Longcarcinoom nieuwe wegen nieuwe kwaliteitsaspecten

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LUNG CANCER Diagnostic, Predictive flow

Cancer in the lung: primary vs metastases

Primary lung cancer: NSCLC – SCLC
Staging M+

NSCLC: adenocarcinoma vs squamous cell carcinoma

Adenocarcinoma **KRAS / EGFR mutation**
KRAS and EGFR negative:
ALK
BRAF?
PIC3CA?

Squamous cell carcinoma FGFR1?
DDR2?
PIC3CA?

Erik Thunnissen WCLC2011

Cancer in the lung: primary vs metastases

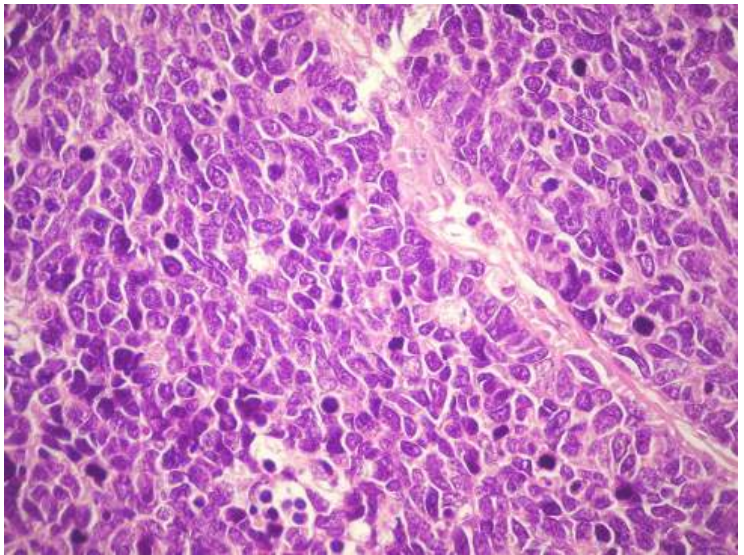
- Clinical information essential:
- PRIMARY LUNG TUMOR: Surfactant prot A, Napsin A, TTF1
- Saving of material not to do additional stains for metastases:
- Colorectal : CK7, CK20, CDX2,
- Prostate: PSA, PAP,
- Breast: ER, PR, GCDFP15, GATA3
- Germ cell: PLAP, AFPHcG, CD30, OCT3/4, Sox2, Sox17
- Melanocyte: Melan A, HMB45, Sox 10, MITF
- Mesothelium: Calretinin, CK5/6, D2-40, WT-1
- Kidney: RCC, CD10, Pax2, Pax8
- Ovary: CA125, Pax5, Pax 8,

Nonaka, Diagnostic Histopathology 2010

Primary lung cancer: NSCLC – SCLC

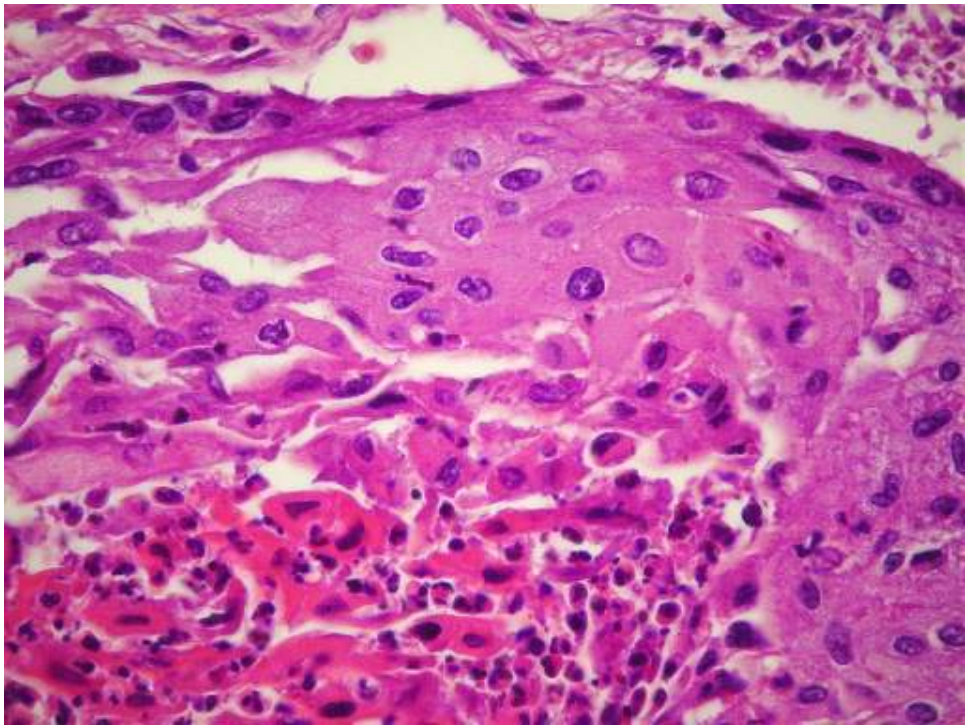
- SCLC: CD56, CHROMOGRANIN, SYNAPTOPHYSIN
- DD SCLC: CD45, KI67

- IN 5% CASES NO DISTINCTION POSSIBLE: BIOLOGY IS NOT BLACK AND WHITE



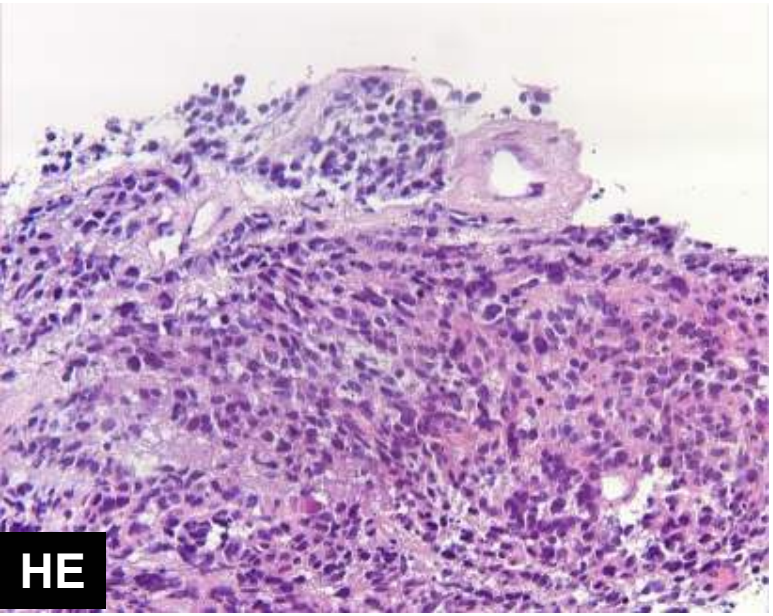
NSCLC: adenocarcinoma vs squamous cell carcinoma

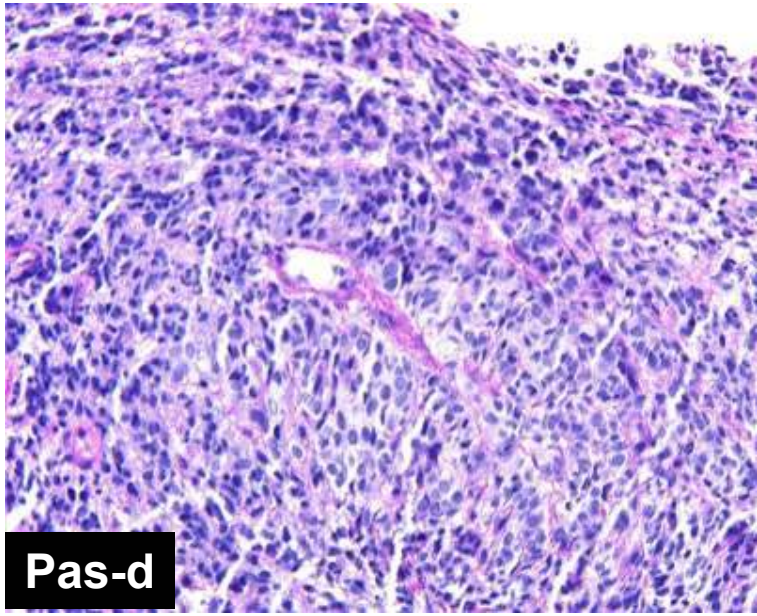
Squamous cell carcinoma

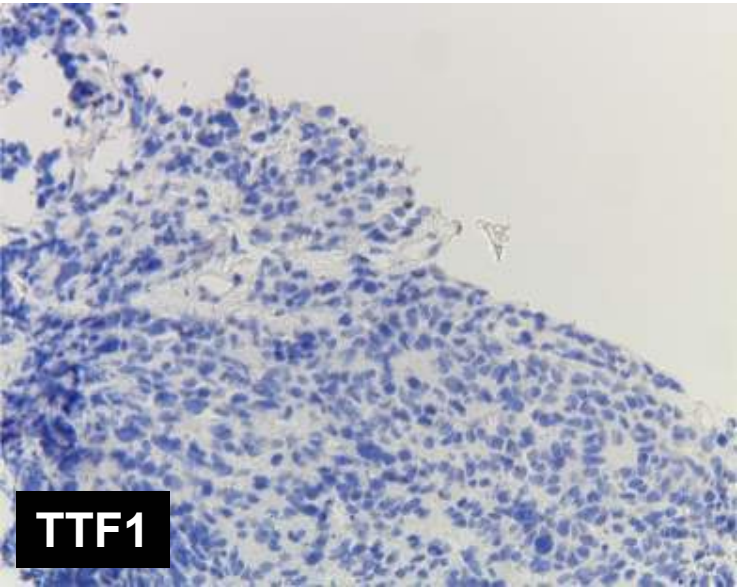


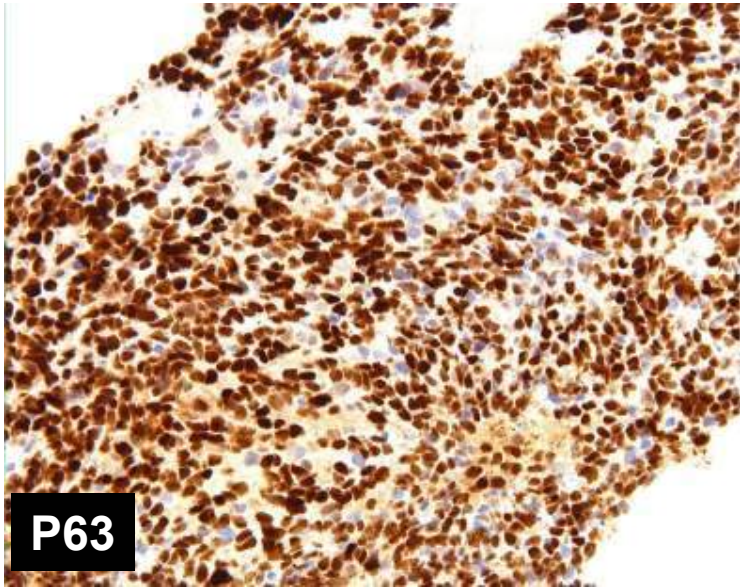
Study: 20% of squamous cell carcinomas were squamoid: IHC TTF1 or mucin +

Biopsy lung tumor

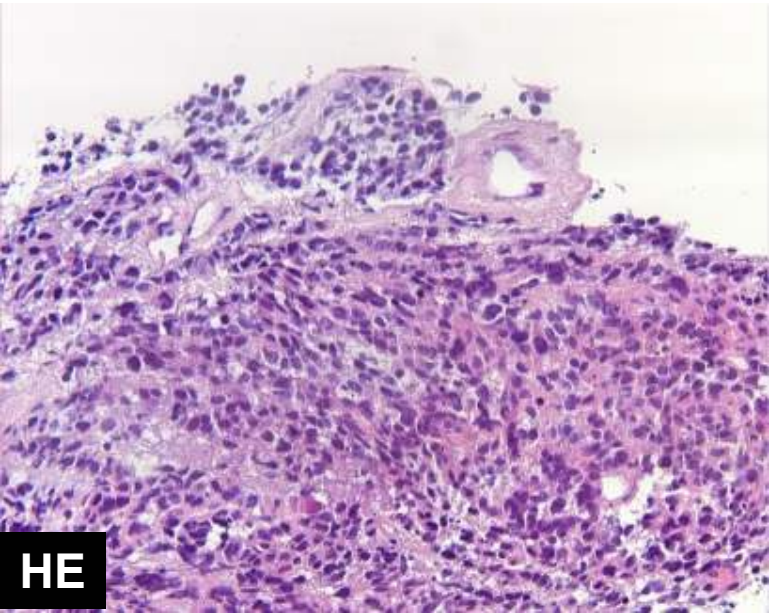


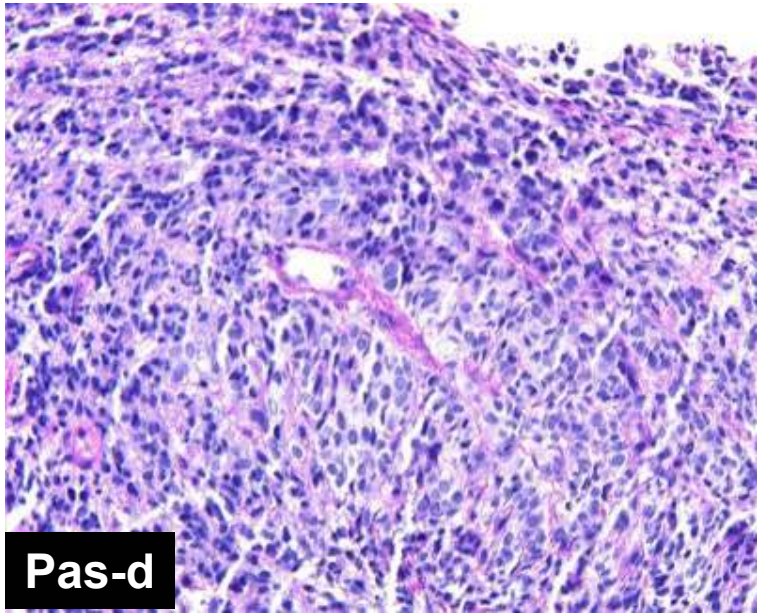


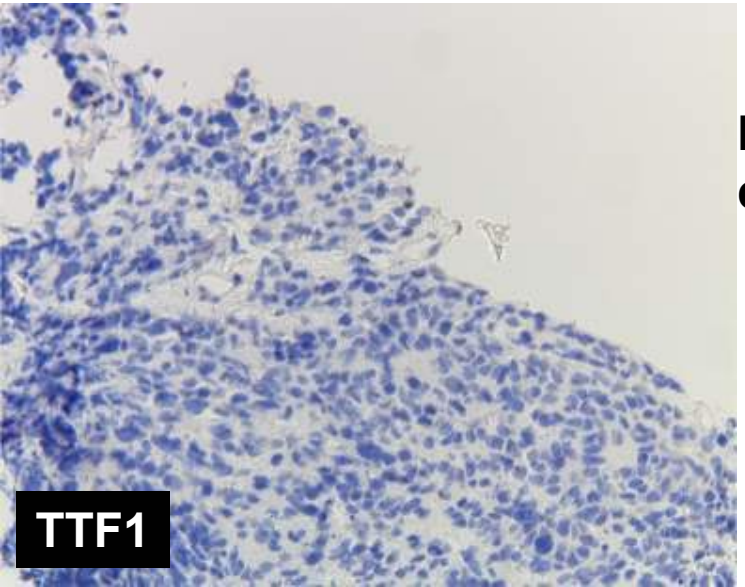




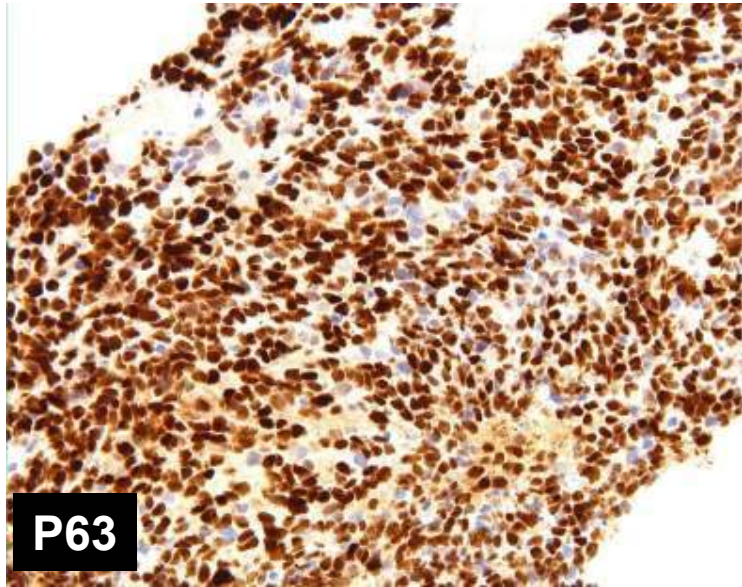
Biopsy lung tumor



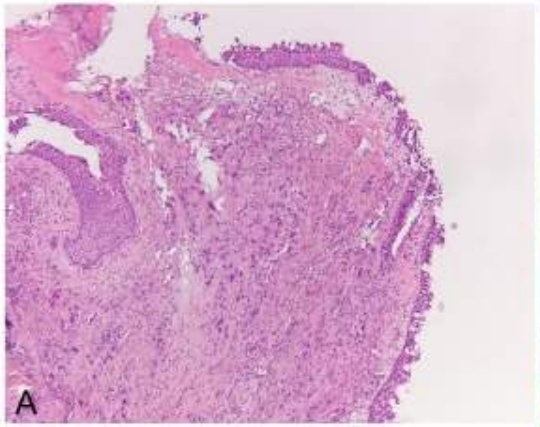


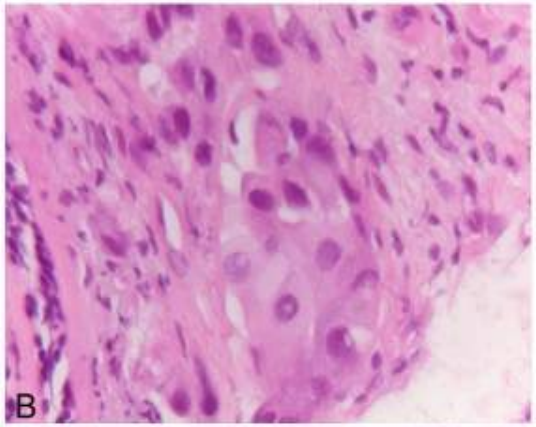


Favour squamous cell carcinoma



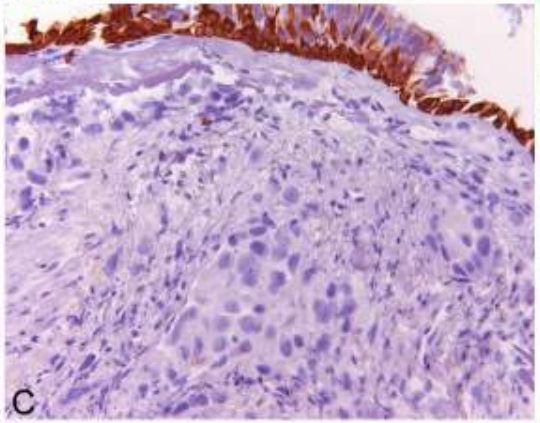
HE

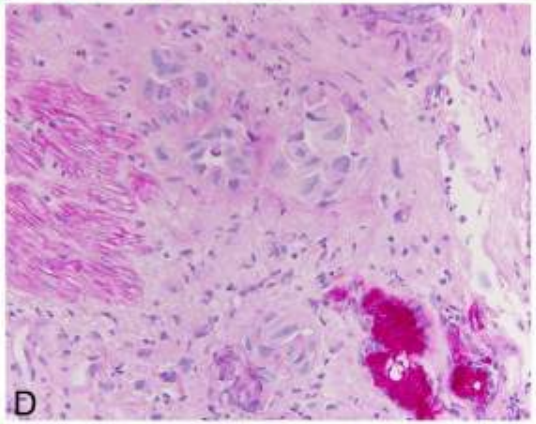




HE

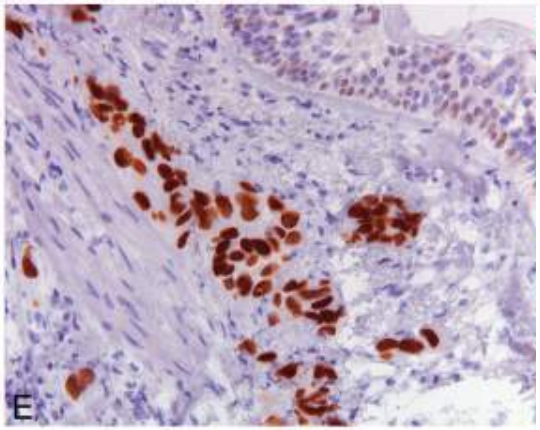
p63





PAS-D

TTF1



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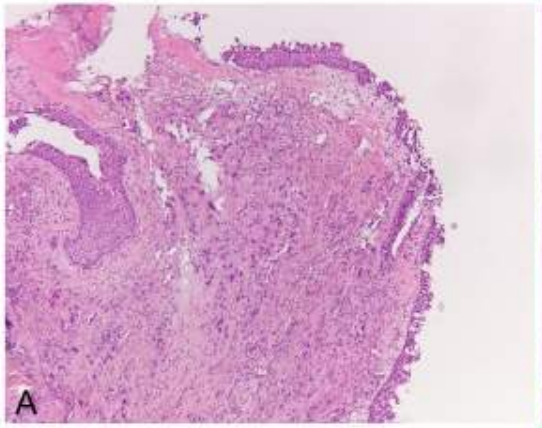
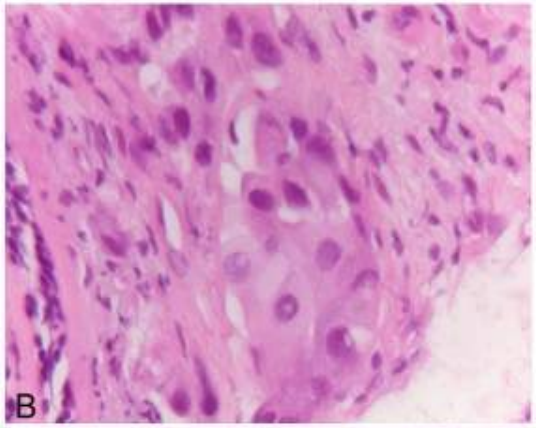
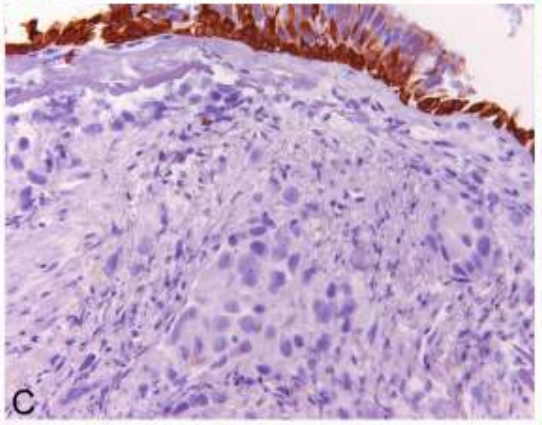
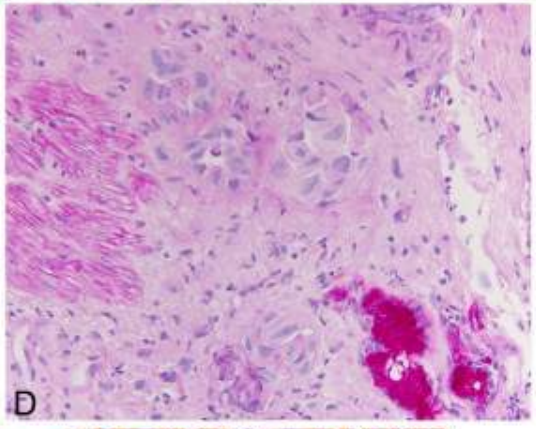




HE

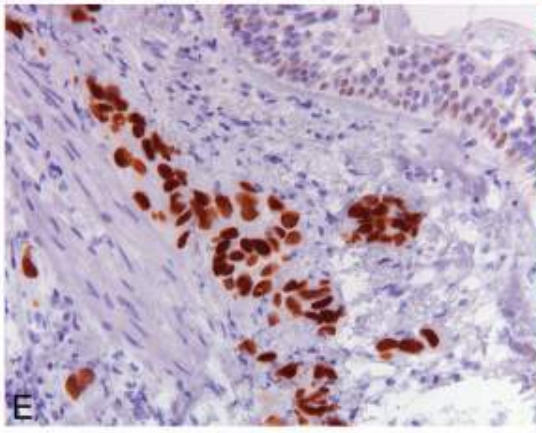
p63





PAS-D

TTF1



Favour adenocarcinoma

NSCLC: adenocarcinoma vs squamous cell carcinoma

	p63	TTF1	mucin
• Squamous cell carcinoma	+++	-	-
• Adenocarcinoma	-/+	+	-
	-/+	-	+
	-/+	+	+
• NOS	-/+	-	-
	+++	+	+

- 85-90% favour adenocarcinoma or squamous cell carcinoma,
- remaining Bx NOS; Rx Large cell / Adenosquamous carcinoma

LUNG CANCER Diagnostic flow

Cancer in the lung: primary vs metastases

↓

Primary lung cancer: NSCLC – SCLC
Staging M+

↓

NSCLC: adenocarcinoma vs squamous cell carcinoma

↓

Adenocarcinoma **KRAS / EGFR mutation**
KRAS and EGFR negative:
ALK

Squamous cell carcinoma FGFR1?

LUNG CANCER Diagnosis

Cancer in the lung: primary vs metastases

↓

Primary lung cancer: NSCLC – SCLC
Staging M+

↓

NSCLC: adenocarcinoma vs squamous cell carcinoma

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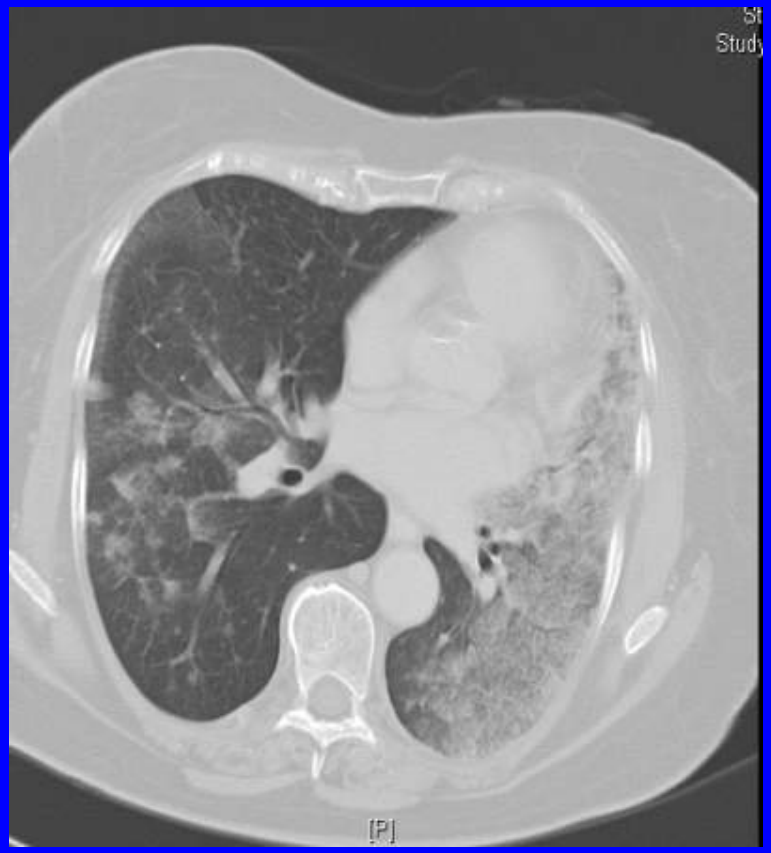
prediction ↓

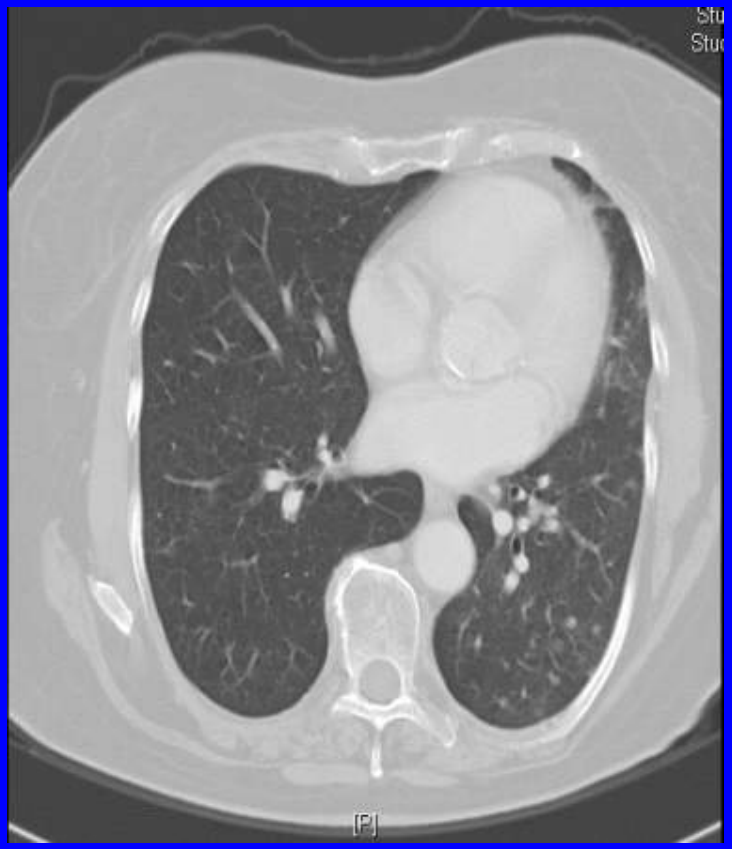
Adenocarcinoma **KRAS / EGFR mutation**
KRAS and EGFR negative:
ALK

Squamous cell carcinoma FGFR1?

“Lazarus Response” to gefitinib: Chemoresistant EGFR mutant adenocarcinoma



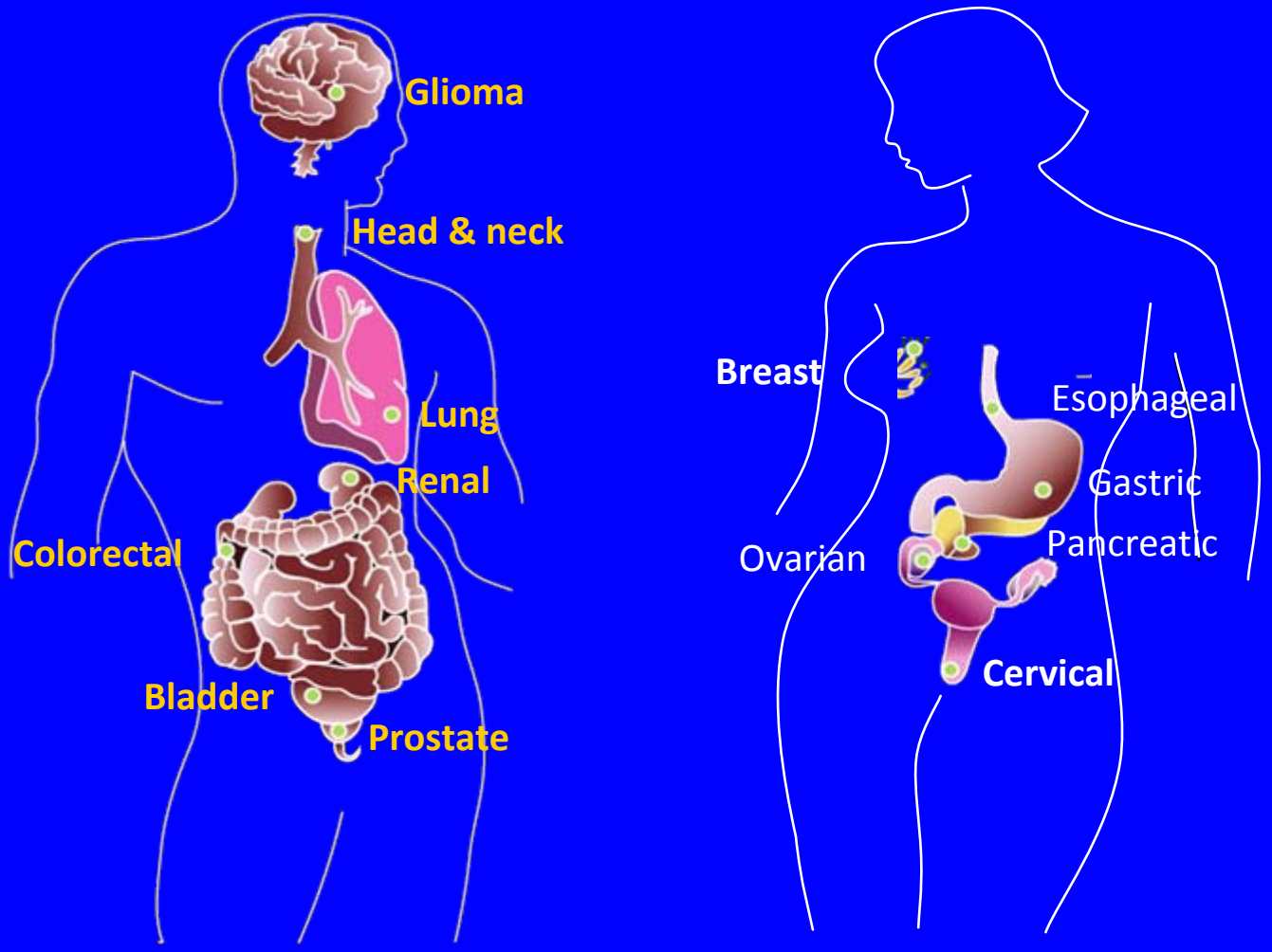
January 2002



October 2004

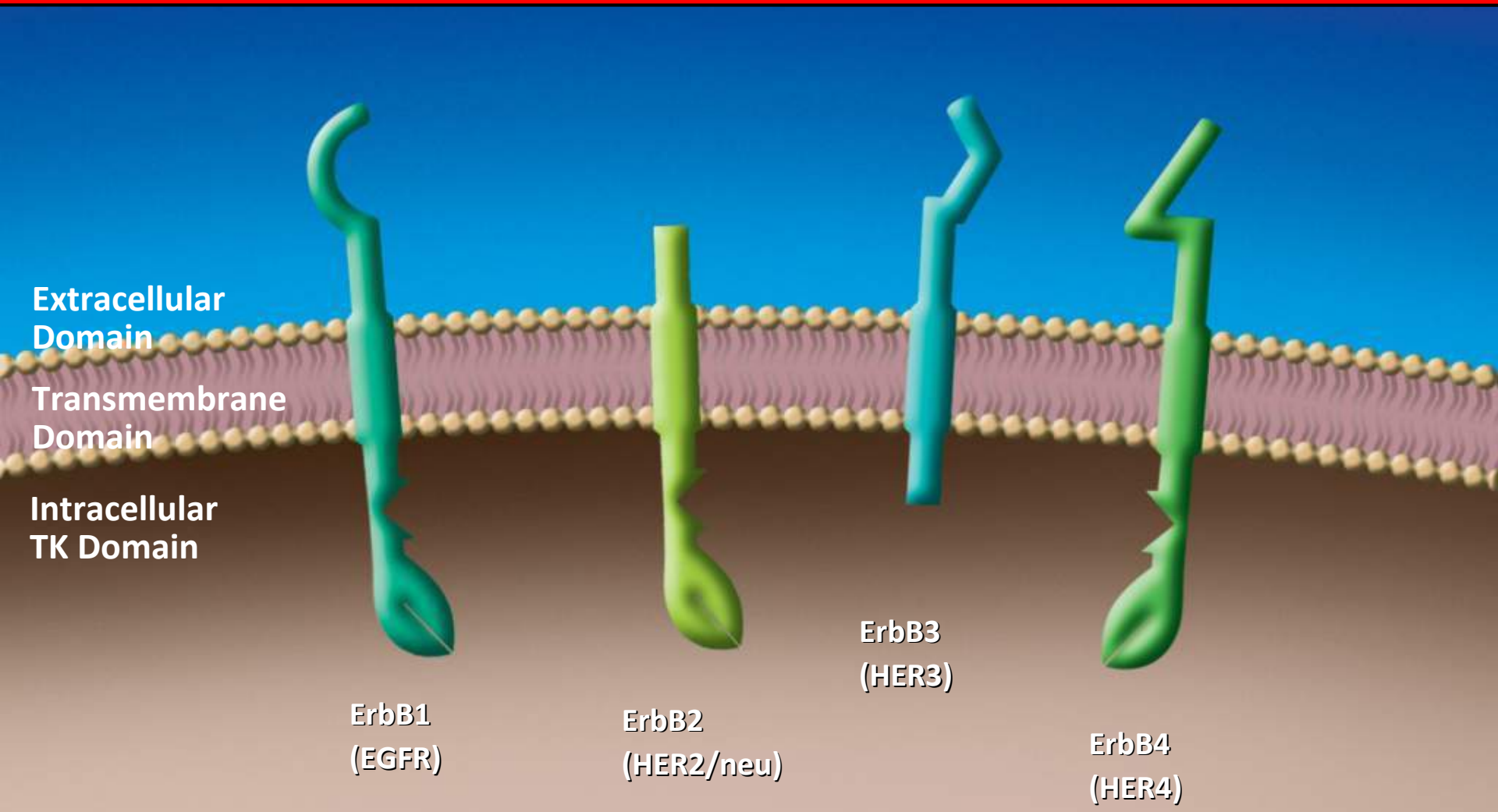
Johnson 2004

EGFR is deregulated in most solid tumors



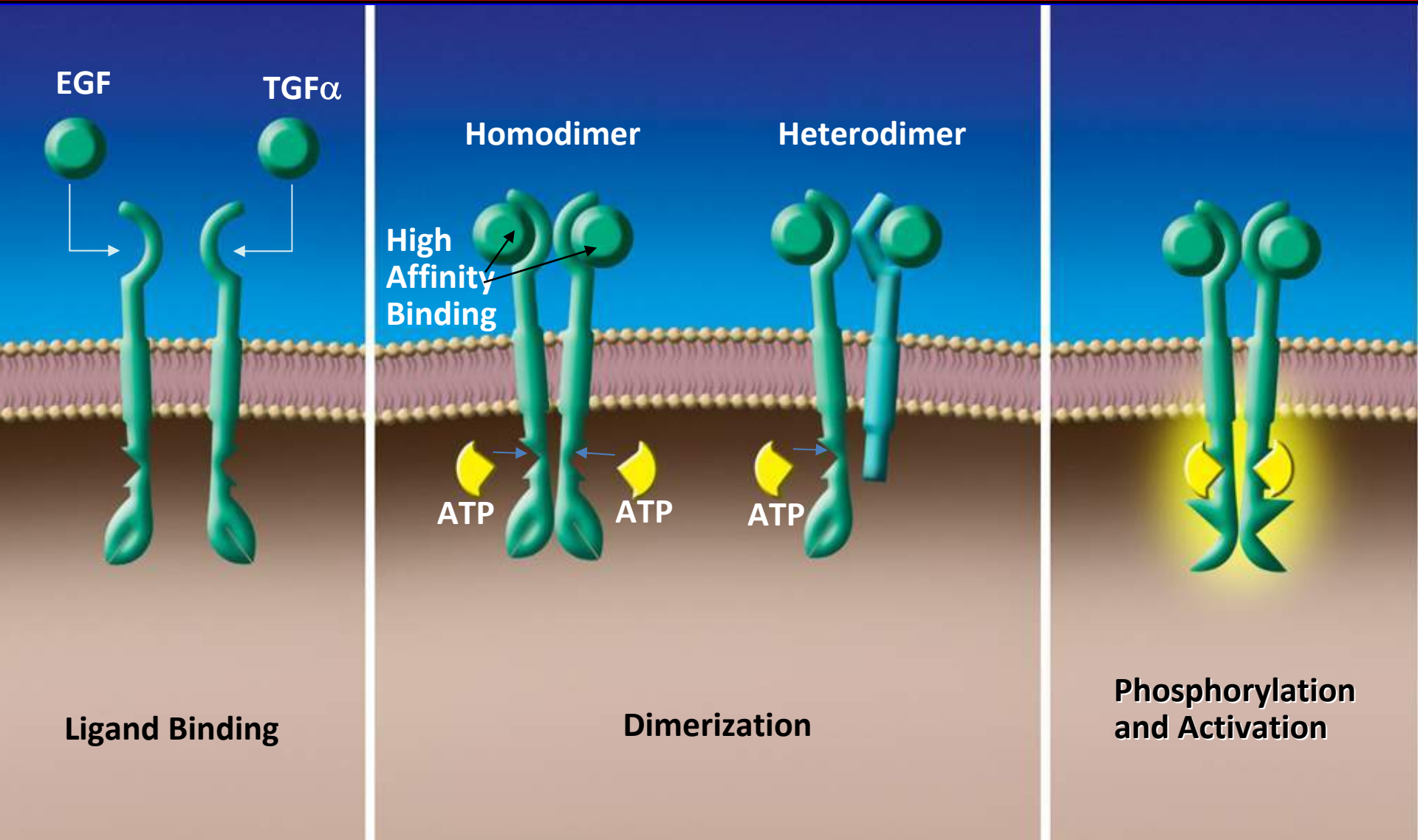
Adapted from Rowinsky 2004

EGFR Belongs to the ErbB Family of Cell Surface Receptors

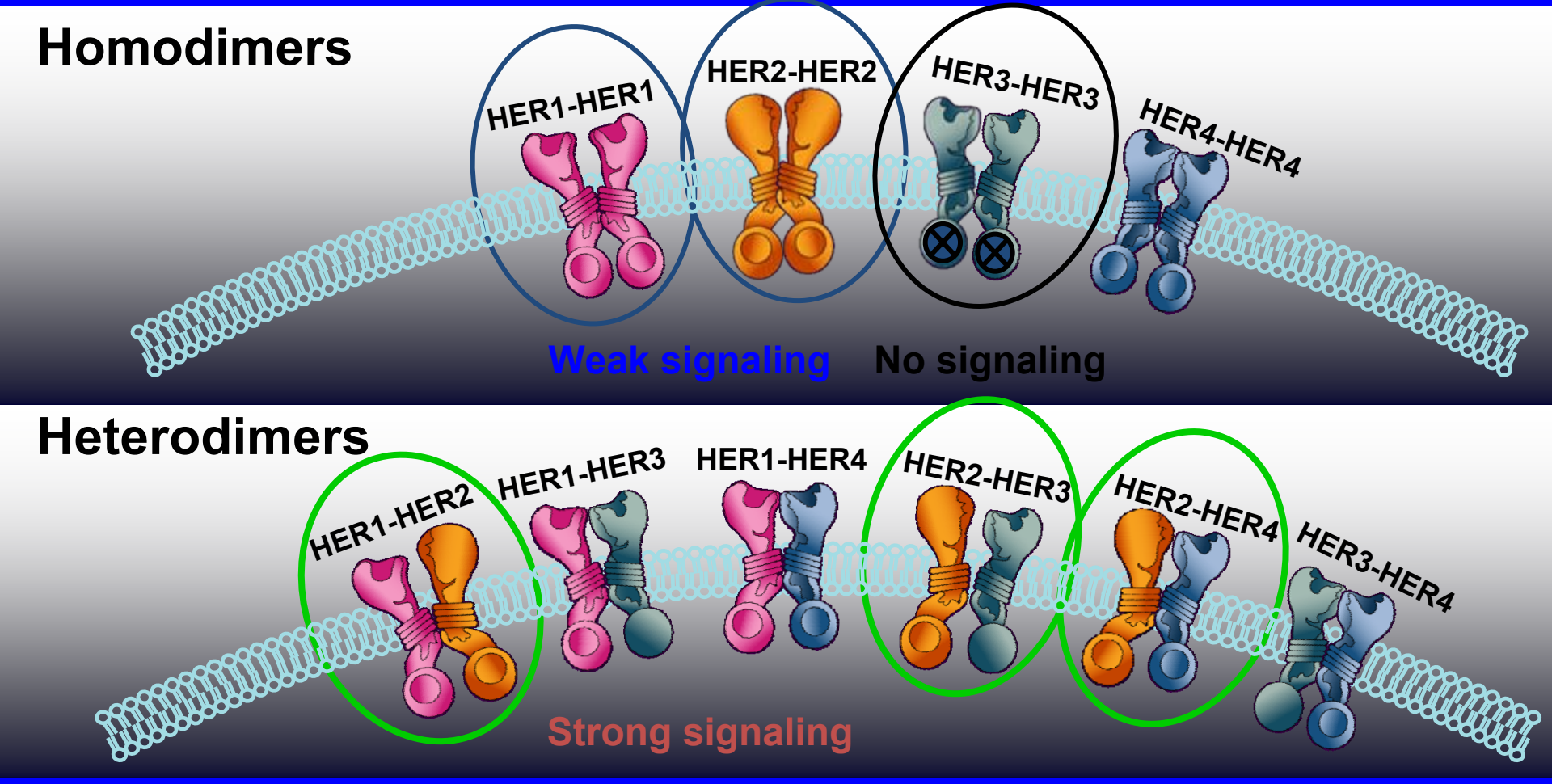


Amgen slide

Ligand Binding and Dimerization Result in TK Activation

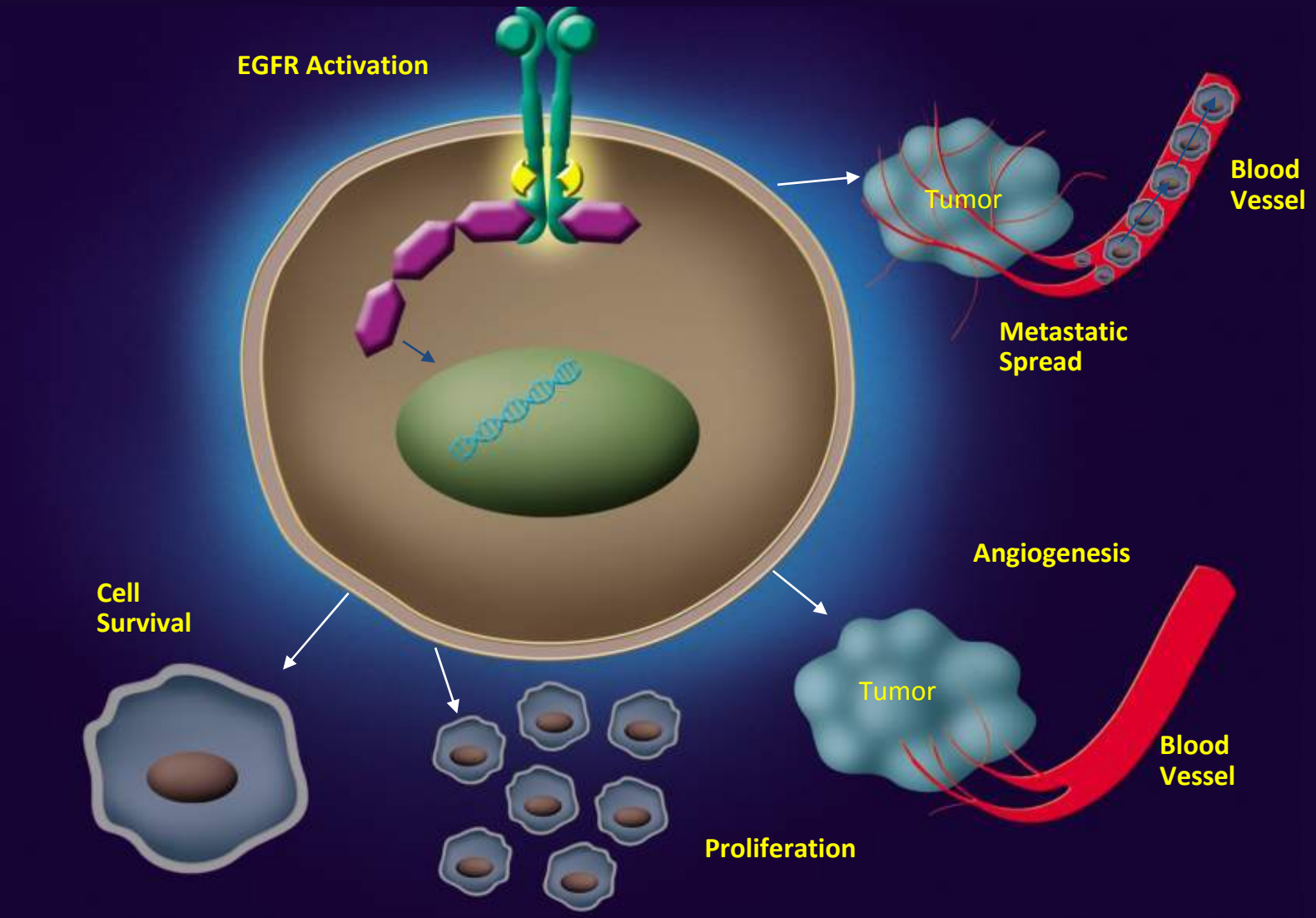


EGFR/HER Family of Surface Tyrosine Kinases



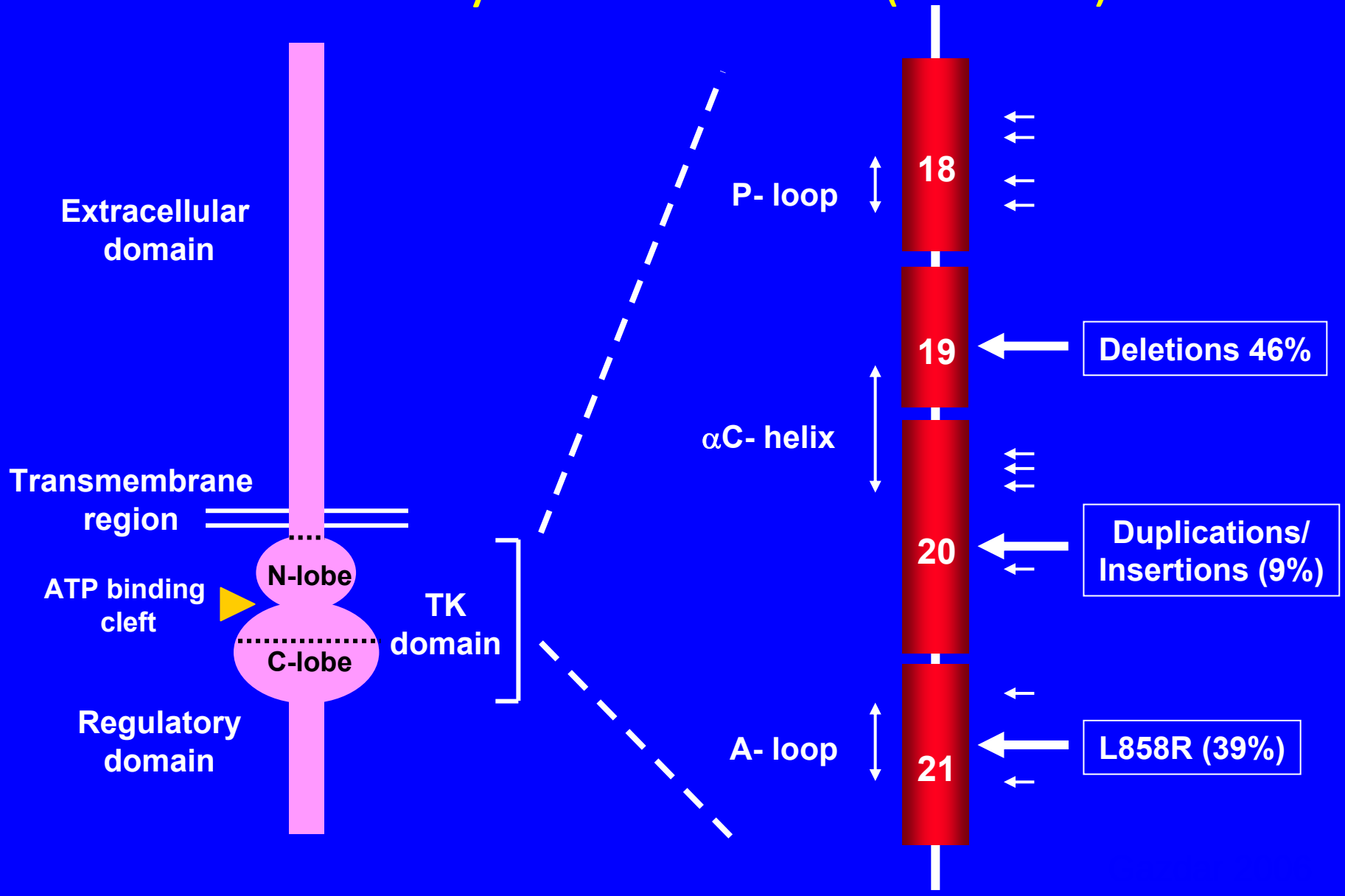
Rowinsky. *Annu Rev Med.* 2004;55:433; Roskoski. *Biochem Biophys Res Commun.* 2004;319:1; Herbst. *Int J Radiat Oncol Biol Phys.* 2004;59(suppl):21.

EGFR Activation Enhances Pathways Important for Tumor Cell Growth

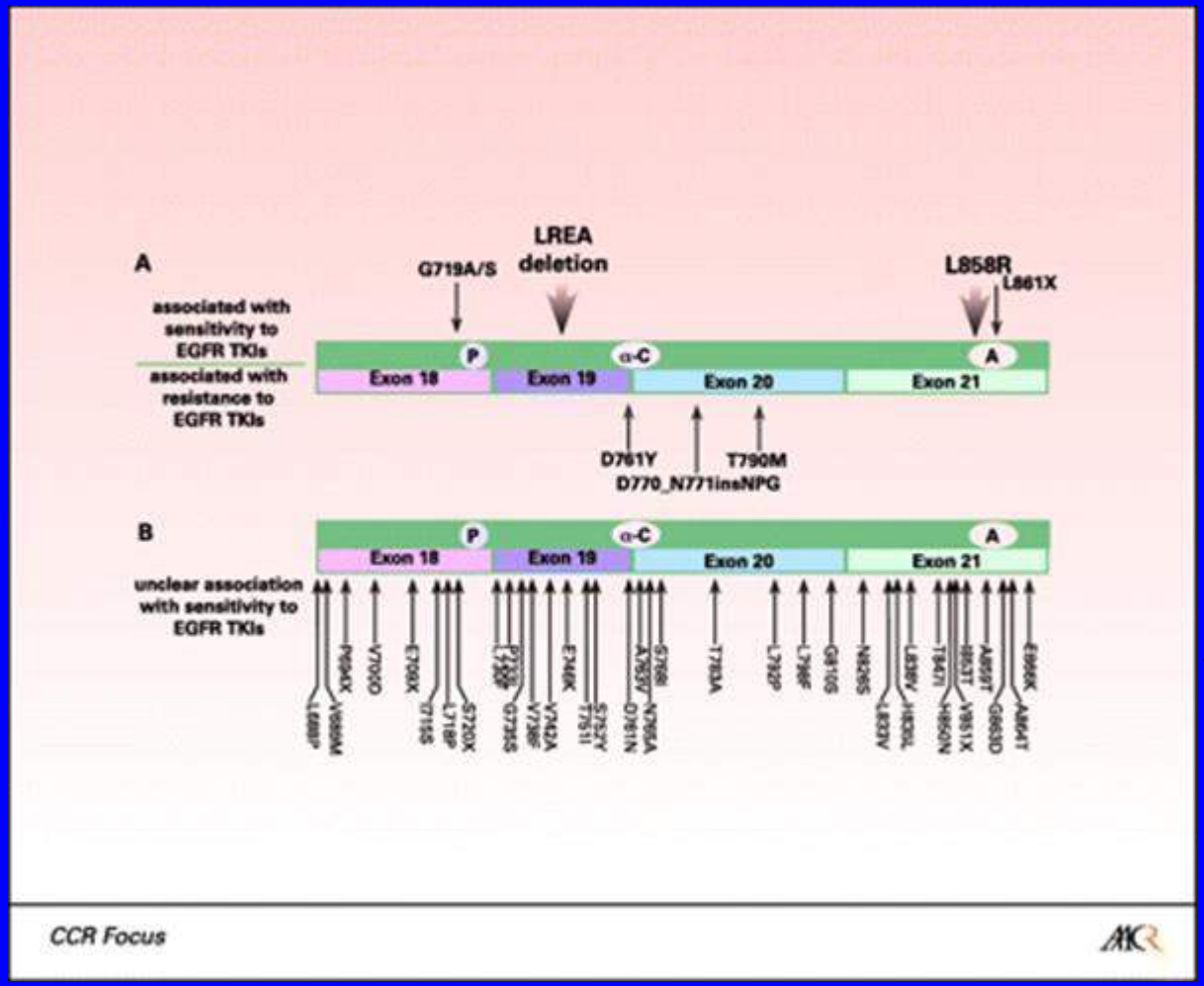


Mutations in the TK domain of EGFR:

Meta analysis of 5 studies (n=1256)



Not all EGFR mutations are created equal



"Activating mutations"

"Resistant mutations"

"Indeterminate mutations"

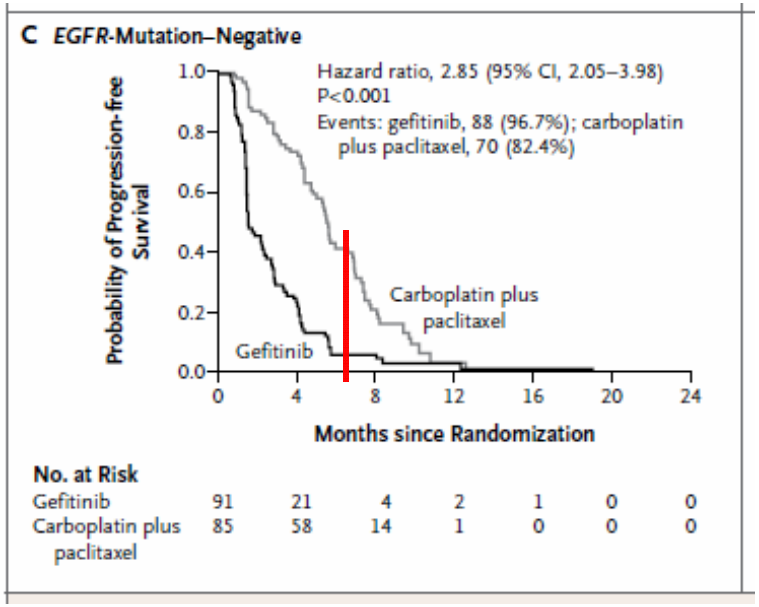
complex mutations = combination of >1 mutation

www.sm-egfr-db

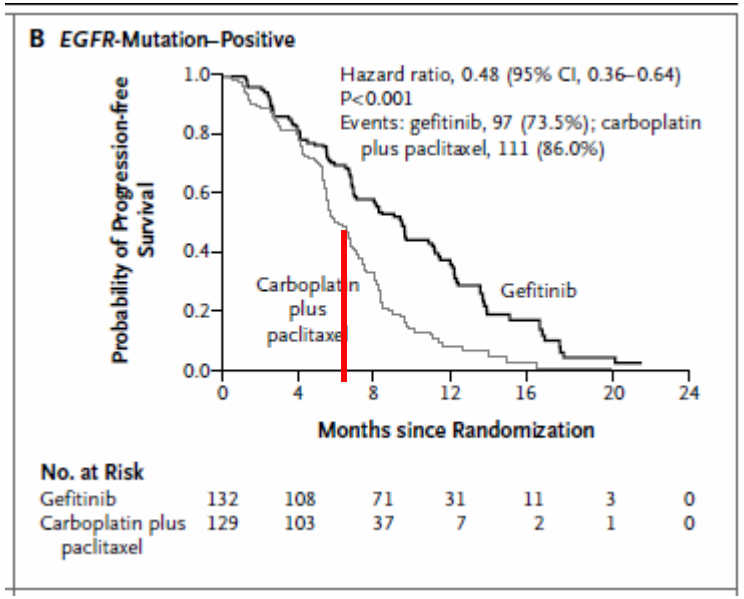
Modified after Riely et al 2006

EGFR mutation

Absent



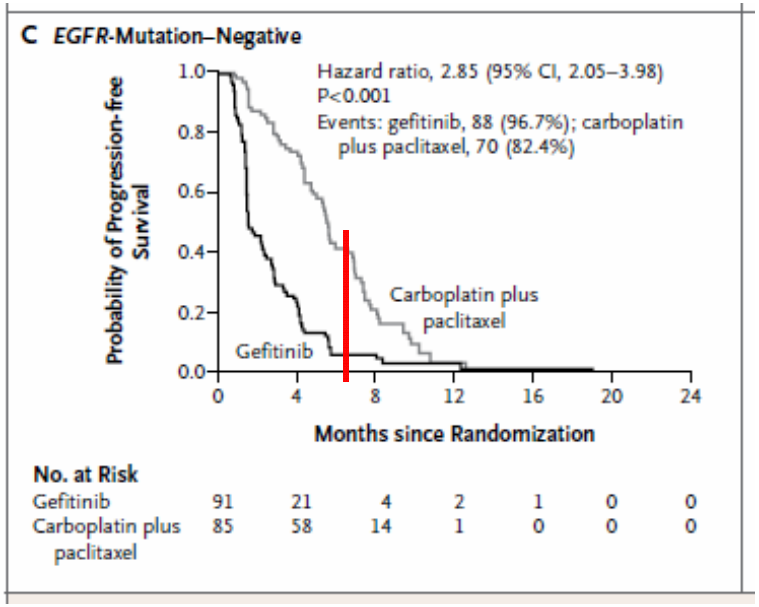
Present



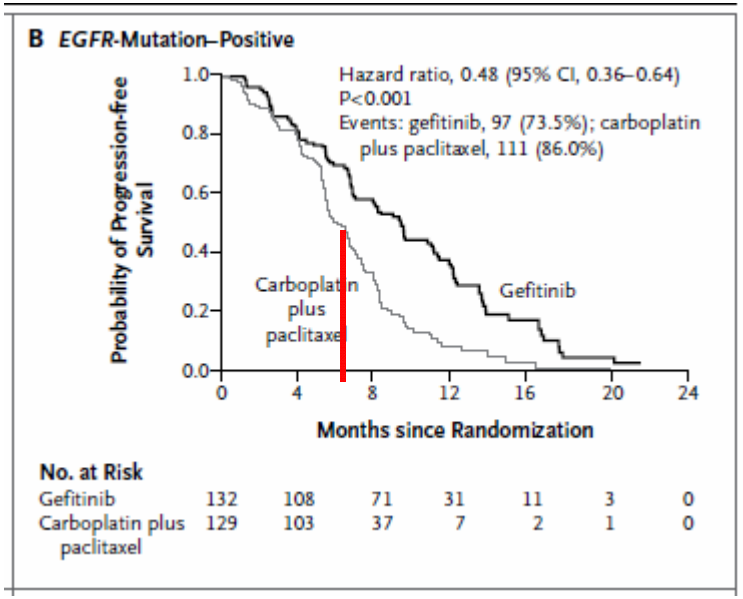
Mok NEJM 2009, Mitsudomi

EGFR mutation

Absent



Present



Conclusion:

Since in patients without EGFR mutations more harm is done with EGFR-TKI than with chemo-x, EGFR-TKI treatment only for patients with EGFR mutations: selection required.

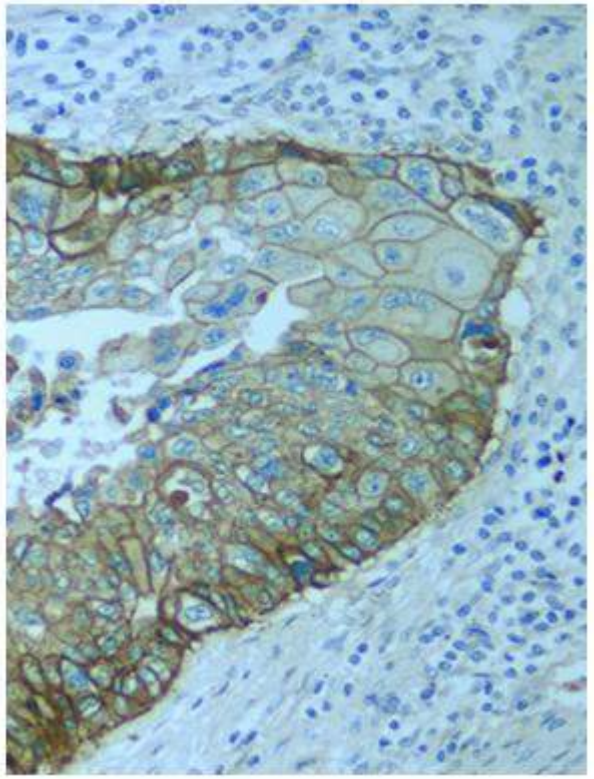
Mok NEJM 2009, Mitsudomi

Flow chart NSCLC

HE	Squamous cell carcinoma	NOS	Adenocarcinoma
Additional stain	P63 + TTF1 - Mucin -	P63 - TTF1 - Mucin - P63 + TTF1 + Mucin +	P63 - TTF1 + and/or Mucin +
Diagnosis	Squamous cell carcinoma	NOS/ AdSq	Adenocarcinoma
		Mutation analysis	EGFR/KRAS

EGFR protein is often over-expressed in NSCLC

- 62% NSCLC
  - 82% squamous cell carcinoma
  - 44% adenocarcinoma
  - 80% adenocarcinoma with BAC features (peripheral adenocarcinomas
- 0% SCLC



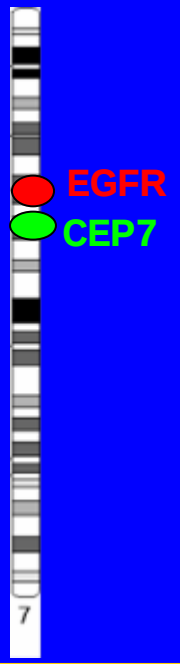
Squamous cell carcinoma

Hirsch et al 2003

EGFR FISH: Colorado Score System

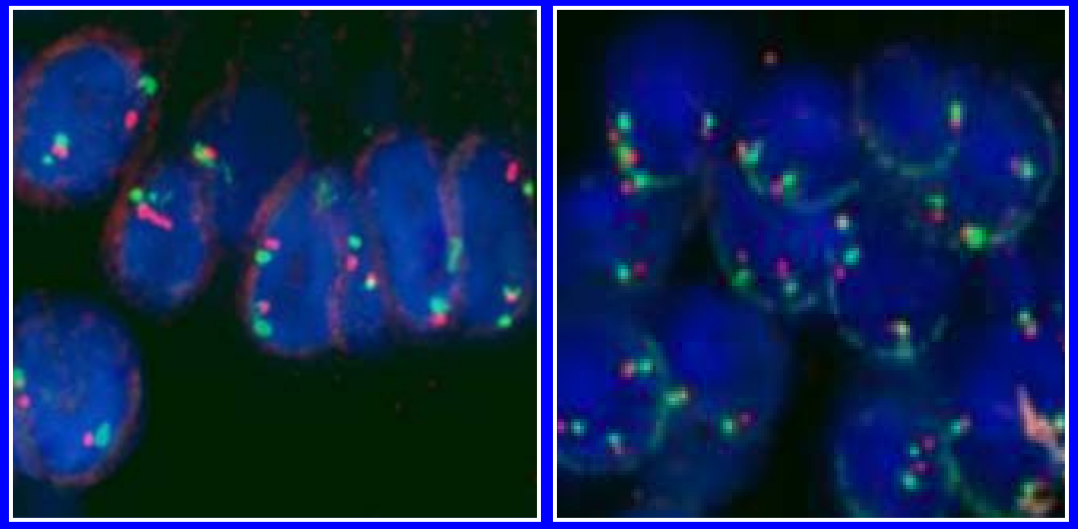
Low copy number

EGFR negative

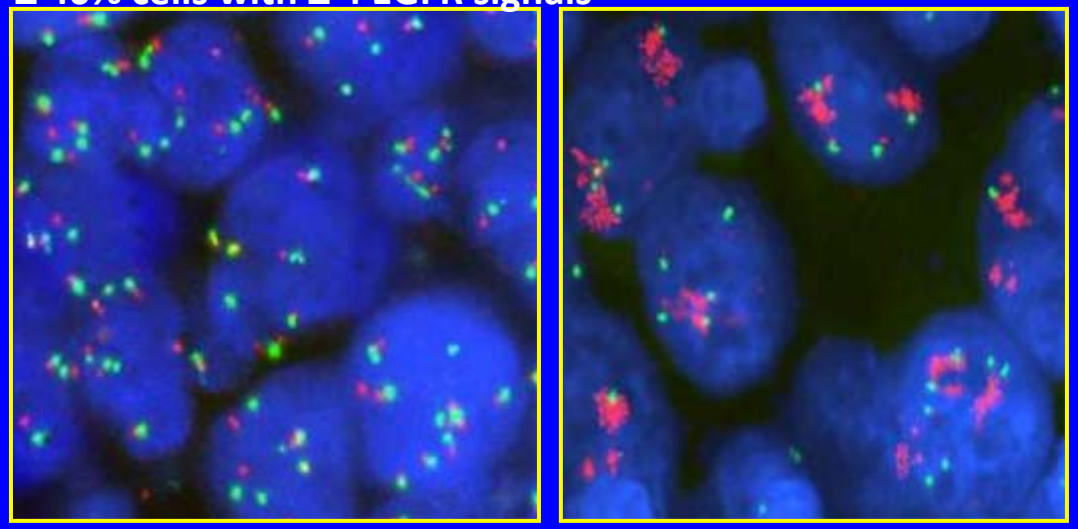


High copy number

EGFR positive

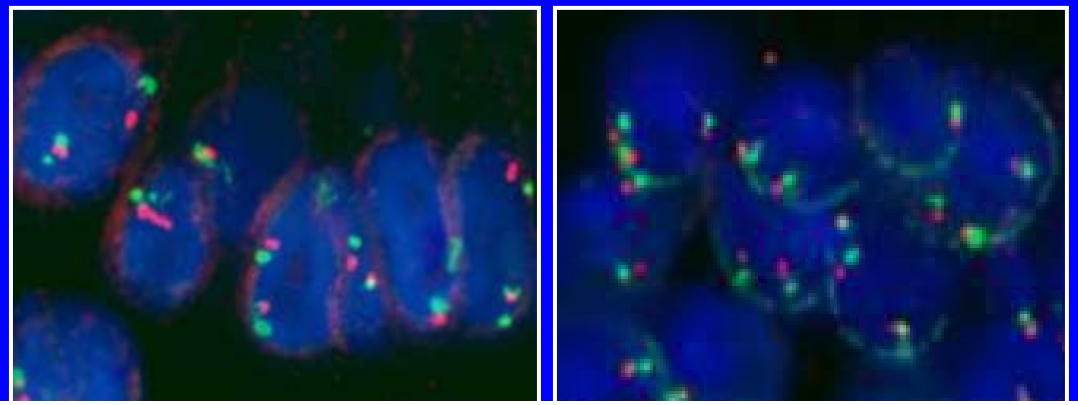


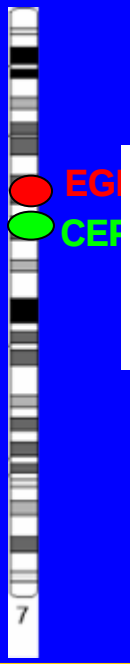
≥ 40% cells with ≥ 4 EGFR signals



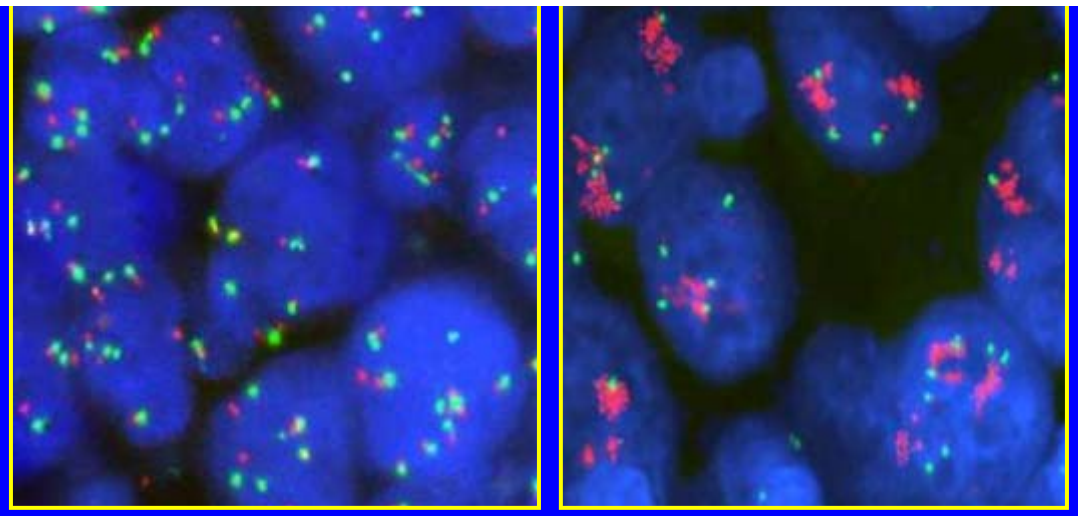
Marileila Garcia

EGFR FISH: Colorado Score System





Mutant allele specific Amplification
Late event



High copy number

EGFR positive

Marileila Garcia

EGFR mutation analysis: WHO?

Is selection based on clinical grounds sufficient?

- **Non-smokers, Women, Asian**
- Rosell NEJM 2009, 2105 cases 350 mutations
- 68% EGFR mutations in non-smokers; 6% current smokers, 26% ex-smokers
- 73% women, 27% men
- 98% Kaukasian

EGFR mutation analysis: WHO?

- Is selection based on clinical grounds sufficient?
- Non-smokers, Women, Race
- Rosell NEJM 2009, 2105 cases 350 mutations
- 68% EGFR mutations in non-smokers; 6% current smokers, 26% ex-smokers
- 73% women, 27% men
- 98% Kaukasian
- Clinical parameters are insufficient to select patients for EGFR mutation analysis

WHO?
Histology as triage for EGFR mutation detection?

- Most frequent ADENOCARCINOMAS ~30-10%
- LARGE CELL CARCINOMAS >2%
- Squamous cell carcinomas 1-2%

RARE

- Small cell carcinoma Rare (combined SCLC-adenocarcinoma)
- Pulmonary salivary gland tumors

WHO?
Histology as triage for EGFR mutation detection?

- Most frequent ADENOCARCINOMAS ~30-10%
- LARGE CELL CARCINOMAS >2%
- Squamous cell carcinomas 1-2%

RARE

- Small cell carcinoma Rare (combined SCLC)
- Pulmonary salivary gland type tumors

- DUTCH guidelines:

  Non-squamous NSCLC

  Not in mucinous AC, LCNEC, carcinoids

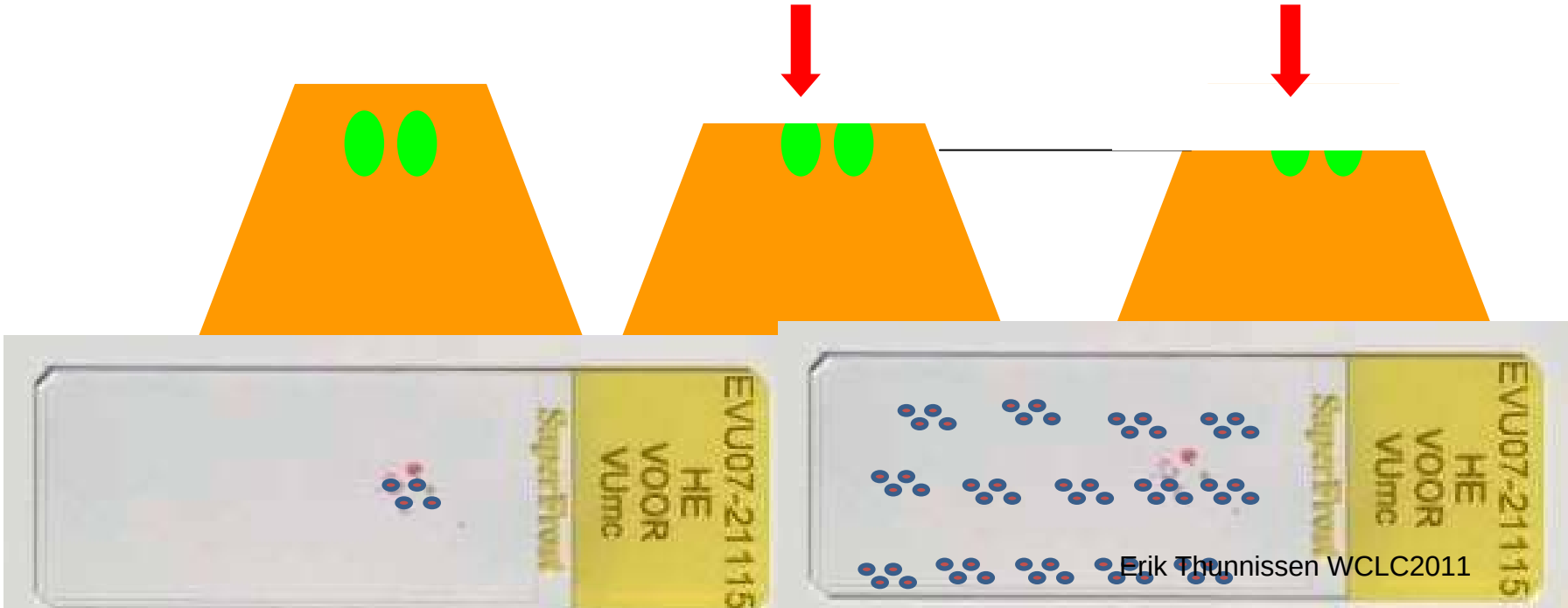
Material is limited

- Direct question of pulmonologist/oncologist:
- In case of malignancy EGFR mutation AND EGFR expression?

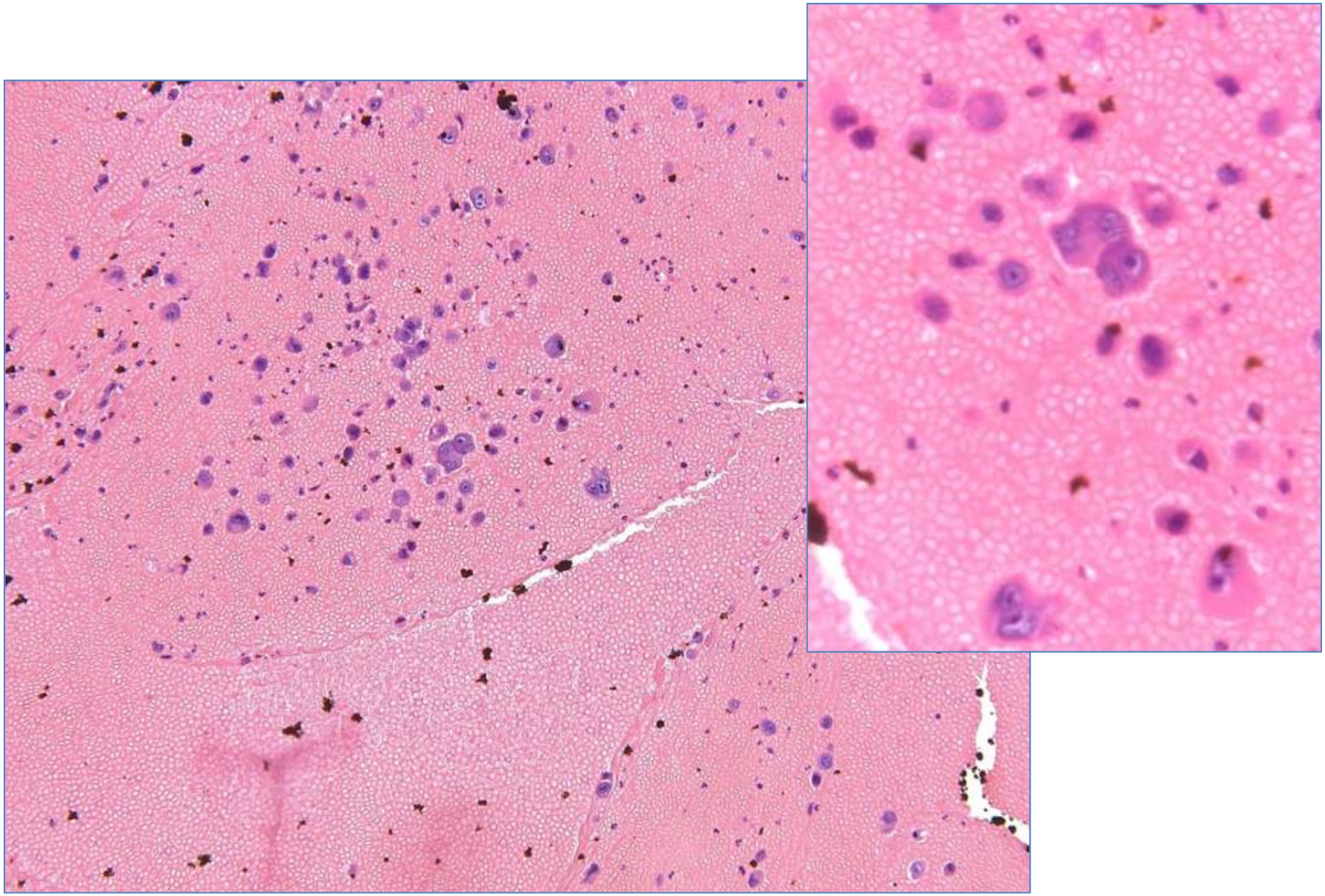
Molecular pathology: balance between pulmonology, pathology and molecular biology and oncology

Pulmonologist: clinical information
questions: diagnosis, “EGFR” if malignant

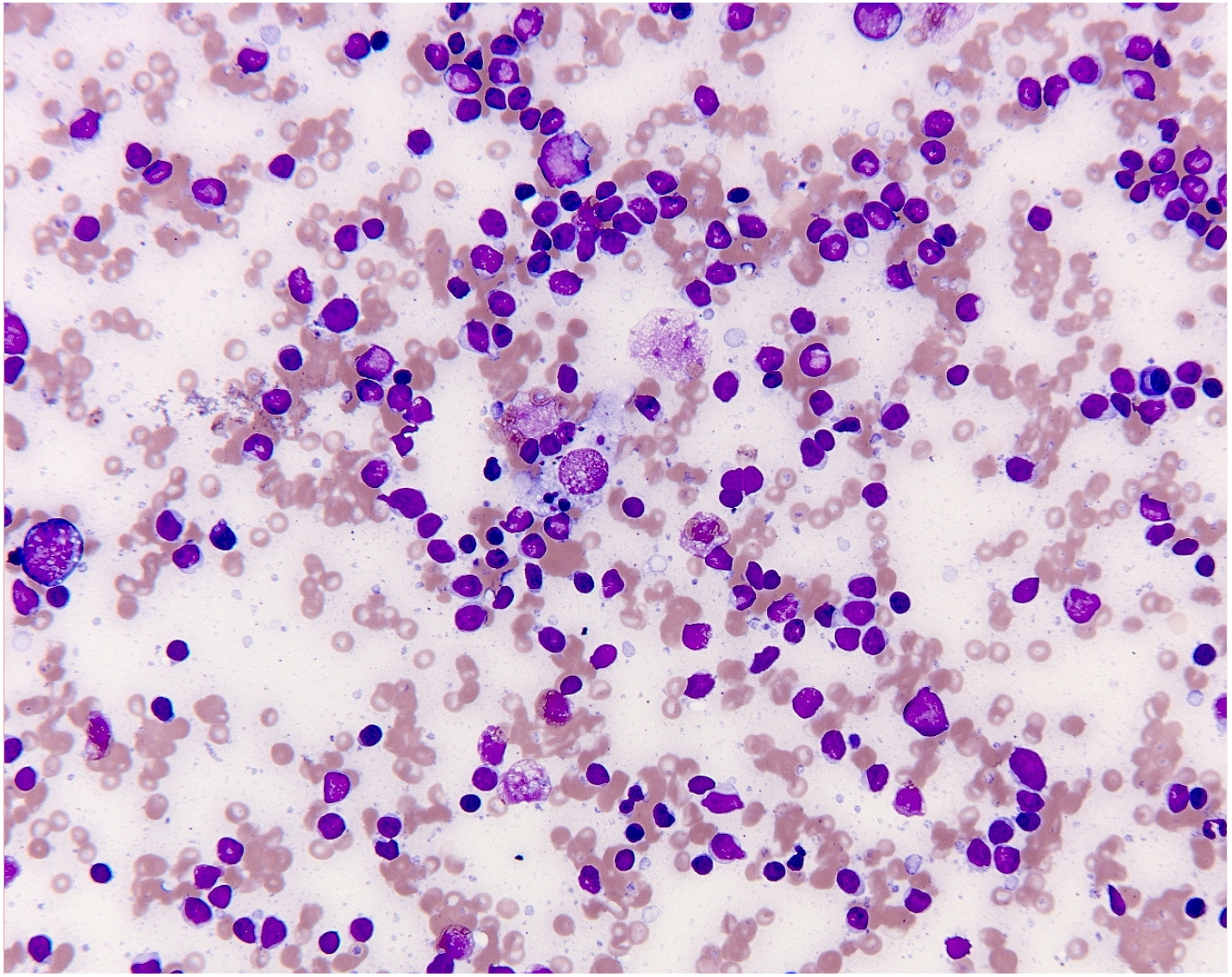
Pathology “EGFR” code: specific handling in contrast to regular



Pleuravocht



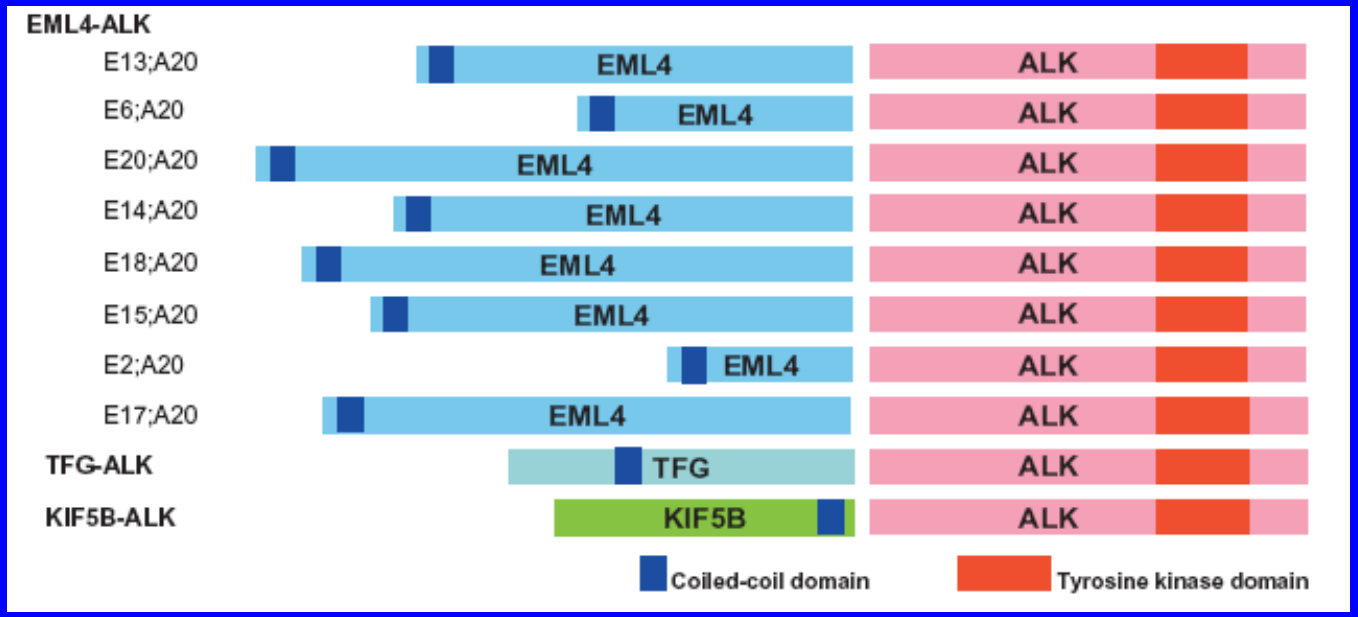
EBUS lymfklier station 7

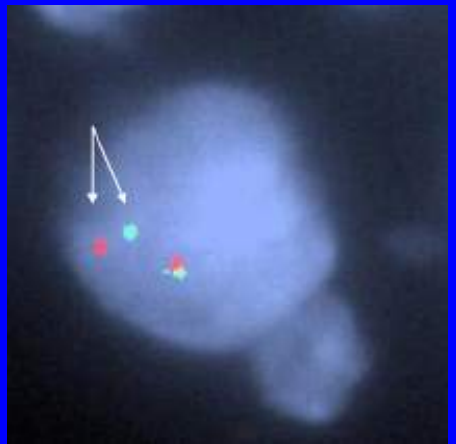


2010

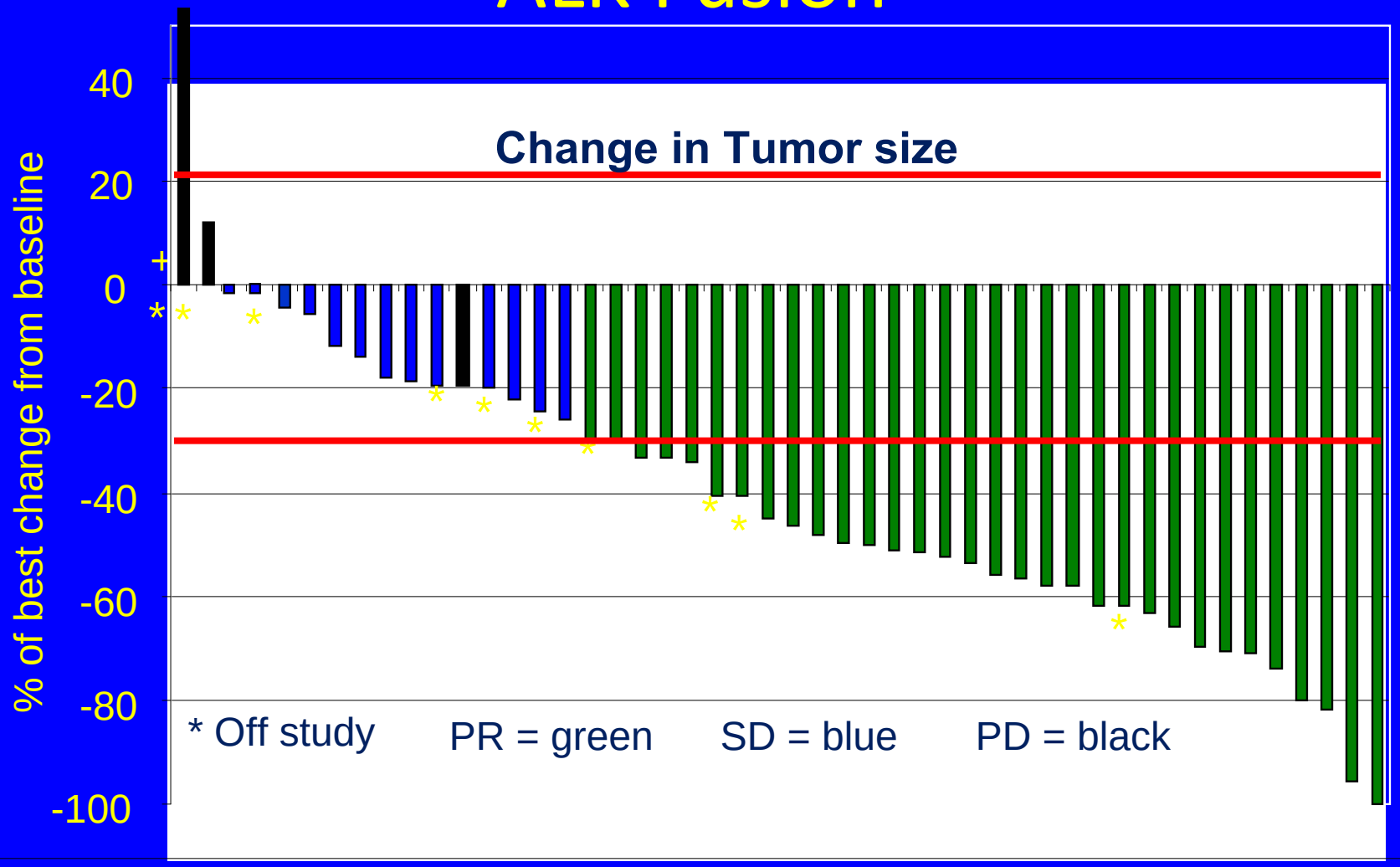
- Another major breakthrough

ALK fusion in NSCLC





Tumor Responses to crizotinib, NSCLC with ALK Fusion



Camidge, DR, et al AACR/IASLC, 2010; Kwak NEJM 2010

Typing – importance treatment consequences

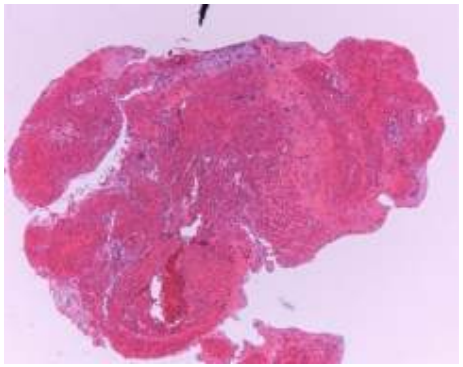
Adenocarcinoma

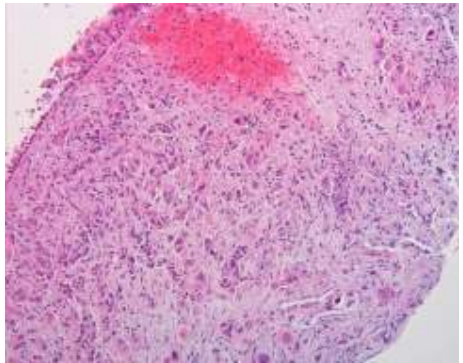
- Pemetrexed + cisplatin survival benefit over GC
- EGFR - erlotinib/gefinitib
- KRAS, B-Raf
- Alk – crizotinib
- cMET

- Squamous cell carcinoma
  - Gemcitabine + cisplatin (GC)
  - Contra Bevacizumab toxicity

PvdV

Pathologist





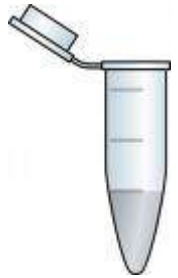
- vital tumor cells

- necrosis
- stroma
- inflammatory cells

Estimation
% vital tumor cells

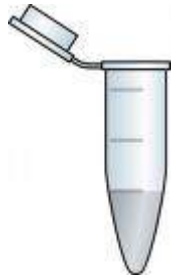
➢2x Threshold mutation technique

EGFR Mutation analysis
Which technique?



DETECTION OF	ANALYTICAL SENSITIVITY	SAMPLE TRANSFER[x]
All mutations		
PCR sequencing	20-30%	5
PCR-HRM/ sequencing	2-5%	2/5
WAVE Surveyor	(2-?)5%	5
Pyrosequencing	1%	
Massive parallel seq.	1%	
Only known mutations		
SARMS *	0.5-1%	1
PNA/LNA Clamp	1%	1
SNAPSHOT (primer extension)	1-5%	5
PCR Fluorescent RFLP	5%	7
ME PCR sequencing	0,1%	7
PCR Invader		3

EGFR Mutation analysis: Which technique?



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Analytical sensitivity relates to required fraction of tumor cells in sample

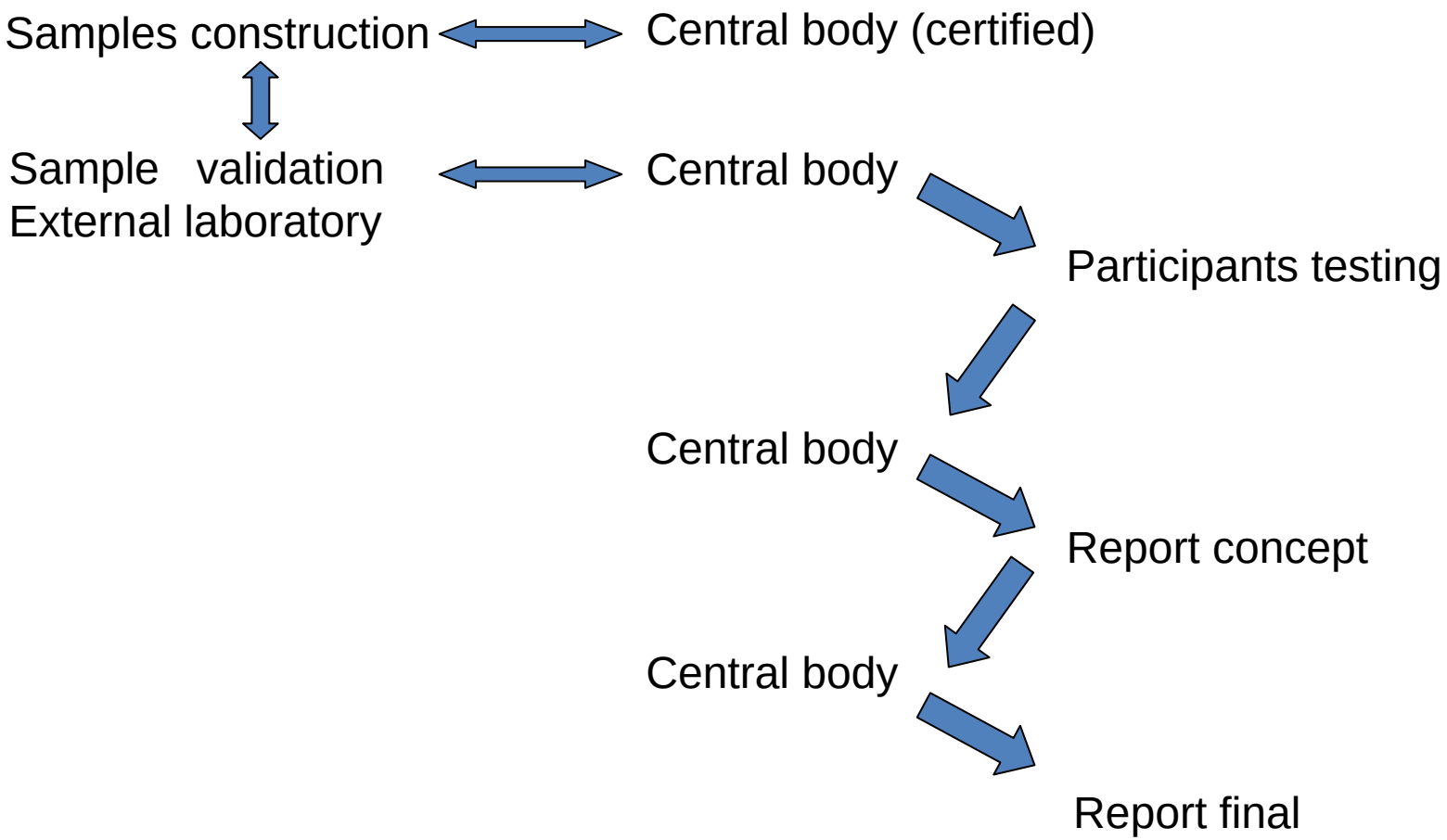
Any of the sensitive methods will do, as long a EQA performance is OK

ORGANISATIONS guidelines
External Quality Assurance (EQA)

- USA CAP-AMP-IASLC
- Europe ESP, ESMO, ETOP, UKNEQAS

- www.EMQN.org
  - Material validated
  - Pilot study (requirement in certified organisation)
  - World wide open **3rd quarter 2011**

EQA ring study = proficiency testing



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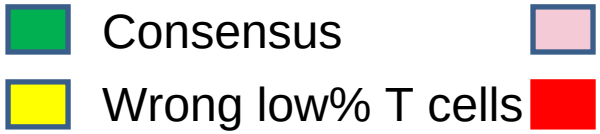
Dutch EQA

	IHC	ISH	Mutation analysis
2008	**TMA sections n=17**	**TMA sections n = 17**	**isolated DNA n=3 from cell lines** **paraffin sections n=2**
2009		**TMA sections n=13**	**isolated DNA n=4 from cell lines** **TMA n=13**

EQA

Test	EGFR IHC	EGFR ISH		EGFR mutation		KRAS Mutation	
	2008	2008	2009	2008	2009	2008	2009
Consensus	4/17	17/17	13/13	5/5	17/17	5/5	17/17
Labs	8	8	9	9	9	10	12
NA cases		15 / 136	3 / 117	1 / 45	2 / 153	4 / 50	2 / 204
Success rate	-	89±27%[1)]	97±4%	98±7%	99±4%	92%±19[2)]	99±4%
Positive cases	-	3	3	2	5	3	6
FN	-	3 / 22	0 /27	0 /18	1 / 44	1 / 26	4 / 71
Sensitivity	-	88±35%[3)]	100±0%	100±0%	98±7%	96±11%	94±11%[4)]
Negative cases	-	14	10	3	12	2	11
FP	-	1 / 99	0 / 87	0 / 26	0 / 107	1 / 20	2 / 131
Specificity	-	96±12%[5)]	100±0%	100±0%	100±0%	95±16%	98±4%
Accuracy		95±10%[6)]	100±0%	100±0%	99±2%	93±16%[7)]	97±4%

Mutation analysis TMA 2009



TMA	ISH 2008 2009	*EGFR* 2009	*KRAS* 2009	A		B		C		D		E		F		G		H		I		K	L	M
	ISH 2009	Mut EGFR	Mut KRSA	Mut EGFR	Mut KRAS	Mut EGFR	Mut KRAS MEGFR	Mut EGFR	Mut KRAS EGFR	Mut EGFR	Mut KRAS EGFR	Mut EGFR	Mut KRAS EGFR	Mut EGFR	Mut KRAS	Mut EGFR	Mut KRAS EGFR	Mut EGFR	Mut KRAS	Mut EGFR	Mut KRAS EGFR	Mut KRAS	Mut KRAS	Mut KRAS EGFR
1			G12C																					
2		-5 AA																						
3			G12D																					
4																								
5			G12D																					
6																								
7																								
8		+3 AA																						
9		-5 AA																						
10	Amp																							
11																								
12		-5 AA																						
13	Amp	-4 AA																						

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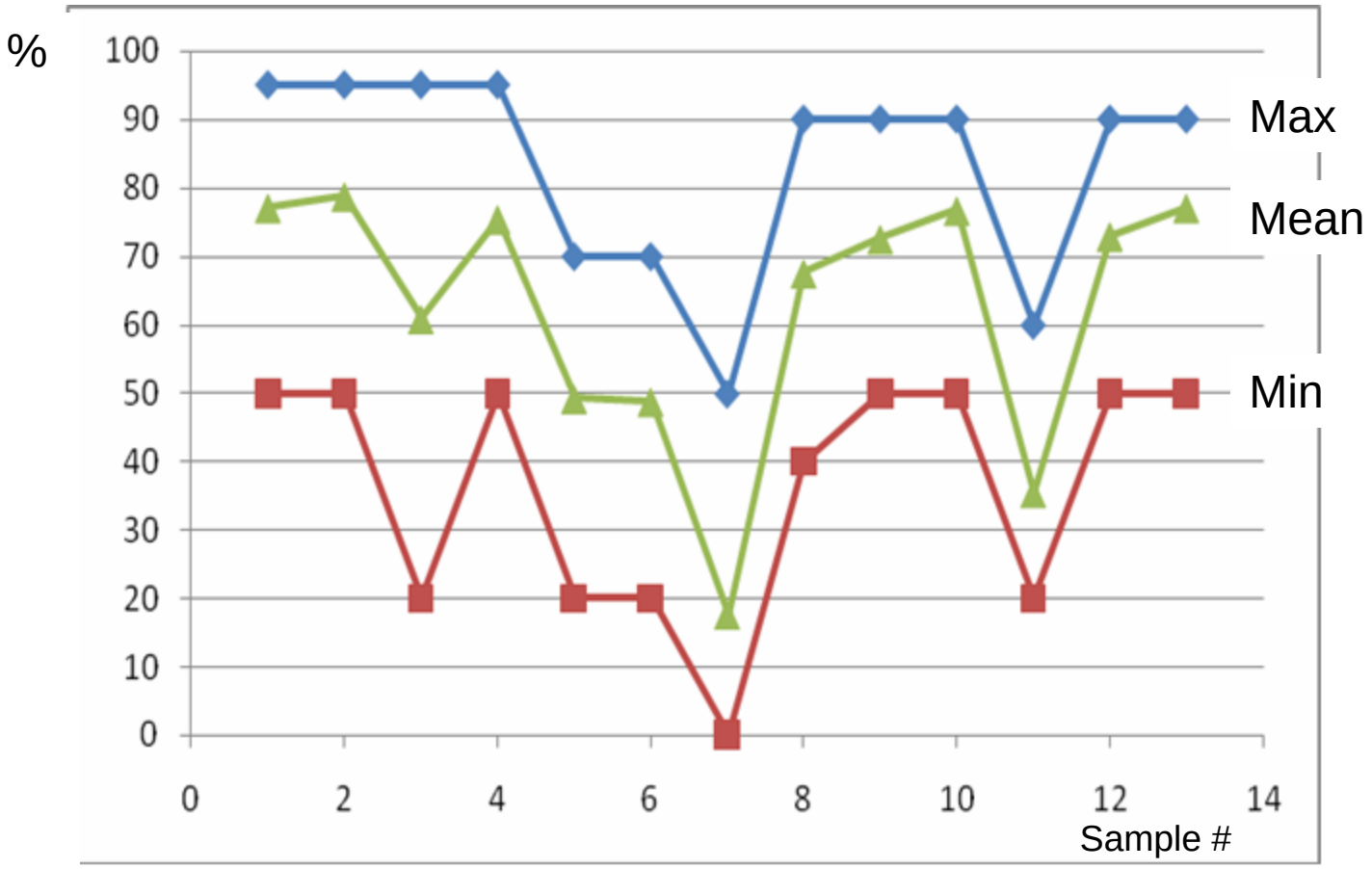
Estimation of % of tumor cells for each TMA sample



Website calibration for estimation % tumor cells available ~2011

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EGFR pilot

- Simon Patton, EMQN
- ETOP sponsored by Astra Zeneca, Roche

- 24 labs **(10ESP Krakow, other connections)**

EGFR pilot

- PCR sequencing n=8 (18-21 n=7, 18,19,21 n=1)
- DXS old n=8, new n=2
- HRM pyroseq. n=3, seq n=1
- Taqman 858 fragment length del 19 n=2

Score per case

- False positive/ negative 0
- No result/ failure to amplify 50%
- One mutation missing 50%
- Error in genotyping or protein typing 0.75%
- Ok = 100%*)

- *) FOR THE FRAGMNENTS TESTED

EGFR mutation score per sample

- 1 79% FN 4,
- 2 95%
- 3 79% FP 5
- 4 77% FP 5
- 5 78% FP 5
- 6 79% FN 5
- 7 93% FN2
- 8 98%
- 9 98%
- 10 98%

EGFR mutation score per sample

- Each sample contained sample number plus block number
- 5 samples >95% most the same result
- 5 samples 77-79%!!

- Changed sample # for block #,
- (reverse: frequently correct outcome): sample registration error 5 labs same mistake

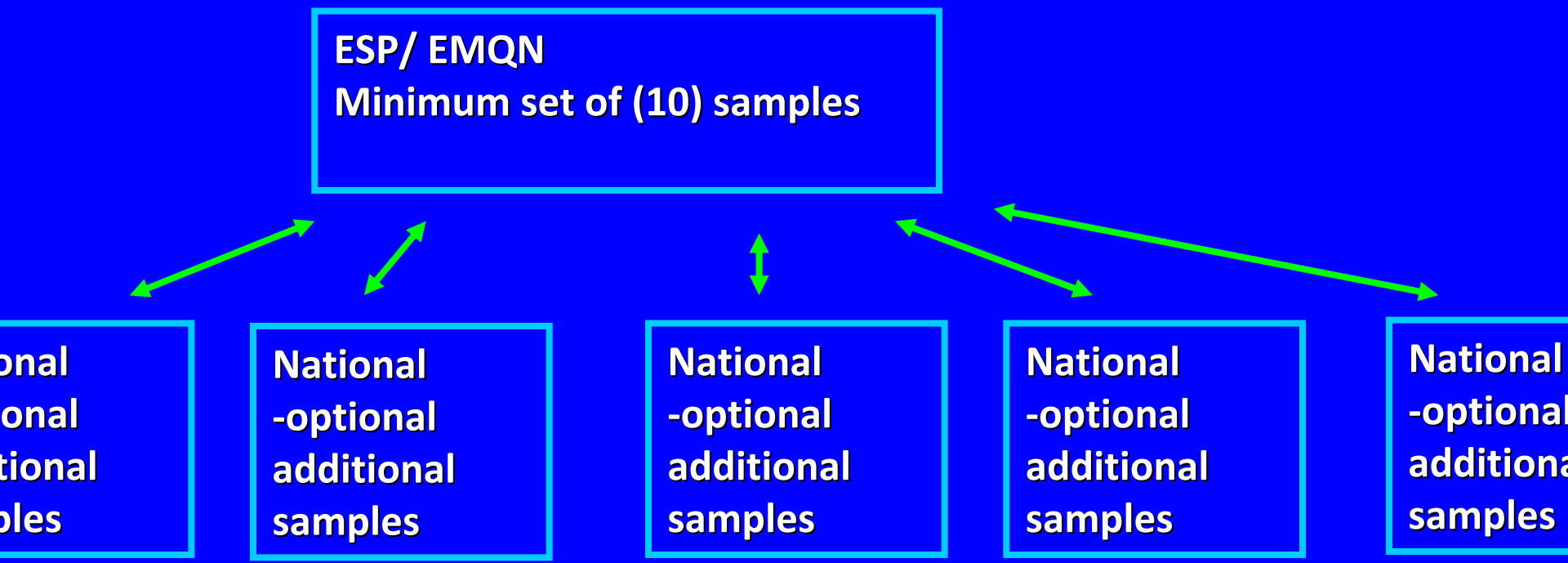
Score per lab

- 10 /10 n = 14
- 9,5 /10 n = 3
- 9 /10 n = 2
- 4-5 /10 n = 5

Interim conclusion

- Handling/ registration issue needs attention 5/24 labs (20%)
- At analytical level well performed 2 FN (0.8%)
- Feed back on reporting at individual level

ESP Proposal European QC EGFR testing krakow:



Questions?

- ?