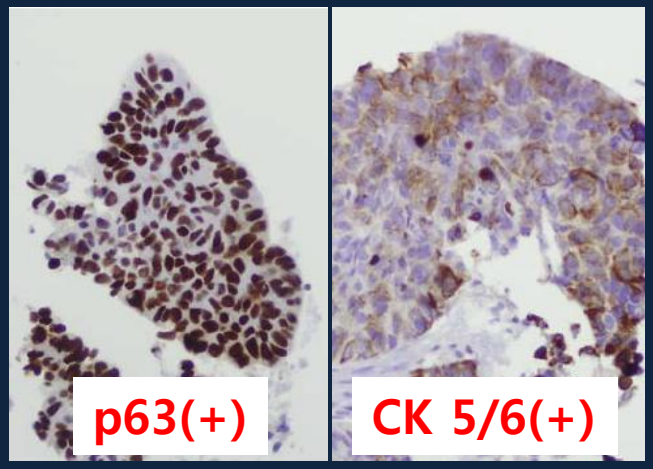


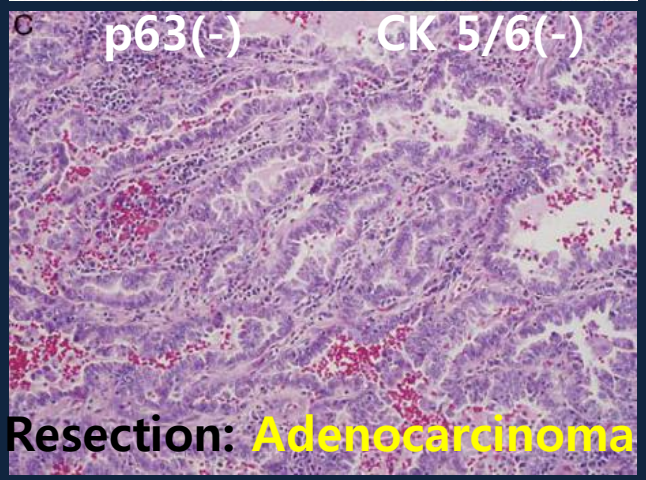
Travis WD et al. J Thorac Oncol 2011;6:244-85.

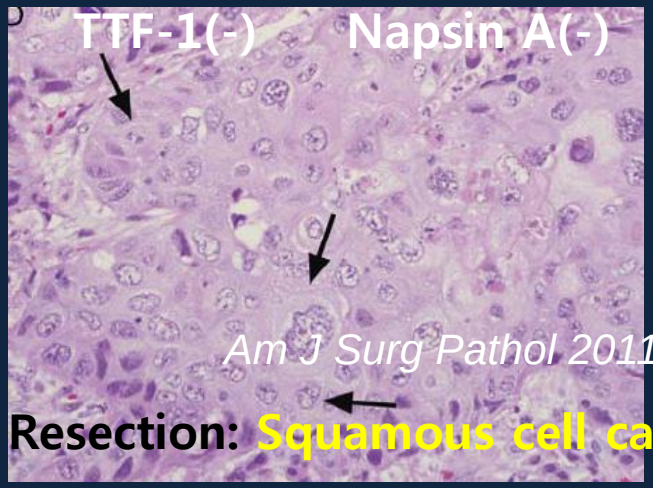












Am J Surg Pathol 2011;35:15-25.

Panels for subtyping poorly diff. NSCLC

- TTF-1
- AB/PAS
- p63
- CK5/6

Loo PS et al.
J Thorac Oncol 2010;5:442-7.

- TTF-1
- Napsin A
- p63
- CK5/6

Mukhopadhyay S & Katzenstein AL
Am J Surg Pathol 2011;35:15-25.

P40, 34ßE12: squamous cell ca.

Predictive testing: Molecular analysis

Guideline

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

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Summary of Recommendations

Lindeman NI et al. J Thorac Oncol 2013;xxx

Guideline questions

1. When should molecular testing for NSCLC be performed?

2. How should *EGFR* testing be performed?

3. How should *ALK* testing be performed?

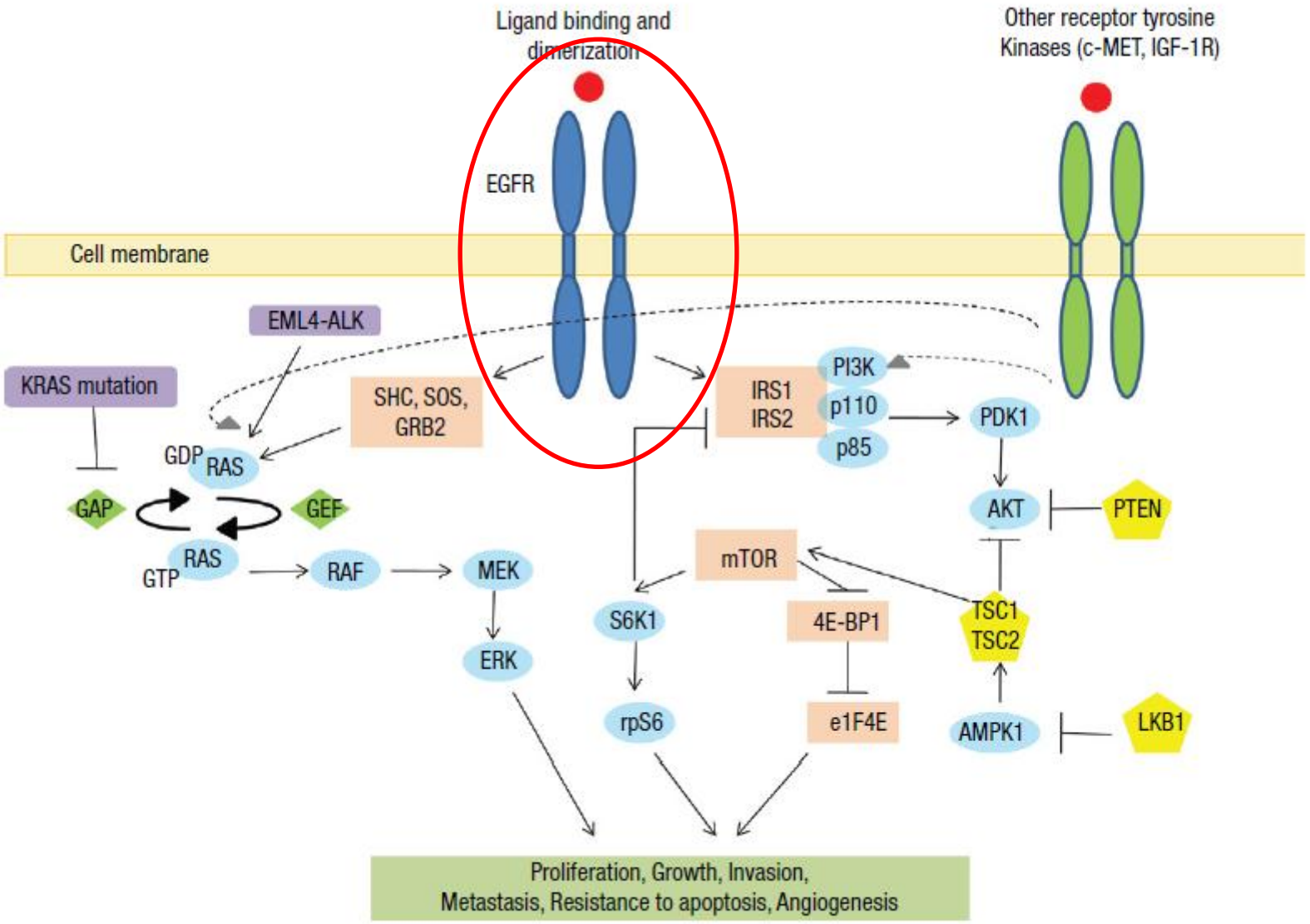
4. Should other genes be routinely tested in lung adenoca?

5. How should molecular testing of lung adenoca be implemented and operationalized?

Lindeman NI et al. J Thorac Oncol 2013;xxx

EGFR mutation

Cell signaling pathway in lung cancer



Clinical Lung Cancer, Vol. 13, No. 4, 252-66 © 2012

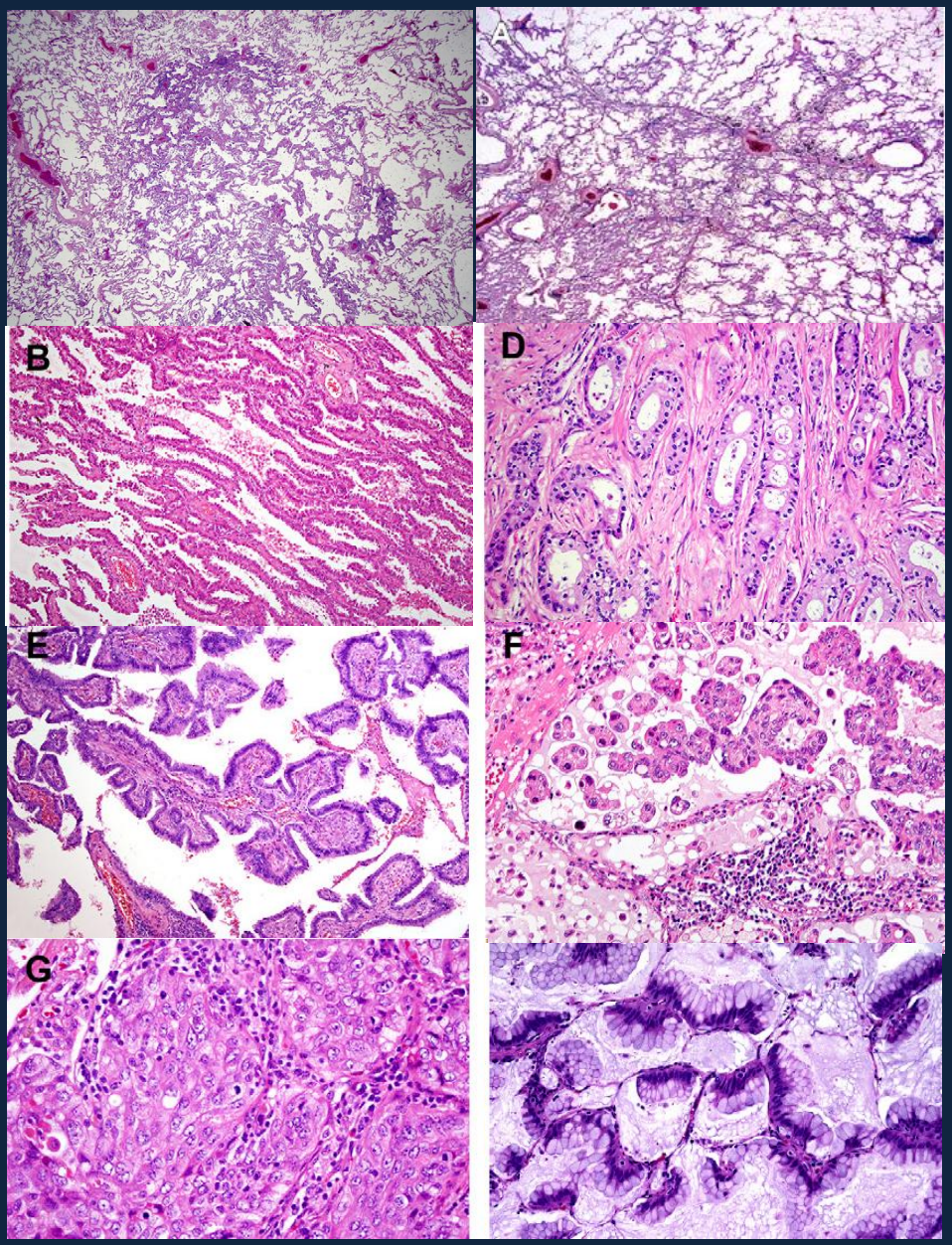
대한병리학회 심폐병리연구회 조사 2009

EGFR mutations

1,753 Korean patients in 15 Hospitals

Clinicopathologic characteristics	%		
Total	34.3		
Adenocarcinoma	43.3		
Never smoker	48.1	19.8	Smoker
Female	50.3	22.3	Male
Female & Never smoker	51.9		
Never smoker & Adenocarcinoma	52.6		
Female & Adenocarcinoma	53.5		
Female, Never smoker, Adenocarcinoma	54.8		

Lung adenocarcinoma histologic subtypes



AIS/ MIA

Lepidic pattern/ Acinar

Papillary/ Micropapillary

Solid/ Mucinous

Travis WD. Clin Chest Med 2011;32:669-92.

EGFR mutation & Adenocarcinoma histologic subtypes

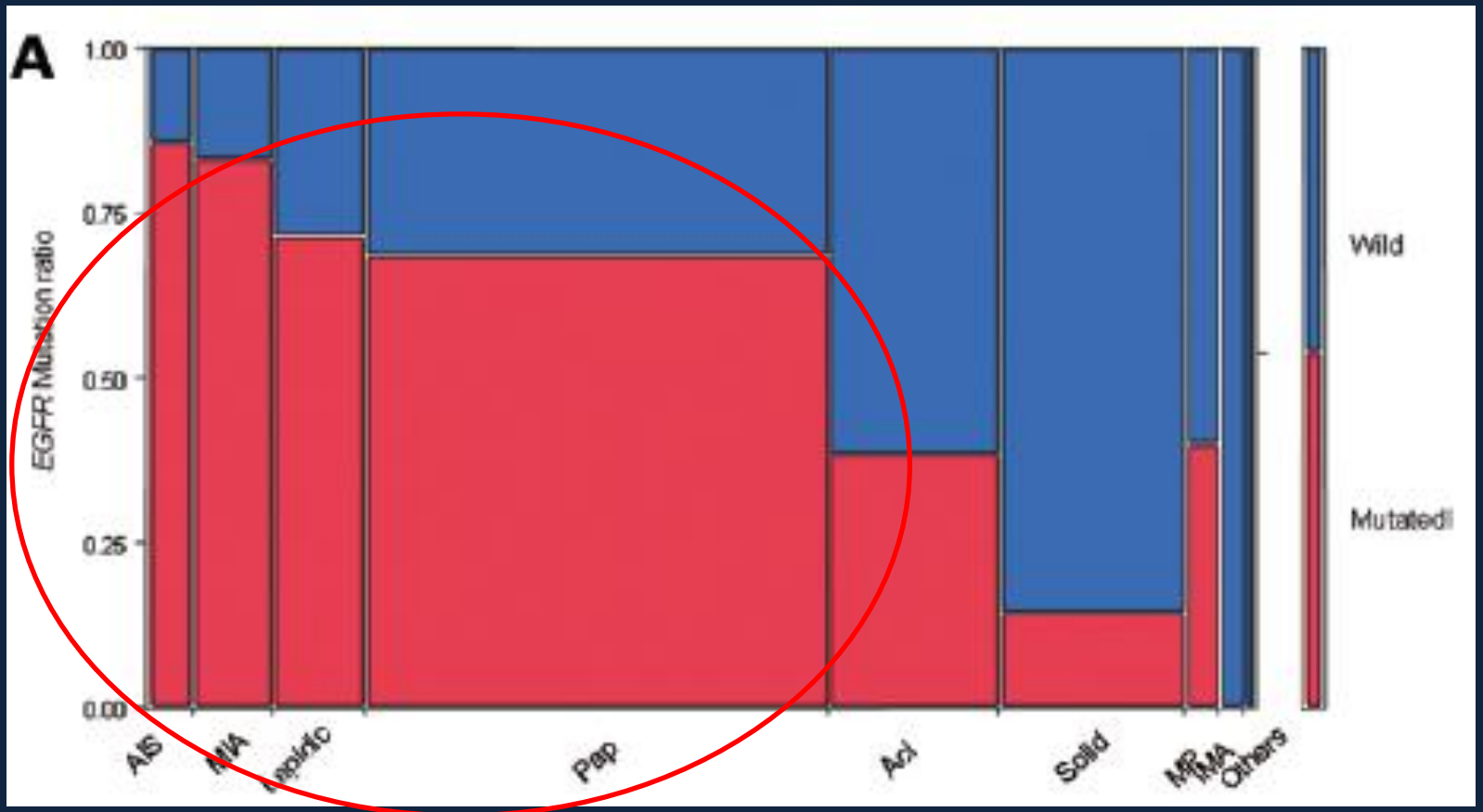
IASLC/ATS/ERS

	TTF-1+	EGFR+	K-RAS +	Other
AIS/MIA (non-mucinous)	100%	10-30% (never smokers)	10-30% (smokers)	--
Lepidic (non-mucinous)	100%	10-30% (never smokers)	10% (smokers)	BRAF mt 5%
Papillary	90-100%	10-30%	No	BRAF mt 5%
Acinar	+/-	<10% (nonsmokers)	20% (smokers)	ALK >5%
Micro-papillary	?	20%	33%	BRAF mt 20%
Solid	70%	10-30% (never smokers)	10-30% (smokers)	ALK >5% MUC1+
Invasive mucinous	0-33%	None	80-100%	MUC 2,5,6+

Travis WD et al. J Thorac Oncol 2011;6:244-85.

EGFR mutation & Adenocarcinoma histologic subtypes

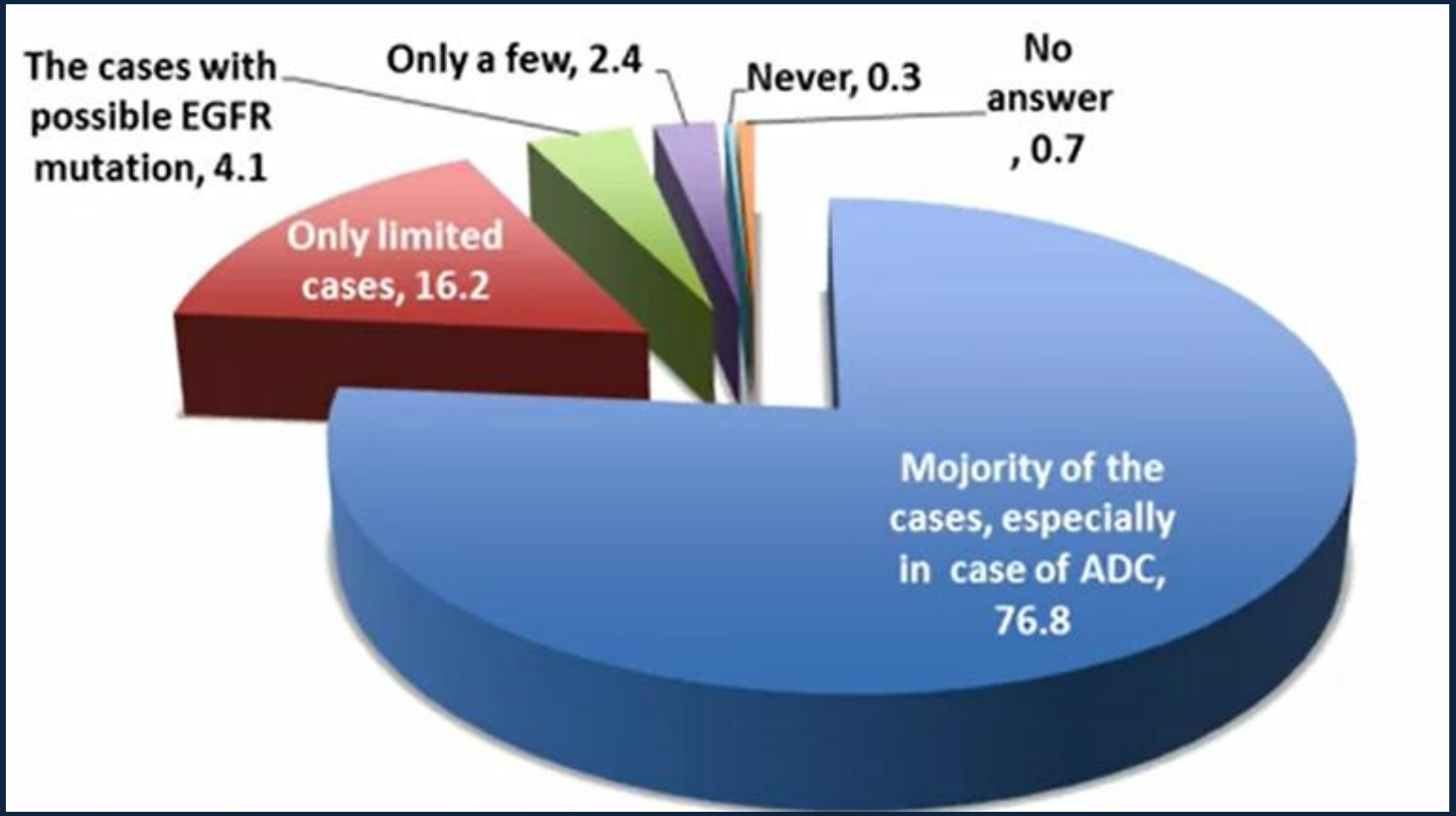
Japan (n=440)



Yoshizawa A et al. J Thorac Oncol 2013;8:52-61.

How many samples do you submit to *EGFR* mutation test?

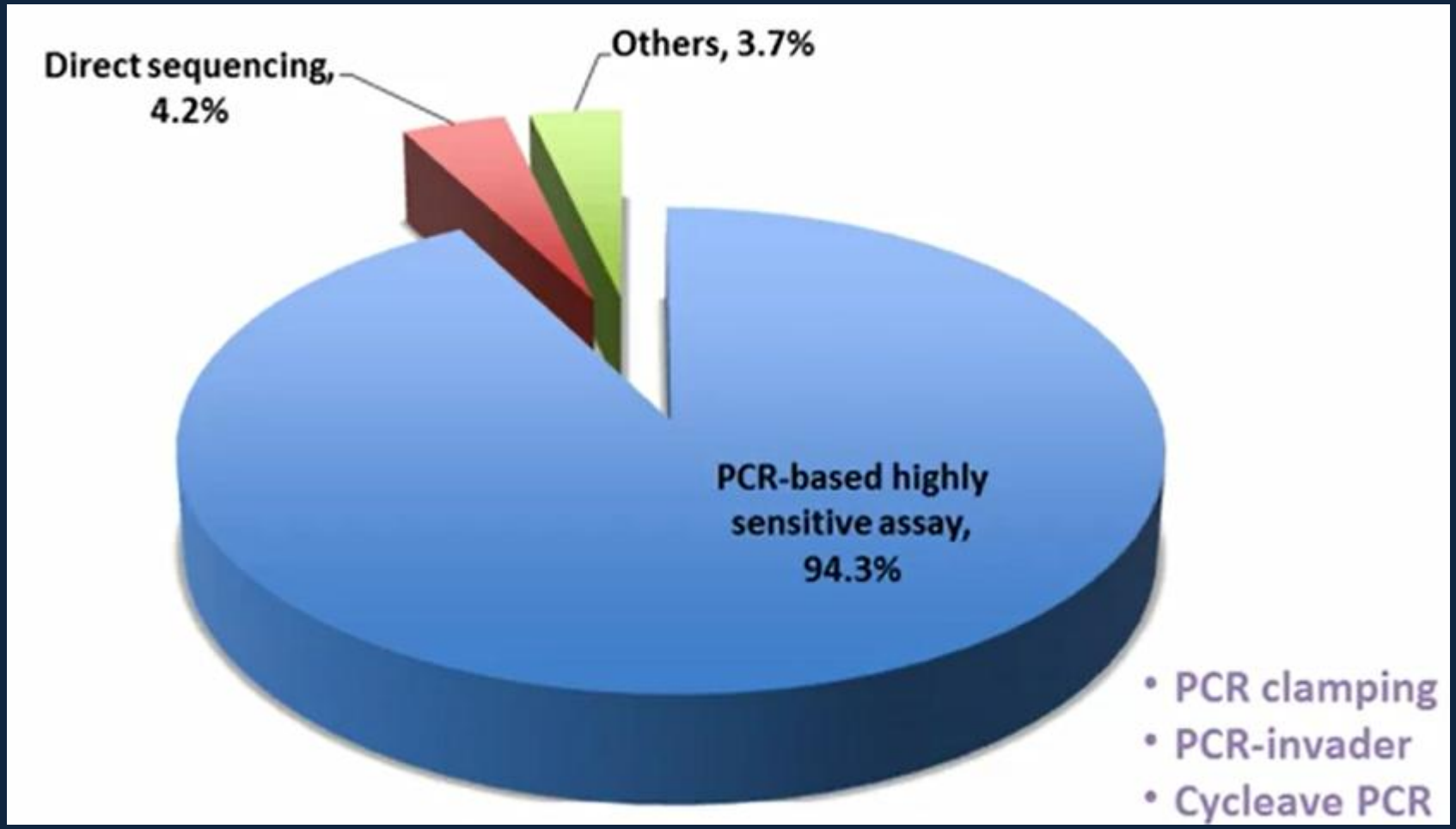
Japan (n=582)



Yatabe Y. ELCC 2012

What method do you use for *EGFR* test?

Japan

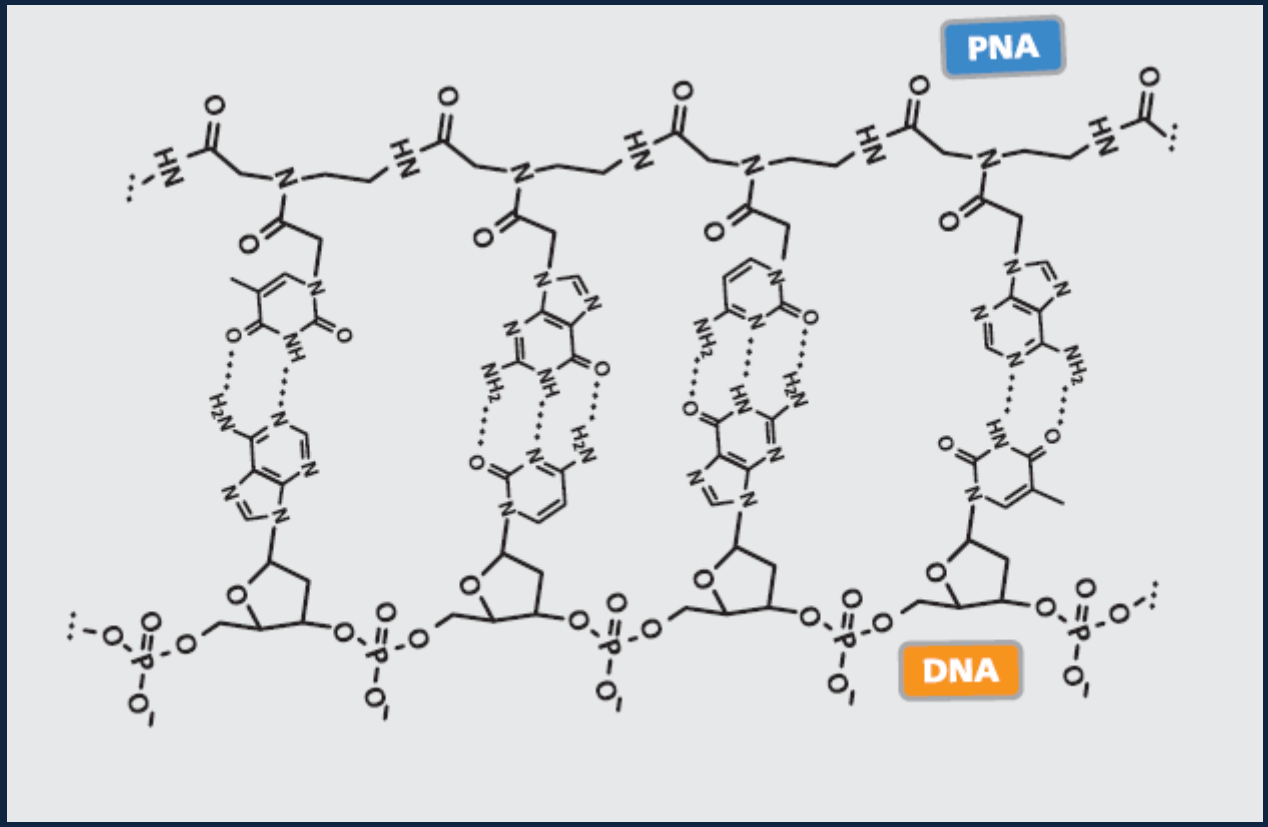


Yatabe Y. ELCC 2012

Examples of different techniques reported for *EGFR* mutation testing

Methods	% of tumoral DNA required in sample	*EGFR* mutations, deletions, insertions detected
Direct sequencing	25%	Known mutations and new
Pyrosequencing	10%	Known mutations and new
PCR-SSCP	10%	Known mutations and new
q-PCR	10%	Known mutations only
PCR, heteroduplex cleave	5-10%	Known mutations and new
PCR, heteroduplex melting	5-10%	Known mutations and new
SnapShot PCR	1-10%	Known mutations only
ARMS	1%	Known mutations only
PNA PCR clamp	1%	Known mutations only
SMAP	0.1%	Known mutations only

PNA (Peptide Nucleic Acid)



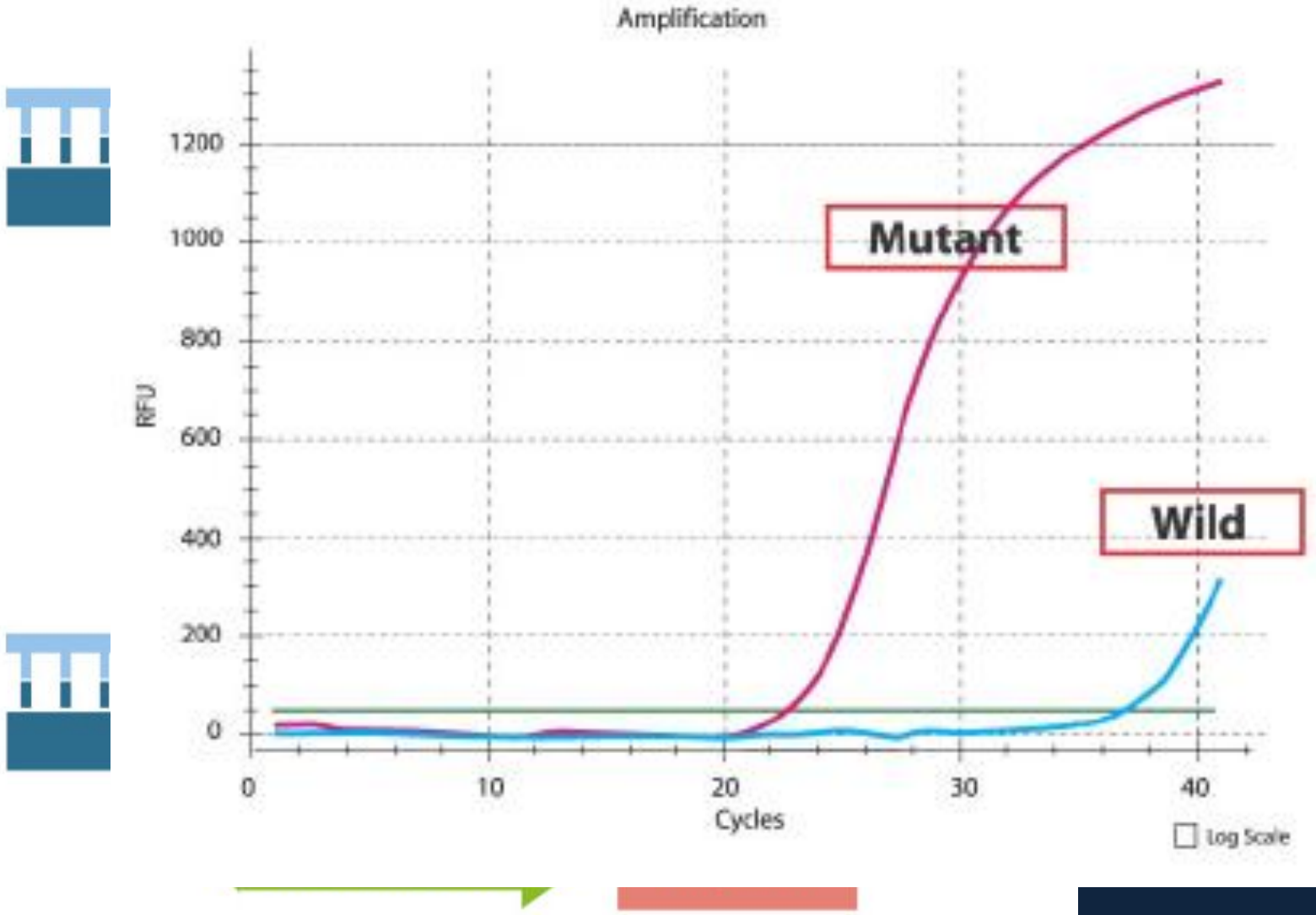
PNA : an artificially created nucleic acid replaced with polyamide backbone of N-(2-aminoethyl) glycine.

: strongly binds to its complementary DNA or RNA sequence

: high specificity, sensitivity and stability as molecular probes

Panagene co.

Principle of PNA clamp technology



Panagene co.

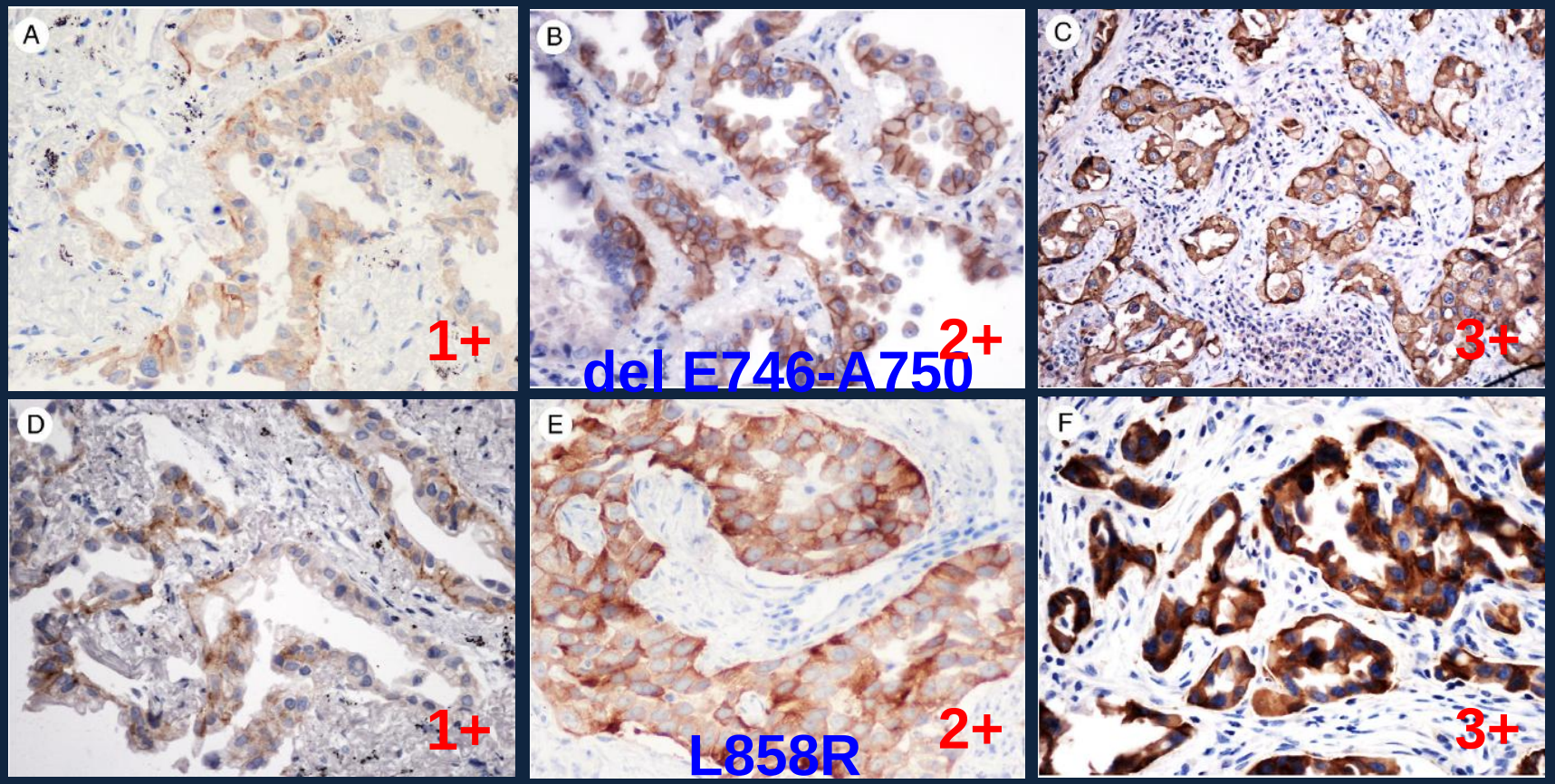
Response to *EGFR*-TKIs to *EGFR* mutational status

Korean (n=240)

Response	EGFR mutation		Total
	PNA+/DS+	PNA+/DS-	
CR/PR	31 (50.8)	7 (31.8)	38 (45.8)
SD	23 (37.7)	10 (45.5)	33 (39.8)
PD	7 (11.5)	5 (22.7)	12 (14.5)
Total	61	22	83

Kim HJ et al. Lung Cancer 2012;75:321-5.

IHC staining with *EGFR* mutation-specific antibodies



Xiangshan F et al. Hum Pathol 2013 in press

IHC methods for *EGFR* mutation testing

Reference	No of sample	EGFR mutations	IHC	Comparator	Sensitivity	Specificity
Kozu et al.	577	Exon 19 del	59 (10%)	135 (23%)	42%	100%
		Exon 21 L858R	139 (24%)	172 (39%)	76%	98%
Brevet et al.	194	Exon 19 del (E746_A750)	22 (11%)	20 (10%)	100%	98.8%
		other Exon 19 del	25 (13%)	31 (16%)	74%	
		Exon 21 L858R	22 (11%)	21 (11%)	95%	99%
Ilie et al.	61	Exon 19 del (E746_A750)	12 (20%)	8 (13%)	23%	
		all Exon 19 del	13 (21%)	10 (16%)		
Kato et al.	70	Exon 19 del (E746_A750)	9 (13%)	11 (16%)	82%	100%
		Exon 21 L858R	11 (16%)	12 (17%)	75%	97%
Nakamura et al.	20	Exon 19 del (E746_A750)	4 (20%)	3 (15%)	92%	100%
		Exon 21 L858R	11 (16%)	12 (17%)		
Simonetti et al.	78	Exon 19 del (E746_A750)	17 (22%)	17 (22%)		
		other Exon 19 del	3 (4%)	12 (15%)		
		Exon 21 L858R	25 (32%)	25 (32%)		
		Exon 21 L861Q	0 (0%)	2 (3%)		

Ellison G et al. J Clin Pathol 2013;8:52-61.

EGFR mutation test using cytology samples

Reference	Cytology samples (no. of samples for mutation analysis (fail data if available))	Method(s) of *EGFR* mutation testing assessed	Authors' conclusions on use of cytology samples for *EGFR* mutation testing
Asano *et al*[29]	Cell-free PLE (n=20), CT-guided needle lung biopsies (n=18)	Mutant-enriched PCR versus non-enriched PCR and direct sequencing	Mutant-enriched PCR detected *EGFR* alterations that were not identified with a non-enriched assay
Fassina *et al*[61]	TTNA samples (n=77)	HRMA versus direct sequencing	HRMA of TTNA samples was accurate, fast, easy, cheap, and reliable for the detection of common *EGFR* mutations
Hlinkova *et al*[62]	Cytological samples obtained by endobronchial brushing (n=53)	HRMA versus direct sequencing (with mutant-enriched PCR if <25% tumour cells)	HRMA in combination with mutant-enriched PCR is a sensitive method for mutation detection in cytology samples
Horiike *et al*[63]	Transbronchial FNA (n=93 (10 fails (11%) with direct sequencing; 0 fails with Scorpion ARMS))	Scorpion ARMS versus direct sequencing	Both methods detected *EGFR* mutations in transbronchial FNA samples although Scorpion ARMS was more sensitive
Kawahara *et al*[63]	PLE (n=21), CSF (n=2), and ascites (n=1)	Immunocytochemistry versus PNA-LNA PCR clamp	*EGFR* mutations were detected in PLE and CSF with 100% sensitivity using antibodies specific for the exon 19 deletion E746_A750 and the exon 21 point mutation L858R
Kimura *et al*[64]	Cell-free PLE (n=43)	Direct sequencing	DNA in PLE can be used to detect *EGFR* mutations
Kimura *et al*[51]	Cell-free PLE (n=24)	Scorpion ARMS versus direct sequencing	DNA in PLE can be used to detect *EGFR* mutations. Scorpion ARMS was more sensitive than direct sequencing
Kozu *et al*[44]	Imprint cytological smears from fresh-cut surface of resected tumour specimens (n=36)	HRMA versus IHC	(Results of cytology sample analyses were combined with those of 541 tissue specimens (see table 2))
Lim *et al*[65]	FNA (n=29)	Whole genome amplification followed by direct sequencing	*EGFR* mutations were identified using direct sequencing of whole genome-amplified genomic DNA from low-volume RNA samples
Lozano *et al*[66]	Primary lung tumour FNA (n=68), metastatic lymph node FNA (n=10), bone metastases FNA (n=3), left adrenal metastasis FNA (n=1), PLE (n=6), PCE (n=1), and bronchoalveolar lavage (n=1)	Direct sequencing	Assessment of *EGFR* mutation in cytology samples is feasible and comparable with biopsy results

Ellison G et al. J Clin Pathol 2013;8:52-61.

EGFR mutation test using cytology samples

Reference	Cytology samples (no. of samples for mutation analysis (fail data if available))	Method(s) of *EGFR* mutation testing assessed	Authors' conclusions on use of cytology samples for *EGFR* mutation testing
Nakajima *et al*[60]	EBUS-TBNA samples from metastatic lymph nodes (n=43)	Loop-hybrid mobility shift assay confirmed by direct sequencing	*EGFR* mutations can easily be detected in metastatic lymph nodes samples by EBUS-TBNA
Oshita *et al*[57]	Cytology samples obtained by transbronchial abrasion (n=52) (2 fails (4%))	Loop-hybrid mobility shift assay	Assessment of *EGFR* mutations in cytological samples is feasible and comparable with biopsy results
Otani *et al*[30]	Biopsy needle wash fluid (n=26)	Mutant-enriched PCR versus non-enriched PCR versus direct sequencing	*EGFR* mutations can be detected in the wash fluid of CT-guided biopsy needles
Rekhtman *et al*[68]	Transbronchial/transthoracic FNA (n=67), extrathoracic FNA (n=29), PLE (n=29), and bronchial brush/wash (n=3) (2 failures (2%))	Length analysis and PCR-RFLP	*EGFR* analysis is feasible in routinely processed cytology samples
Savic *et al*[69]	Transbronchial FNA (n=35), PLE (n=16), bronchial washing (n=15), bronchial brushes (n=13), and bronchoalveolar lavage (n=5)	PCR-direct sequencing	*EGFR* analyses are applicable to cytology specimens
Schuurbiers *et al*[54]	EBUS-/EUS-FNA samples (n=35 (8 fails (23%))	Direct sequencing	Molecular analysis for *EGFR* mutations can be performed routinely in EBUS-/EUS-FNA samples
Soh *et al*[50]	Cell-free PLE (n=61)	Direct sequencing versus mutant-enriched PCR versus non-enriched PCR versus PNA-LNA PCR-clamp	Some discrepancies between the results of the four assays were noted. Mutant-enriched PCR detected the most mutations
Takano *et al*[23]	Bronchial brushing/washing (n=43), PLE (n=40), transbronchial FNA (n=9), PCE (n=8), superficial lymph node FNA (n=7), tumour FNA (n=6), and sputum (n=4)	HRMA versus direct sequencing	Exon 19 deletions and the exon 21 point mutation L858R can likely be detected from archived Papanicolaou-stained cytology slides with sensitivity of ca. 90% and specificity of ca. 100%
van Eijk *et al*[56]	EBUS-TBNA/EUS-FNA samples (numerous samples from 43 patients)	Real-time PCR with hydrolysis probes	All mutations detected in matched histological samples were also identified in the cytology samples
Yasuda *et al*[70]	ELF (n=23)	PNA-LNA PCR clamp	Sensitivity for detecting mutations in ELF was 58%
Zhang *et al*[71]	PLE cells and matched cell-free PLE (n=26)	Mutant-enriched PCR versus direct sequencing	Direct sequencing may miss a significant proportion of mutations in PLE samples. Mutant-enriched PCR may be more reliable
Smits *et al*[72]	Cytology and FFPE samples (n=816; 719 samples had interpretable result)	Direct sequencing or HRMA	(Results of cytology sample analyses were combined with those of FFPE specimens)

Ellison G et al. J Clin Pathol 2013;8:52-61.

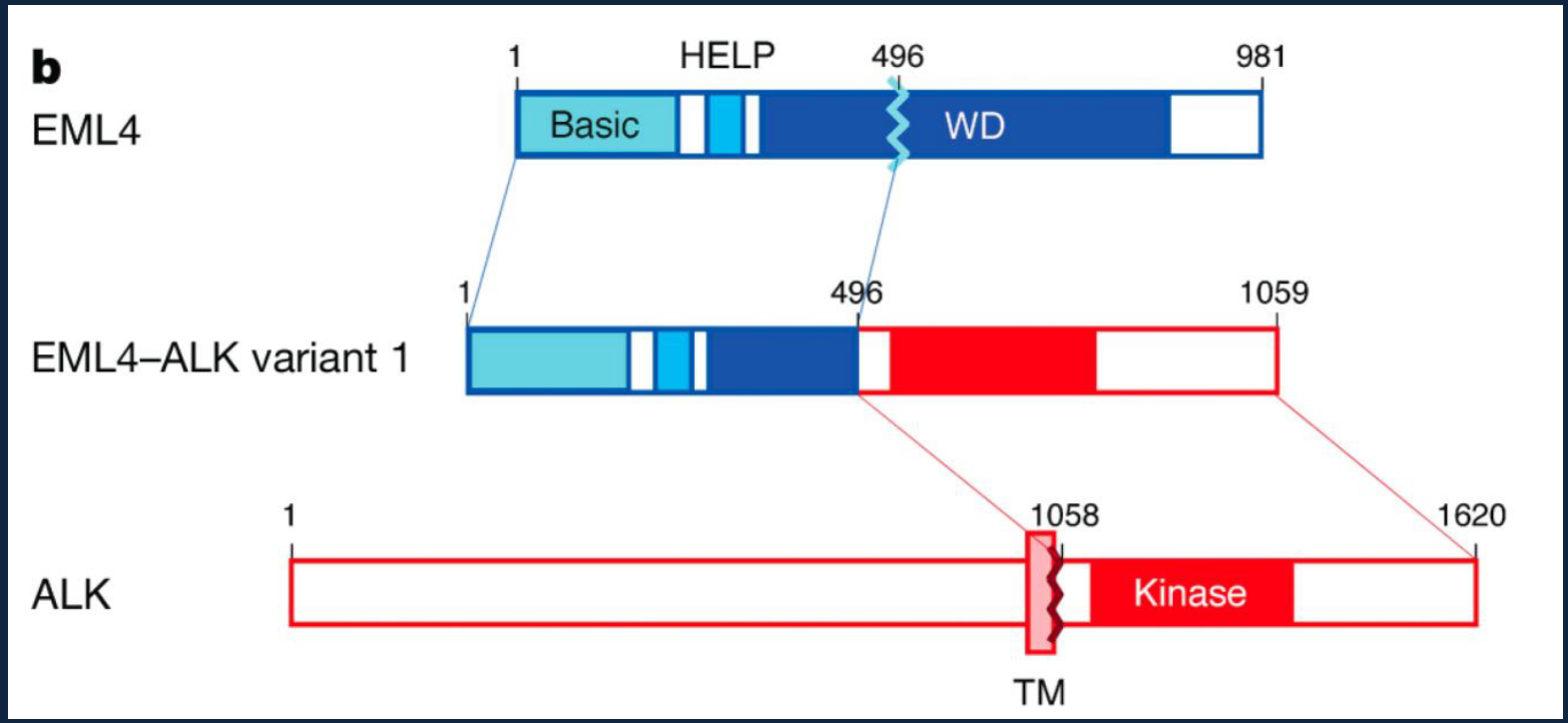
EGFR mutation test using cytology samples

Tsai *et al*[73]	PLE (n=78)	IHC versus direct sequencing	*EGFR* mutations were detected in PLE with 71% and 88% sensitivity using antibodies specific for the exon 19 deletion E746_A750 and the exon 21 point mutation L858R, respectively Correlation of TKI response rate with *EGFR* mutation status was comparable when determined by IHC and direct sequencing (67% vs 72%)
Navani *et al*[67]	EBUS-TBNA samples (n=774)	ARMS or MassARRAY	EBUS-TBNA cytology samples are suitable for *EGFR* analysis
Aisner *et al*[74]	Cytology cell blocks, including FNA of primary and metastatic lung lesions and exfoliative cytology specimens (n=42)	PCR-sequencing	Cell block specimens provide an alternative DNA source to surgical specimens for *EGFR* analysis
Zhuang *et al*[65]	CT-guided FNA biopsy (n=43)	Direct sequencing	CT-guided FNA biopsy is a feasible and safe method to provide samples for *EGFR* analysis
Santis *et al*[68]	EBUS-TBNA lymph node samples (n=131; successful analysis of 126 samples)	COLD-PCR	EBUS-TBNA samples provide sufficient tumour material for *EGFR* mutation analysis COLD-PCR is a robust screening assay for *EGFR* mutations
Malapelle *et al*[75]	LBC (n=42)	Direct sequencing	LBC samples can be used for *EGFR* mutation analysis; however, direct sequencing requires micro-dissection to provide sufficient sample DNA
Betz *et al*[76]	Romanowsky-stained direct cytology smears (n=33)	Direct sequencing	Following micro-dissection, direct smears can be used as a specimen source for *EGFR* analysis when cell blocks exhibit insufficient cellularity
Cho *et al*[77]	Body fluid specimen (n=32: pleural fluids (n=29), CSF (n=1), pericardial (n=1), and ascites (n=1))	Direct sequencing	Combined direct sequencing and cytological analysis might be clinically useful and sensitive for the detection of *EGFR* mutations
Tsai *et al*[52]	PLE (n=150)	Direct sequencing of cell-derived RNA versus genomic DNA	Sequencing of RNA improves sensitivity for *EGFR* mutation detection in PLE samples compared with genomic DNA
Lozano *et al*[66]	Cytology samples (n=150: Papanicolaou smears (n=120), Fresh/liquid (n=14), cell block (n=10), ThinPrep tests (n=6))	Direct sequencing	*EGFR* analysis using cytological samples is feasible and comparable with biopsy results
Nakajima *et al*[69]	EBUS-TBNA metastatic lymph node samples (n=156)	PNA-LNA PCR clamp	EBUS-TBNA samples can be used for multi-gene mutational analysis

Ellison G et al. J Clin Pathol 2013;8:52-61.

ALK rearrangement

EML4-ALK fusion gene in NSCLC



Soda M et al. Nature 2007;448:561-6.

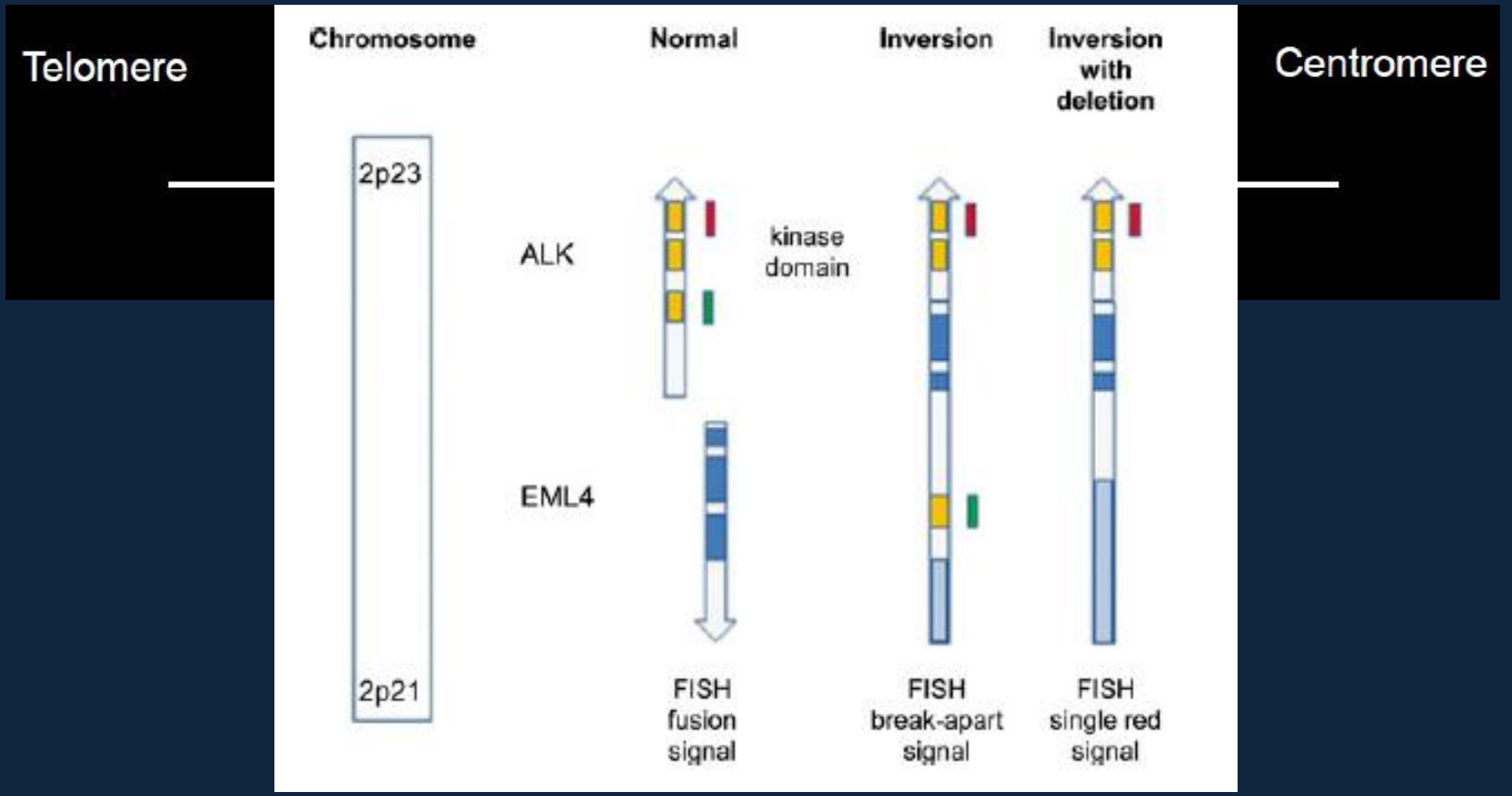
Frequency of *EML4-ALK* mutation

Table 2 Frequency of *EML4-ALK* Mutation and Demographic Characteristics of Selected Studies

Author	N	Alk+[a]	% of ADC	Mean Age	Smokers	Female
Soda et al[6]	33	9.1%	9.1%	n/a	n/a	n/a
Takeuchi et al[68]	253	4.4%	4.4%	n/a	n/a	n/a
Koivunen et al[67]	305	3.0%	n/a	n/a	n/a	n/a
Perner et al[69]	603	2.6%	n/a	n/a	n/a	n/a
Inamura et al[71]	149	3.4%	3.4%	52	40%	60%
Shaw et al[66]	141	13.0%	14.0%	52	0%	42%
Takahashi et al[70]	313	1.6%	2.4%	70	0%	80%
Kwak et al[74]	1500	5.4%	n/a	51	24%	48%

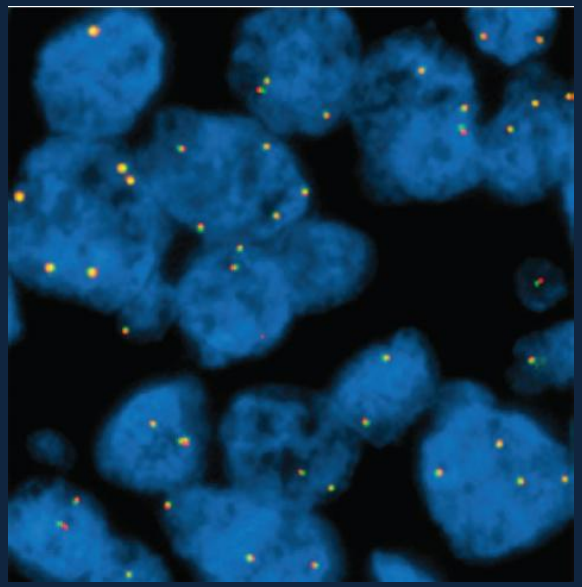
Clin Lung Cancer 2011;12:360-8.

Break-apart FISH assay for *ALK* fusion genes

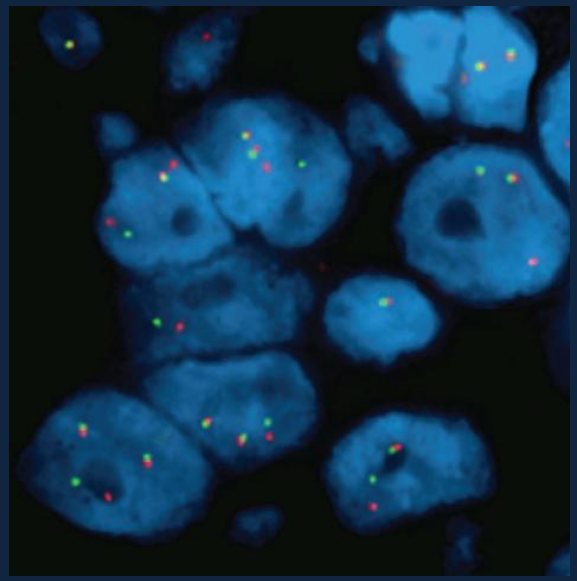


Thunnissen E et al. Virchows Arch 2012;461:245-57.

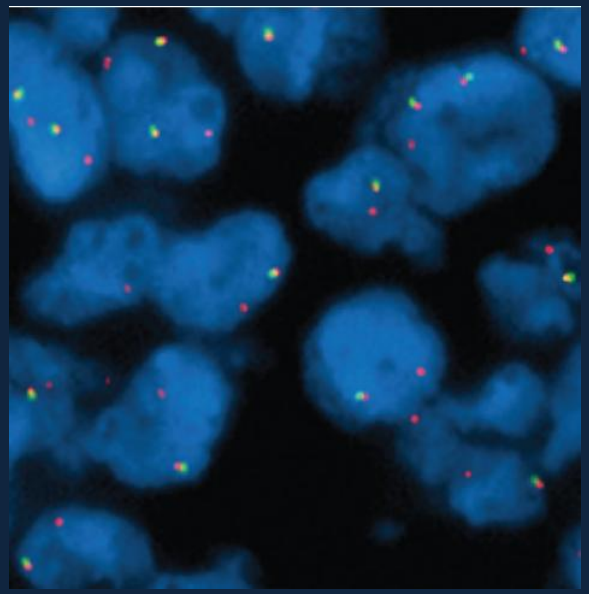
Different break-apart FISH testing patterns



Fused (negative)

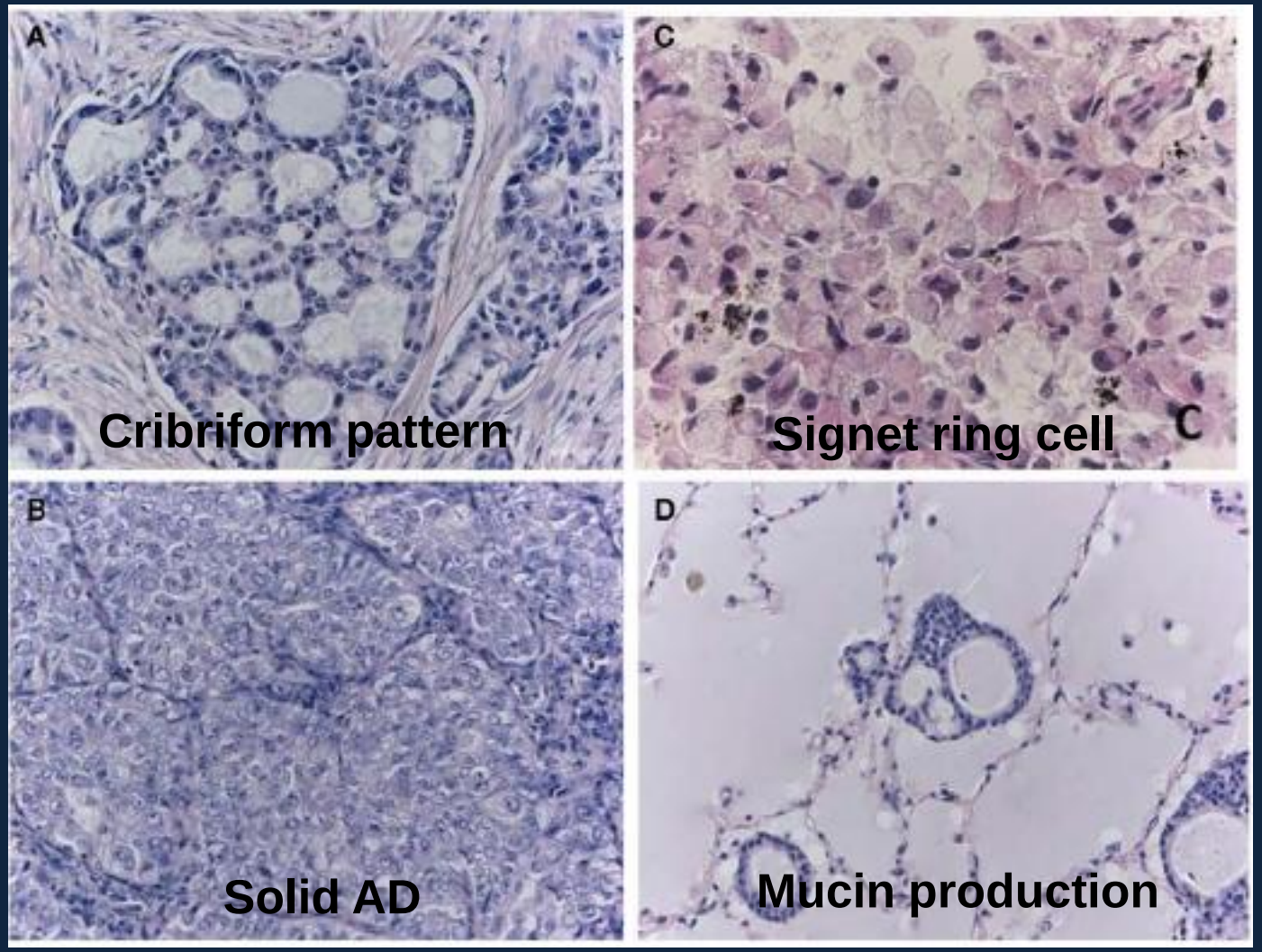


Split (positive)



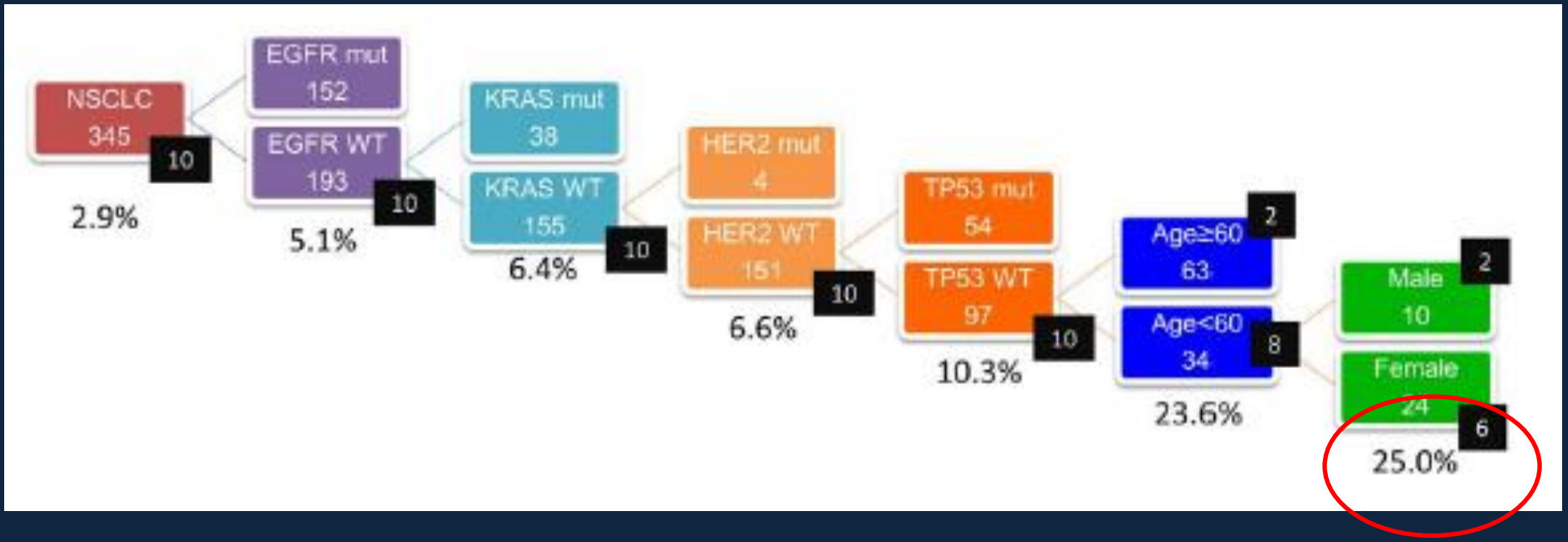
Single red (positive)

Morphological characteristics: *ALK*+ lung cancer



Murakami Y et al. Front Oncol 2012;2:1-9.

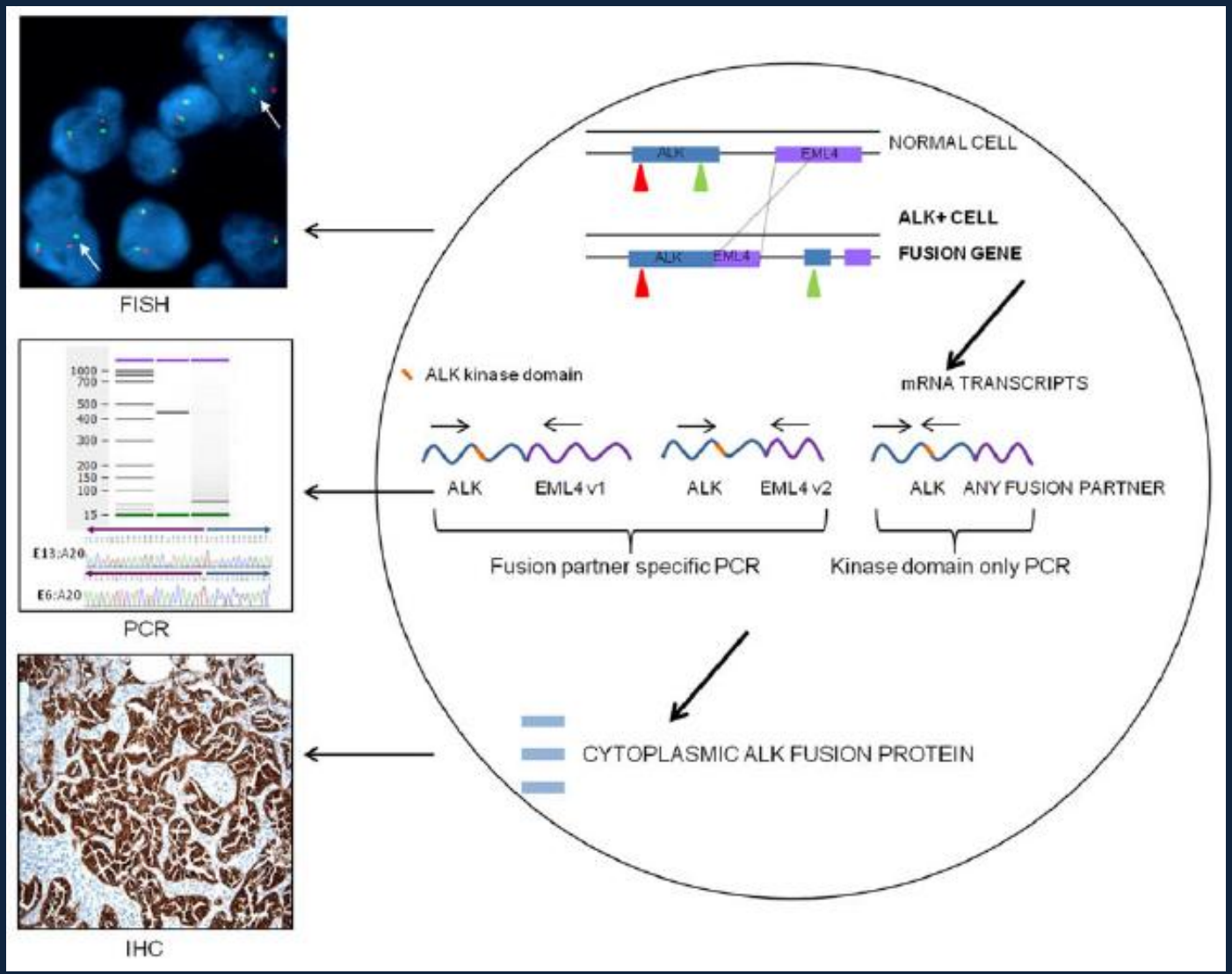
EML4-ALK translocation: Clinicopathologic features



“Mutually exclusive”

Murakami Y et al. Front Oncol 2012;2:1-9.

Schematic of ALK testing methodologies



Weickhardt AJ et al. Cancer 2012 online.

Advantages and disadvantages of ALK detection methods

	Pros	Cons
RT-PCR	A potentially rapid diagnostic method Very sensitive More accurate	Difficult to obtain high-quality of RNA Not applicable for unknown partners Difficult to confirm the presence of tumor cells Difficult to apply to archival tissues
FISH	Applicable for any partners Screening method in clinical trials Established in many labs Applicable to archival tissues	Expensive Relative long turnaround time Less sensitive
IHC	Applicable for any partners Rapid turnaround time Established in many labs Applicable to archival tissues Cheap	Indirect demonstration of the fusion gene Occasional false negative results High dependence on antibody clones and detection methods

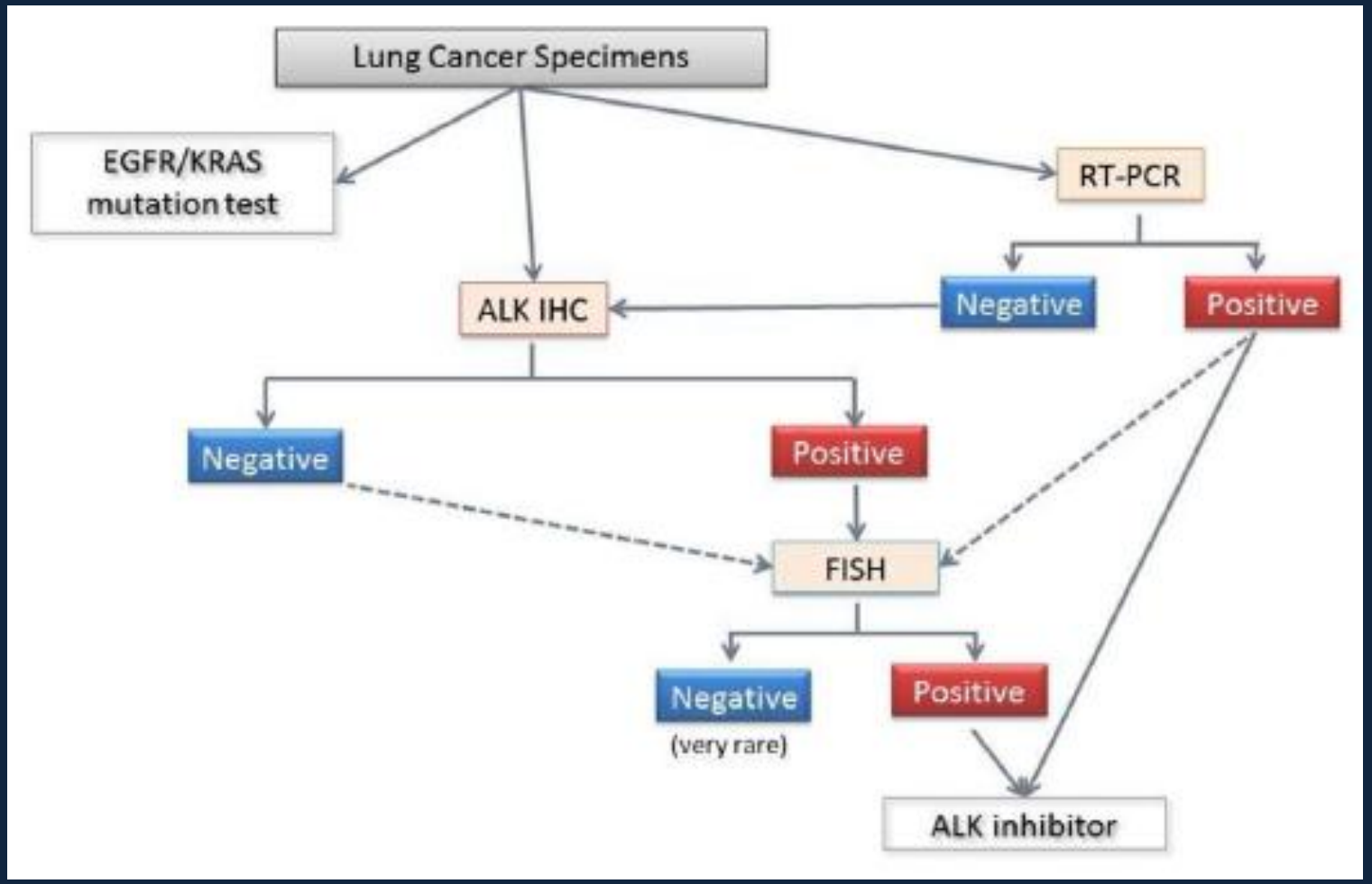
Published articles comparing *ALK* anaylsis by FISH & IHC

Reference	Antibiody	Samples, No.	ALK+		ALK-		Sensitivity %	Specificity %
			FISH	IHC	FISH	IHC		
Shaw et al.	ALK1	NSCLC, 141	19 (13%)	19 (13%)	NA	NA		
Boland et al.	ALK1	NSCLC, 335	6 (1.8%)	6 (1.8%)	NA	NA		
Rodig et al.	ALK1	AD, 358	20 (5.6%)	8/10 (available)	NA	NA	80	NA
Mino-Kenudson et al.	ALK1	AD, 153	22 (14.3%)	NA	NA	NA	67	97
	D5F3	AD, 153	22 (14.3%)	NA	NA	NA	100	99
Yi et al.	ALK1	AD, 101	10 (9.9%)	11 (10.9%)	91 (90.1%)	90 (89.1%)	90	98
Paik et al.	5A4	NSCLC, 453	19 (4.2%)	26 (5.7%)	434 (95.%)	427 (94.3%)	100	92.5
Paik et al.	5A4	NSCLC, 735	28 (3.8%)	35 (4.8%)	707 (96.2%)	700 (95.2%)	100	96.2
		AD, 395	27 (6.8%)	NA	368 (93.2%)	NA		
McLeer-Florin et al.	5A4	AD, 441	NA	29 (6.5%)	NA	NA	100	95
Yang et al.	ALK1	AD, 300	22/216 (10.2%)	32 (10.7%)	194/216 (89.8%)	268 (89.3%)		

Weickhardt AJ et al. Cancer 2012 online. Thunnissen E et al. Virchows Arch 2012;461:245-57.

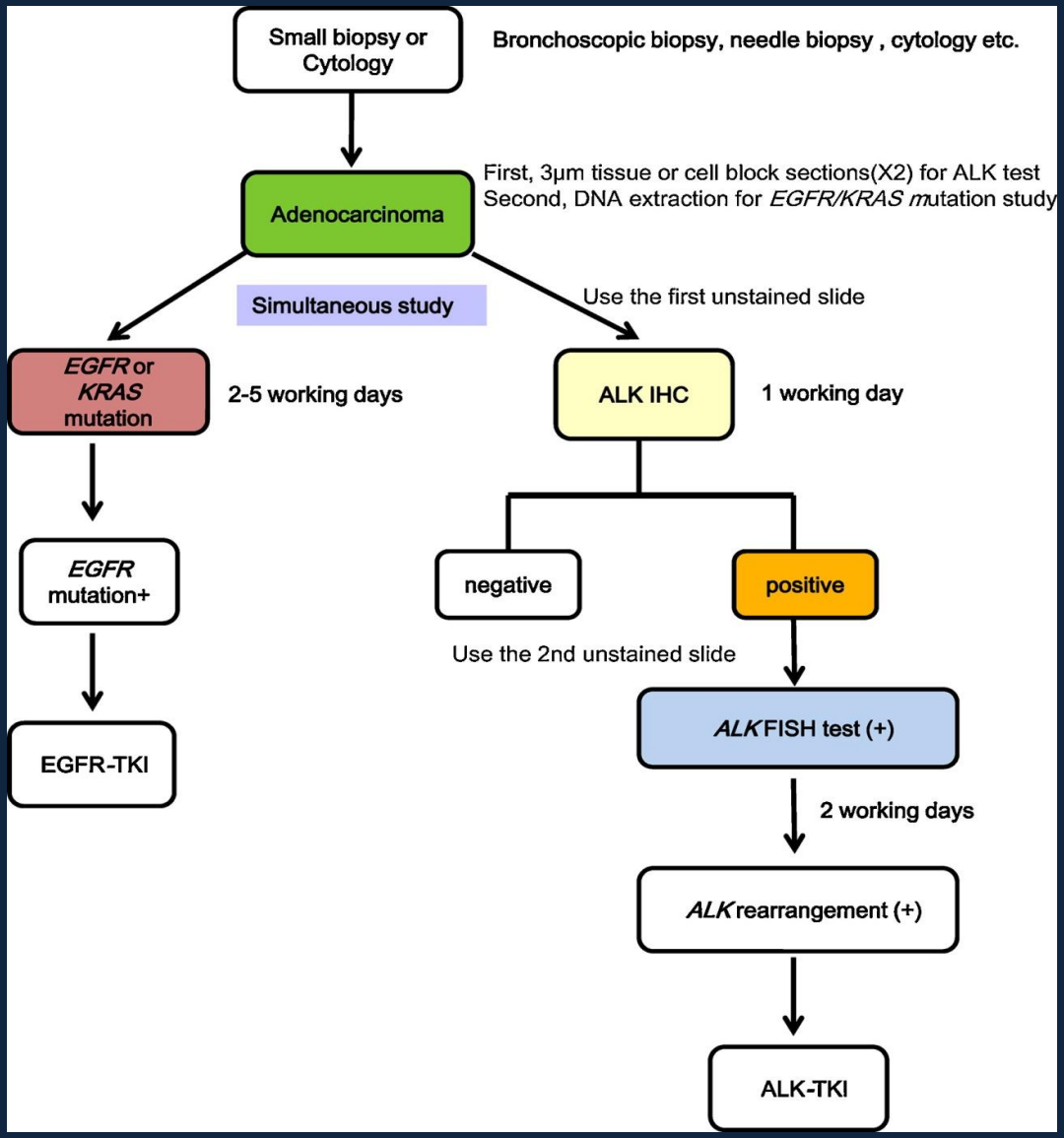
ALK gene test for lung cancer patients

Japan



Japan Lung Cancer Society. Aug 1, 2011

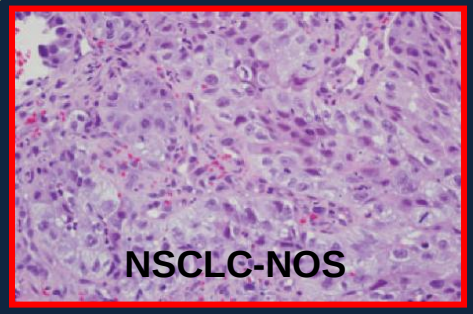
Chung's SNUBH adenocarcinoma molecular test



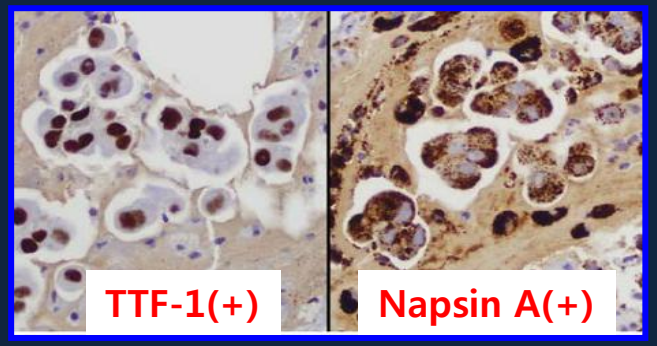
Paik JH et al. Lung Cancer 2012;76:403-9.

NSCLC diagnosis & molecular analysis

1st H&E Histology

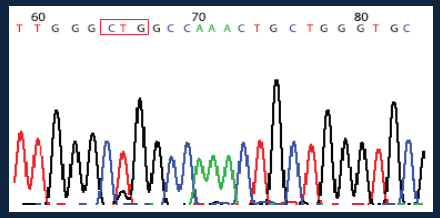


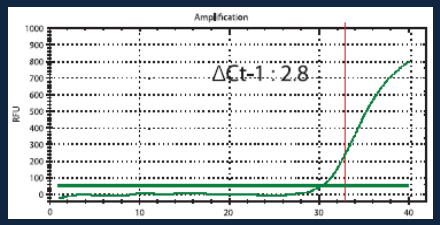
2nd IHC



3rd Molecular analysis

EGFR mutation

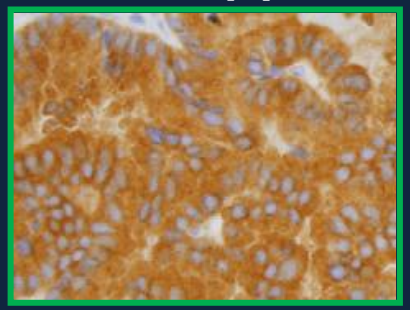




Wild -type

ALK rearrangement

IHC(+)



FISH