GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

BIOPSY - KEY TO THE DIAGNOSTICS INITIALLY AND AT PROGRESSION

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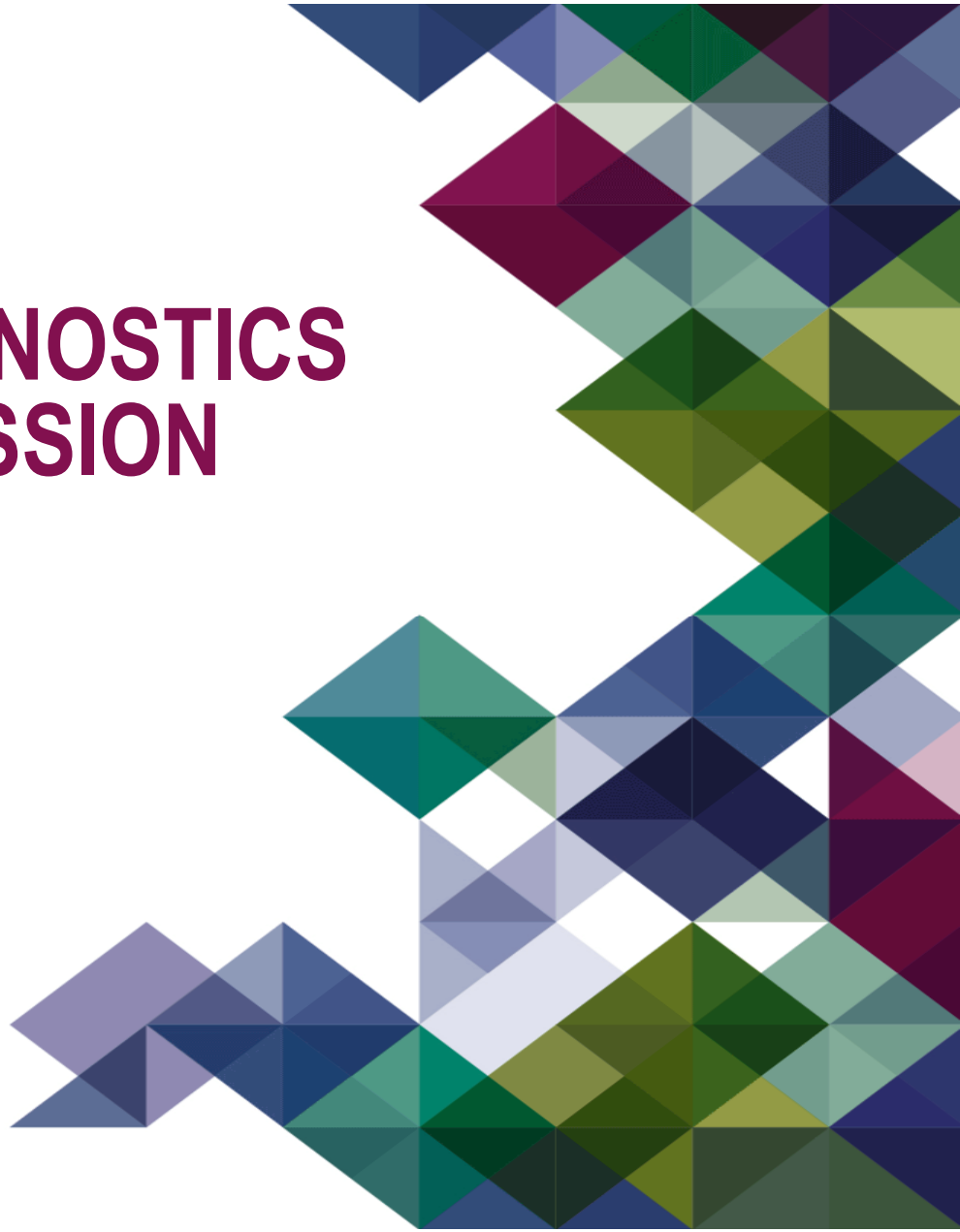


Disclosures

- I have acted as consultant for MSD, Pfizer, Clovis, BMS, AZ
- I have received honoraria for speaker AZ, Pfizer, Roche trainer SP142
- The VUmc received Grants: IIR Pfizer, Astra Zeneca

Diagnosis of lung cancer

- Large cell carcinoma
- Small samples

Predictive analysis

- EGFR
- ALK
- ROS1
- MET
- PD-L1

Tissue management

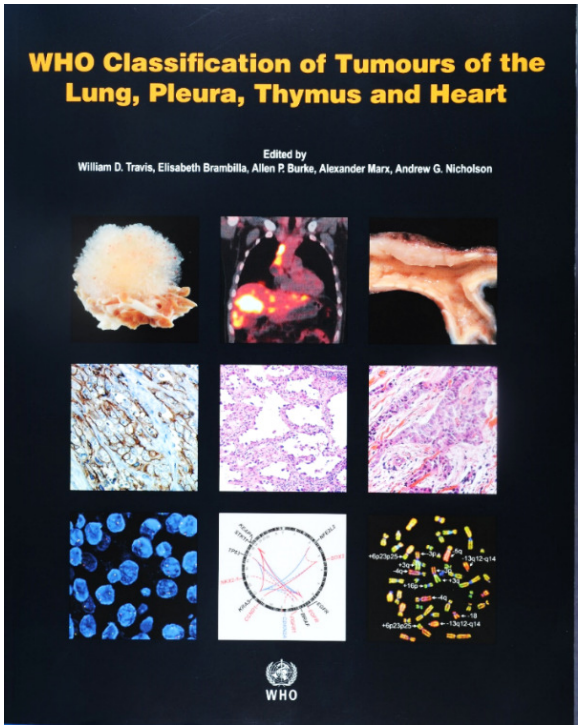
Lung Cancer Classification and sample type

WHO 1967-2015: intended for, and only applicable to, resected cases

- Small Cell Carcinoma
- Squamous Cell Carcinoma
- Adenocarcinoma
- Large cell carcinomas
- Sarcomatoid carcinomas
- Adenosquamous carcinomas
- Carcinoid tumours
- Salivary-type carcinomas

WHO 2015: a simplified classification intended for small sample diagnosis

- Small Cell Carcinoma
- Squamous Cell Carcinoma
  - Probable Squamous Cell Ca
- Adenocarcinoma
  - Probable Adenocarcinoma
- NSCLC-NOS
  - NSCLC-NOS (null IHC)
- Carcinoid tumour
- Salivary-type (occasionally)



2015

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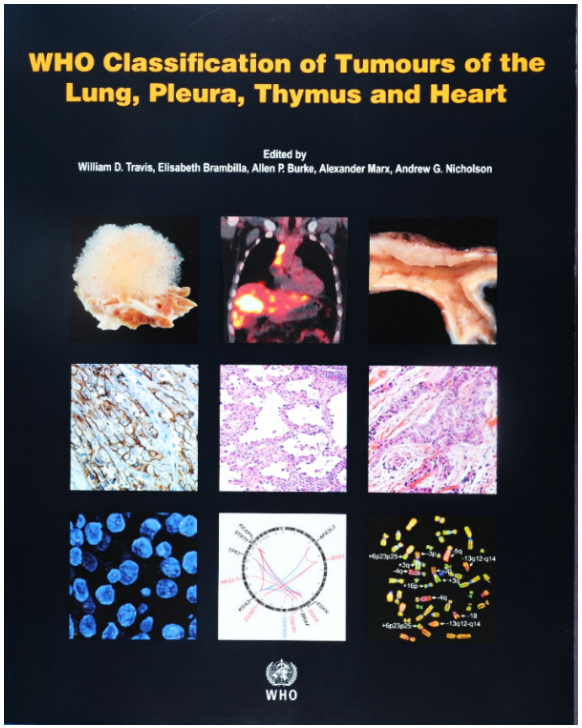
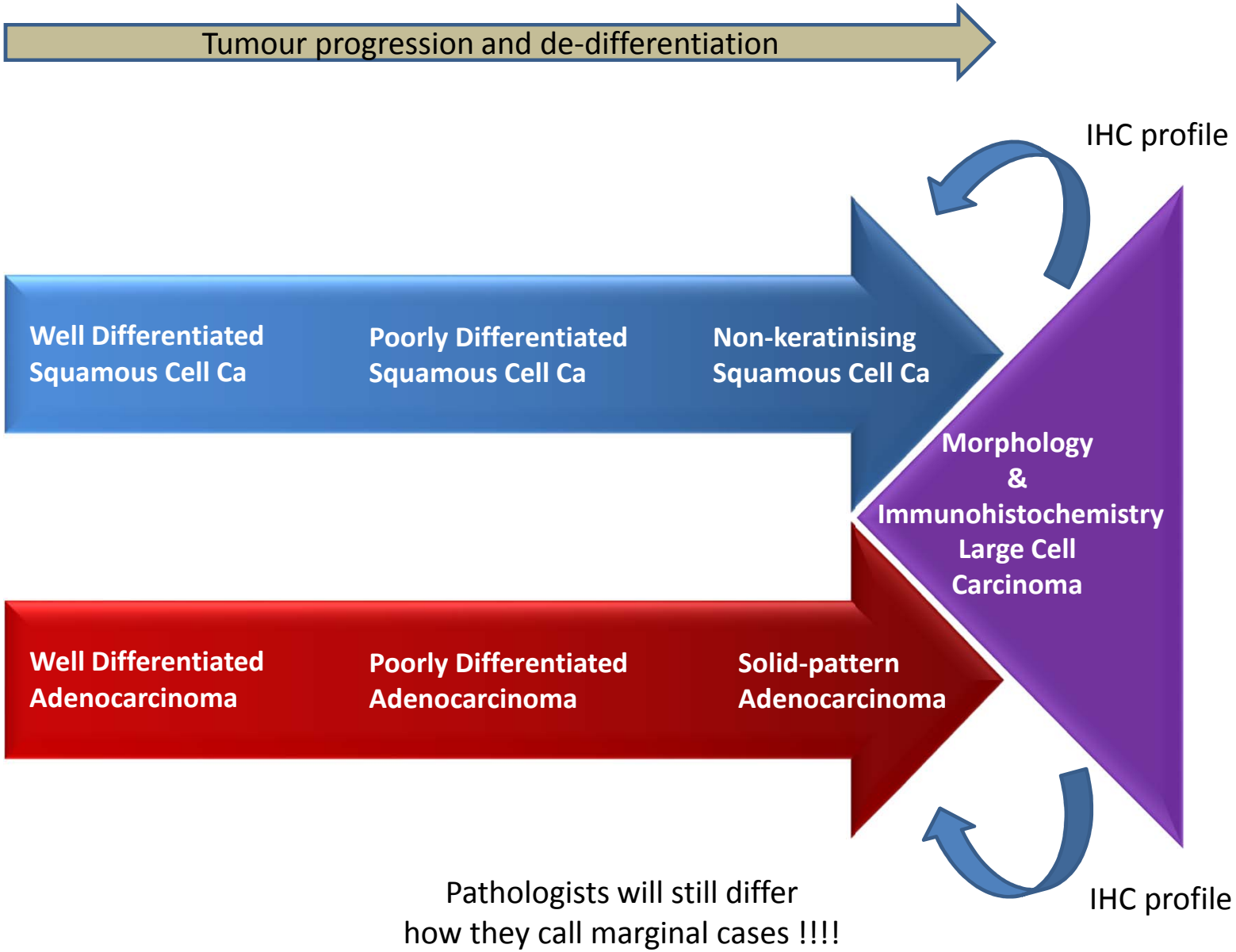
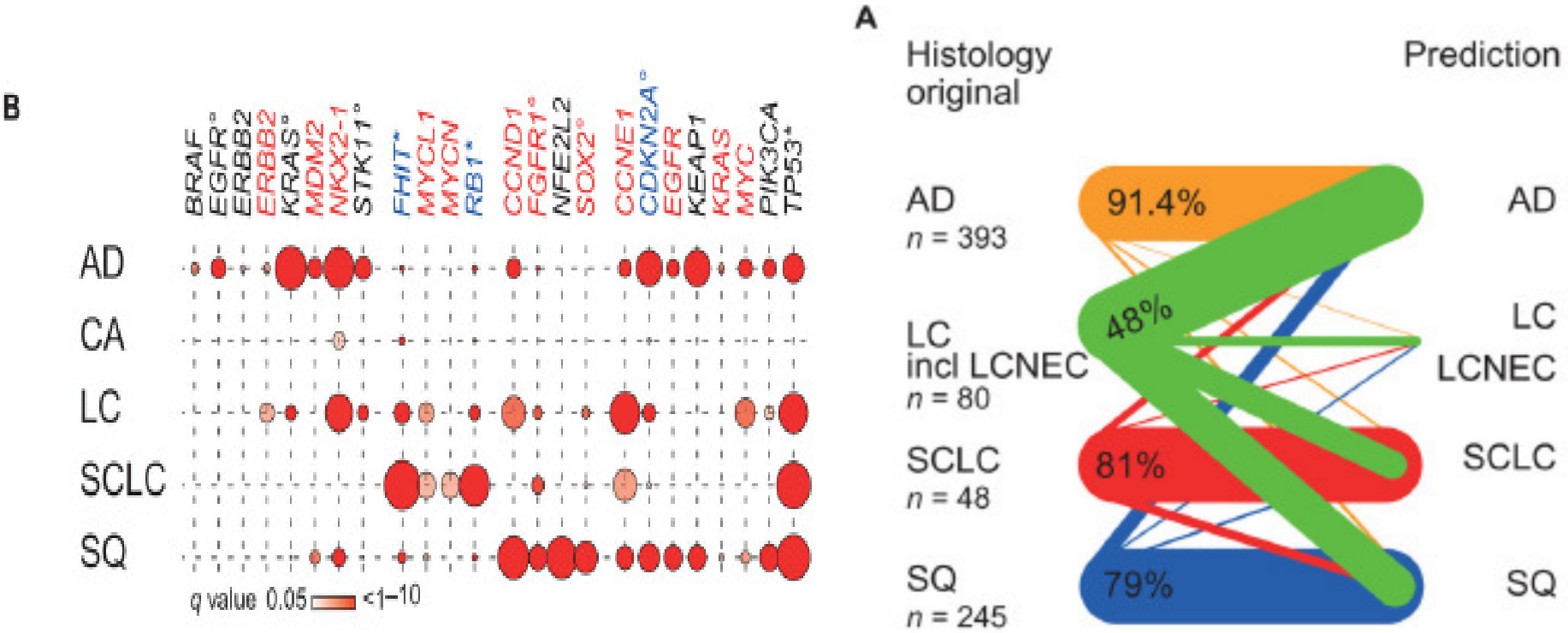


2015


Tumour progression and de-differentiation
IHC profile
Well Differentiated Squamous Cell Ca
Poorly Differentiated Squamous Cell Ca
Non-keratinising Squamous Cell Ca
Morphology & Immunohistochemistry
Large Cell Carcinoma
Well Differentiated Adenocarcinoma
Poorly Differentiated Adenocarcinoma
Solid-pattern Adenocarcinoma
IHC profile
Pathologists will still differ how they call marginal cases !!!!

A Genomics-Based Classification of Human Lung Tumors

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



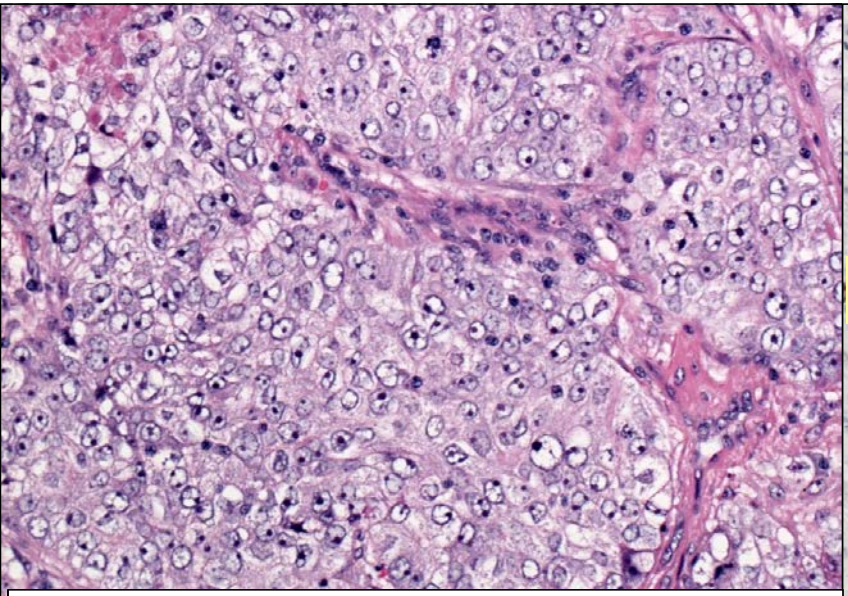
Sci Transl Med. 2013 October 30; 5(209): 209ra153. doi:10.1126/scitranslmed.3006802.

Diagnosis of Large Cell Carcinoma: 2015

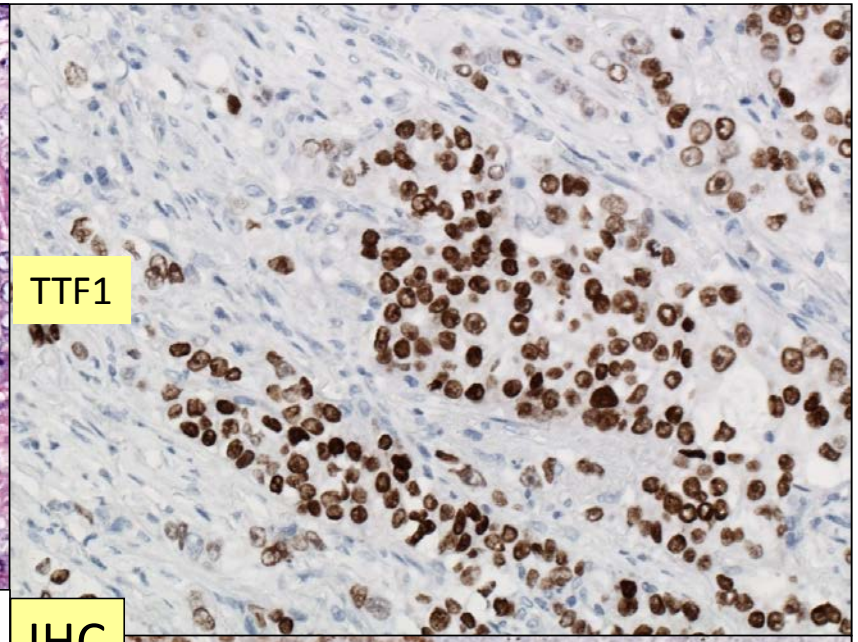
Definition

Large cell carcinoma is an undifferentiated non-small cell carcinoma (NSCC) that lacks the cytological, architectural, and **immunohistochemical features** of small cell carcinoma, adenocarcinoma, or squamous cell carcinoma. The diagnosis requires a thoroughly sampled resected tumour, and cannot be made on non-resection or cytology specimens.

The diagnosis of large cell carcinoma is only made on resection specimen when additional staining (*Immunohistochemistry and/or mucin stains*) is **negative, unclear, or not available**.

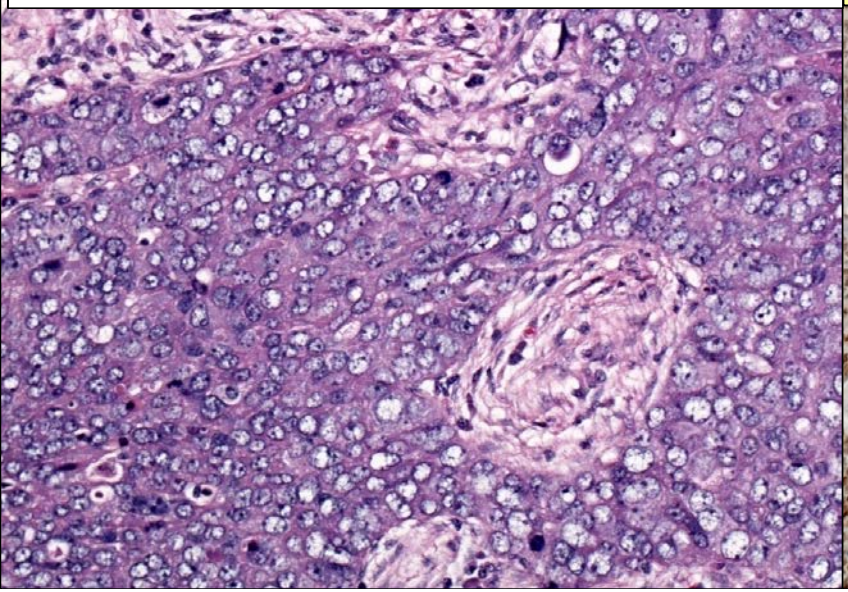


TTF1

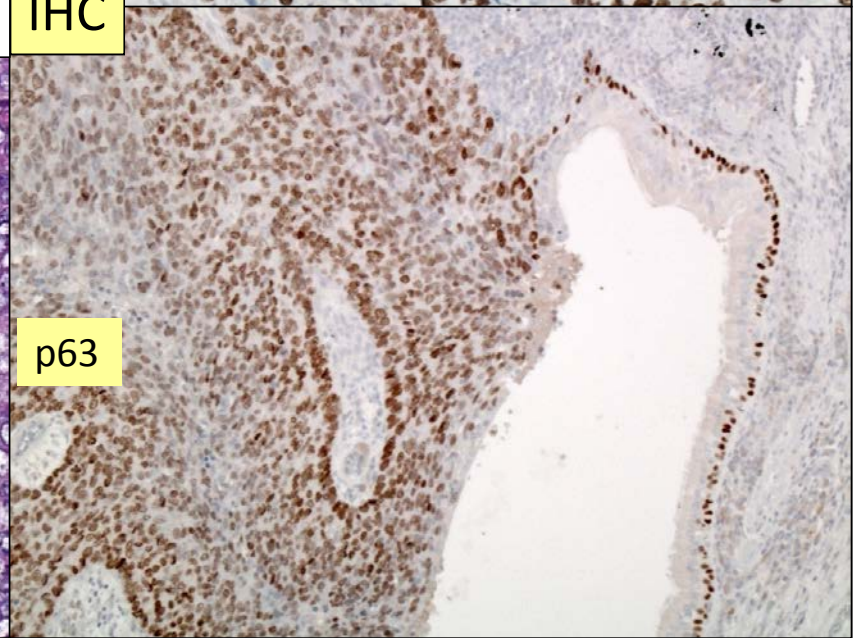


Large cell carcinoma becomes

IHC



p63



Resection specimen

Adenocarcinoma:
Solid subtype

Non-keratinizing
Squamous cell
carcinoma

Clinical impact

- Large cell category gets smaller
- Classification shift may reduce post-operative 5 year survival for squamous cell and adenocarcinomas
- No impact on molecular testing practice
- New nomenclature

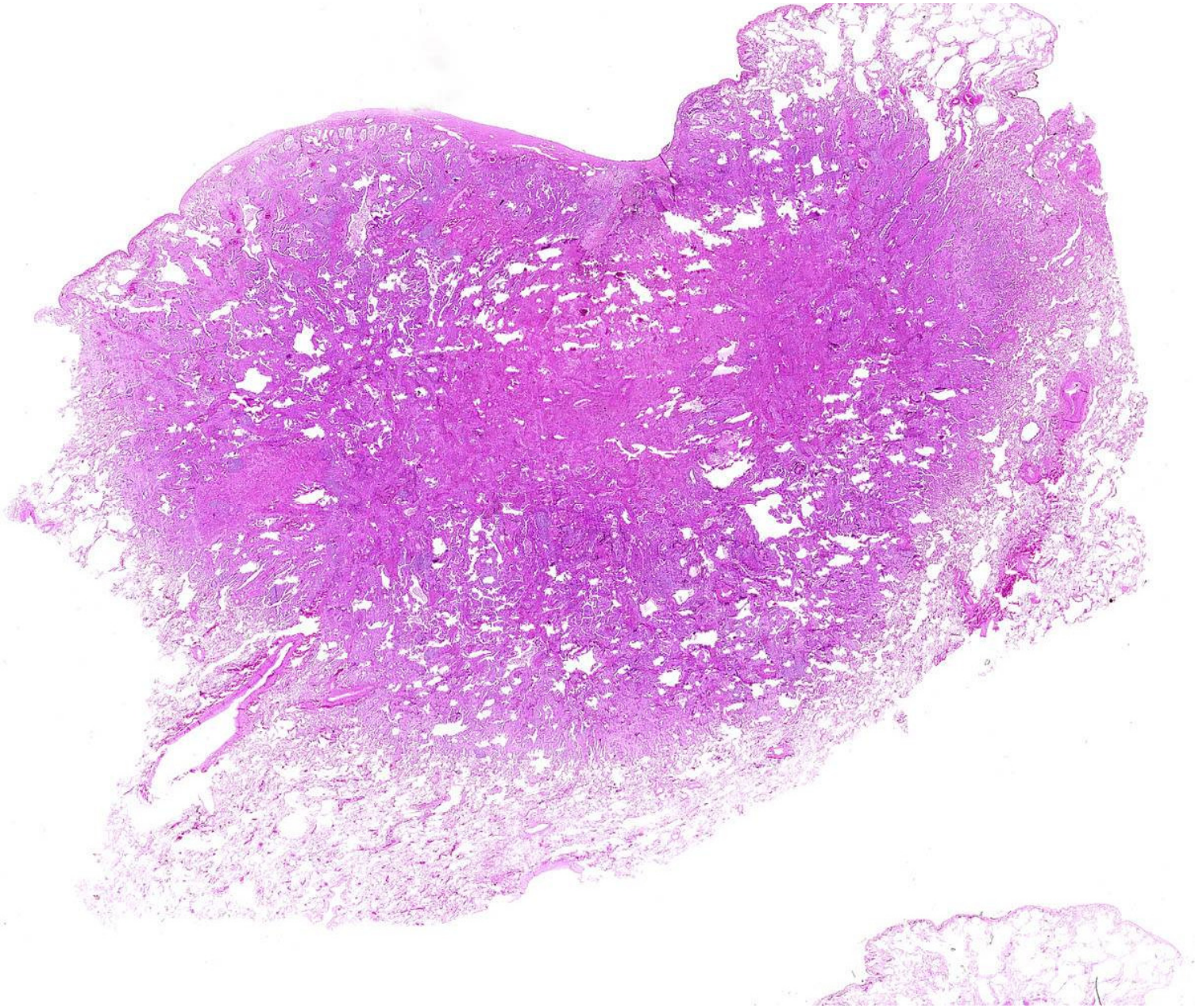
WHO classification of lung cancer

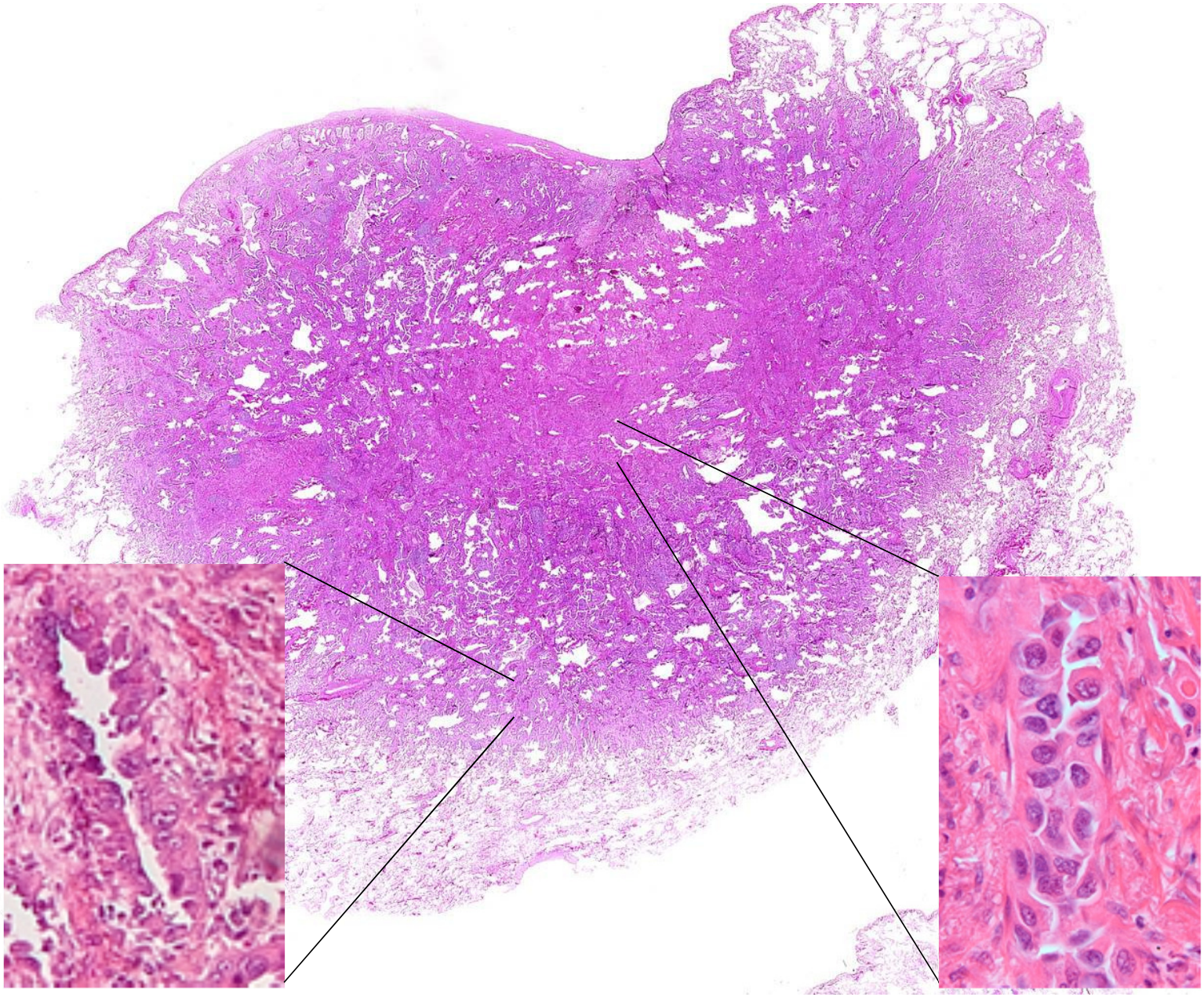
- Large cell carcinoma
- **Small samples**

Predictive analysis

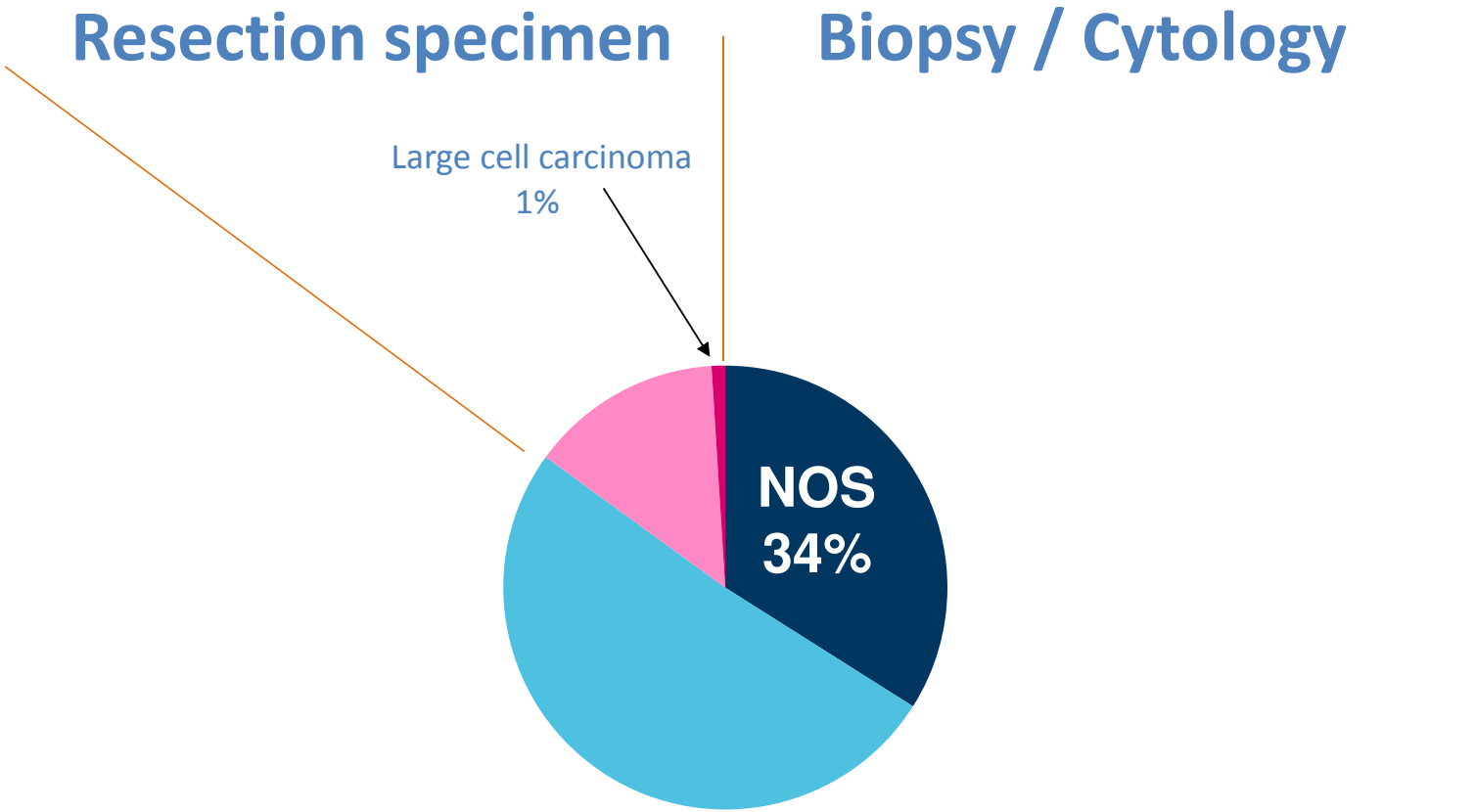
- EGFR
- ALK
- ROS1
- MET
- PD-L1

Tissue management





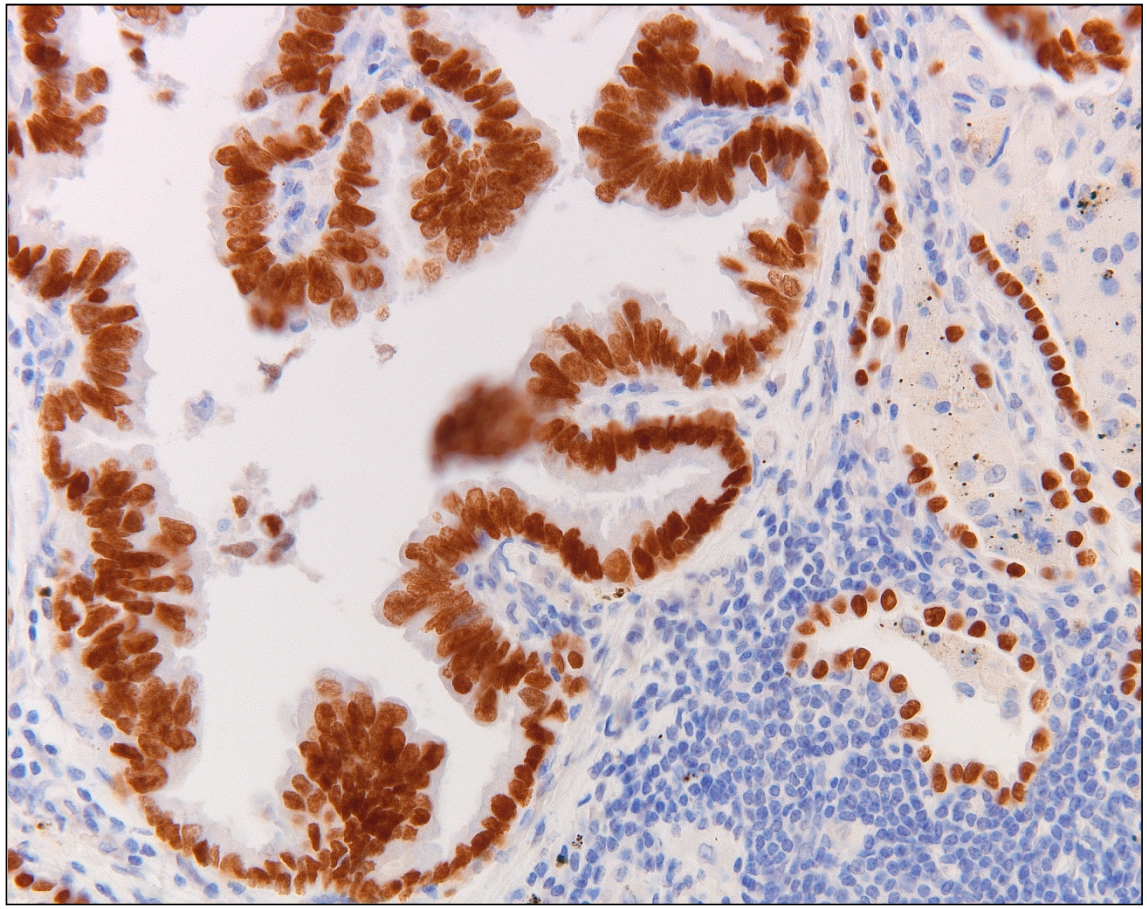
Uncertainty of H&E in NSCLC



Travis et al. Multidisciplinary classification of lung adenocarcinoma, JTO 201, 6(244-285)

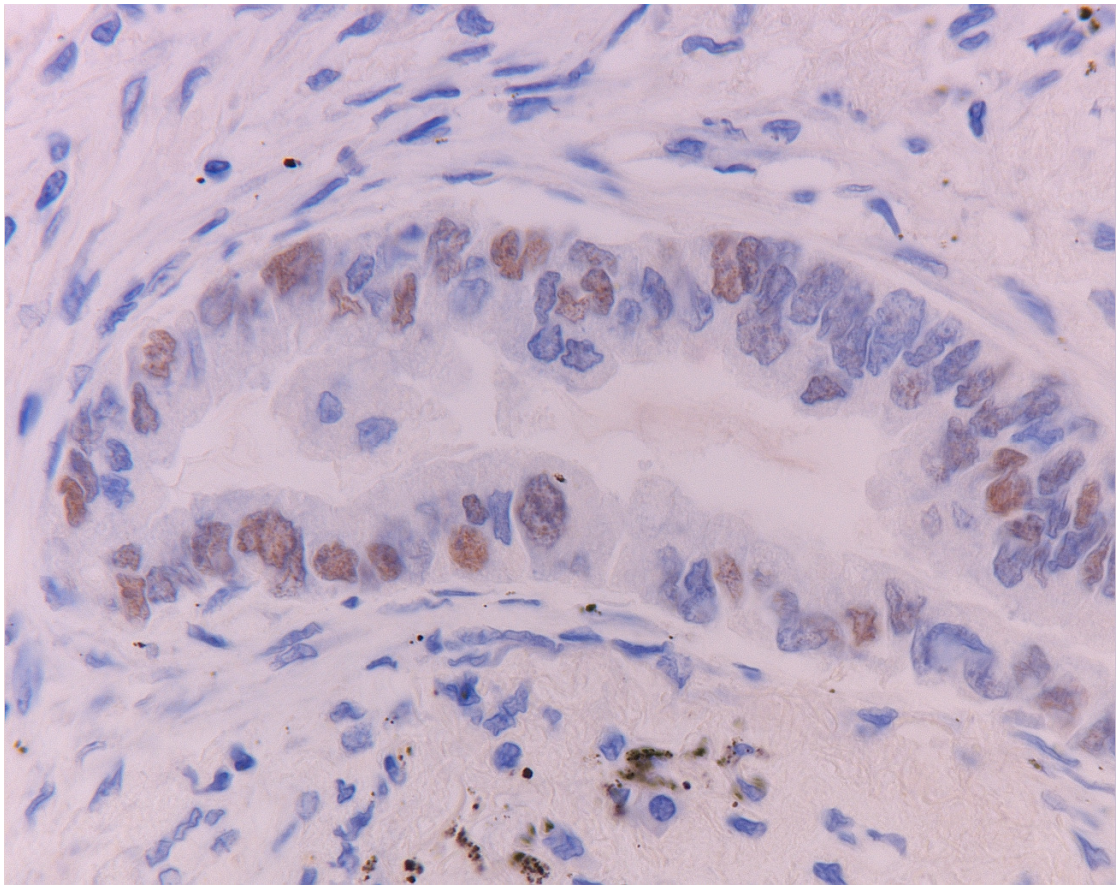
Adenocarcinoma

IHC TTF1 (NKX2-1) (clone 8G7G3/1)



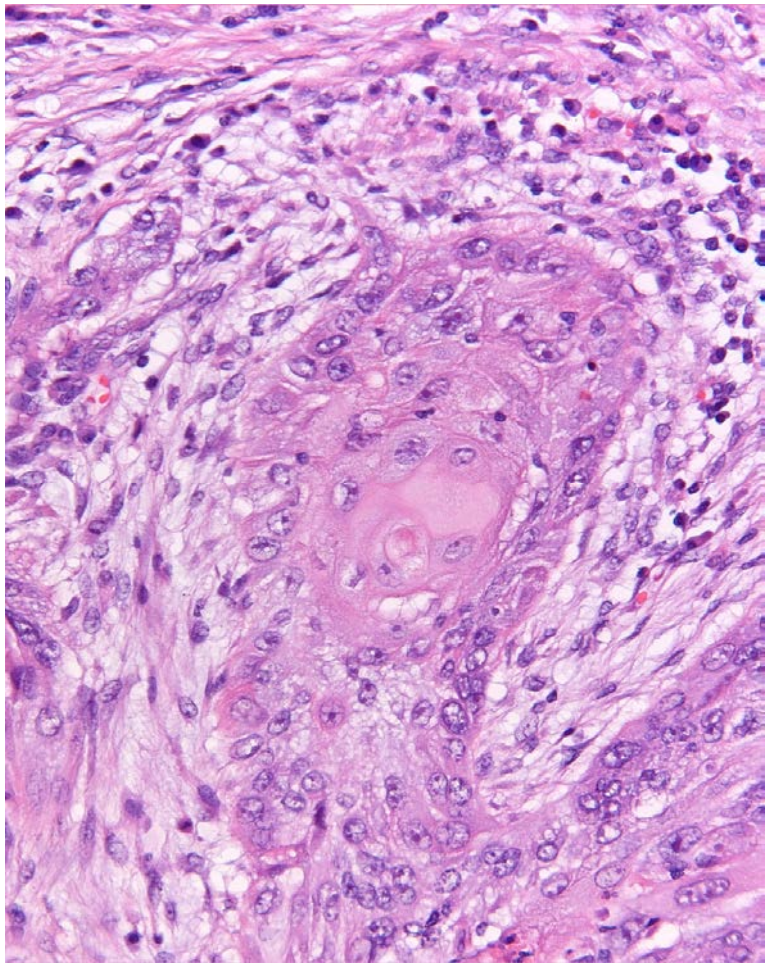
Adenocarcinoma

IHC TTF1

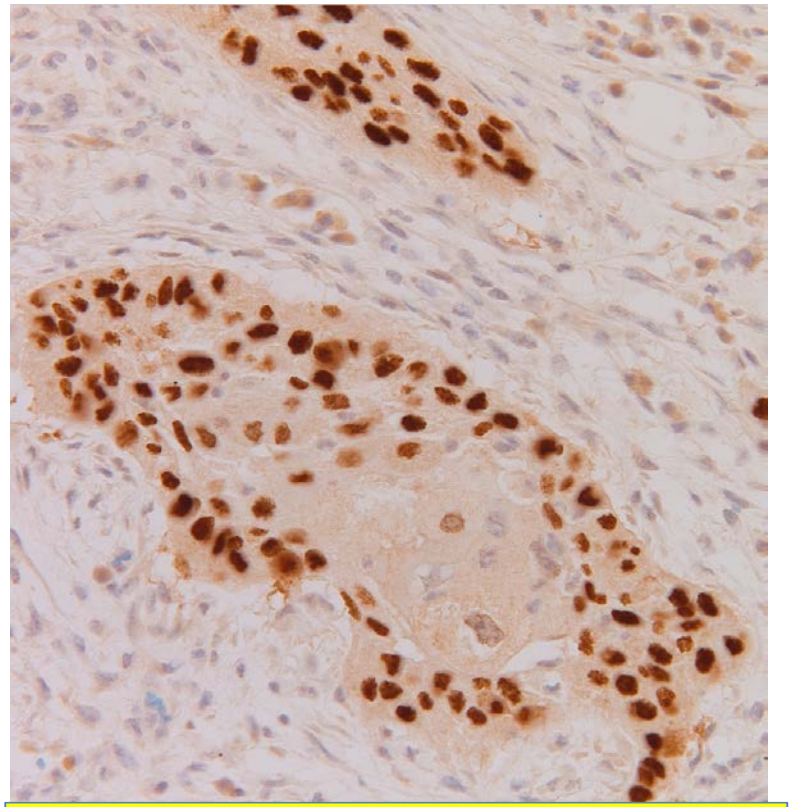


TTF1 Threshold weak is also positive

H&E



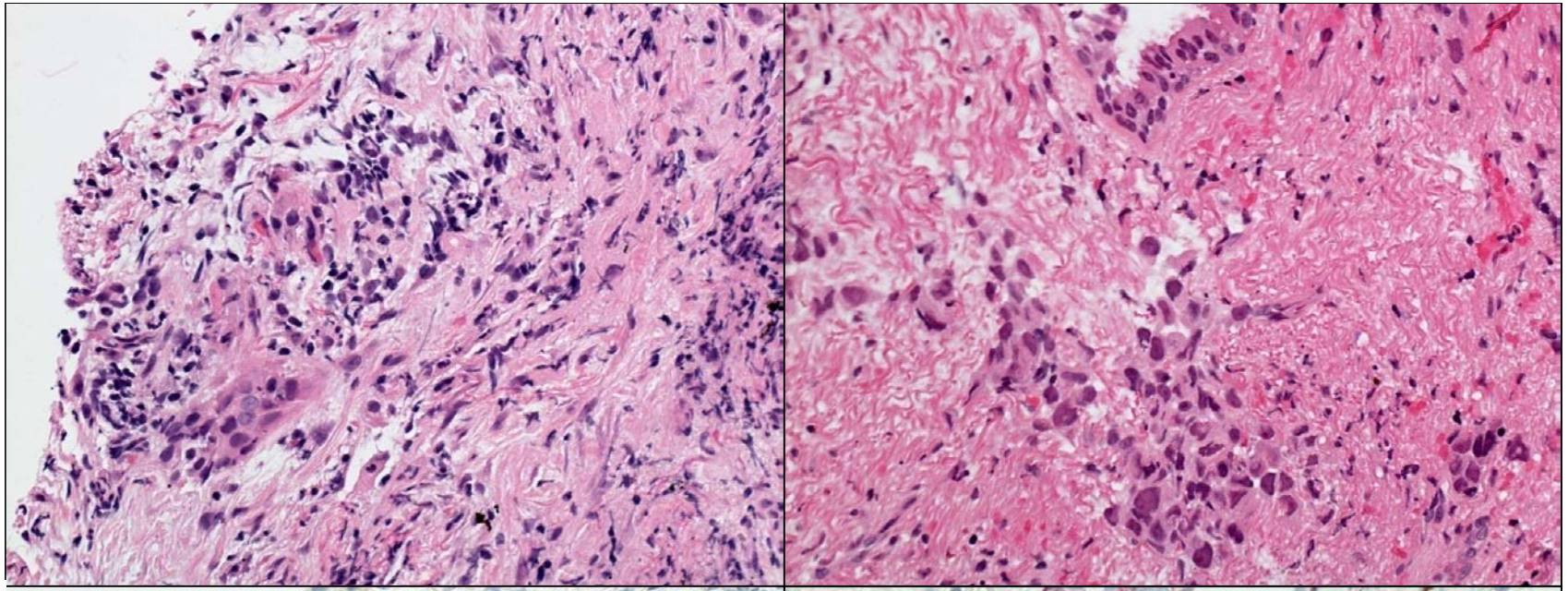
IHC p63



Threshold
Strong positivity in all undifferentiated cells

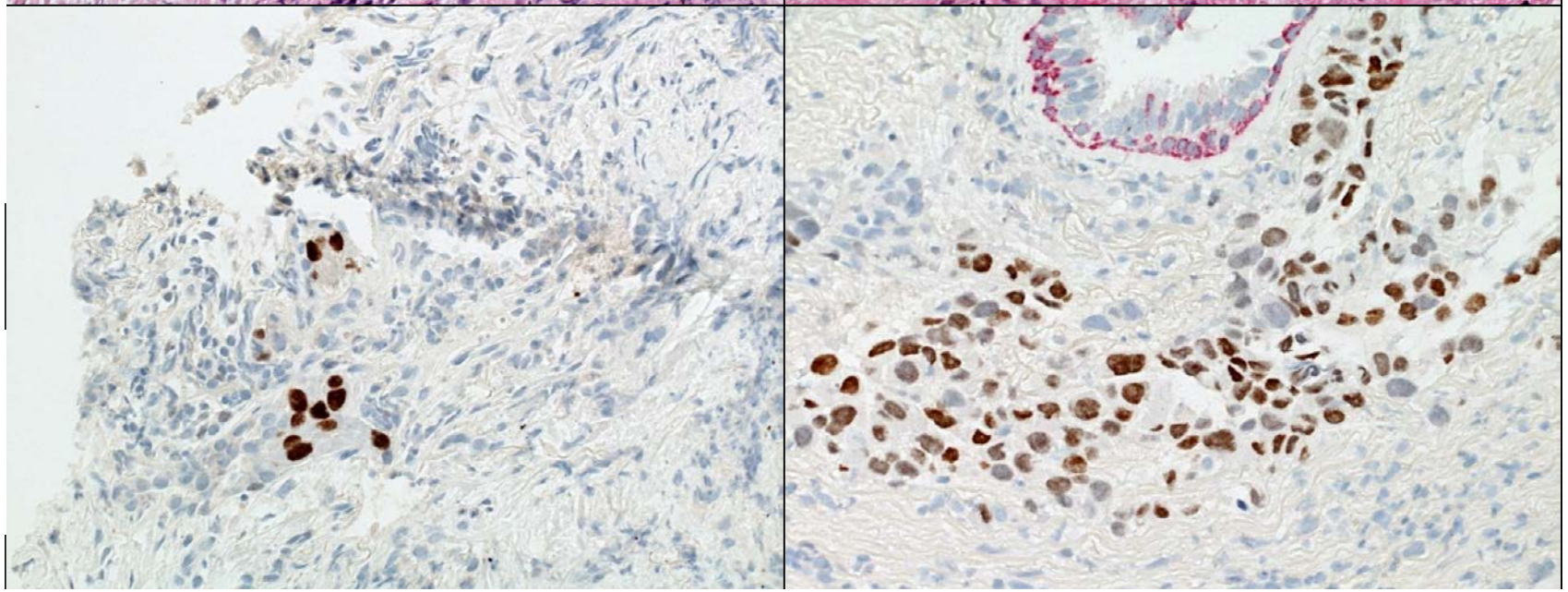
P63/p40

NSCLC-NOS



NSCLC-NOS

Tumour cells express Nuclear p63 Or p40

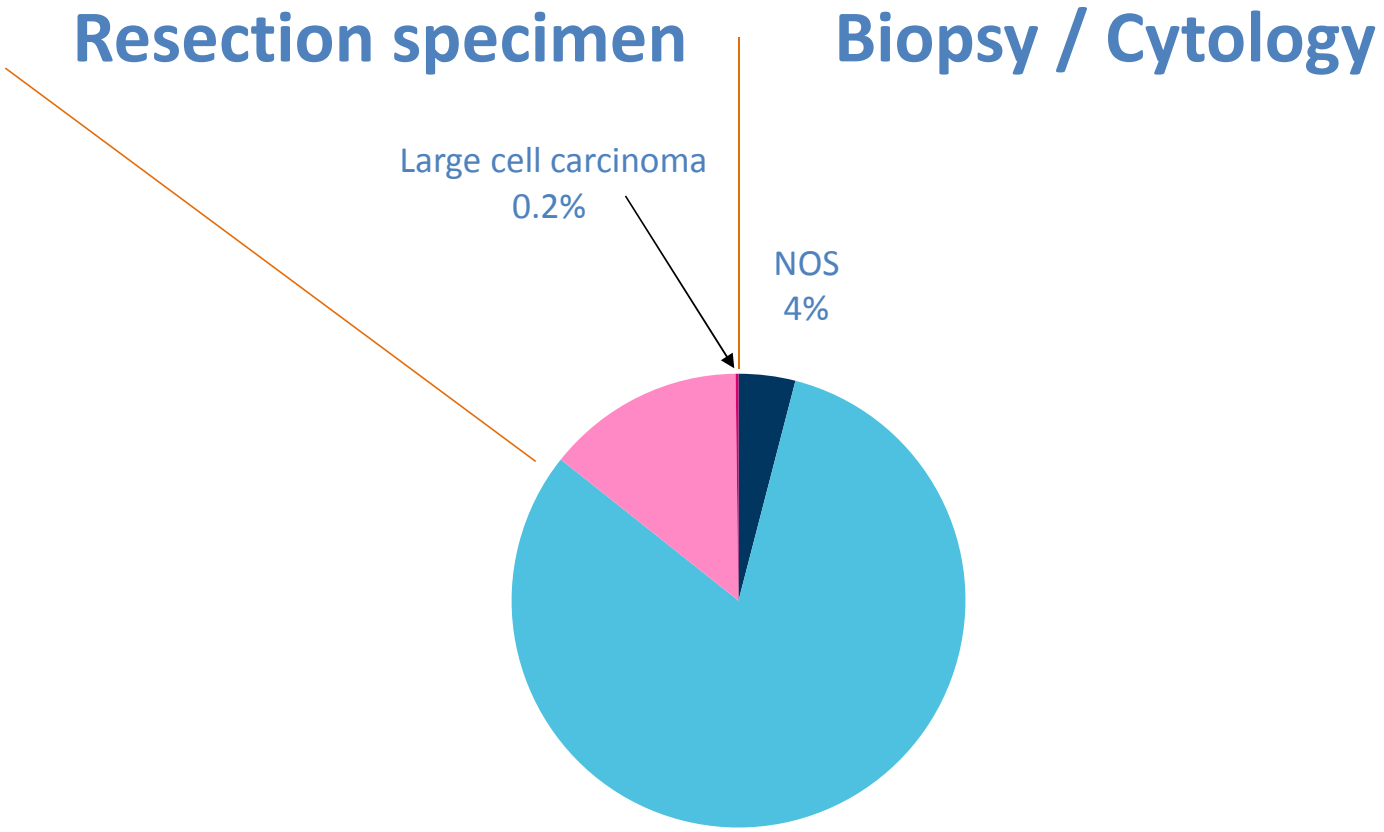


TTF1 positive in tumour cell nuclei

NSCLC- probably squamous cell

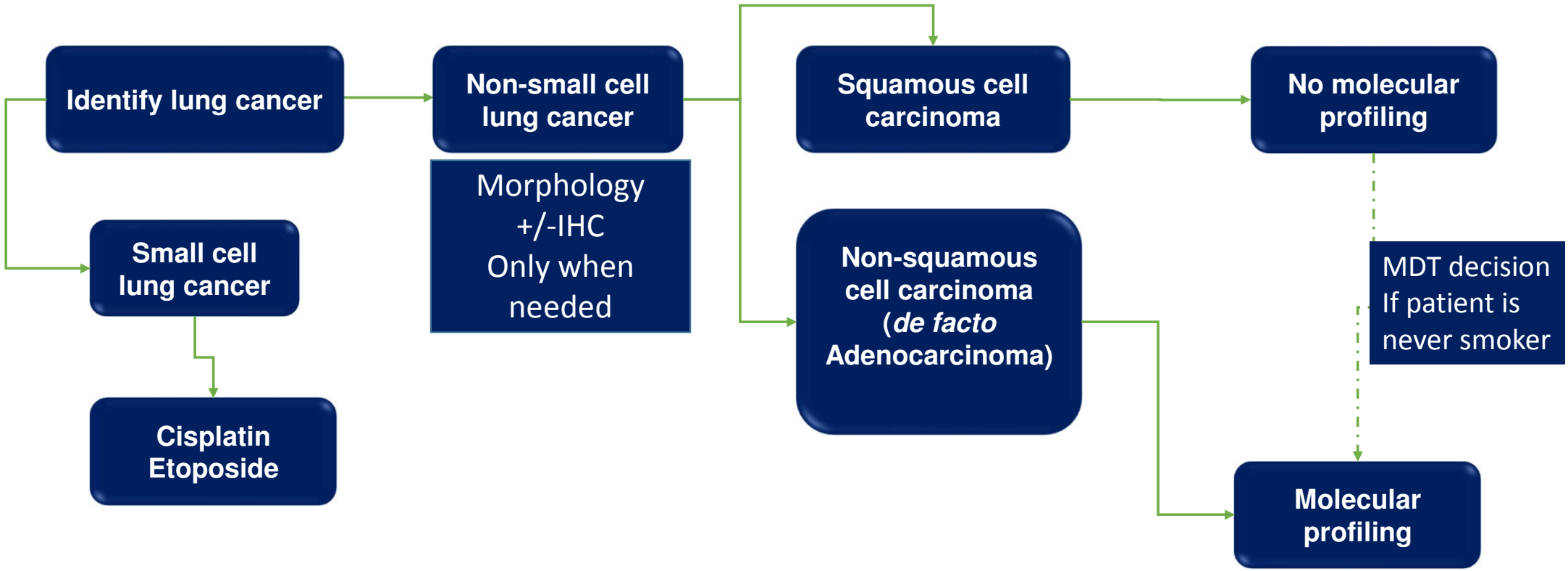
NSCLC – probably adeno-carcinoma

NSCLC H&E + stain

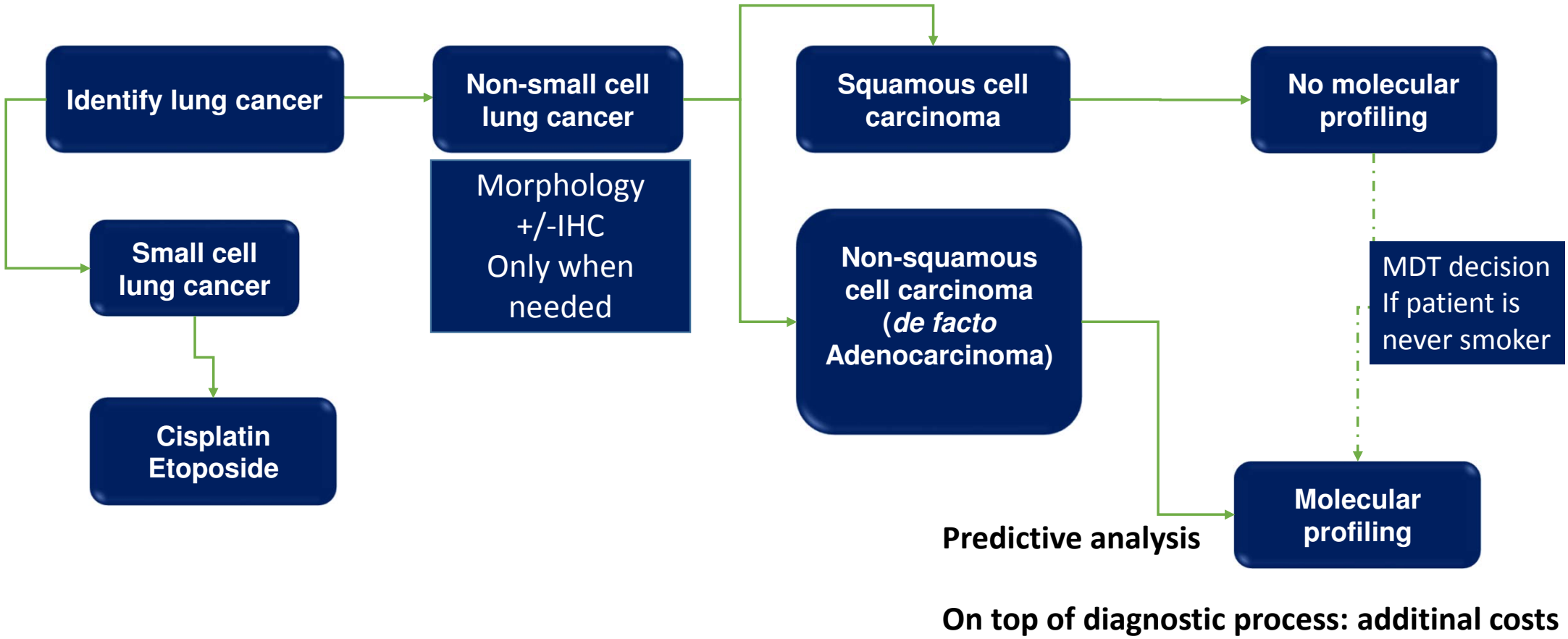


WHO lung cancer classification 2015

Evolving Diagnostic Algorithm: Small sample diagnosis



Evolving Diagnostic Algorithm: Small sample diagnosis



WHO 2015: Histology and mutation

Cell type	EGFR mutation	KRAS mutation	BRAF mutation	ALK fusion
Adenocarcinoma	**15%**	**30.9%**	**2.5%**	**12.5%**
Probable Adenoca (by IHC)	**5.9%**	**41.8%**	**0**	**0**
Squamous Carcinoma	**0**	**0**	**0**	**0**
Probable Squamous ca (by IHC)	**0**	**0**	**0**	**0**
NSCLC-NOS	**7.3%**	**31%**	**2.7%**	**0**
Small cell carcinoma	**0**	**0**	**0**	*1 case**

Kret A et al. Lung Cancer Jan, 2015; suppl 1

WHO classification of lung cancer

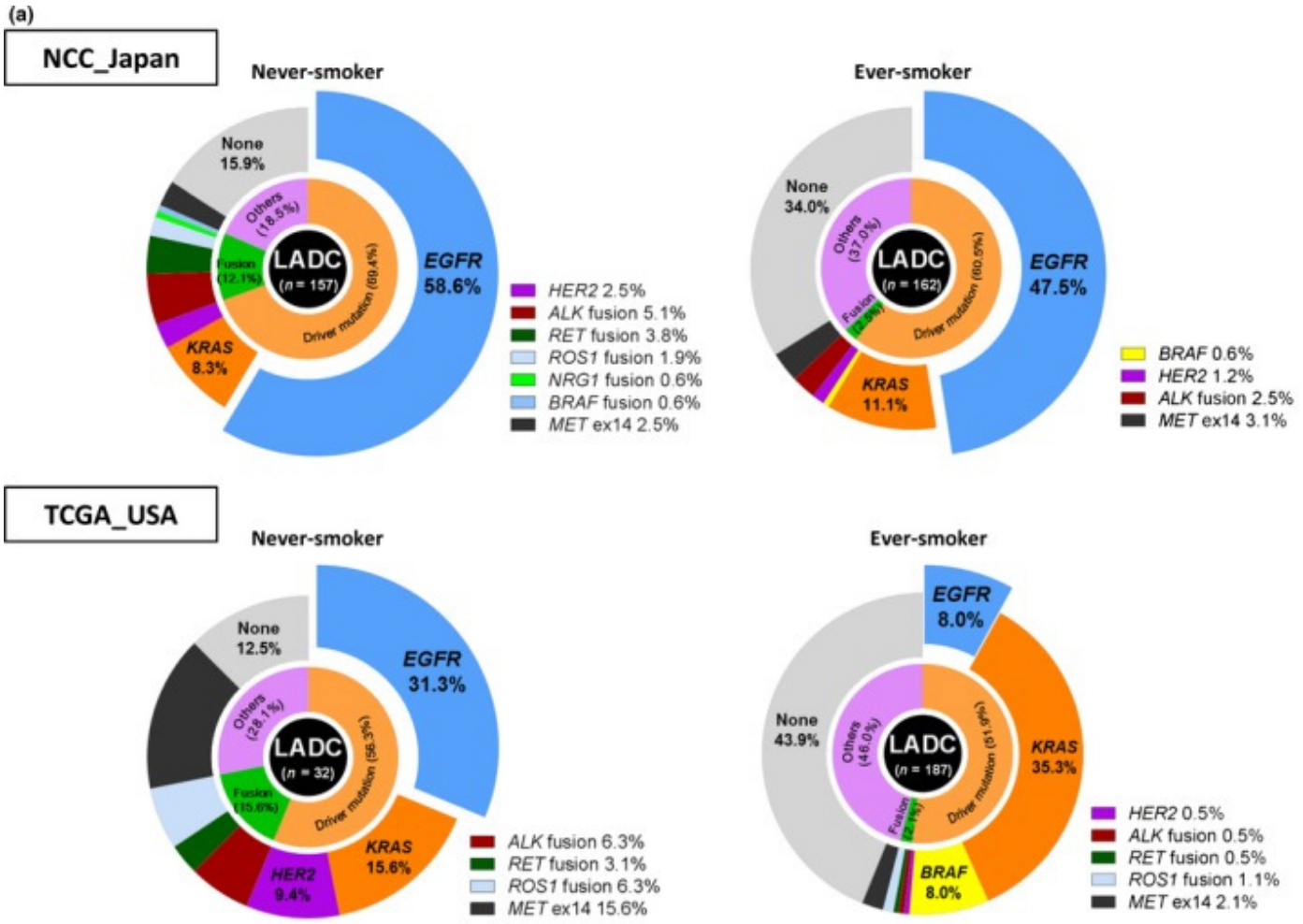
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis

- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management

Frequency of driver oncogene aberrations in lung adenocarcinoma, according to smoking status



Saito, Cancer Sci 2016, 107 (713-20)

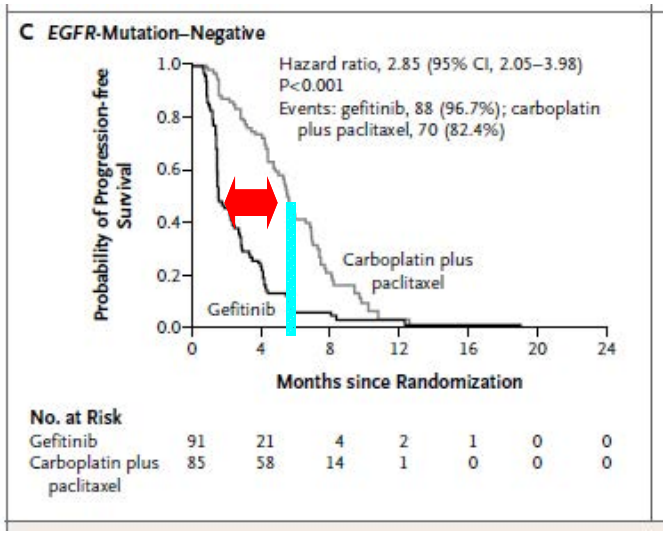
Biomarker Prediction: treatment related

		IHC	FISH	mut/indel/ transl
EGFR mut	erlo-, gefi-, afatinib	-/+	-	+
ALK	crizotinib	+	+	+
ROS1	crizotinib	+	+	+
MET ex14/ampl	met mab	-	+	+
BRAF	sora/vemurafenib	+?	-	+
PIK3CA	ever/temsirolimus	-	-	+
HER2	trastuzumab/lapatinib/ dacomatinib	+	+	-
EGFR ihc	cetuximab	+	+/-	-
RET	Sunitinib, sorafenib, vandetanib cabozantinib	+	-	-
NRAS	vemurafenib	-	-	+
cKIT	imatinib/sunitinib	-	-	+
KRAS	selumetinib, docetaxel	-	-	+
DDR2	dasatinib	-	-	+
FGFR1	ponatinib	-	+	-

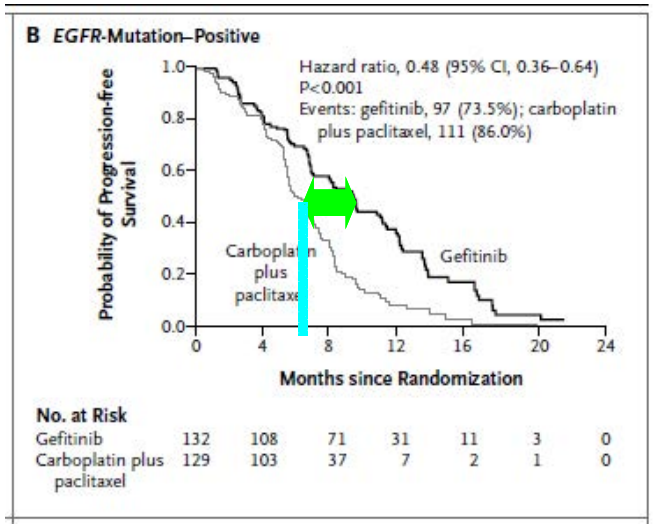
2008/9
2013
2016

Effect of gefitinib in case of present or absent EGFR mutation

Absent



Present

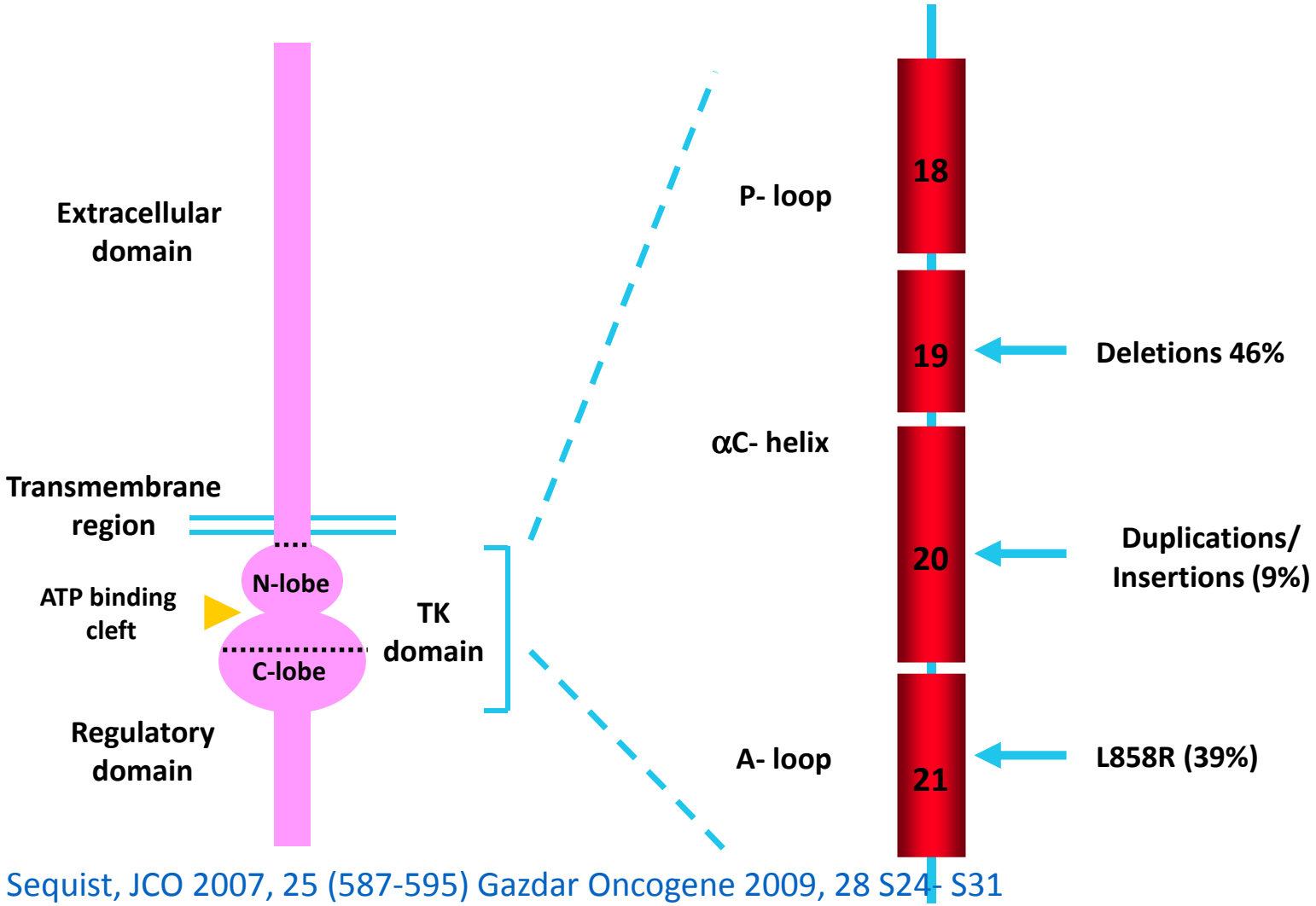


Conclusion:
Since in patients without EGFR mutations more harm is done with EGFR-TKI than with chemo-x, EGFR-TKI treatment only for patients with EGFR mutations: selection required.

Mok NEJM 2009, 361 (947-957)

Mutations in the TK domain of EGFR:

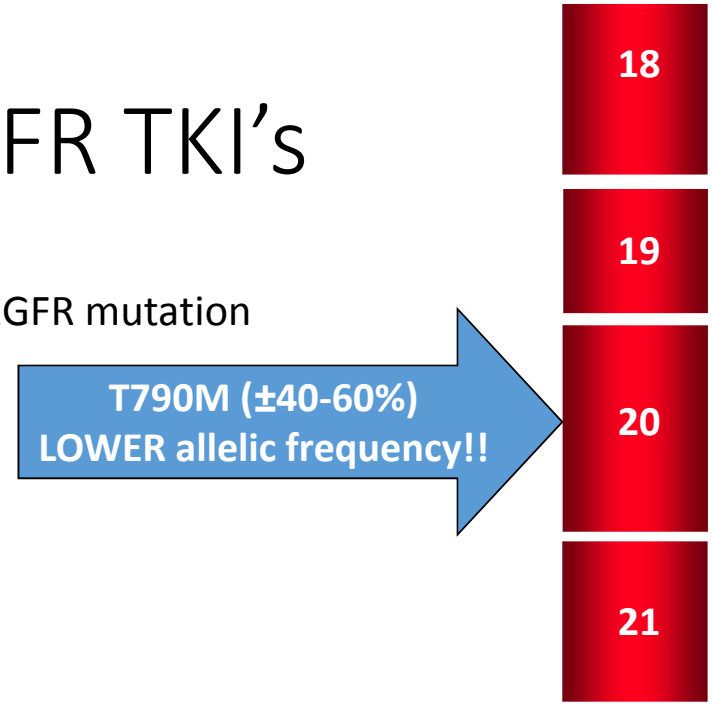
Meta analysis of 5 studies (n=1256)



Sequist, JCO 2007, 25 (587-595) Gazdar Oncogene 2009, 28 S24- S31

Resistance to first line EGFR TKI's

- 37 Bx after recurrence: all retain original EGFR mutation
- T790M (48-63%),
- Part MET amplification (5%)
- Part Her2 amplification (13%)
- Part PIK3CA, EGFR amplification
- Part EMT
- Part SCLC (<2-4%)
- Induction of FGF2 and FGFR1
- Acetylcholine receptor



- Repeatedly assessing cancer throughout the course of disease

- Sequist Sci Transl Med. 2011, 3 (75ra); Wang JTO 20 13,8(719-25); Ware Oncogenesis 2013,2^{e}39;
- Yu CCR 2013, 19(2240-7)

3rd generation EGFR TKI (afatinib): resistance

Table 1 Patients with NSCLC who developed resistance to third-generation EGFR TKIs

Case report	Pretreatment EGFR mutations	Prior treatments	Third generation EGFR TKI	Gene status at resistance	Reference
60 YF	del.19	Chemo, erlotinib Radiotherapy, afatinib/cetuximab Chemo/erlotinib	AZD9291 PFS = 9 months	del.19 and T790M and C797S and TSC2N486I	[25]
57 YF	del.19	Gefitinib, chemo Afatinib/nimotuzumab	HM61713 PFS = 17 months	del.19 and T790M and C797S	[27]
71 YF	L858R	Gefitinib	AZD9291 PFS = 13 months	L858R and T790M and L718Q	[29]
71 YF	del.19	Chemo, erlotinib	CO-1686	del.19 and T790M and HER2 A	[30]
64 YF	L861Q and T790M and HER2 A	NA	AZD 9291 PFS = 7 months	EGFR L861Q and T790M	[30]
54 YF	del.19 and T790M and MET amp	Chemo, erlotinib	CO-1686 PFS = 3 months	NA	[30]
57 YM	L858R and T790M	Gefitinib, chemo	AZD9291 PFS = 4 months	EGFR L858R and T790M and MET amp	[30]
60 YM	del.19 and T790M	Erlotinib, chemo Radiotherapy	AZD9291 PFS = 18 months	del.19 and T790M and MET amp and HER2 A	[30]
56 YF	del.19 and T790M	Gefitinib, radiotherapy Erlotinib, afatinib, afatinib/cetuximab Pemetrexate/erlotinib	AZD9291 PFS = 22 months	del.19 and T790M and C797S and MET amp	[30]
51 YF	del.19 and T790M	Gefitinib, chemo Afatinib, afatinib/cetuximab	AZD9291 PFS = 9 months	del.19 and KRAS G12S	[30]

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; del. 19, EGFR exon 19 deletion; amp, amplification; chemo, chemotherapy; NA, not available; PFS, progression free survival.

Wang Front Med 2016

WHO classification of lung cancer

- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis

- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

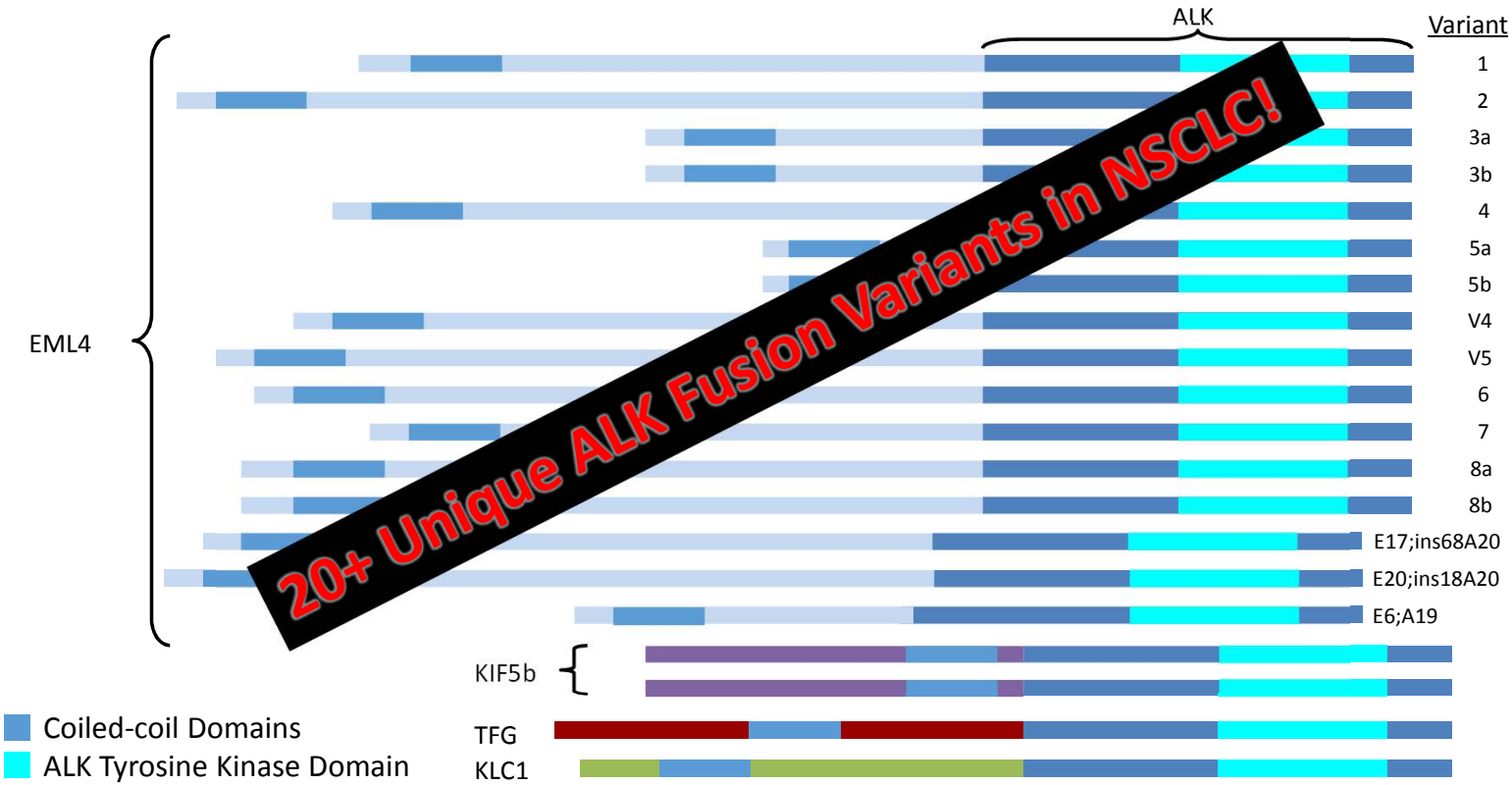
Tissue management

Frequent ALK genetic alterations in cancer

Tumor type	*ALK* alteration	Incidence
IMT	TPM3-ALK TPM4-ALK	50–60% 50–60%
Neuroblastoma	Point mutations and/ or amplification	6–8% / 4.4%
ALCL	NPM-ALK TPM3-ALK	60–80% 12–18%
Lung cancer	EML4-ALK KIF5B-ALK TFG-ALK more	3–7% Rare Rare

Ref: *Mol Cancer Ther* 2011;10:569-579

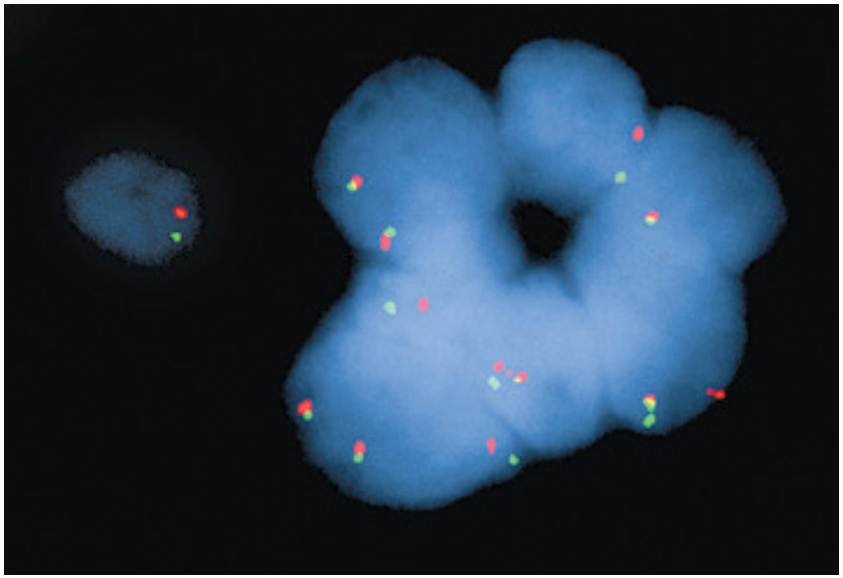
ALK Fusions in NSCLC (2007–Present)



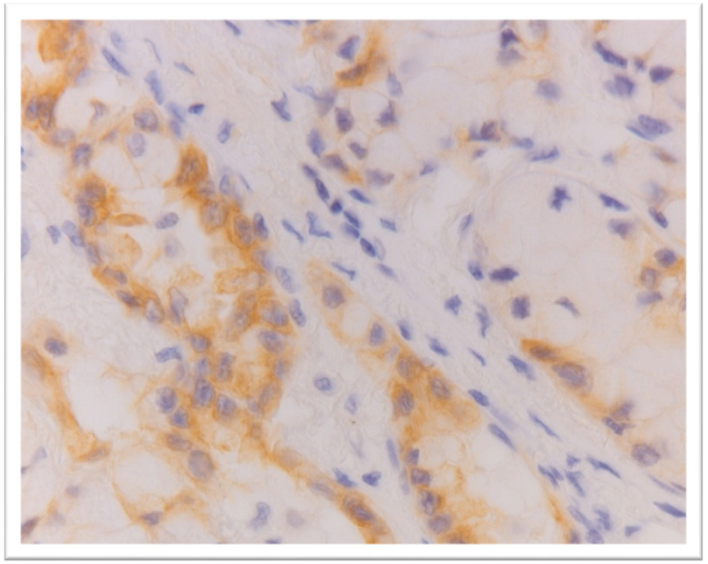
Sanders et al. Can Gen 2011;204:45–52; Togashi et al. PLoS One 2012;7:e31323; Wong, et al. Cancer 2011;117:2709–2718; Penzel et al. J Thorac Oncol 2012;7:1198–1199; Sasaki et al. Euro J Can 2010;46:1773–1780

Demonstrating ALK

FISH



Immunohistochemistry (IHC)



2–8% in adenocarcinomas

Shaw et al. J Clin Oncol 2009;27:4247–4253; Yip et al. J Thorac Oncol 2013;8:408–414, Sholl et al. J Thorac Oncol. 2013;8:322-8.

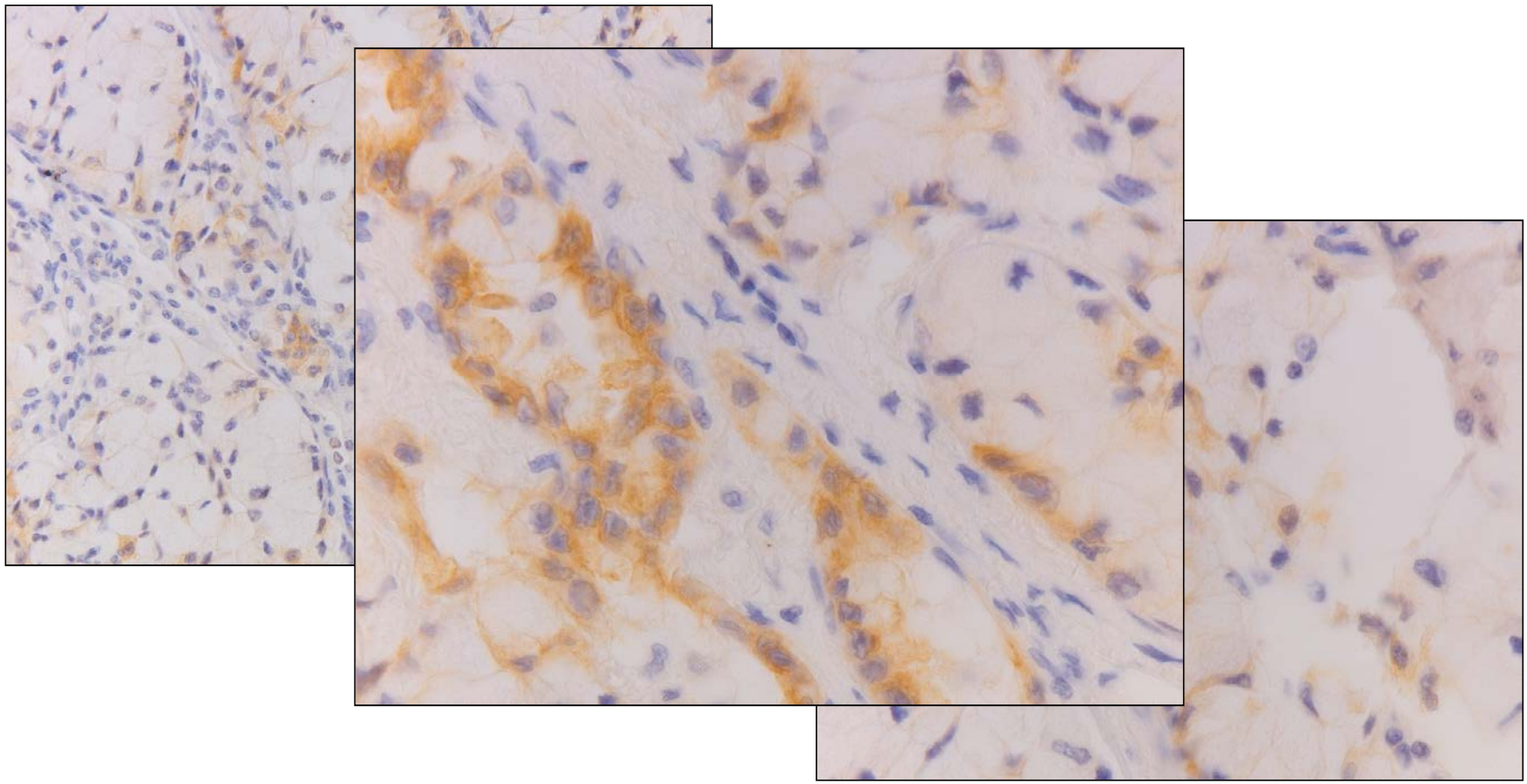
Break apart fluorescence *in situ* hybridisation

- Positive: (typical) split apart / (atypical)single 3’
- Start first 50 nuclei
  - Positive 25 out of 50
  - Negative <5 positive
  - Else equivocal then
- Second step additional 50
  - Positive: ≥ 15%
  - Else not rearranged

  - Borderline cases False Positive or False Negative; von Laffert Lung Cancer 2015, 90 (465-71)

ALK IHC cytoplasm

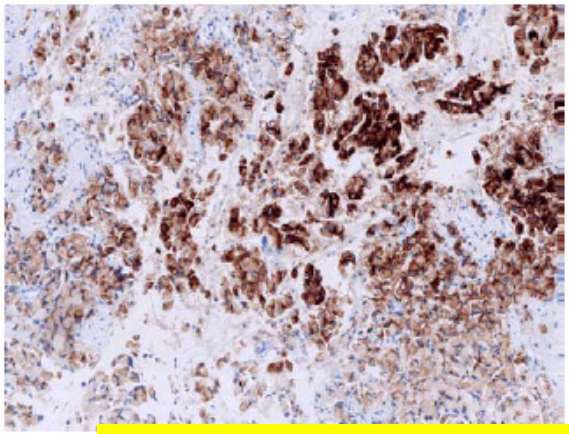
ETOP protocol 5A4 Novocastra
Novolink detection system
IHC H-score

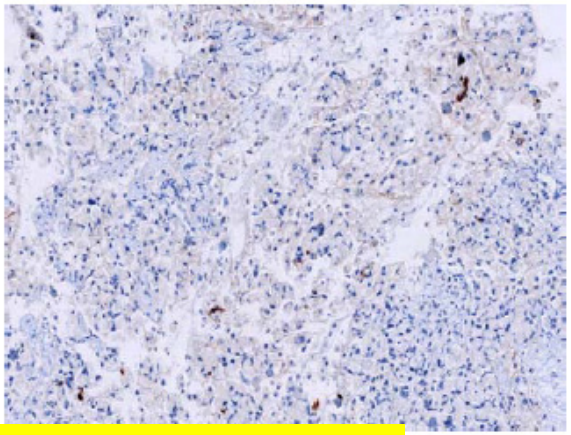


Blackhall et al. J Clin Oncol 2014;32:2780–2787

Two validated ALK IHC protocols (A)

- Ventana ALK (D5F3 cell signaling)
- Tyramide enhancement system
- Score yes/no





Case 3: ALK IHC good screening method ining is still pres

http://www.uclad.com/newsletters/ALK-LUNG-IHC-INTERPRETATION-GUIDE.pdf; Wynes et al. J Thorac Oncol 2014;9:631–638

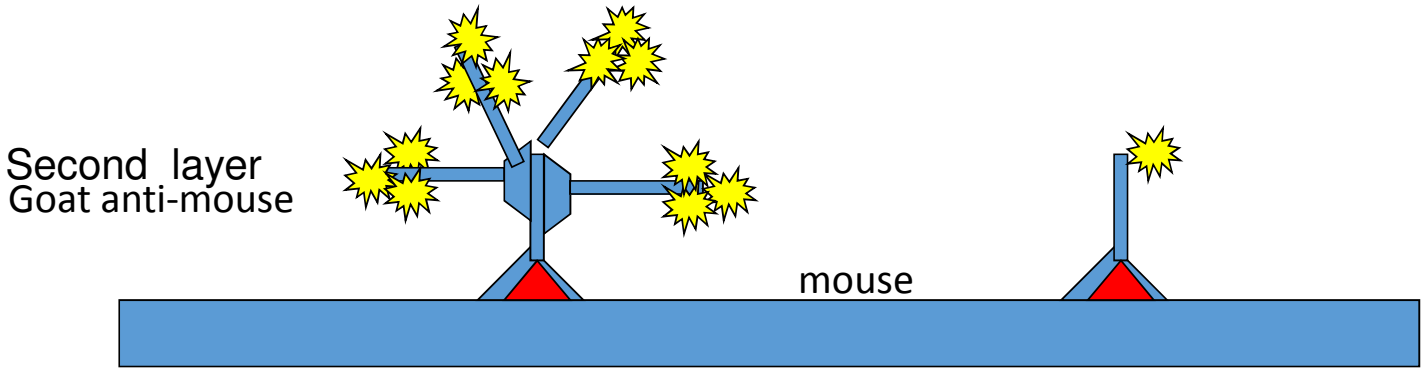
Immunohistochemistry detected/ visible

Indirectly labeled

Visible with
lower epitope concentration

Directly labeled

Visible with
high concentration of epitopes

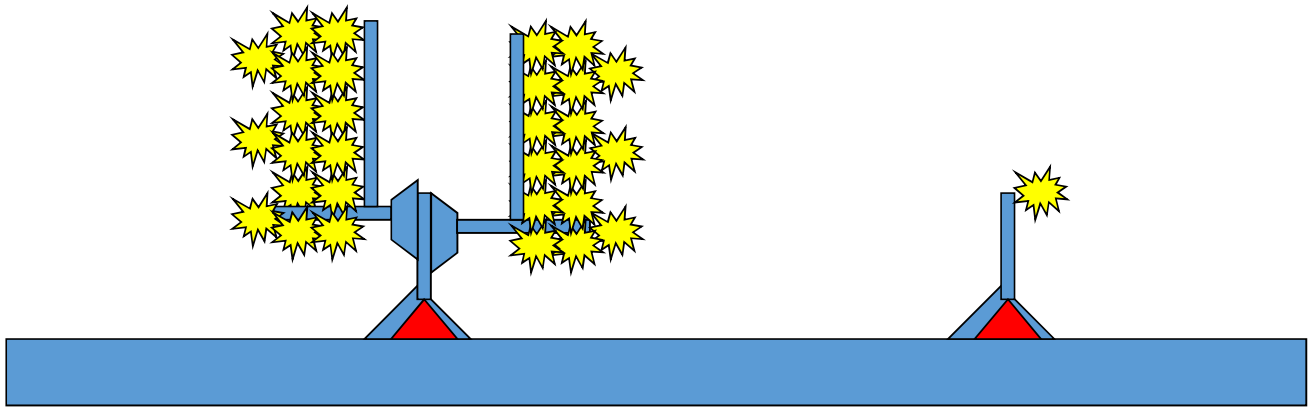


Indirectly labeled

Directly labeled

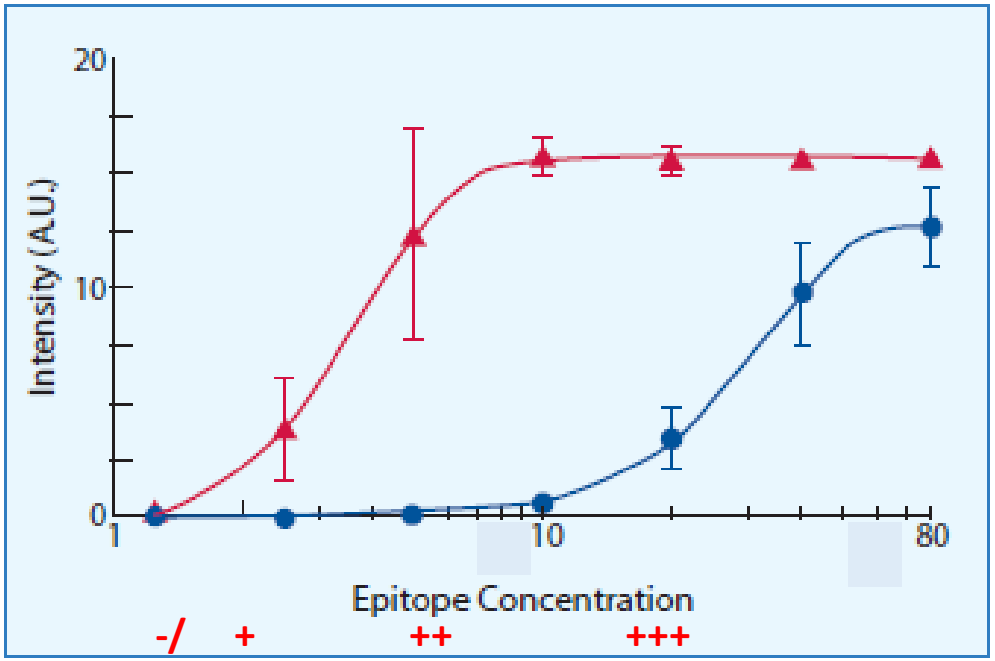
Immunohistochemistry

Indirectly labeled polymer, 5–20x



Indirectly labeled **Directly labeled**

Relation signal intensity and epitope concentration

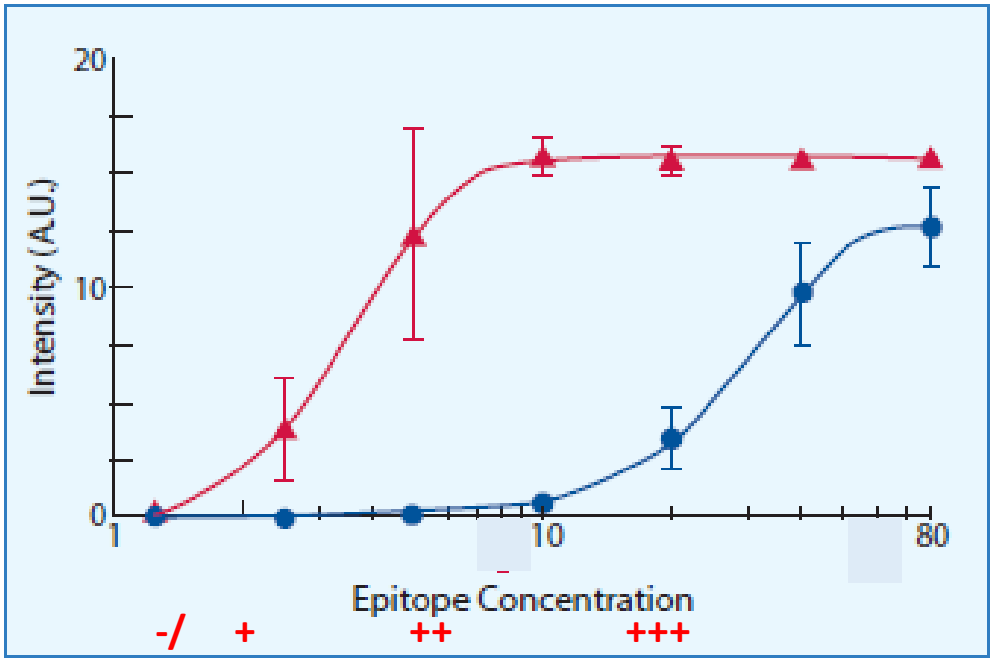


Polymer

Direct labeling

Modified from Prinsen CF, Appl Immunohistochem Mol Morphol. 2003;11:168-73.

Relation signal intensity and epitope concentration



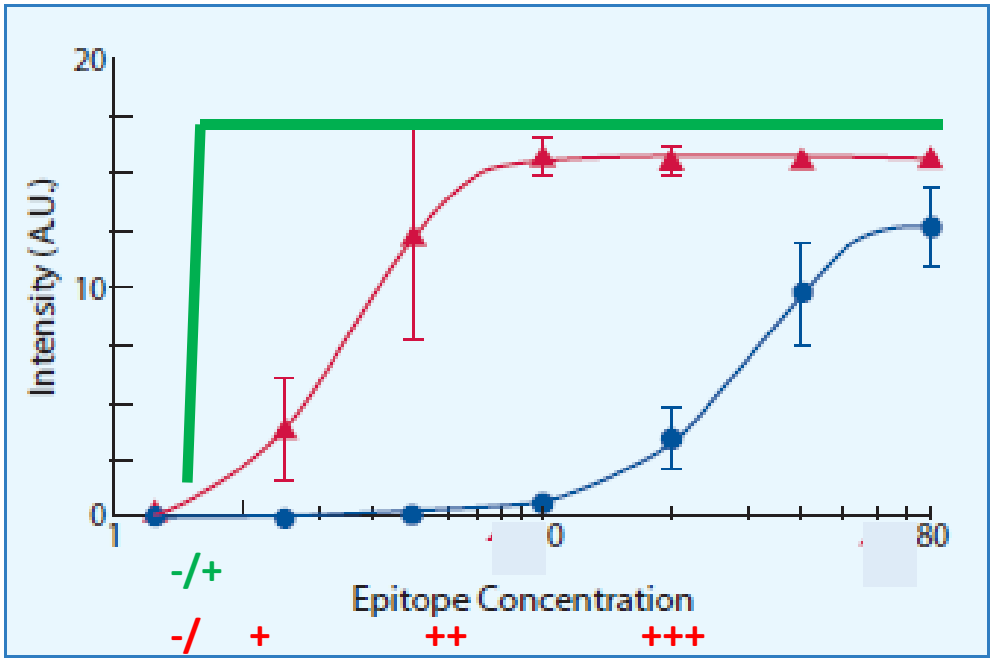
Polymer

Direct labeling

IHC
- Epitope concentration
- Signal enhancement system
- Intensity plateau
- Small linear dynamic range (factor 2-4)
- Lack of standard for epitope concentration
- Eye vs machine…….

Modified from Prinsen CF, Appl Immunohistochem Mol Morphol. 2003;11:168-73.

Relation between epitope concentration and signal enhancement in IHC



Tyramide amplification
Polymer

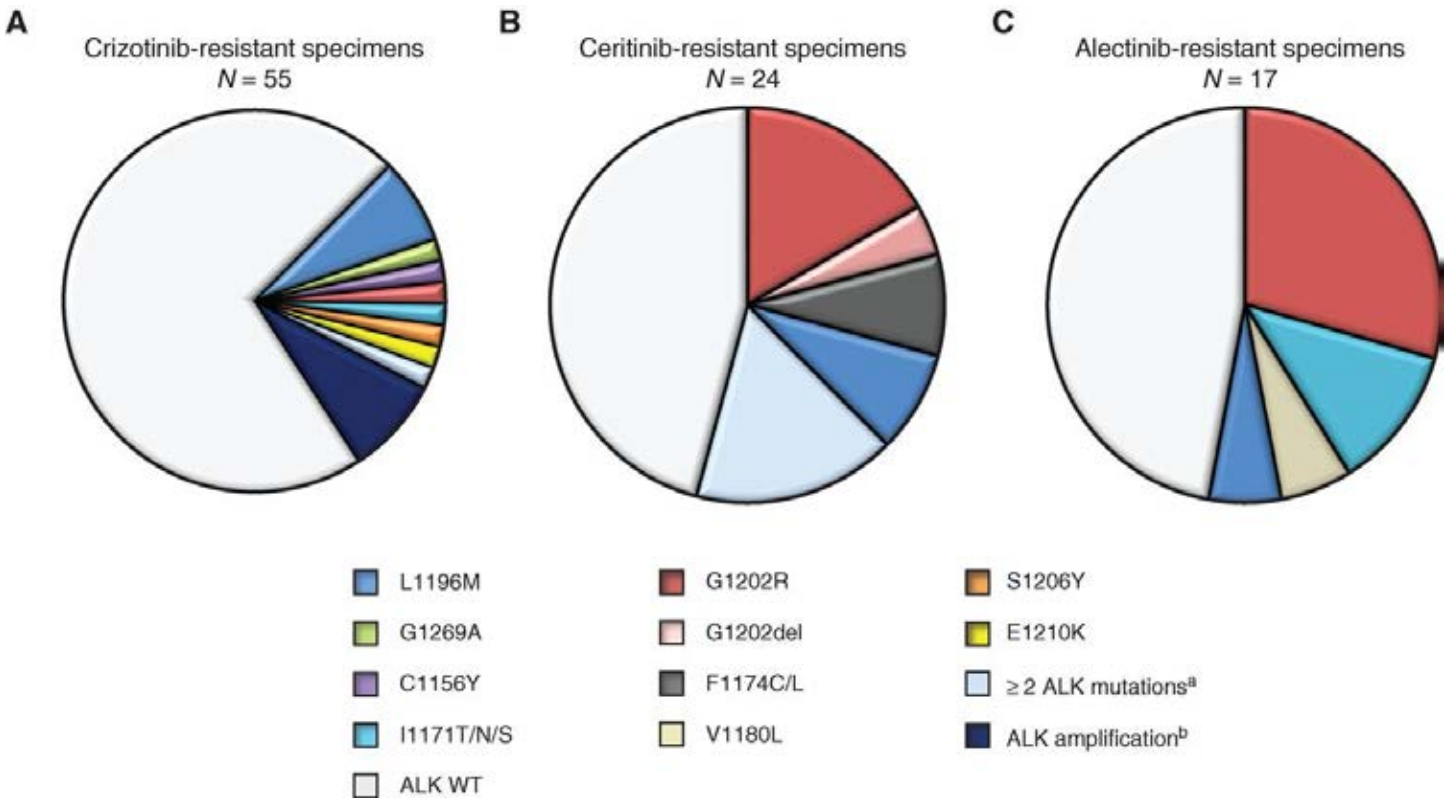
Direct labeling

Modified from Prinsen CF, Appl Immunohistochem Mol Morphol. 2003;11:168-73.

ALK IHC adequate for screening of ALK IHC pos lung cancer

Overview of on-target mechanisms of resistance among ALK-positive specimens obtained from patients progressing

‘ First line’ A crizotinib ‘ Second line’ B cerotinib C alectinib



Gainor et al. Cancer Discov 2016;6:1118-1133

WHO classification of lung cancer

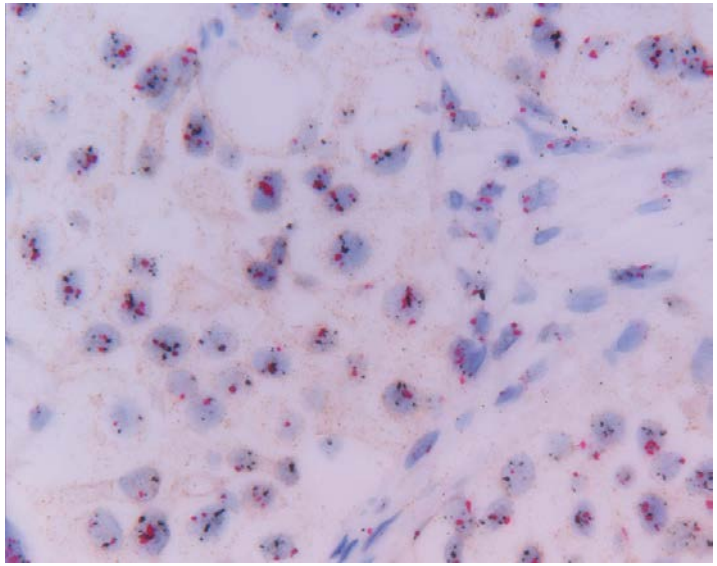
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis

- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management

MET Amplification



MET SISH
- Red Chr 7, black MET

Ratio
- Normal 1.8 – 2.2
- Low 2.2 – <5
- High >5

Amplification ~ 10%

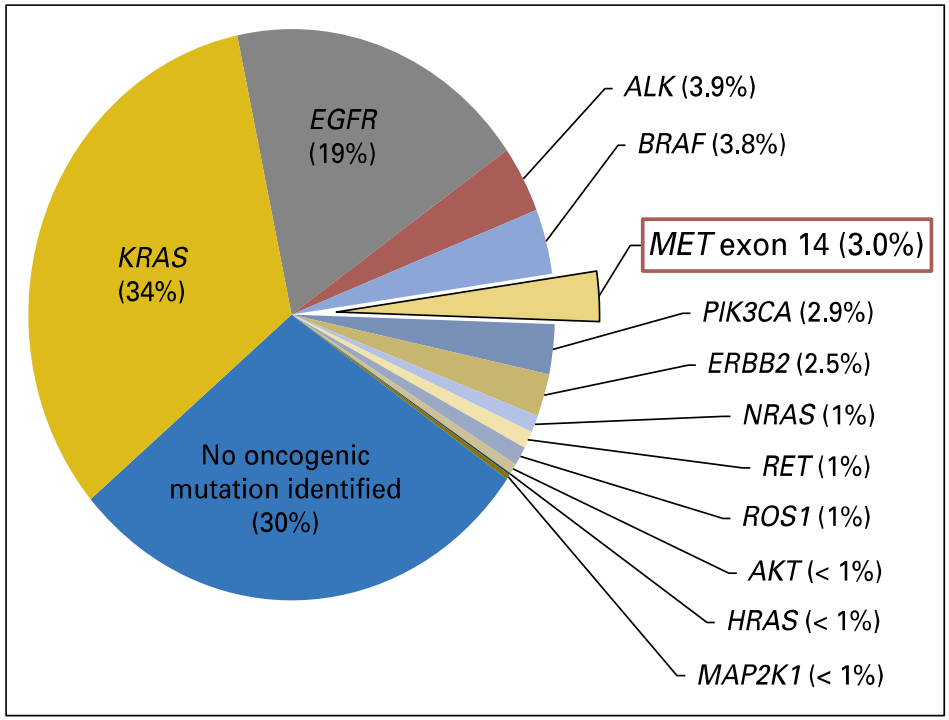
Camidge, ASCO 2014 abs 8001

Prevalence 3% high level gains in NSCLC
Equally distributed between squamous cell and adenocarcomas

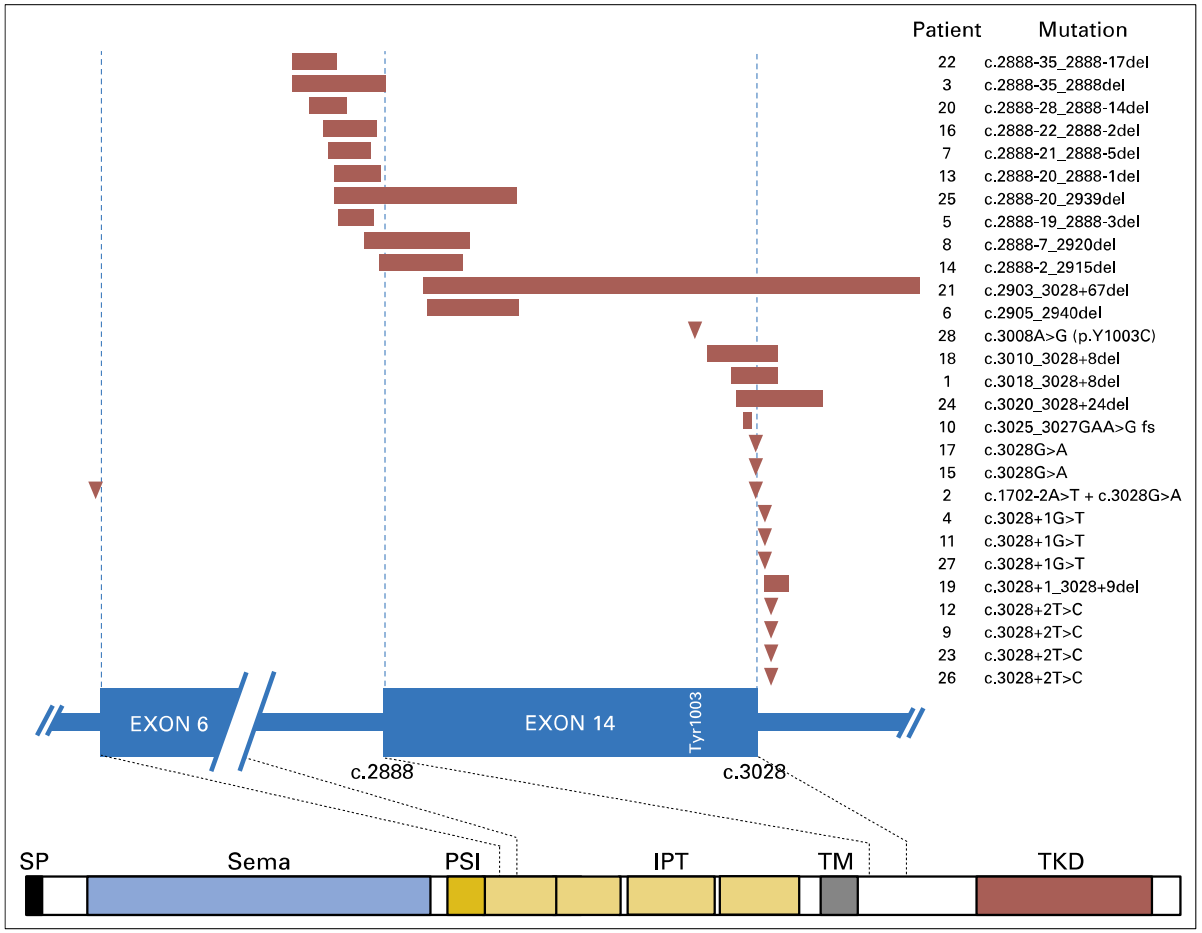
Schildhaus 2015

MET exon 14 mutations

- NGS large panel (282 genes)
- Frequency 1-3% non-squamous NSCLC
- Function exon 14 binding site of CBl E3 ubiquitin ligase
- May be early event in carcinogenesis
- Mutally exclusive with EGFR, ALK, ROS, RET, BRAF, Her2, NRAS
- Slightly older women
- Adenocarcinoma, sarcomatoid carcinoma, SqCC?
- TX with Met inhibitor
- Co-occurence KRAS rapid recurrence
- Resistance 2nd acquired mutation D1228N TKI domain, Y1230C



Paik, Cancer Disc, 2015, 5(842-9)Frampton, cancer Disc 2015, 5 (850-9); Awad, JCO 2016, 34 (721-30); Li, JTO 2016; Heist JTO 2016, 8 (1242-5); OU, JTO 2016; Liu, JTO 2016, 9(1503-10); Liu, JCO 2016, 34(794-802);



Awad, JCO 2016, 34 (721-730)

WHO classification of lung cancer

- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis

- EGFR
- ALK
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- ROS1
- PD-L1
- Resistance biopsy

Tissue management

ROS1 biology

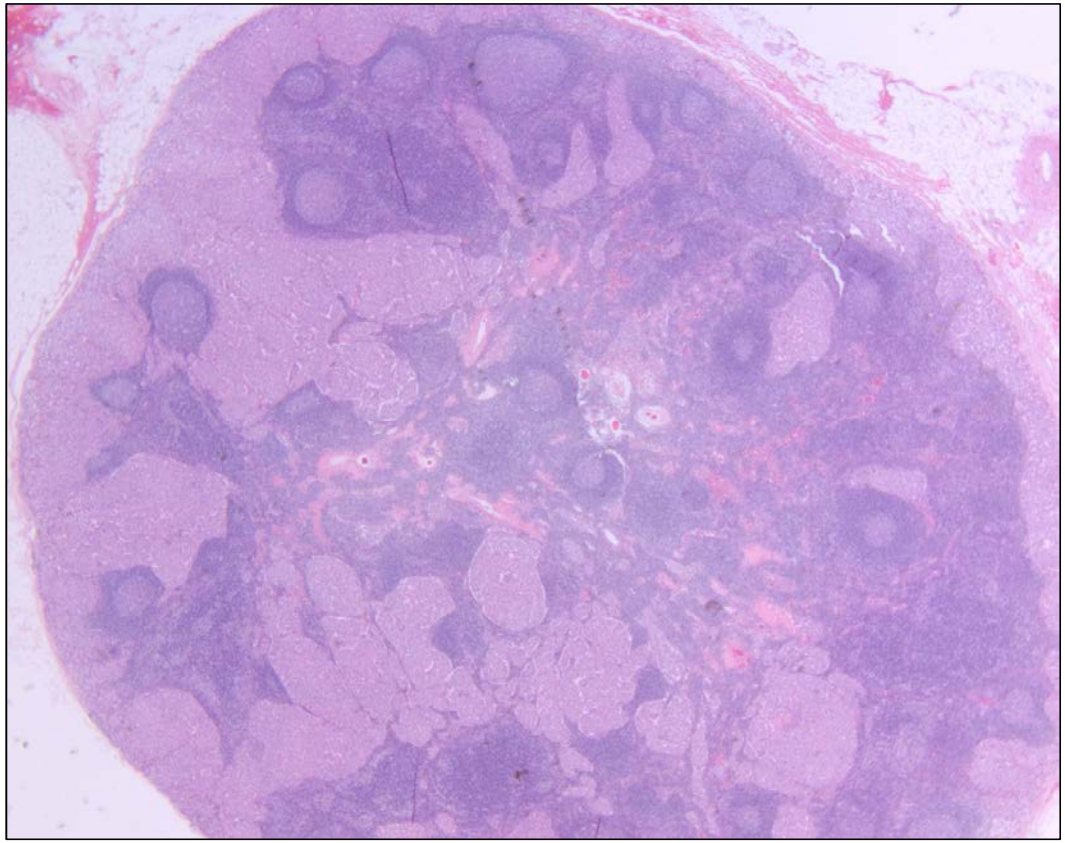
- v-ROS1 = avian sarcoma virus (chicken retrovirus) [1)]
- Receptor tyrosin kinase family
- Human ROS1 chromosome 6 (6q22) [3)]
- Protein: N-terminal domain 1800 amino acids [2)]
- Ligand for ROS1 unknown!

1) Shibuya, J. Virol 1982, 42 (143-52);
2) Acquaviva, Biochem Biophys Acta 2009, 1795 (37-52);
3) Bergethon, JCO 2012, 30 (863-70)

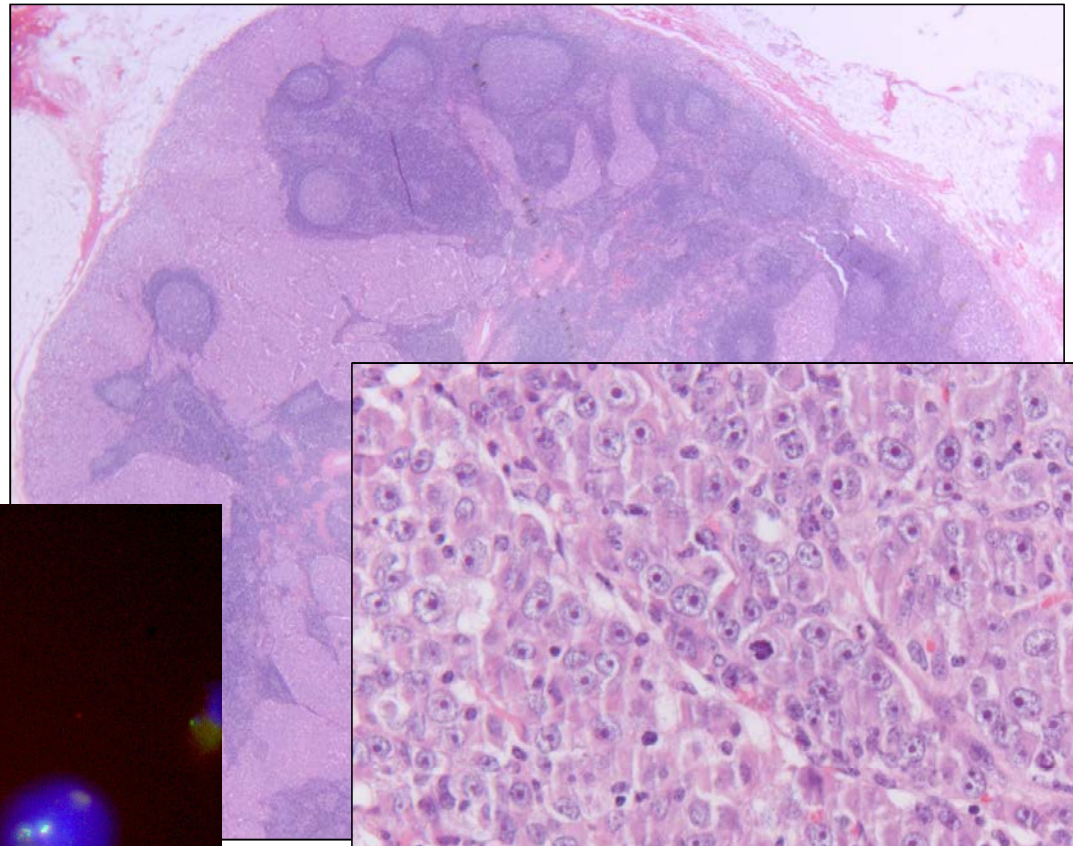
ROS1 fusion partners

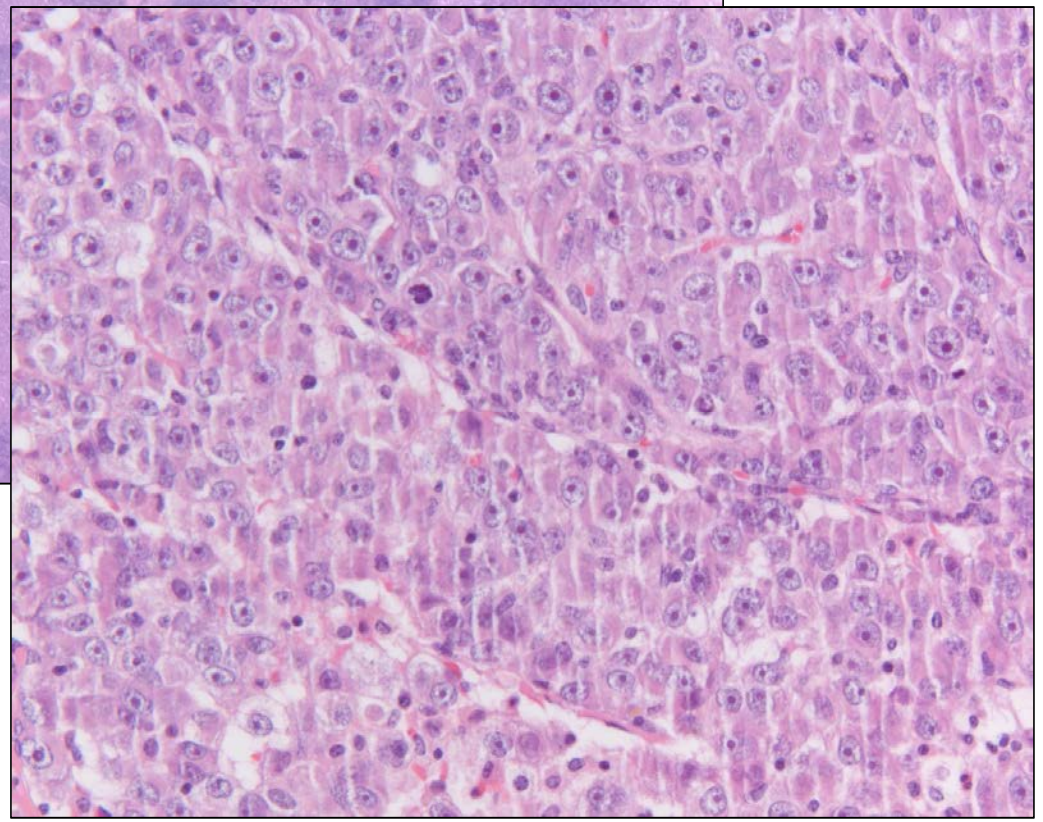
Chin JTO 2012, 7 (1625-30)

HE

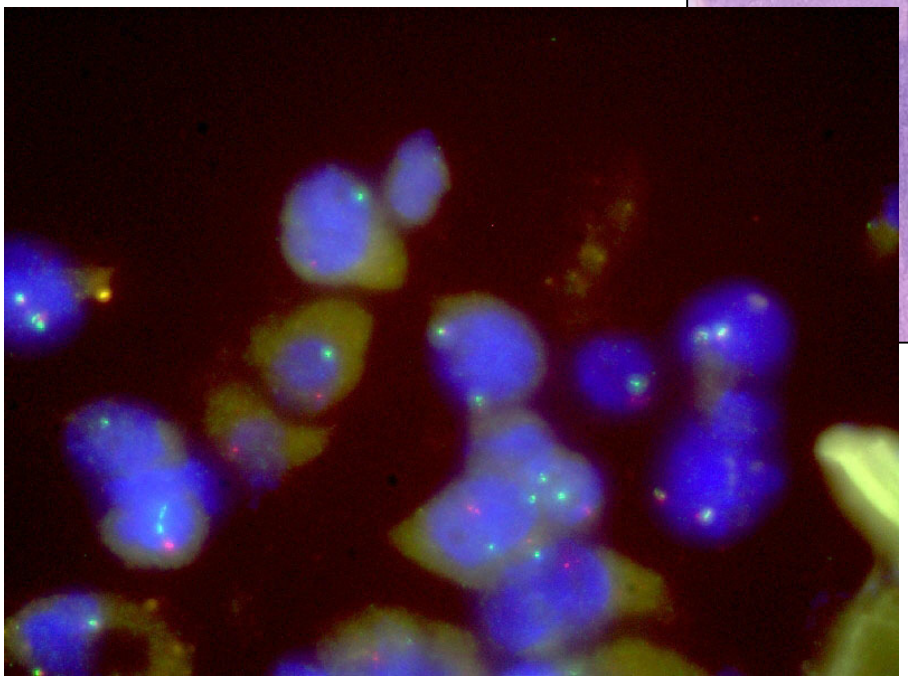


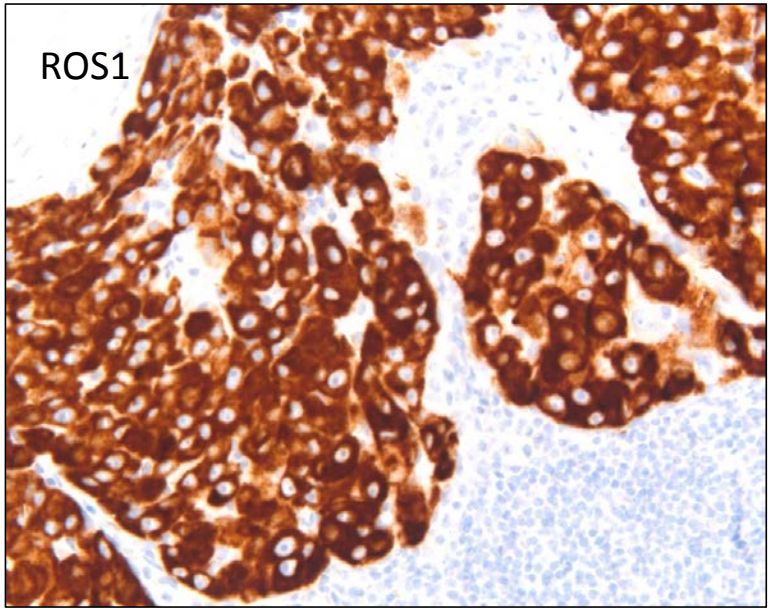
HE

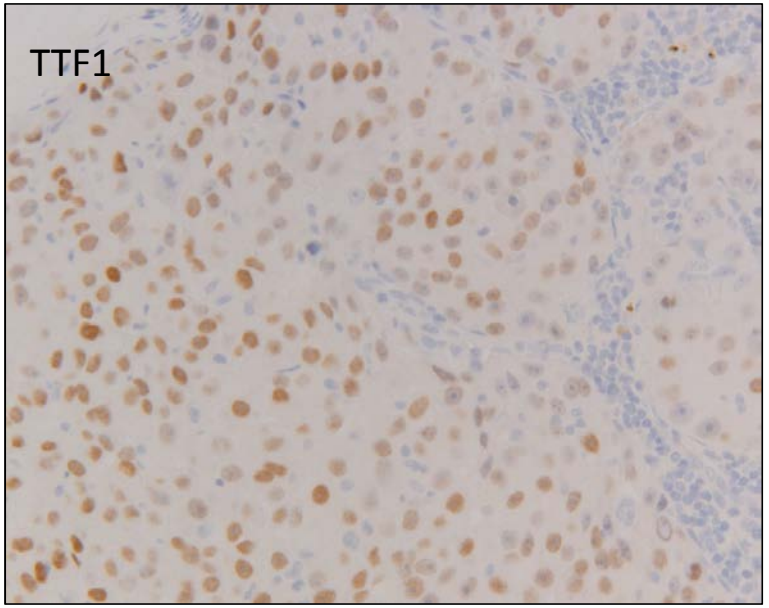


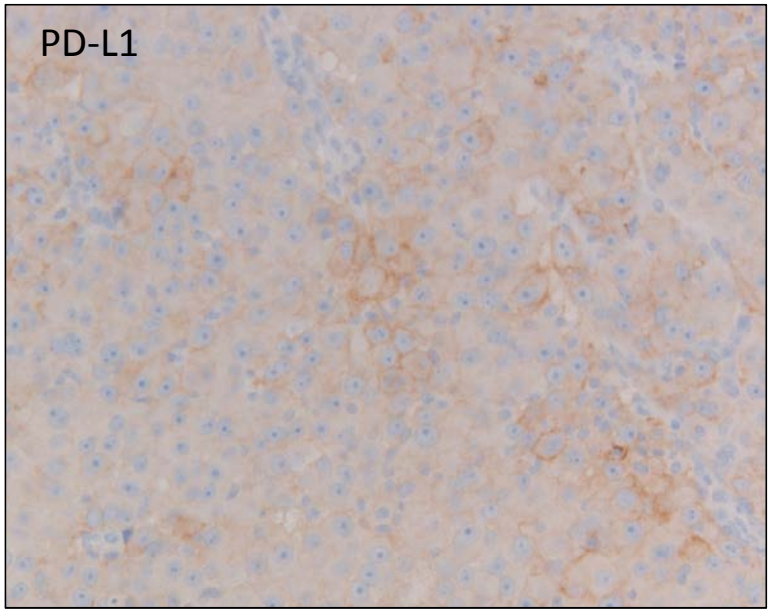


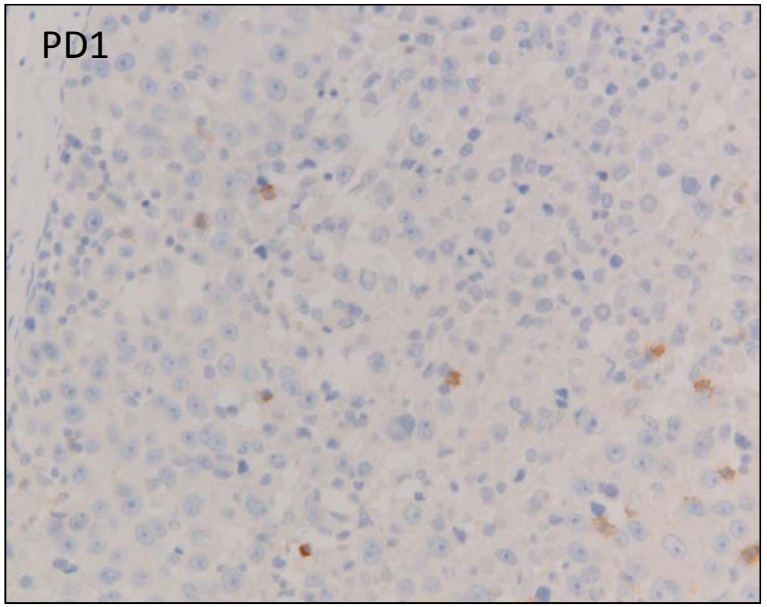
ROS FISH




ROS1


TTF1

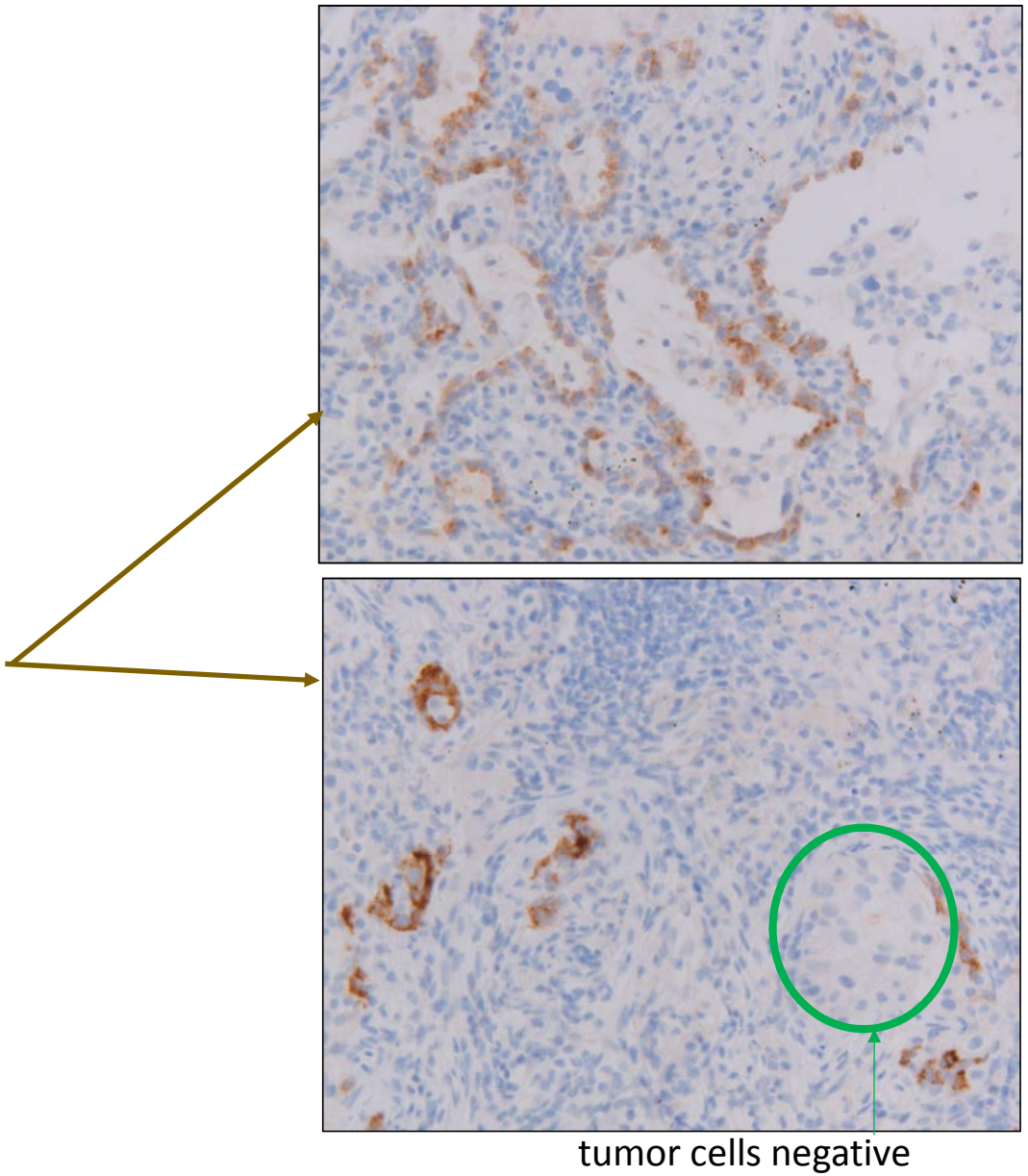

PD-L1


PD1

ROS1 IHC clone D4D6

- In NSCLC
  - fine granular- globular;
  - intensity varies between tumor cells

- Reactive type II pneumocytes
- Alveolar macrophages
- Osteoclast type giant cells
- Mucin = negative



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- ALK
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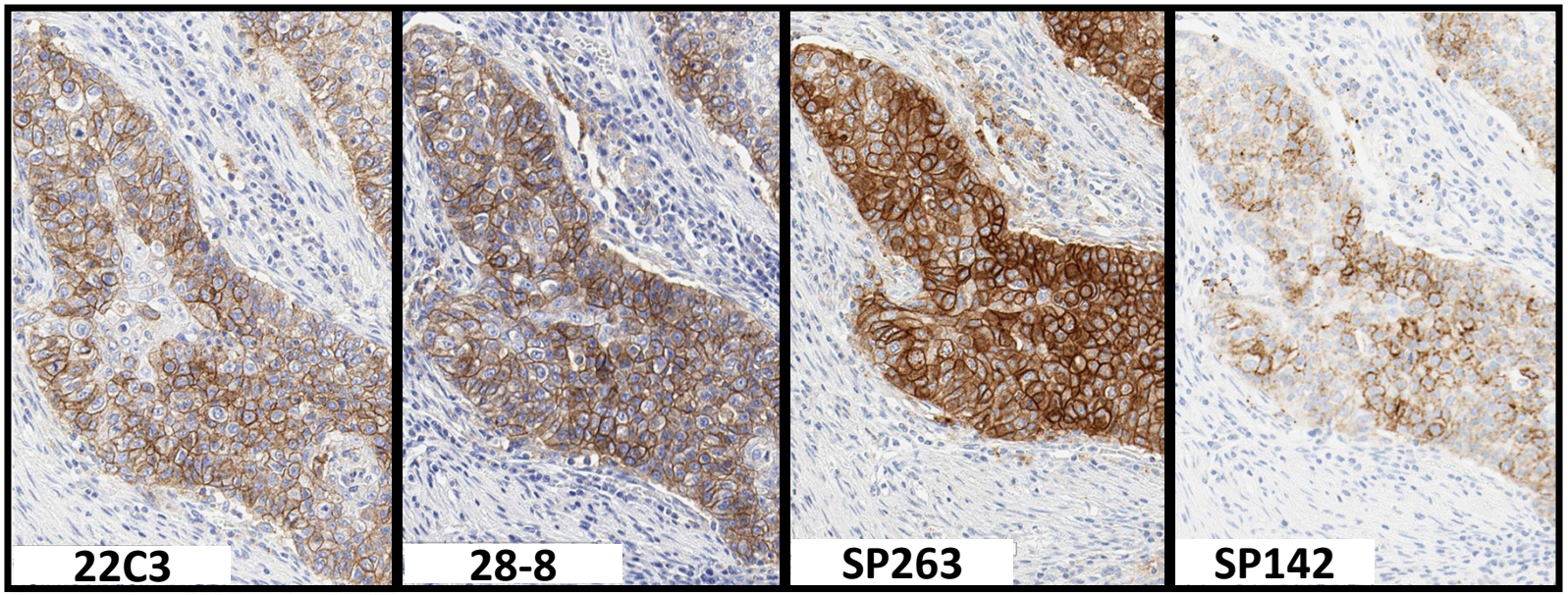
- ROS1
- PD-L1
- Resistance biopsy

Tissue management

Drug	Company	Ab clone	PD-L1 Binding domain	Platform	2nd line Criteria for PDL1 positive
Nivolumab	Bristol-Myers Squibb	28-8 (Dako)	Extra-cellular	Link 48 Autostainer	≥ 1% tumor cells
Pembrolizumab	Merck	22C3 (Dako)	Extra-cellular	Link 48 Autostainer	≥ 50% tumor cells
Atezolizumab	Genentech/Roche	SP142 (Ventana)	Cytoplasmic	Ventana	Tumor cells / tumor infiltrating immune cells
Durvalumab	Astra-Zeneca/ MedImmune	SP263 (Ventana)	Extra-cellular [1)]	Ventana	≥ 25% tumor cells
Avelumab	Pfizer/ Merck Serono	73-10 (Dako)			≥ 1% tumor cells

Example of PD-L1 Tumor Expression



Not only technical validation, also clinical validation required
Not all animals are created equal

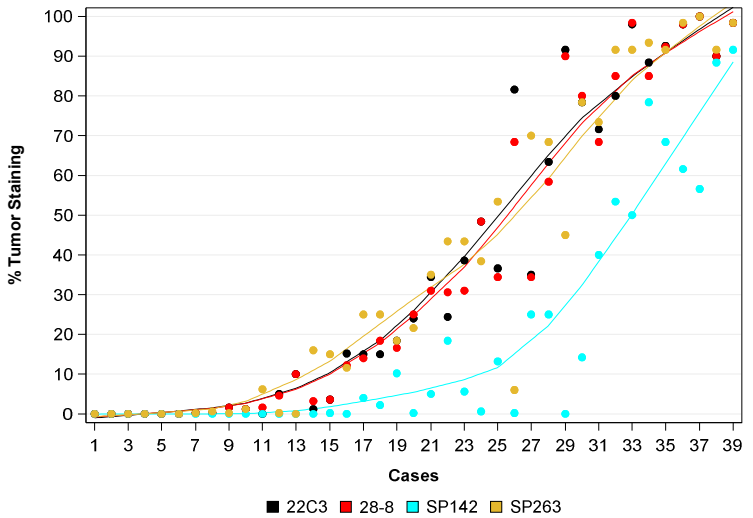
IHC	Technical validation	Clinical validation
Pre-analytical steps within limits	YES	YES
Analytical steps robust	YES	YES
Minimal number of positive cases for validation [1)]	10	10
Minimal number of negative cases for validation [1)]	10	10
Minimal number covering linear dynamic range [2)]	-	10
Requires treatment outcome in study group for a certain drug	NO	YES
Test needs to be constant in time to maintain predictive value	NO	YES

1) According to CAP guidelines [Fitzzgibbon, 2014, 24646069]

2) For clinical validation the samples covering the linear dynamic range (see figure1) are the most relevant, as the threshold for positivity is within this range.

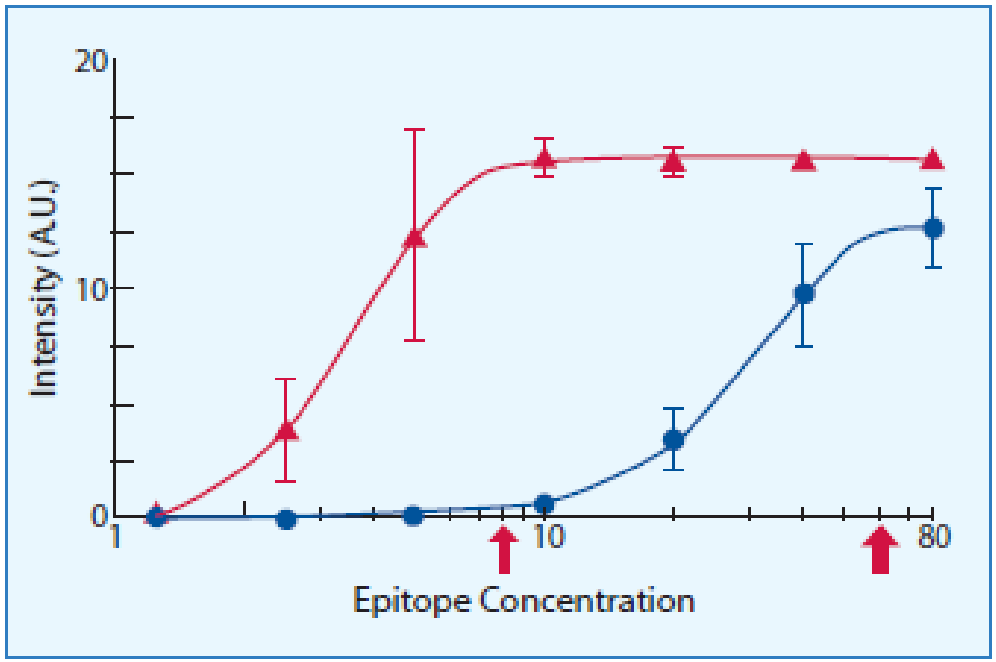
Analytical Evaluation Results: Mean Tumor Proportion Score (TPS) per case based on three readers

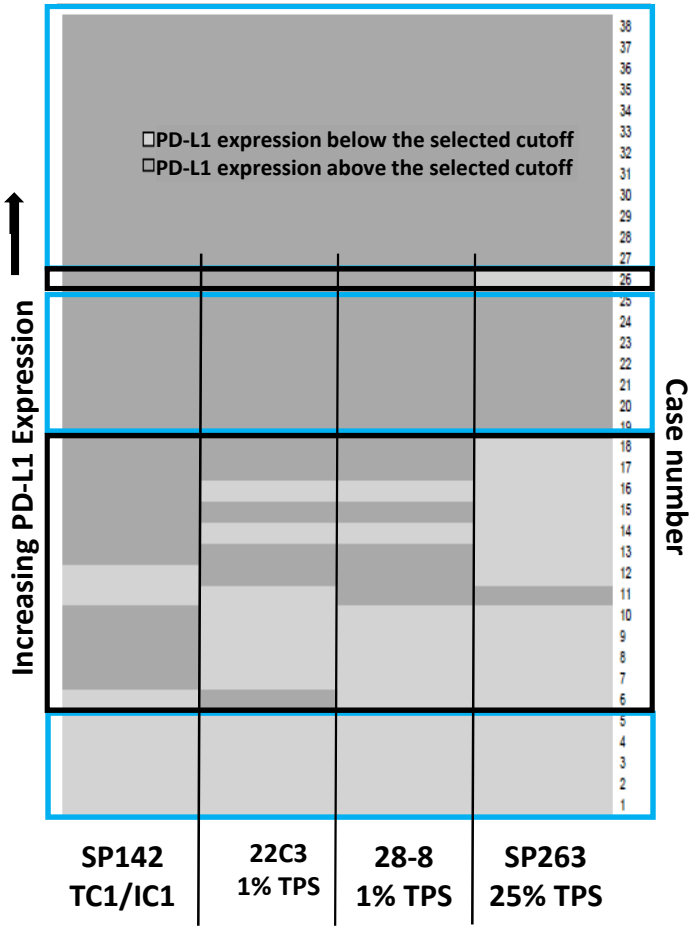
- Analytical comparison of % tumor cell staining (Tumor Proportion Score), by case, for each assay
- Data points represent the mean score from three pathologists for each assay on each case
- Superimposed lines / points indicate identical TPS values
- No clinical diagnostic cut-off applied
- **Conclusion:** 3 of 4 assays are analytically similar for tumor cell staining.

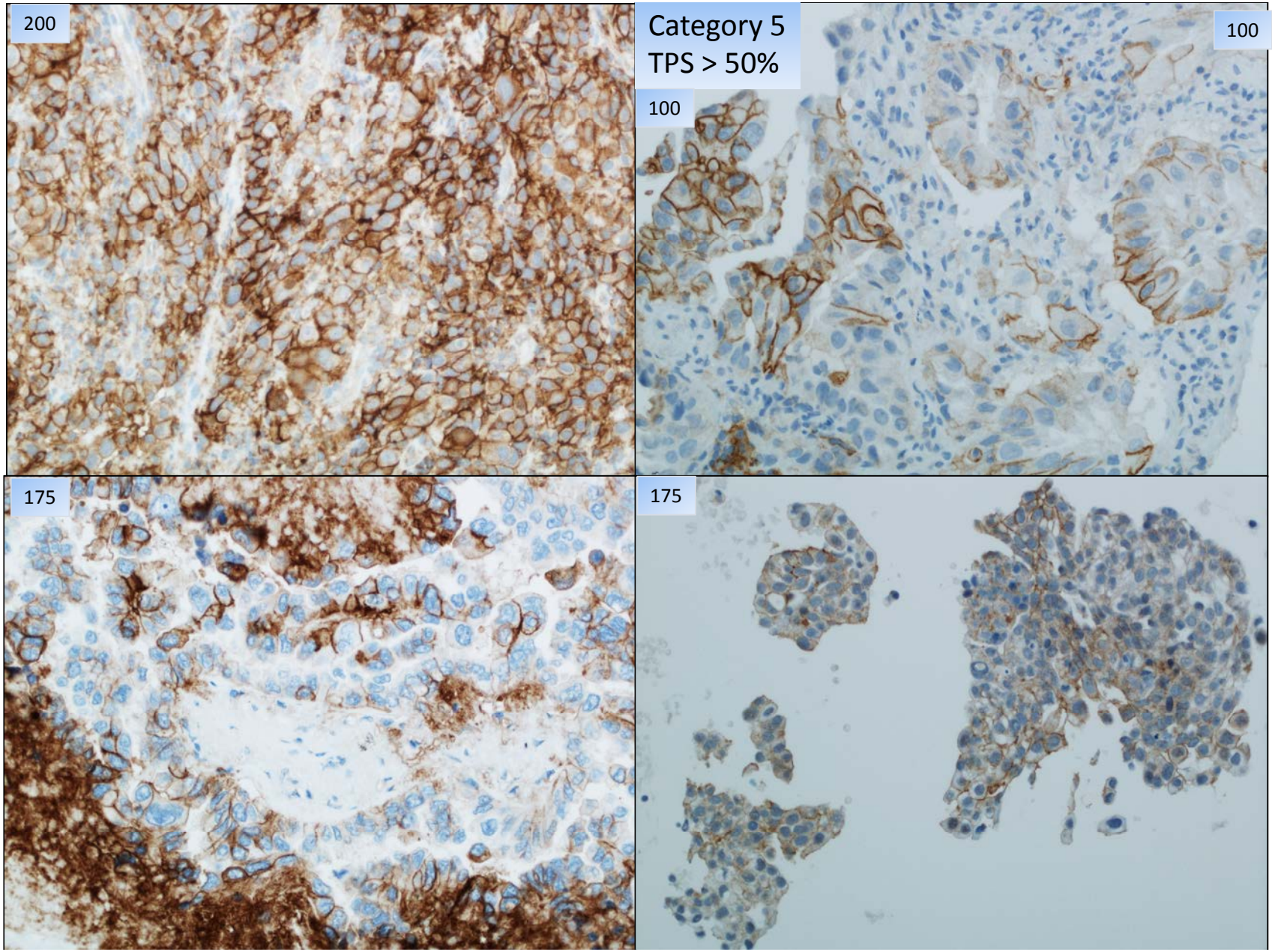


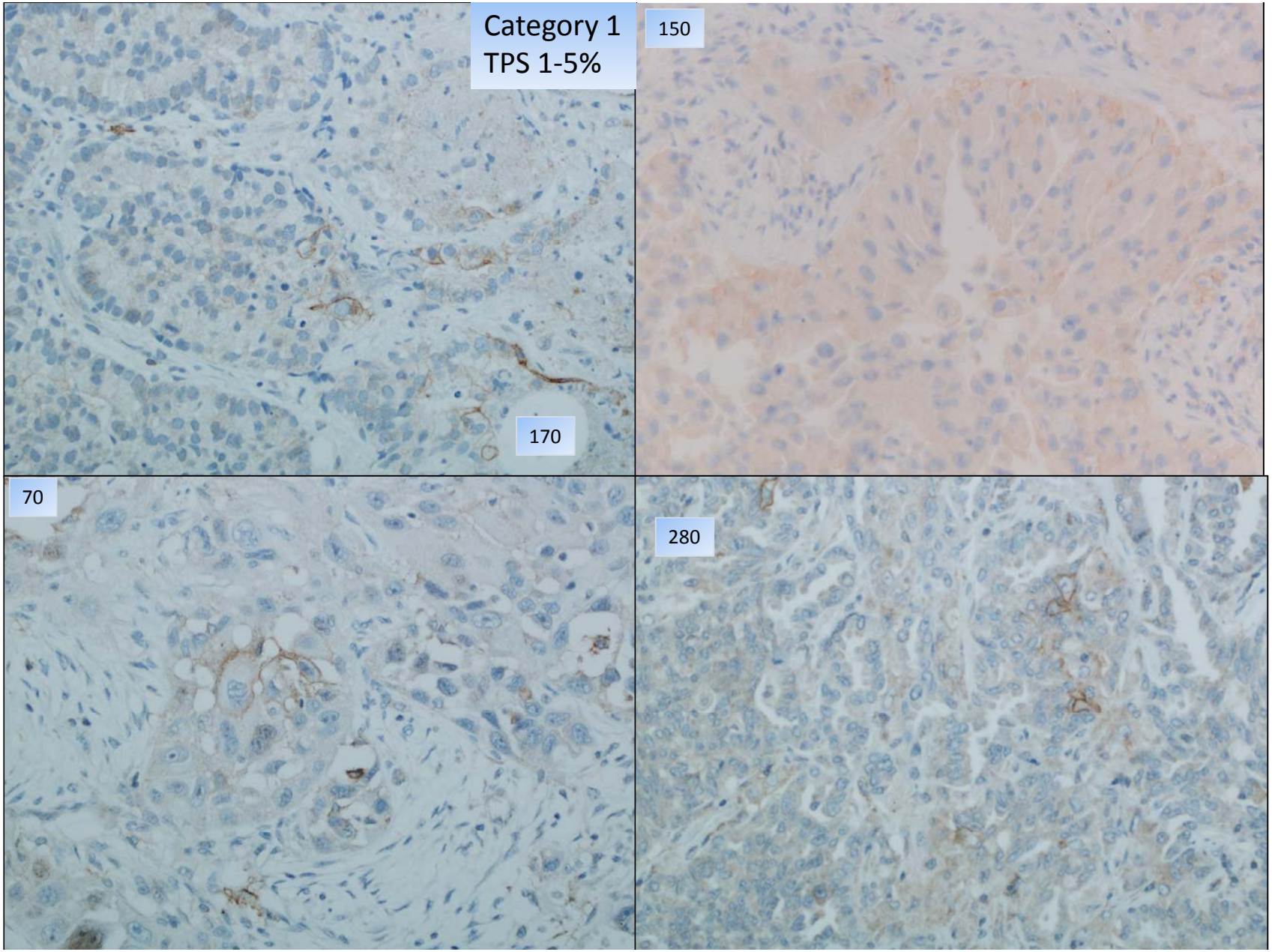
Concordance based on PD-L1 expression using matched assay / algorithm (N=38 cases)

- Cases are rank ordered from lowest to highest PD-L1 expression (above and below respective cut-offs)

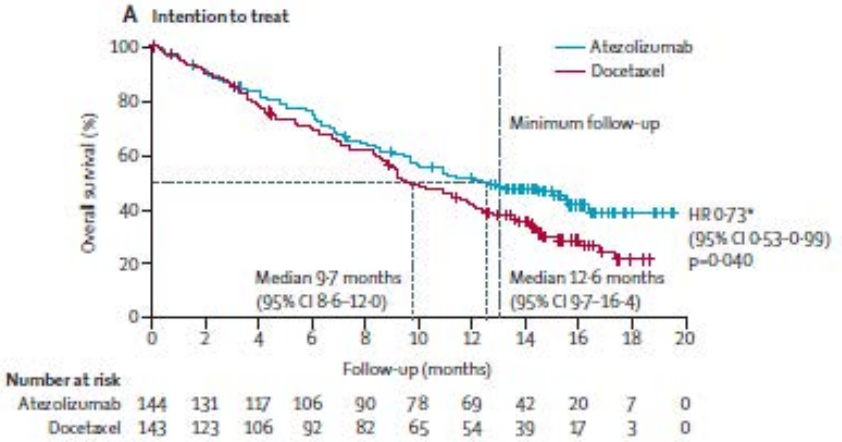





200
Category 5
TPS > 50%
100
100
175
175

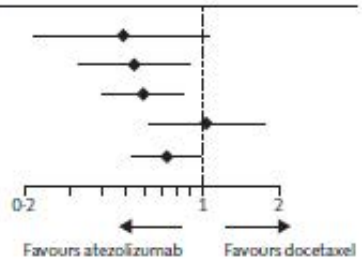

Category 1
TPS 1-5%
170
150
70
280

POPLAR Phase 2 RCT Atezolizumab vs Docetaxel



B

	n (%)	HR*	95% CI	p value	Median overall survival (months [95% CI])	
					Atezolizumab (n=144)	Docetaxel (n=143)
TC3 or IC3	47 (16%)	0·49	0·22–1·07	0·068	15·5 (9·8–NE)	11·1 (6·7–14·4)
TC2/3 or IC2/3	105 (37%)	0·54	0·33–0·89	0·014	15·1 (8·4–NE)	7·4 (6·0–12·5)
TC1/2/3 or IC1/2/3	195 (68%)	0·59	0·40–0·85	0·005	15·5 (11·0–NE)	9·2 (7·3–12·8)
TC0 and IC0	92 (32%)	1·04	0·62–1·75	0·871	9·7 (6·7–12·0)	9·7 (8·6–12·0)
Intention to treat	287	0·73	0·53–0·99	0·040	12·6 (9·7–16·4)	9·7 (8·6–12·0)

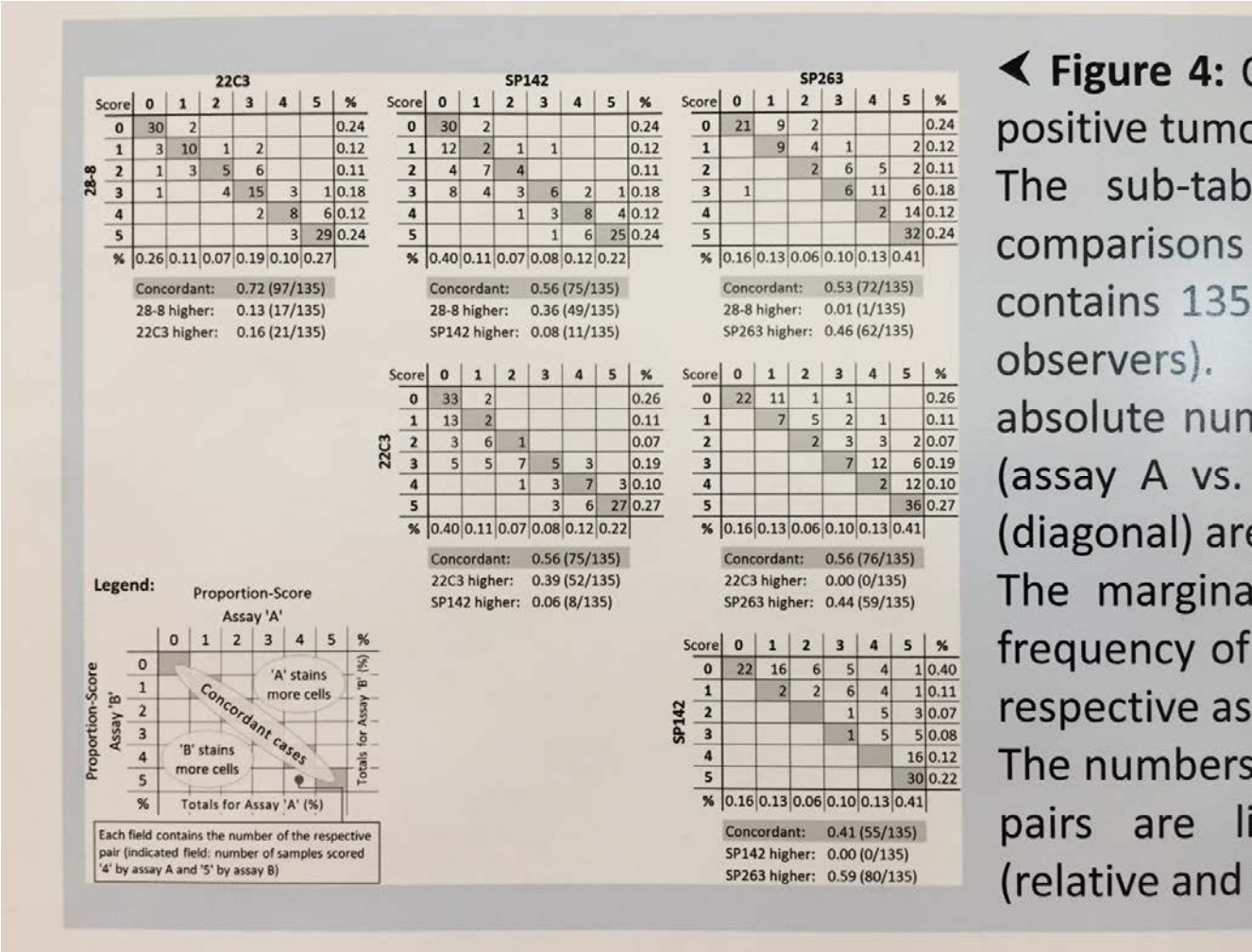


Fehrenbacher, Lancet 2016

PD-L1 scores compared by 2 pathologists/ methods

After calibration

Variation around threshold :
~ 8-9%



22C3 (28-8)

Score	0	1	2	3	4	5	%
0	30	2					0.24
1	3	10	1	2			0.12
2	1	3	5	6			0.11
3	1		4	15	3	1	0.18
4				2	8	6	0.12
5					3	29	0.24
%	0.26	0.11	0.07	0.19	0.10	0.27	

Concordant: 0.72 (97/135)
28-8 higher: 0.13 (17/135)
22C3 higher: 0.16 (21/135)

SP142 (28-8)

Score	0	1	2	3	4	5	%
0	30	2					0.24
1	12	2	1	1			0.12
2	4	7	4				0.11
3	8	4	3	6	2	1	0.18
4			1	3	8	4	0.12
5				1	6	25	0.24
%	0.40	0.11	0.07	0.08	0.12	0.22	

Concordant: 0.56 (75/135)
28-8 higher: 0.36 (49/135)
SP142 higher: 0.08 (11/135)

SP263 (28-8)

Score	0	1	2	3	4	5	%
0	21	9	2				0.24
1		9	4	1		2	0.12
2			2	6	5	2	0.11
3	1			6	11	6	0.18
4					2	14	0.12
5						32	0.24
%	0.16	0.13	0.06	0.10	0.13	0.41	

Concordant: 0.53 (72/135)
28-8 higher: 0.01 (1/135)
SP263 higher: 0.46 (62/135)

SP142 (22C3)

Score	0	1	2	3	4	5	%
0	33	2					0.26
1	13	2					0.11
2	3	6	1				0.07
3	5	5	7	5	3		0.19
4			1	3	7	3	0.10
5				3	6	27	0.27
%	0.40	0.11	0.07	0.08	0.12	0.22	

Concordant: 0.56 (75/135)
22C3 higher: 0.39 (52/135)
SP142 higher: 0.06 (8/135)

SP263 (22C3)

Score	0	1	2	3	4	5	%
0	22	11	1	1			0.26
1		7	5	2	1		0.11
2			2	3	3	2	0.07
3				7	12	6	0.19
4					2	12	0.10
5						36	0.27
%	0.16	0.13	0.06	0.10	0.13	0.41	

Concordant: 0.56 (76/135)
22C3 higher: 0.00 (0/135)
SP263 higher: 0.44 (59/135)

SP263 (SP142)

Score	0	1	2	3	4	5	%
0	22	16	6	5	4	1	0.40
1		2	2	6	4	1	0.11
2				1	5	3	0.07
3				1	5	5	0.08
4						16	0.12
5						30	0.22
%	0.16	0.13	0.06	0.10	0.13	0.41	

Concordant: 0.41 (55/135)
SP142 higher: 0.00 (0/135)
SP263 higher: 0.59 (80/135)

Legend:

Each field contains the number of the respective pair (indicated field: number of samples scored '4' by assay A and '5' by assay B)

Figure 4: C
positive tumo
The sub-tab
comparisons
contains 135
observers).
absolute nun
(assay A vs.
(diagonal) are
The margina
frequency of
respective as
The numbers
pairs are li
(relative and

Scheel Modern Pathology 2016

Field in development

- PD-L1 protein heterogeneous
- 8-9% (biopsy) negative react on immunotherapy

- Biomarker PD-L1 IHC never higher “predictive” than 90%.

- On 18 October, 2016, the US Food and Drug Administration (FDA) approved atezolizumab (TECENTRIQ, Genentech Oncology) for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose disease progressed during or following platinum-containing chemotherapy.

- Patients with EGFR or ALK genomic tumour aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving atezolizumab.

- On October 24, 2016, the U.S. Food and Drug Administration (FDA) approved pembrolizumab (Keytruda) for the treatment of patients with metastatic non–small cell lung cancer (NSCLC) whose tumors express programmed cell death ligand 1 (PD-L1) as determined by an FDA-approved test.
- This is the first FDA approval of a checkpoint inhibitor for the first-line treatment of lung cancer. This approval also expands the indication in second-line treatment of lung cancer to include all patients with PD-L1–expressing NSCLC.

1st line

- Patients with metastatic NSCLC whose tumors have high PD-L1 expression (tumor-proportion score ≥ 50%) as determined by an FDA-approved test, with no *EGFR* or *ALK* genomic tumor aberrations and no prior systemic chemotherapy treatment for metastatic NSCLC.

2nd line

- Patients with metastatic NSCLC whose tumors express PD-L1 (tumor-proportion score ≥ 1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with *EGFR* or *ALK* genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab.

WHO classification of lung cancer

- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis

- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

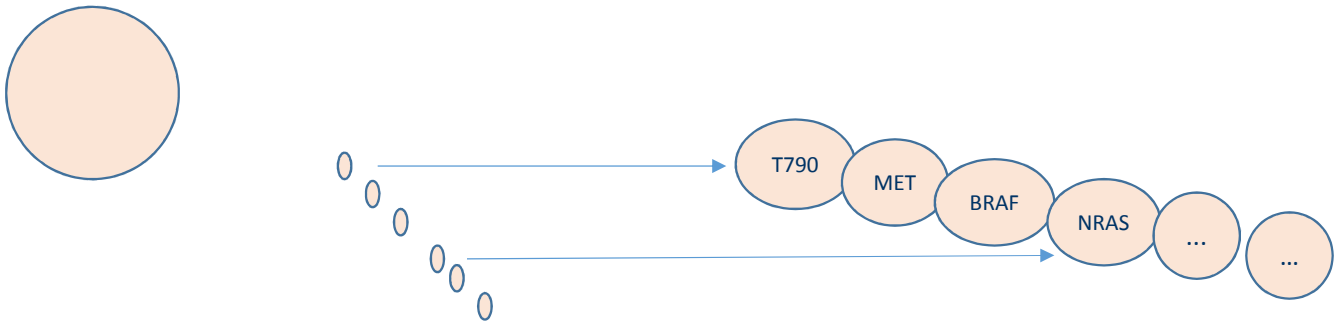
Tissue management

Concepts of resistance mechanisms

- Resistance due to initially present 2nd acquired mutation?
  - Resistance mutation possibly detectable in initial biopsy
- Resistance mechanisme by (induced) stress
  - Resistance mutation not present in initial biopsy

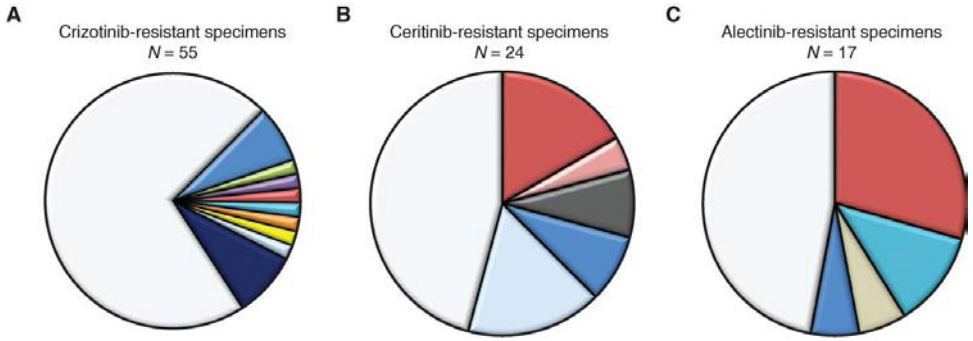
Diverse resistance due to treatment (= stress)

- May derive from a single cell clone (EGFR PC9)
- Crashed with drug (erlotinib)
- Evolving persister cells (0.5%) under same selective pressure
- Diverse drug mechanisms (T790M, MET amplification, MAPK pathway [NRAS mutation], BRAF, …)



Ramirez, Nat Comm. 2016, 10690

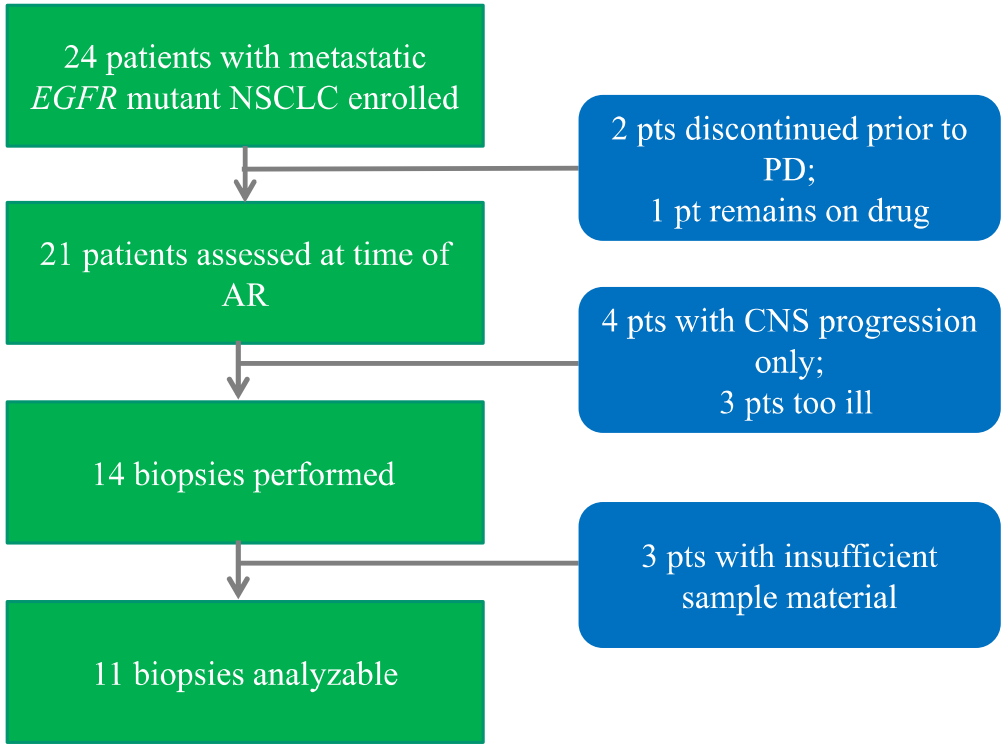
Concept TKI resistance



- The drug related resistance patterns support the stress hypothesis.
- It is not excluded that by the evolved resistance mechanism the tumor cells get more dependent of the escape route.
- In anticipation of (any) treatment: a recurrence is likely to occur, unless a synthetic lethal drug (combination) is given.
- BIOPSIES of recurrent lesion are needed to uncover the resistance mechanism and give the patient a (still small) chance.

CONSORT diagram

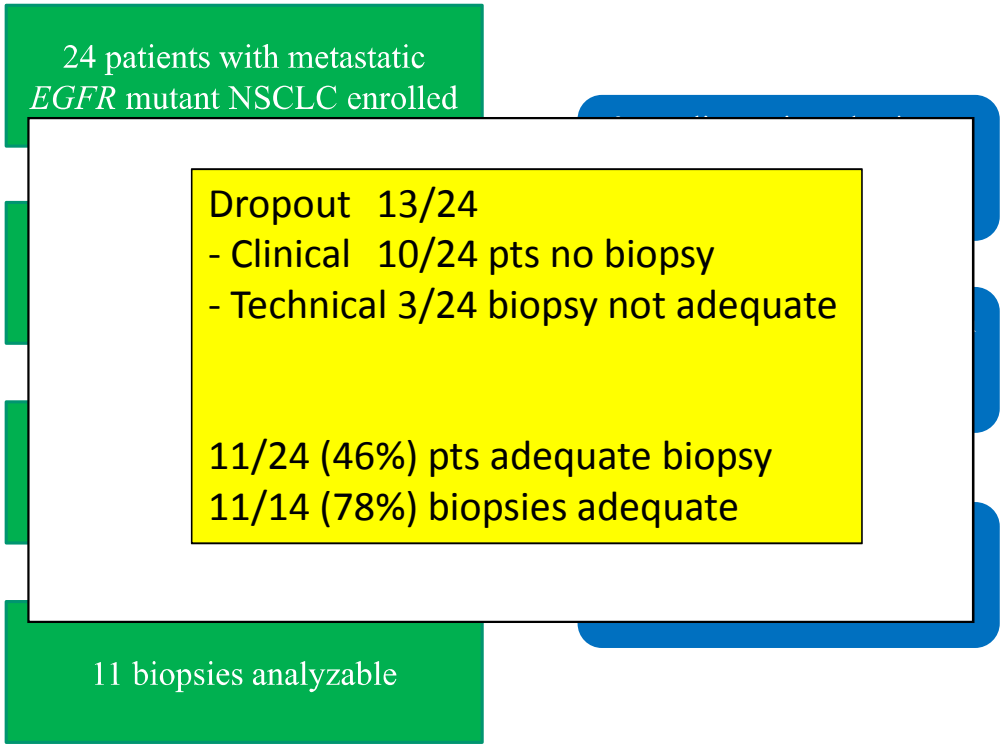
Flow of 24 patients who consented to repeat biopsy.



pts, patients; pt, patient; PD, progressive disease; AR, acquired resistance; CNS, central nervous system.

Campo, JTO, 2016 11(2022-26)

Flow of patients who consented to repeat biopsy.



pts, patients; pt, patient; PD, progressive disease; AR, acquired resistance; CNS, central nervous system.

Campo, JTO, 2016 11(2022-26)

Algorithm for prediction

(In transition, VUmc Amsterdam)

- Prediction step 1
  - IHC ALK, ROS1, (PD-L1 on request)
  - NGS: EGFR, RAS, BRAF, Her2, MET
- Prediction step 2
  - FISH ALK, ROS1, RET
  - SISH MET

WHO classification of lung cancer

- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis

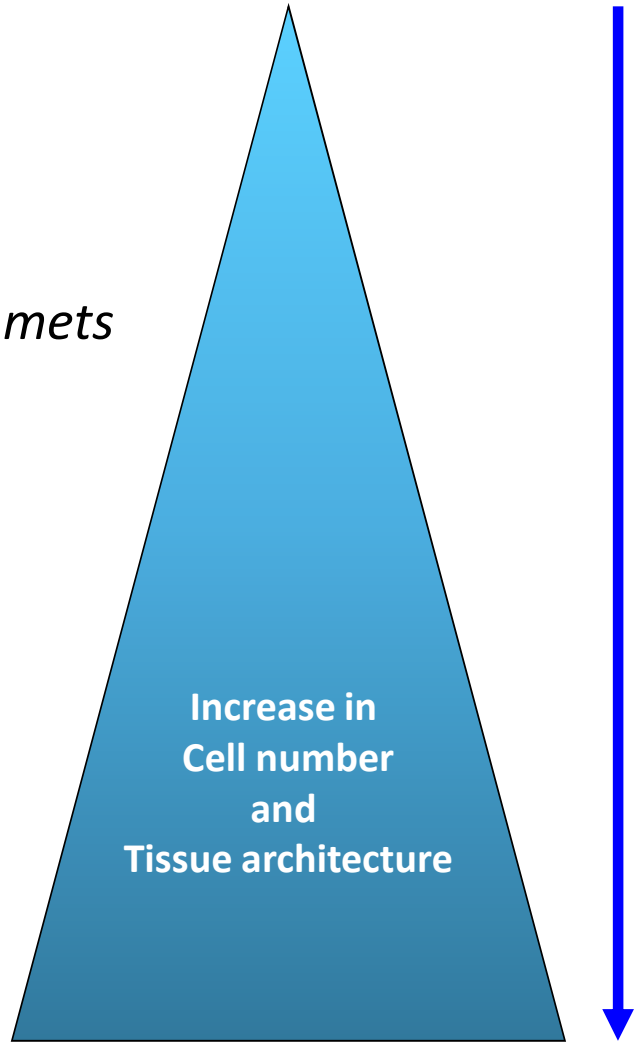
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management

‘Biopsy’ techniques in lung cancer diagnosis

- *Sputum cytology*
- *Bronchial brushings and washings*
- *Fluids*
- *FNA cytology/EBUS-EUS – primary or mets*
- Transbronchial biopsy
- Bronchial biopsy
- Pleural biopsy
- Core biopsy – primary or mets
- **Mediastinoscopy**
- **Lymph node excision**
- **VATS biopsy / resection**
- **Thoracotomy tumour excision**

Courtesy of Keith Kerr



‘Biopsy’ techniques in lung cancer diagnosis

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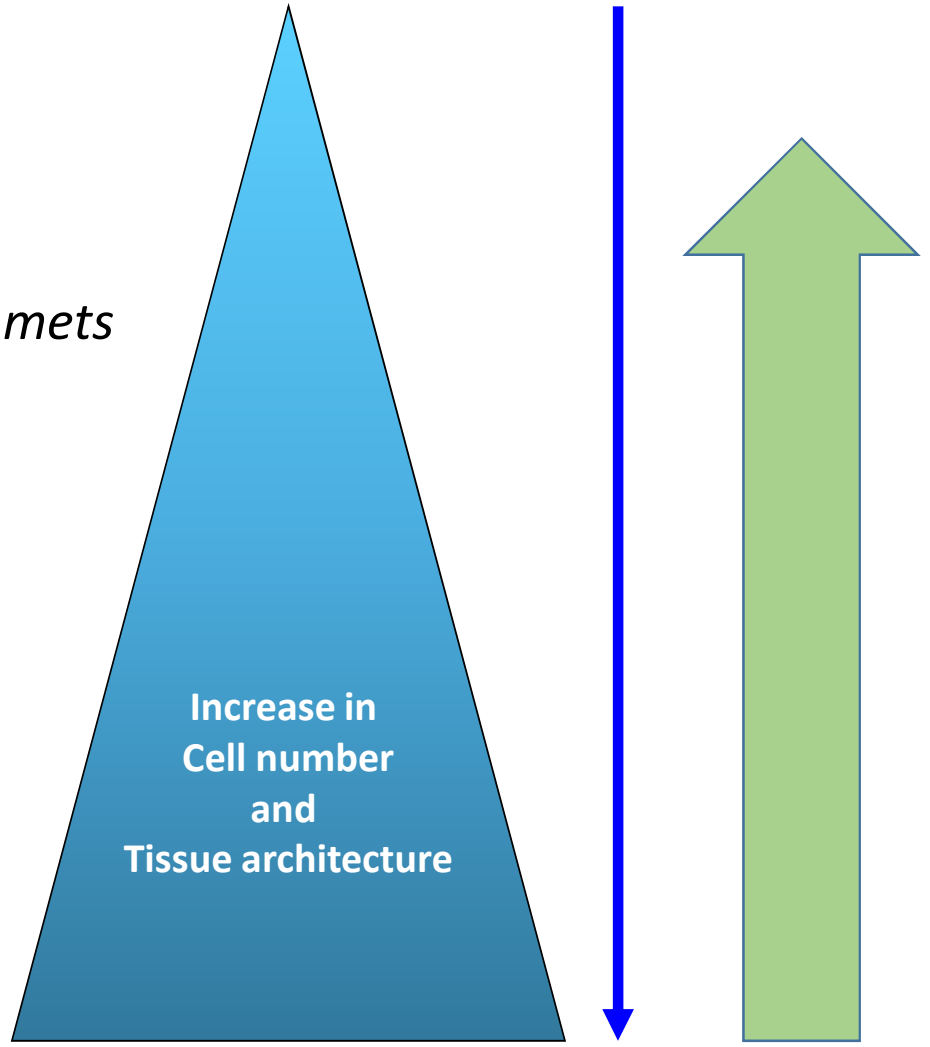


Advances in Imaging, Endoscopy, & other Interventional techniques

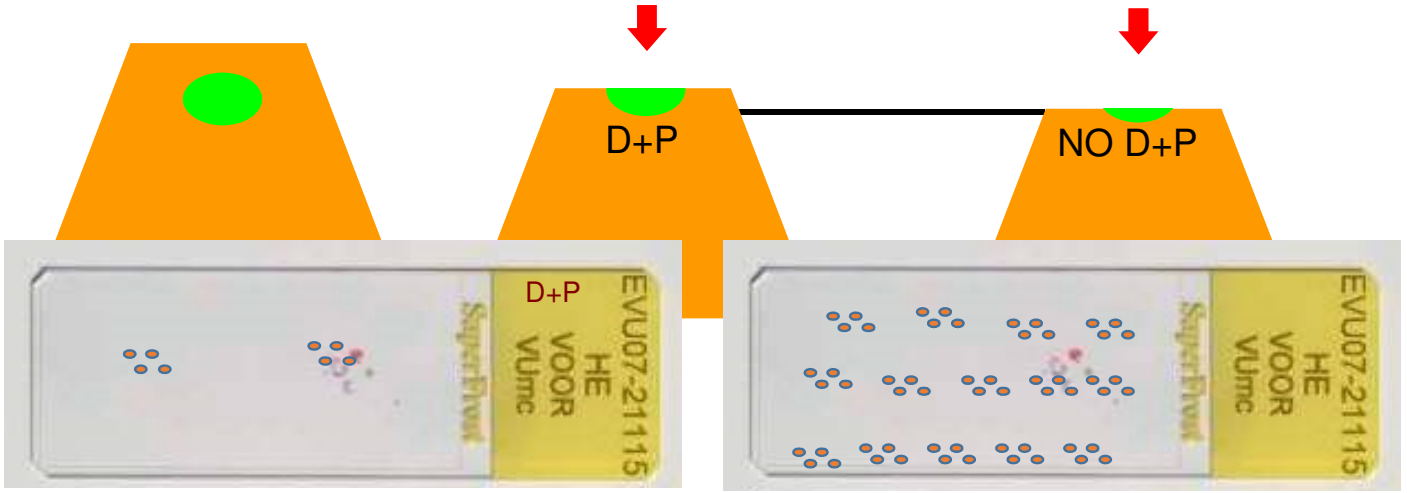
General Tendency Towards less Invasive Techniques To secure A diagnosis

Samples getting smaller……

Tissue management

Pulmonologist: **Clinical information**
Questions: diagnosis, prediction "D+P", if malignant

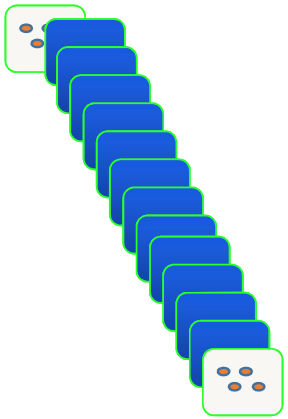
Pathology **"D+P" code: specific handling in contrast to regular**



NOT performing these additional cuts saves material...

Adequate clinical information is essential : D+P

More analysis from one biopsy?



HE first
Diagnostic stain TTF1
Diagnostic stain mucin
Diagnostic stain P63/p40
Predictive stain ALK IHC
Predictive stain ALK FISH
DNA isolation EGFR
DNA isolation
DNA isolation

HE last

NGS

- miseq 40-250 ng
- Ion torrent 10 ng

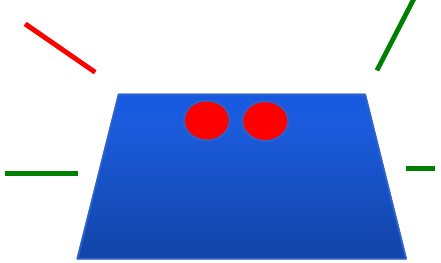
Tissue management = interaction between pulmonologist/oncologist/surgeon/radiologist and pathologist

Sample collector:

Sampling more (≥4) biopsies/ tumor tissue

Clinical request for diagnosis + prediction?

Clinical suspicion of metastases y/n?



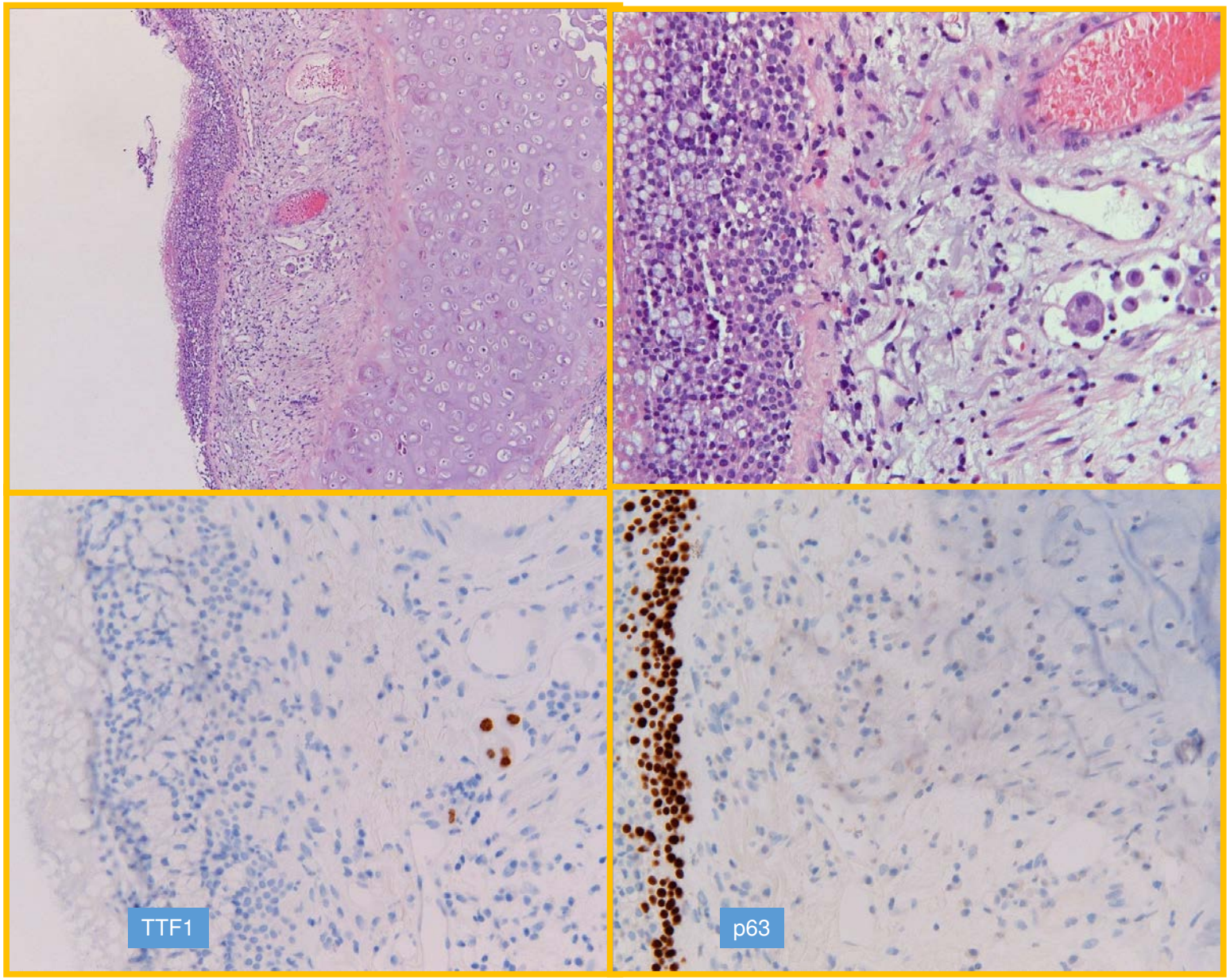
Pathology :

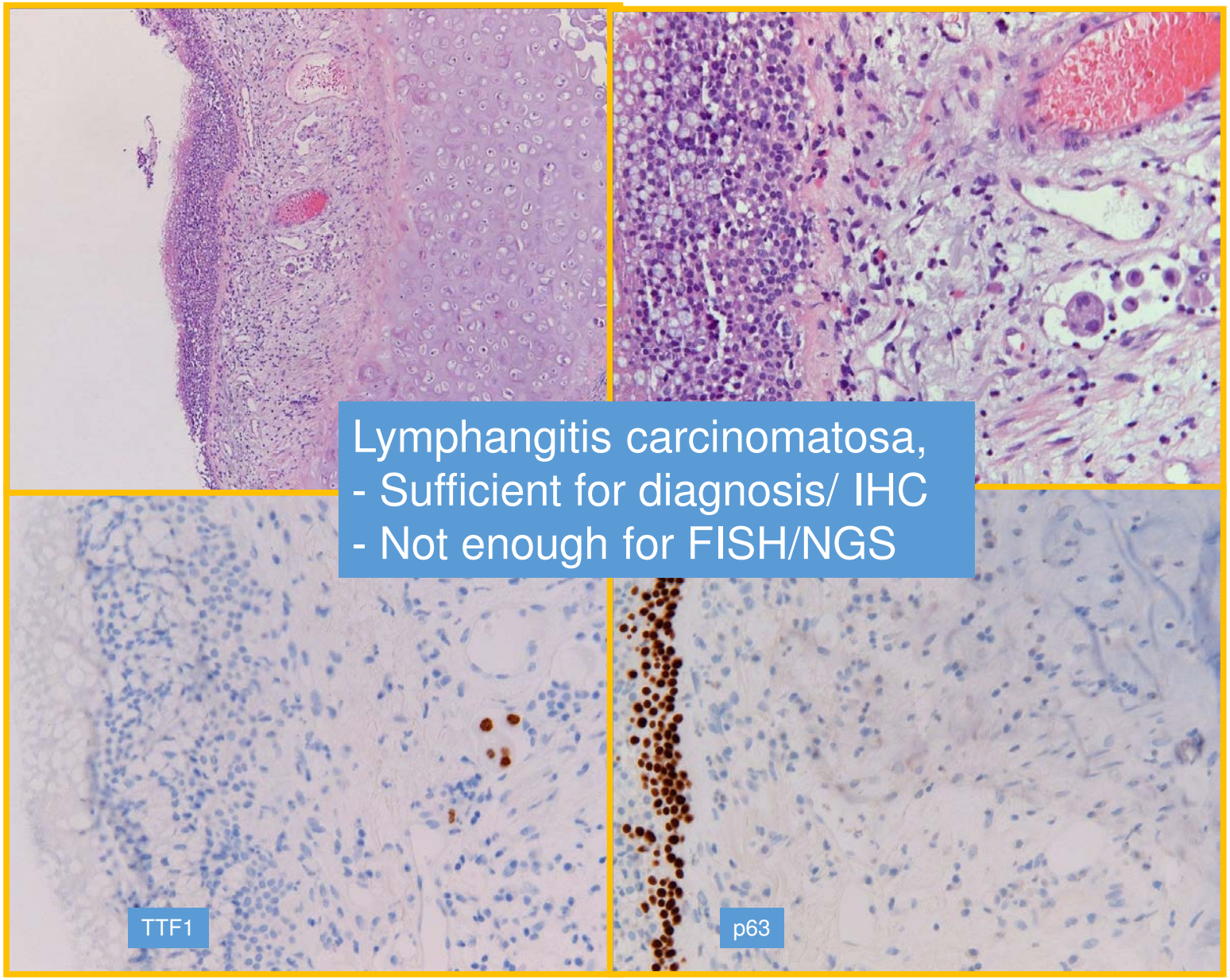
Distribute samples over >1 block

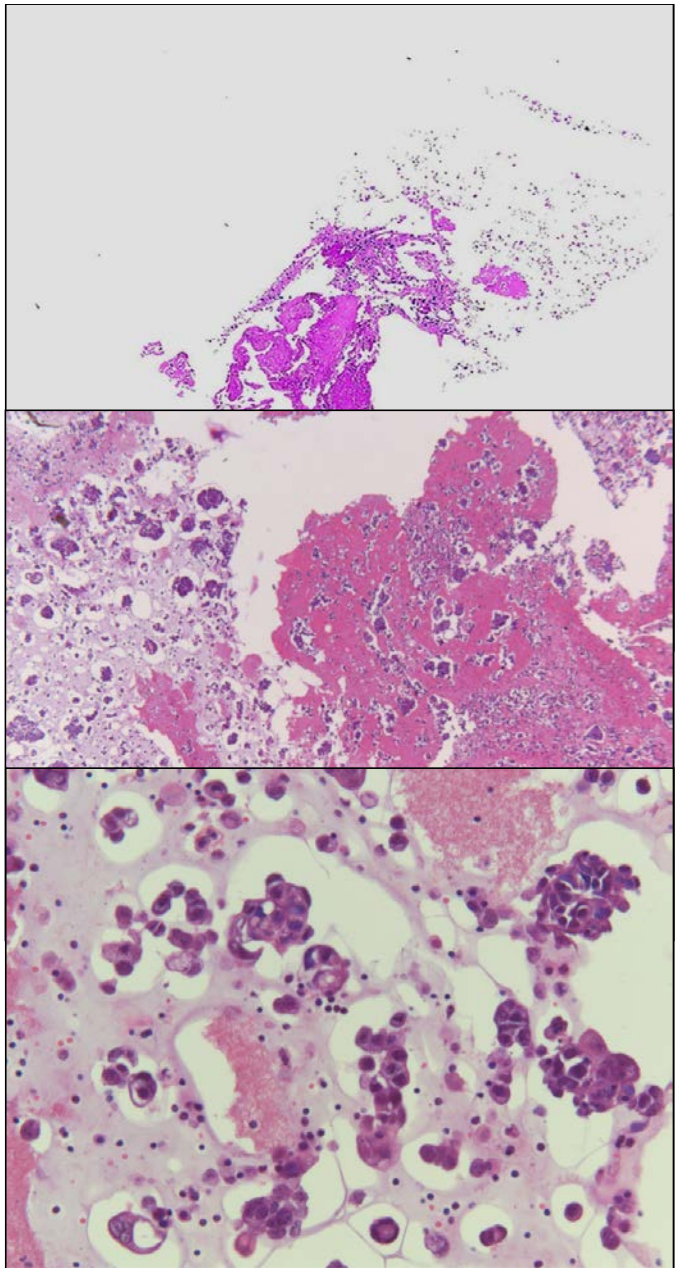
Careful initial cut

Spare section for reflex analysis

Thunnissen, Virch. Arch 2014,464(347-58)


TTF1
p63


Lymphangitis carcinomatosa,
- Sufficient for diagnosis/ IHC
- Not enough for FISH/NGS
TTF1
p63

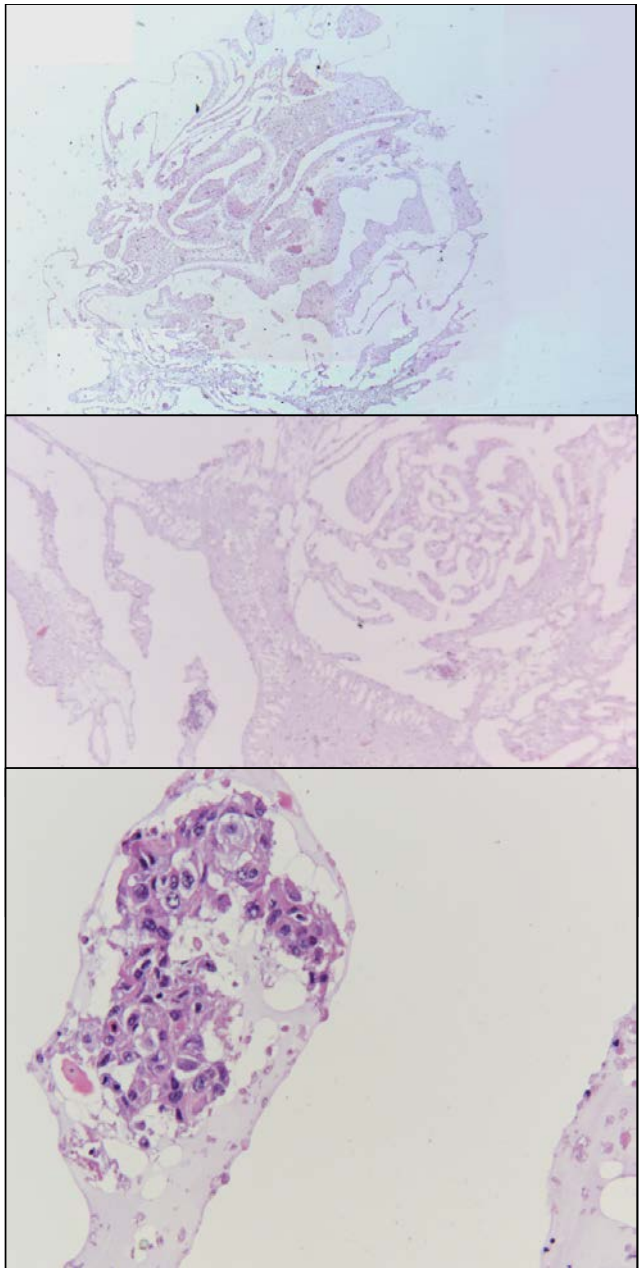


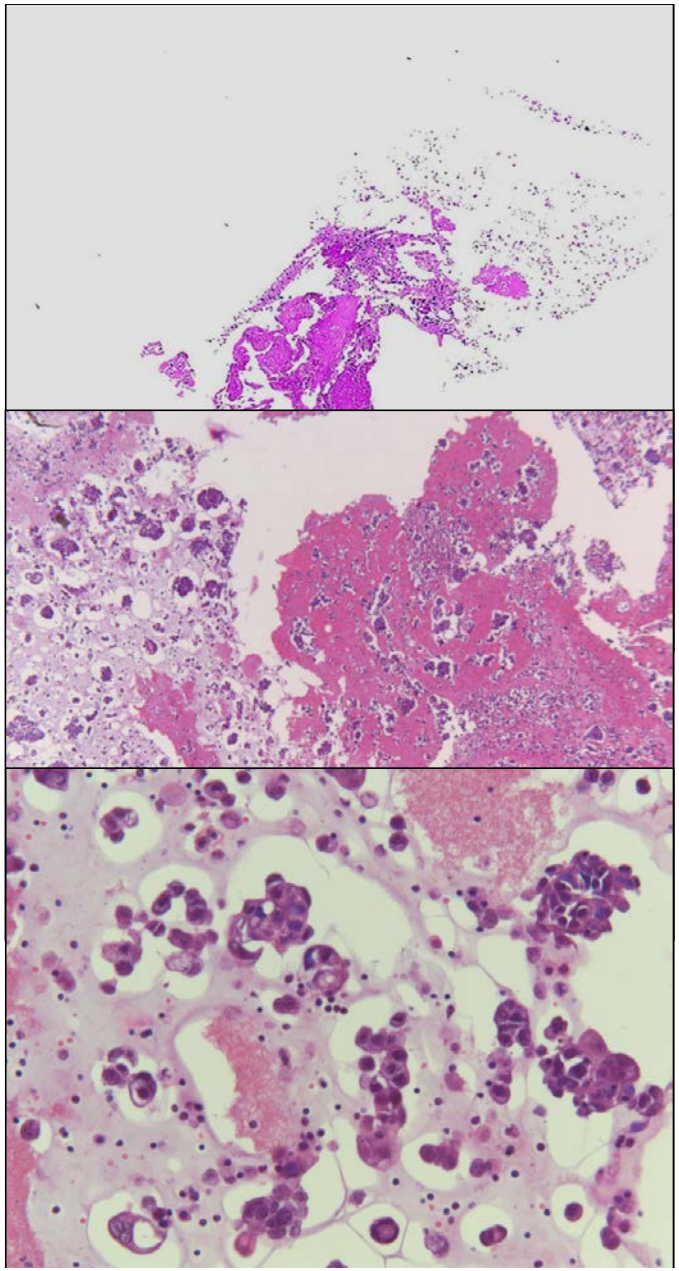
Cytology Cell Block Tumor cellularity

2.5x obj

10x obj

20x obj





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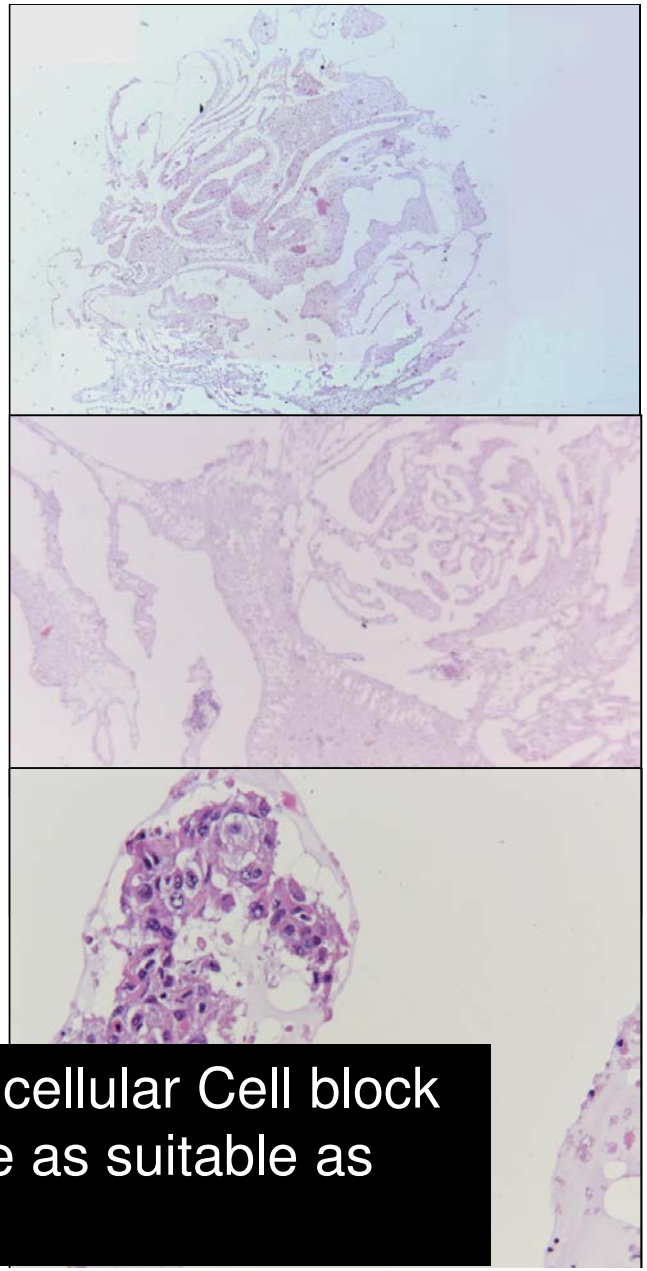
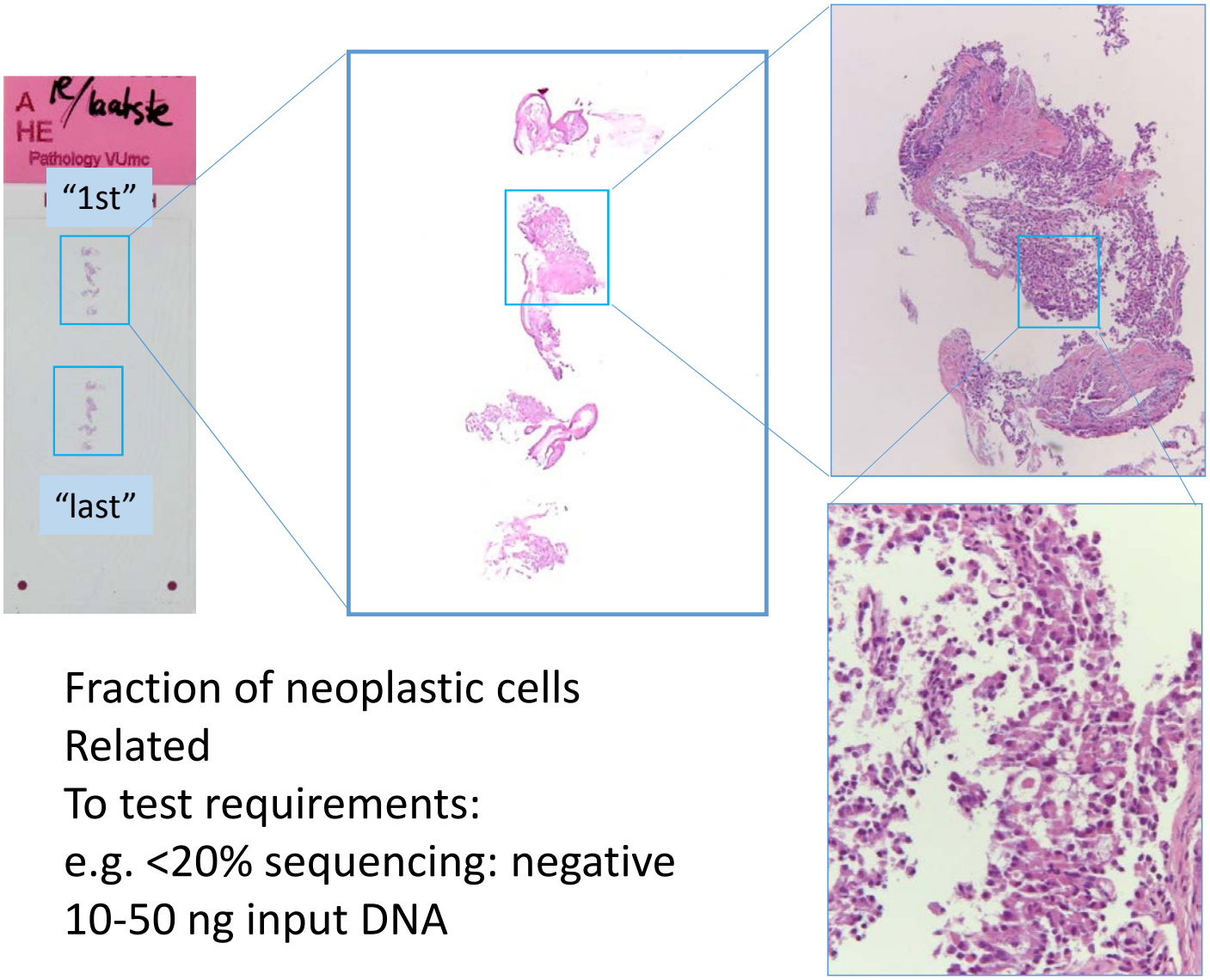


Highly cellular Cell block may be as suitable as tissue



Fraction of neoplastic cells
Related
To test requirements:
e.g. <20% sequencing: negative
10-50 ng input DNA

Dietel M, Thorax. 2016 ,71:177-84.

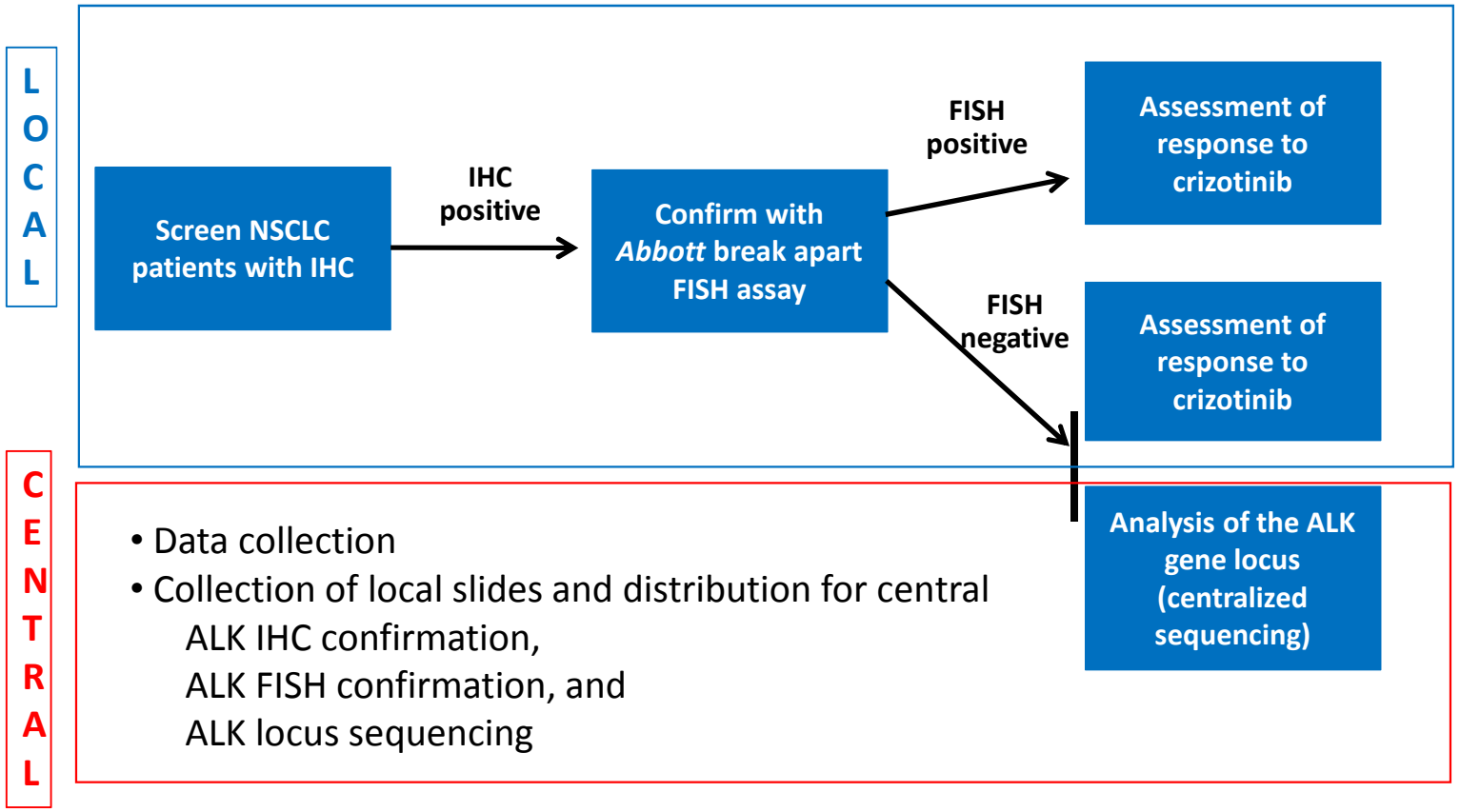
Molecular tumourboard

- Participants
  - Pulmonologists (treats in NL Lung cancer)
  - Pathologists
  - Molecular biologists
- Special cases (not average)
- Frequency: 2x/ month

STUDY DESIGN:

Prospective Multicenter Registry study of ALK IHC+ NSCLC

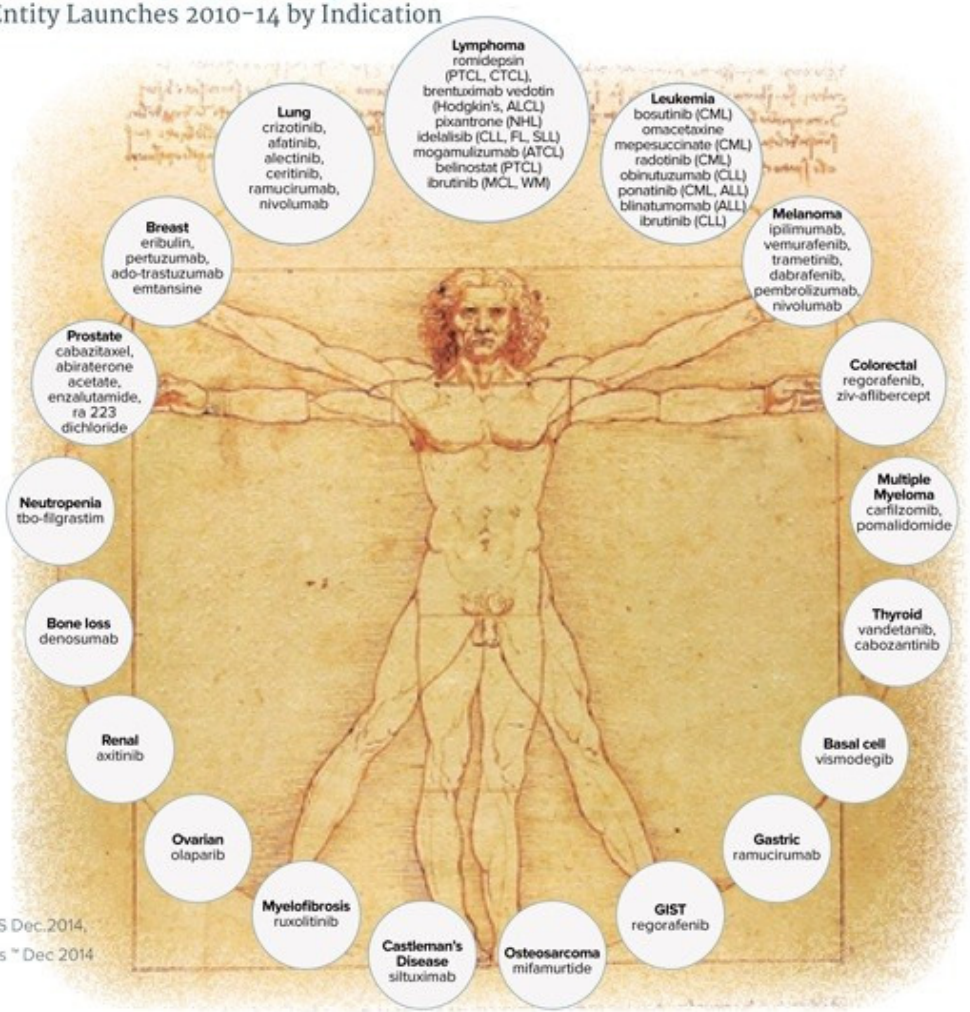
CONTACT: Erik Thunnissen (e.thunnissen@vumc.nl)



Thank you
Questions?

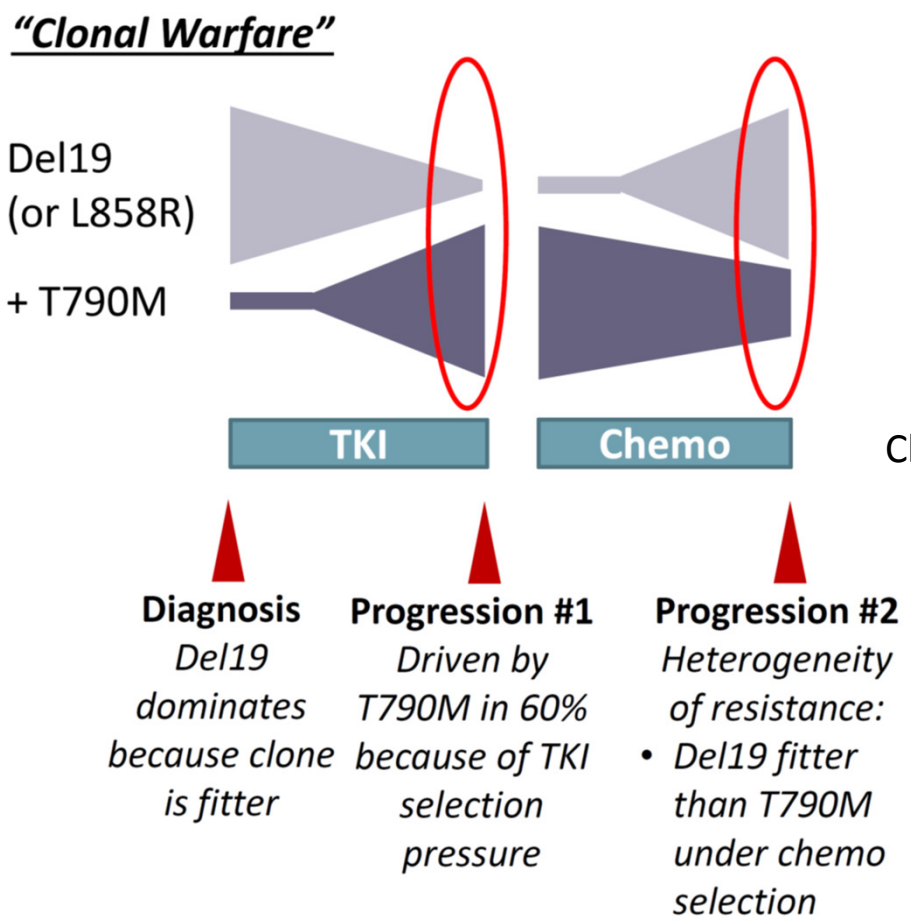
Multiple tumor types are being treated with new medicines launched over the past five years

New Molecular Entity Launches 2010–14 by Indication



Source: IMS Health MIDAS Dec.2014, IMS Lifecycle ™ R&D Focus ™ Dec 2014

Chart notes: Molecules listed had initial global launch in the period 2010 -14. Molecule indications based on approval by one or more regulatory bodies. Excludes sipuleucel-T, an autologous cell procedure not classified by IMS Health as a fully identifiable substance



Clonal selection due to treatment

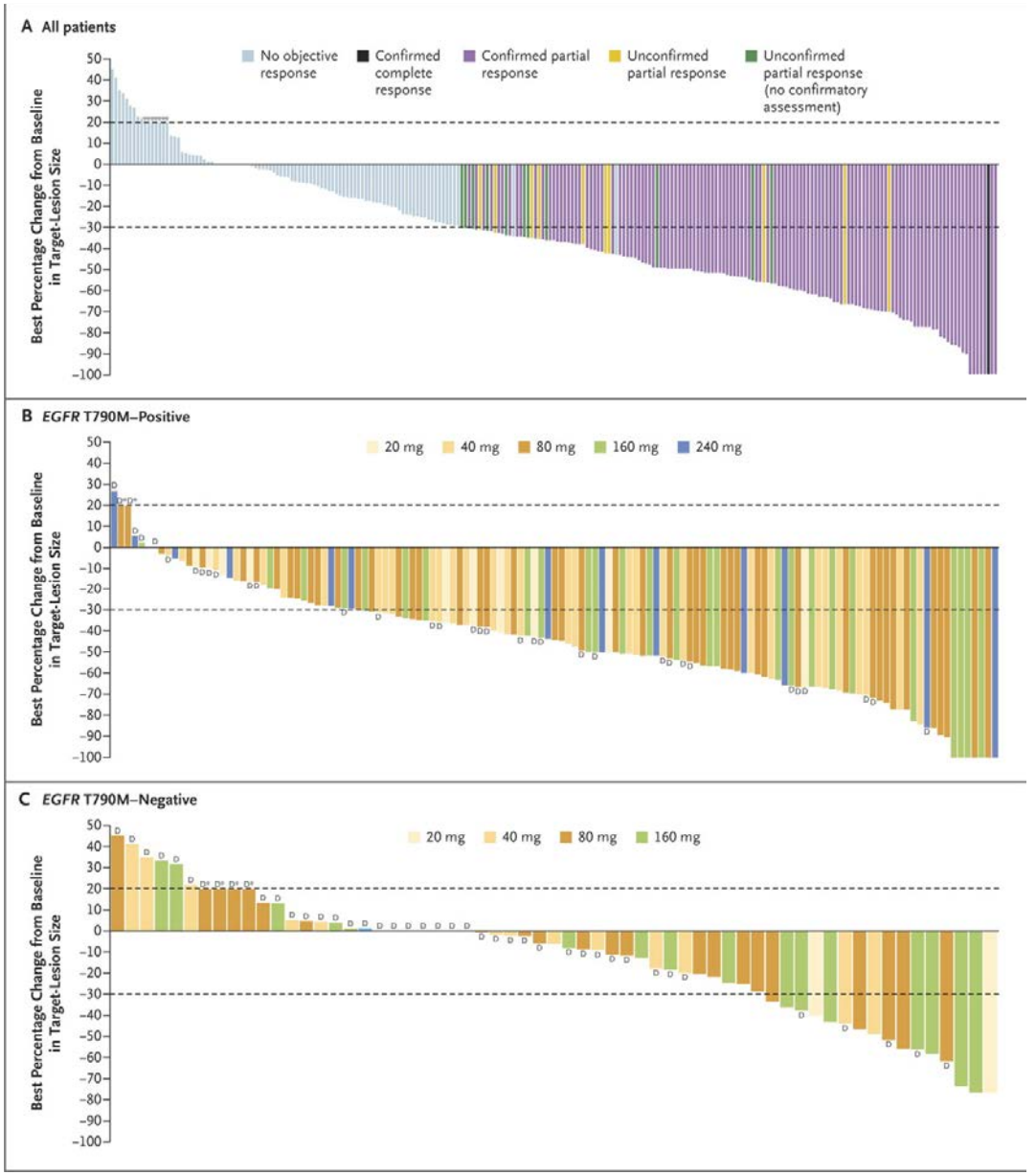
Subclone may be present at time of diagnosis

High T790M in FFPE primary tumor: artifact? Ye JTO 2013

Kuiper, Lung cancer 2014

PFS longer for T790M+

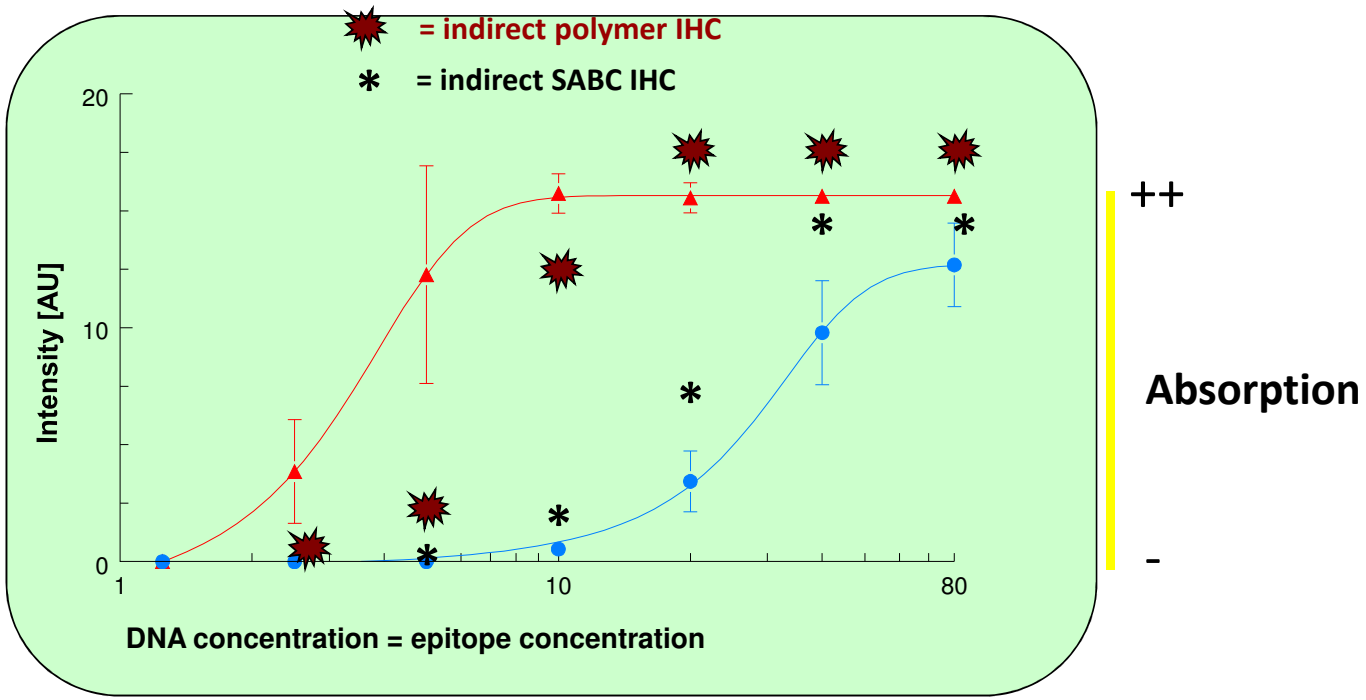
Third generation EGFR TKI: Tagrisso



Janne NEJM 2015, 372(1689-99)

Model Quantitation immunohistochemistry

Indirect tyramide/polymer IHC vs. indirect SABC IHC

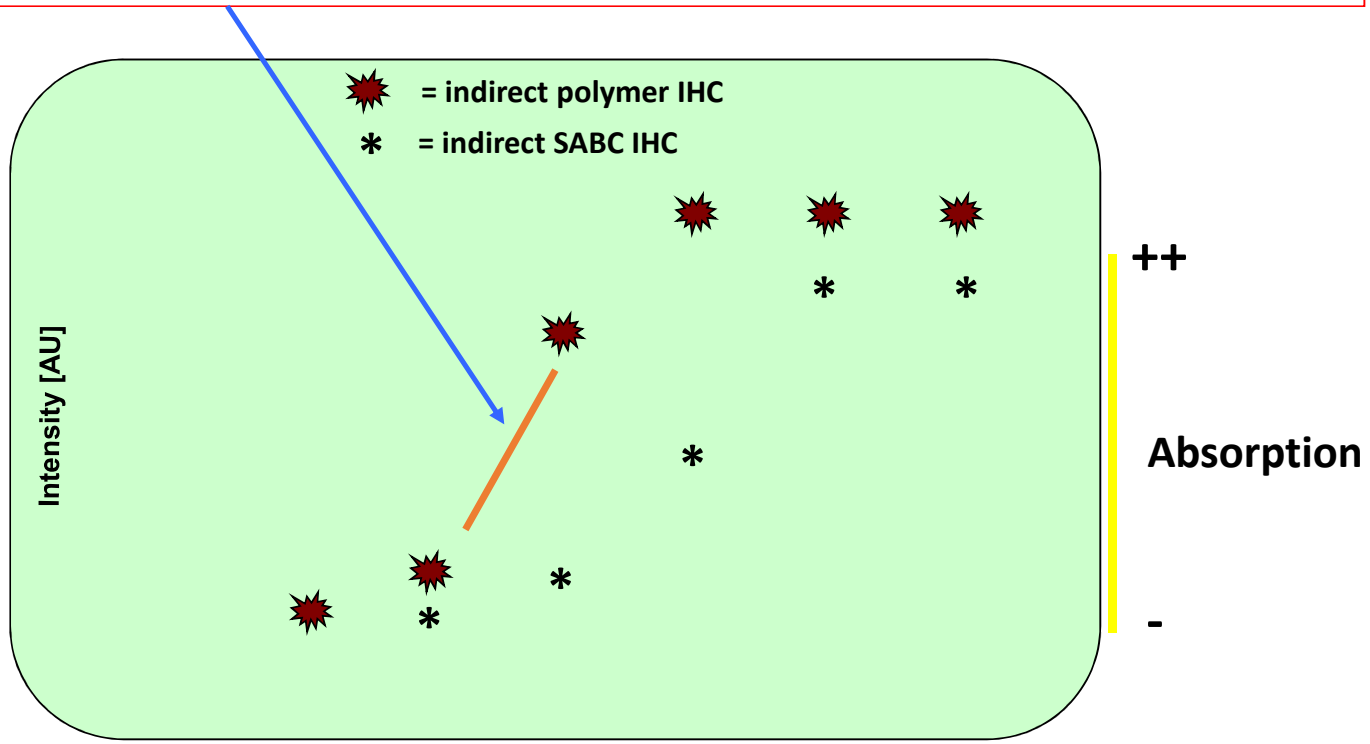


Semi Q: - - - +/- + ++ ++

Absorption

Modified from Prinsen CF, et al. Appl Immunohistochem Mol Morphol. 2003 ;11:168-73.

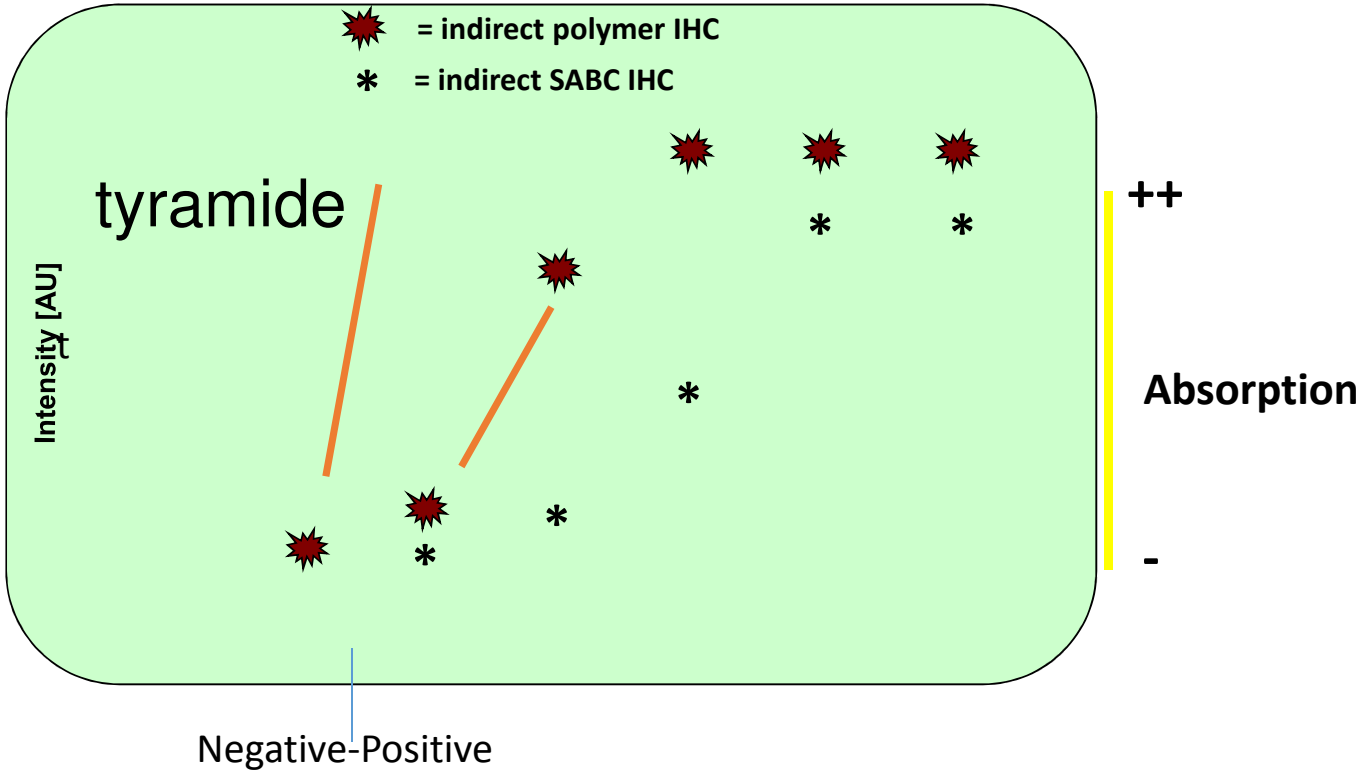
Linear dynamic range = quantitative range,
more signal enhancement:
closer to black and white; positive/ negative



Difference between positive and negative: factor 2 in concentration

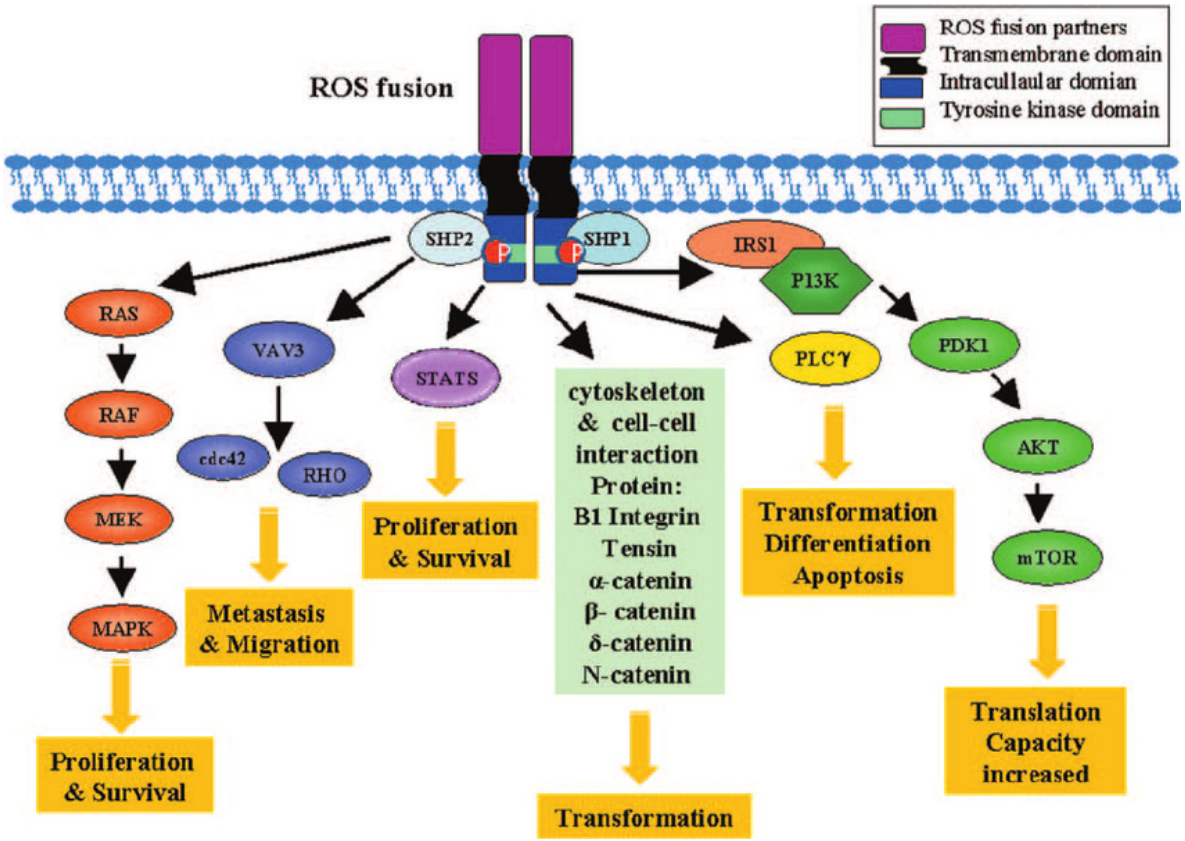
Modified from Prinsen CF, et al. Appl Immunohistochem Mol Morphol. 2003 ;11:168-73.

Linear dynamic range = quantitative range,
More signal enhancement: closer to black and white; positive/ negative



Modified from Prinsen CF, et al. Appl Immunohistochem Mol Morphol. 2003 ;11:168-73.

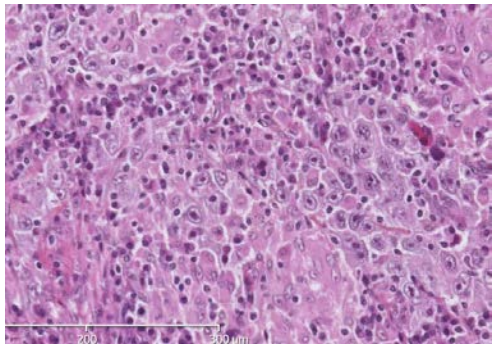
ROS1 signaling pathways



Chin JTO 2012, 7 (1625-30)

Cancer in the lung: Primary vs metastases

- **Clinical information / PALGA essential:**
- **PRIMARY PULMONARY ADENOCARCINOMA: Surfactant prot A, Napsin A, TTF1**
- **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 , 16 (581-592)

Cancer in the lung: Primary vs metastases

- Clinical information / PALGA essential:
- PRIM… …, TTF1
- Met…
- Colo…
- Pros…
- Brea…
- Gerr…
- Mela…
- Mes…
- Kidn…
- Ovary: CA125, Pax5, Pax 8

Discussion between pulmonologist/ oncologist and pathologist whether clinical suspicion is present on metastases or not is required.

Only performing these stains when clinically relevant..

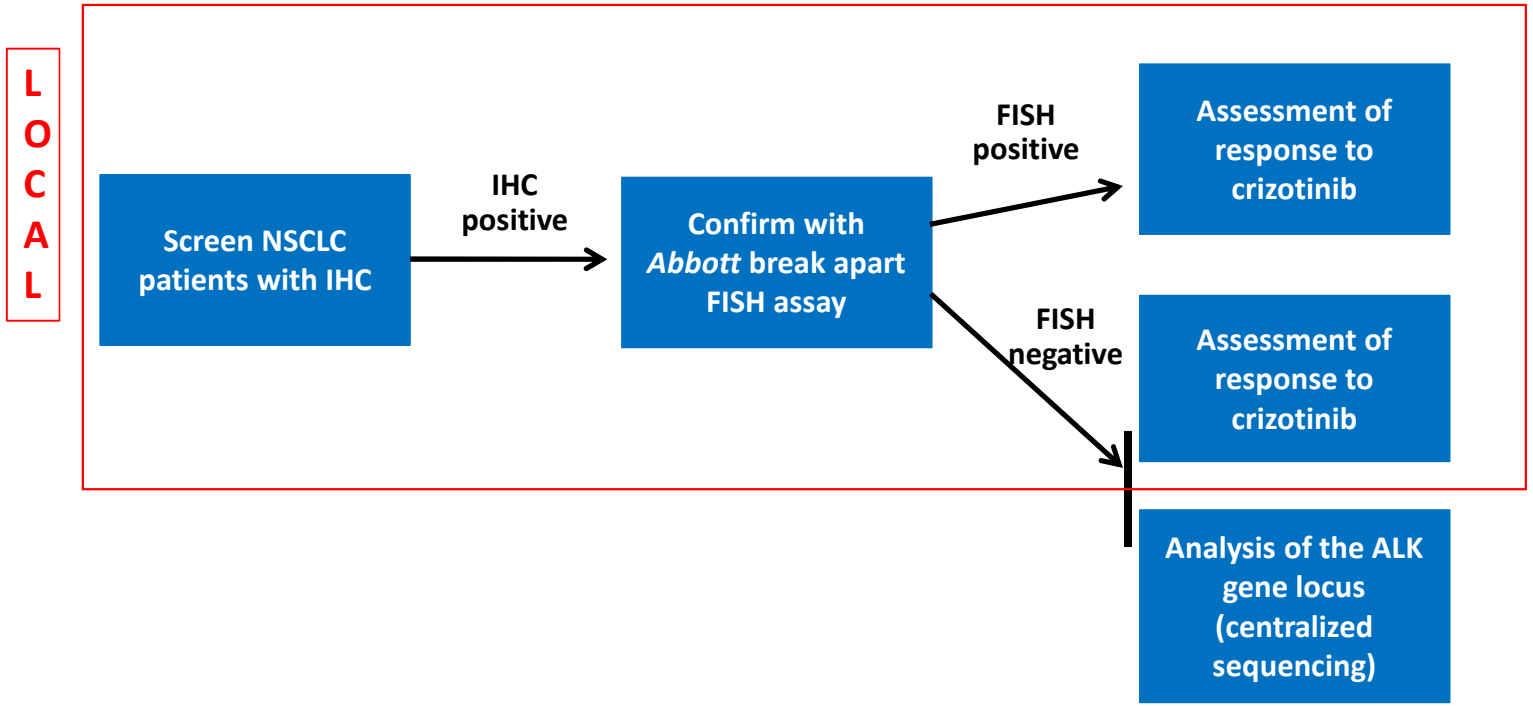
Only a few markers have 100% specificity with limited sensitivity

Nonaka, Diagnostic Histopathology 2010 , 16 (581-592)

STUDY DESIGN: IIR Pfizer

‘European’ Prospective Multicenter Registry study of ALK IHC+ NSCLC

CONTACT: Erik Thunnissen (e.thunnissen@vumc.nl)



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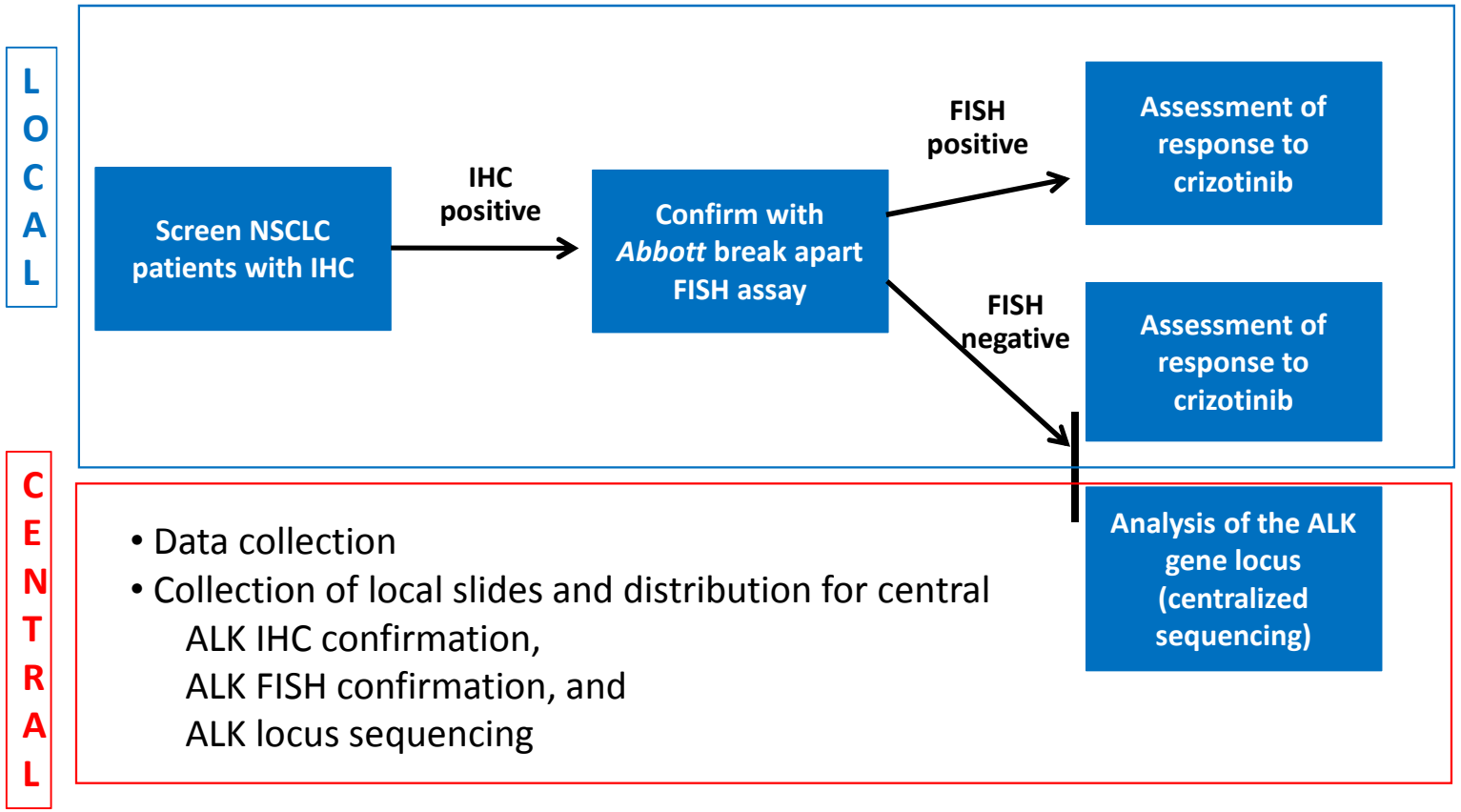
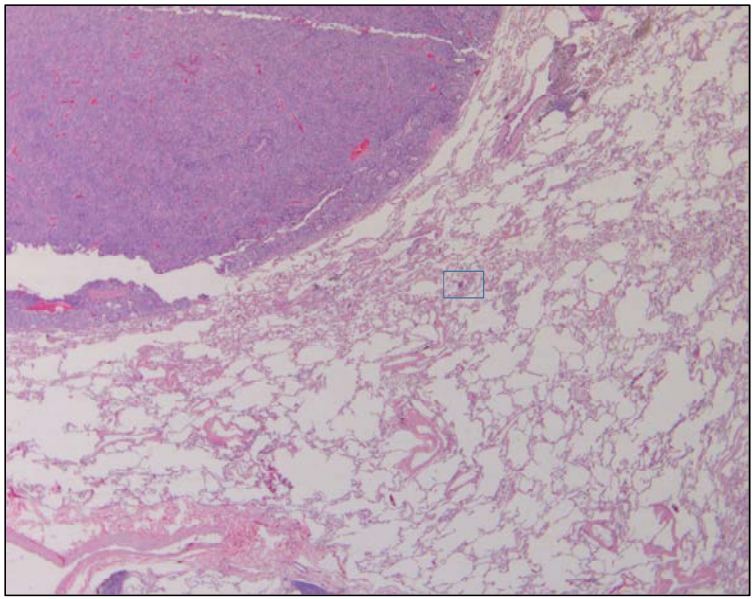
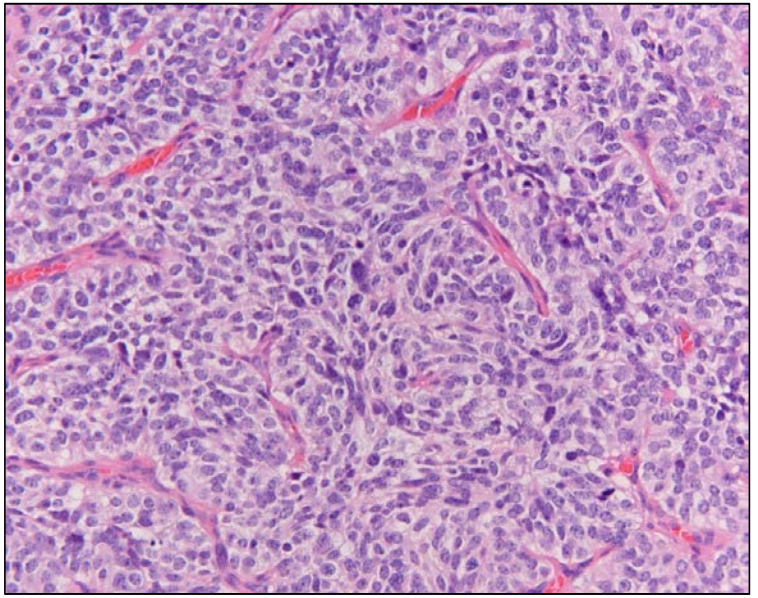
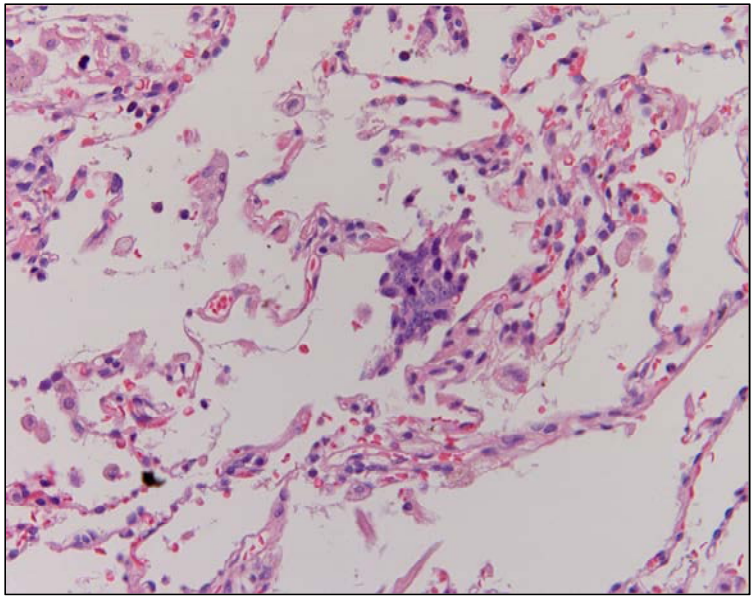


STAS





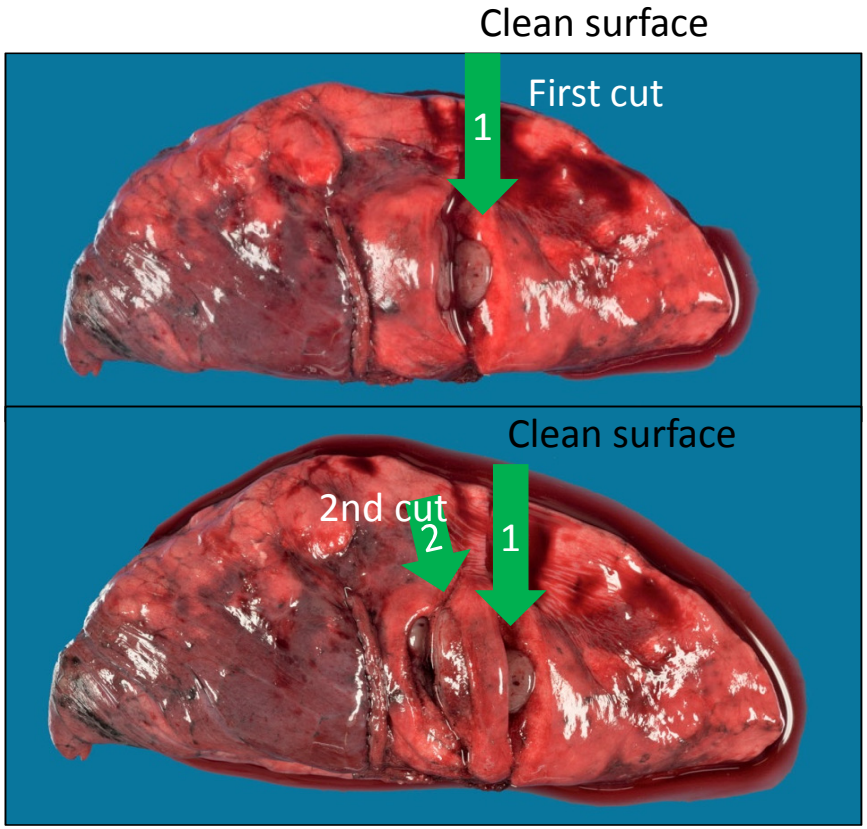
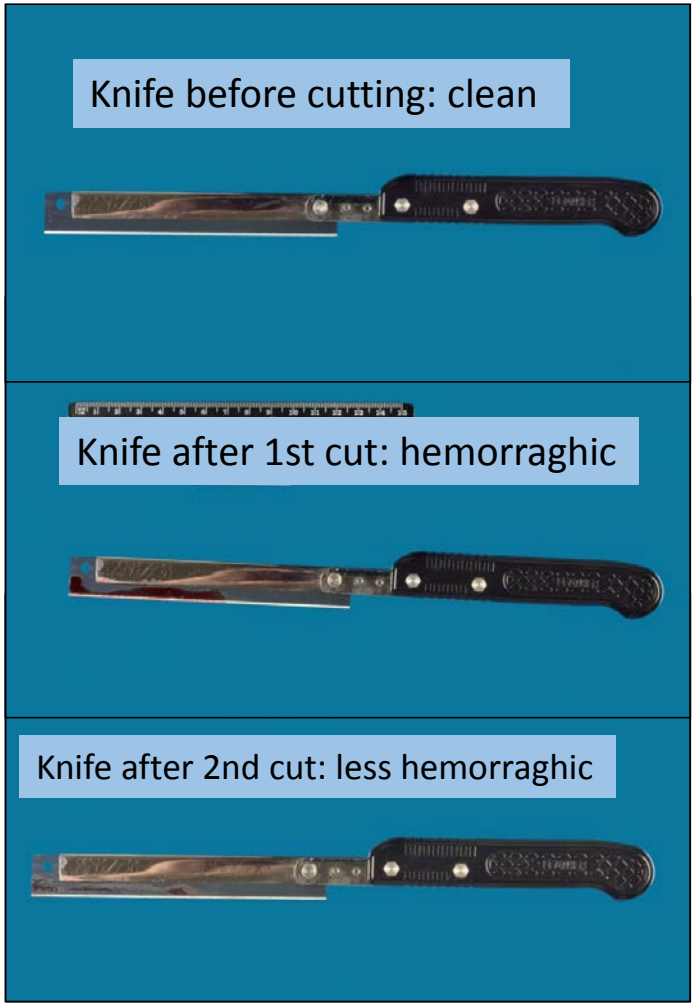


Loose tumour fragments called STAS: spread through airways
STAS = an assumption

More frequent in micropapillary carcinoma.

Could this be artifact?

Gross cutting



Hemorraghic surface tumor and pleura after 2nd cut