

International Symposium on Immunohistochemistry

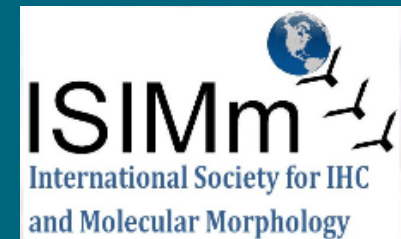
January 4th - 7th, 2018

Hosted by Dept. of Histopathology, Tata Medical Center, Kolkata, India

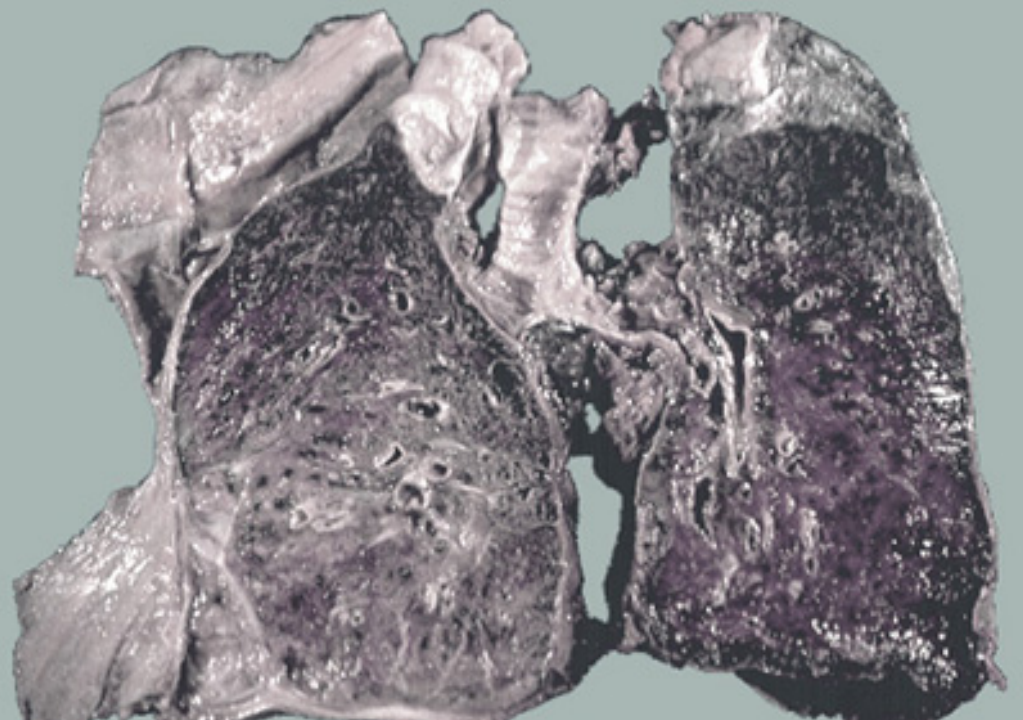
In collaboration with NordiQC, Alborg, Denmark and ISIMM, California, USA



Diagnostic IHC in lung and pleura pathology

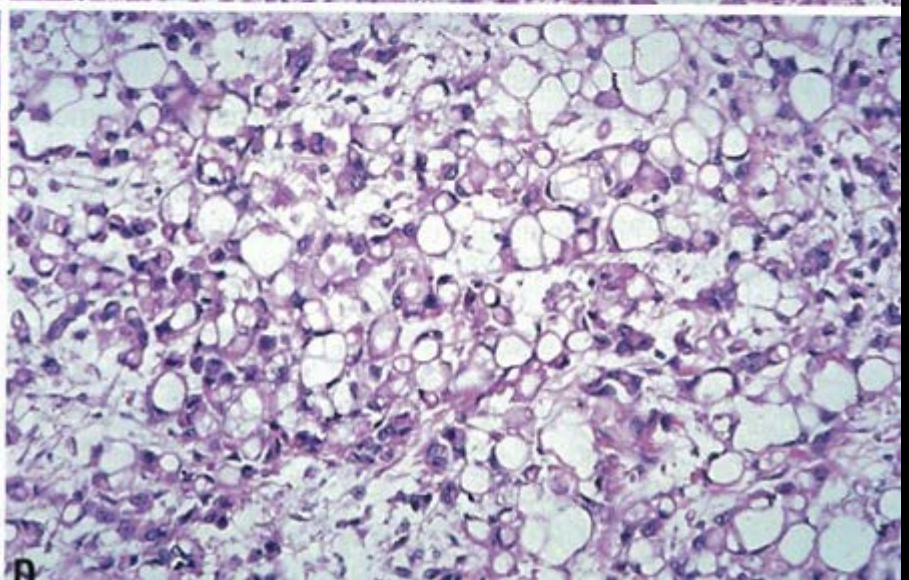
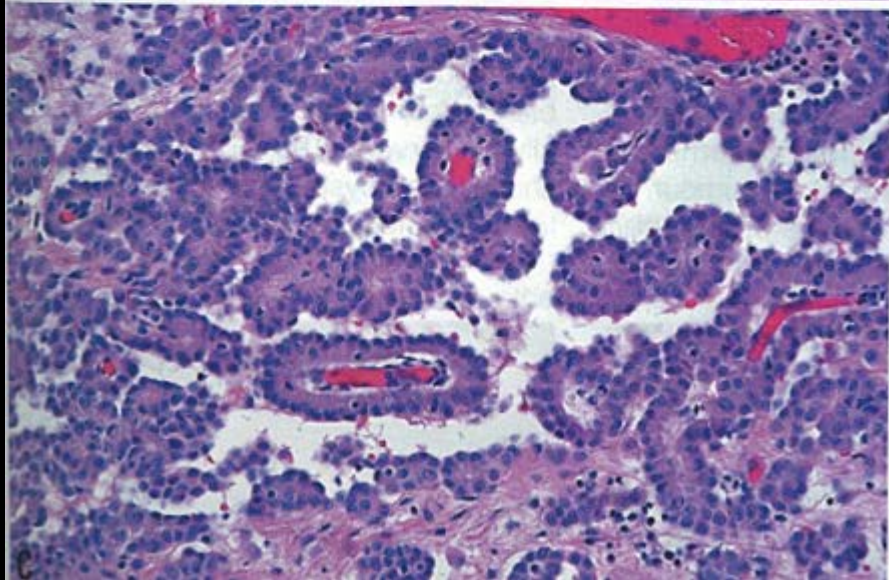
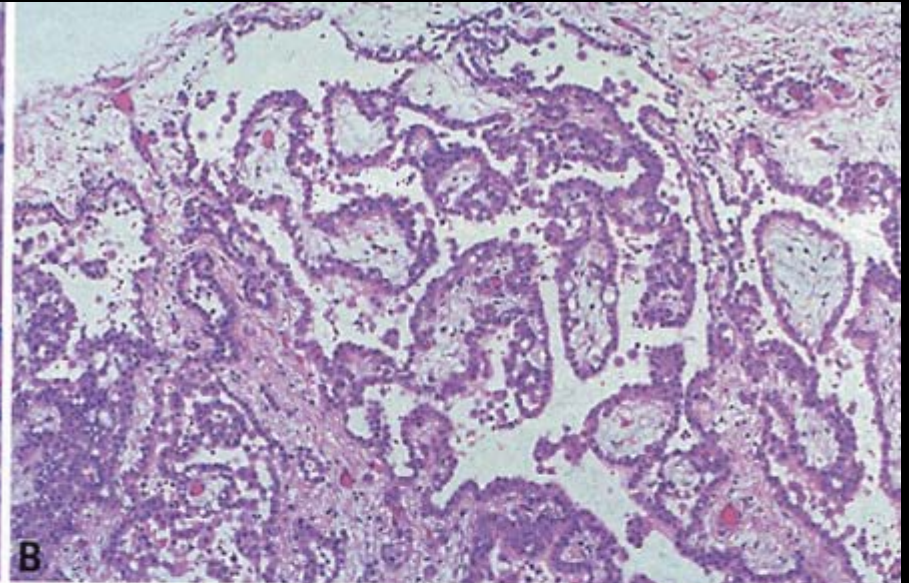
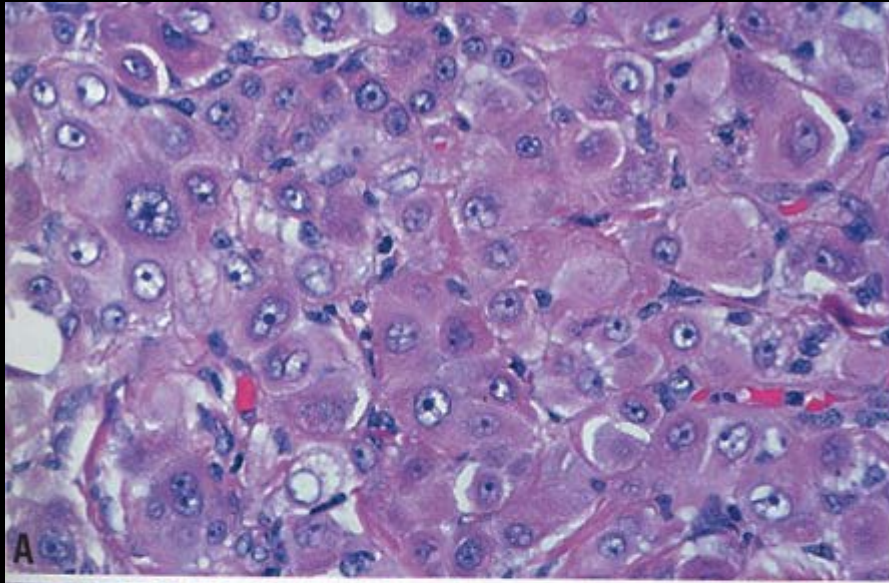


Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark

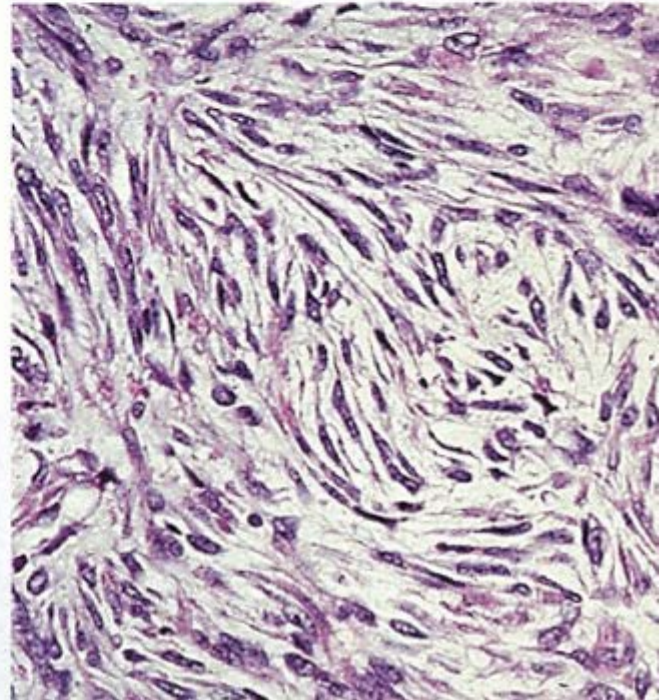
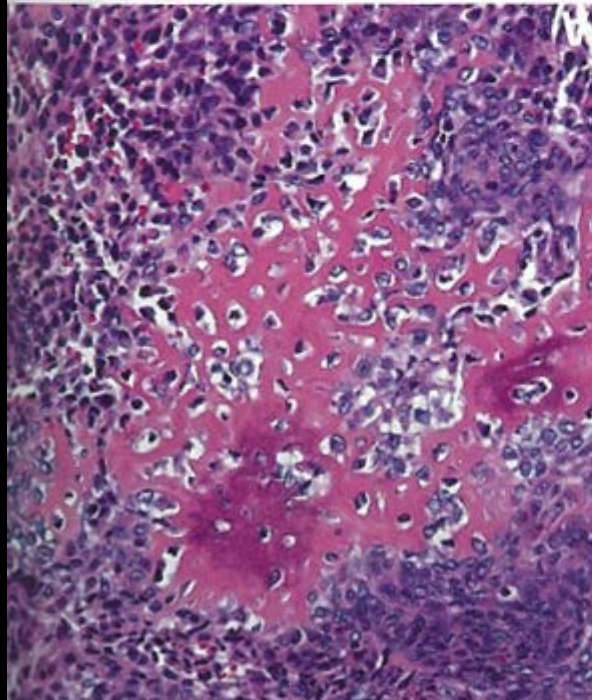
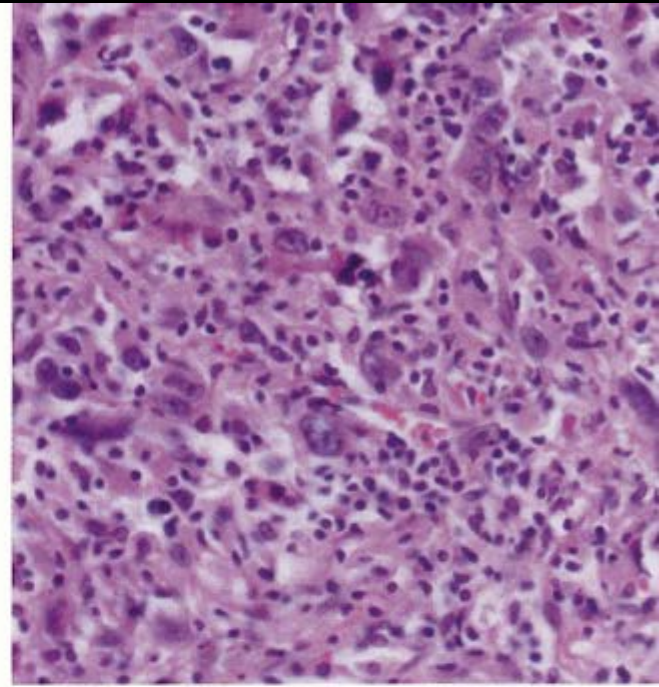
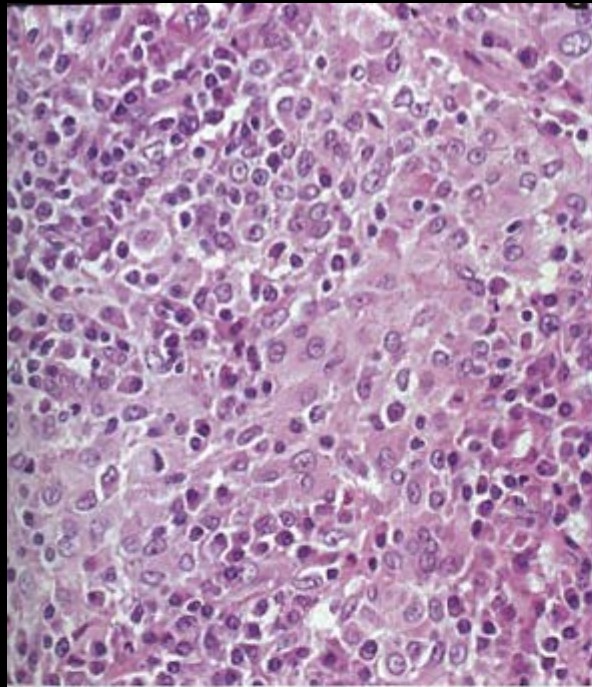


Epithelioid mesothelioma

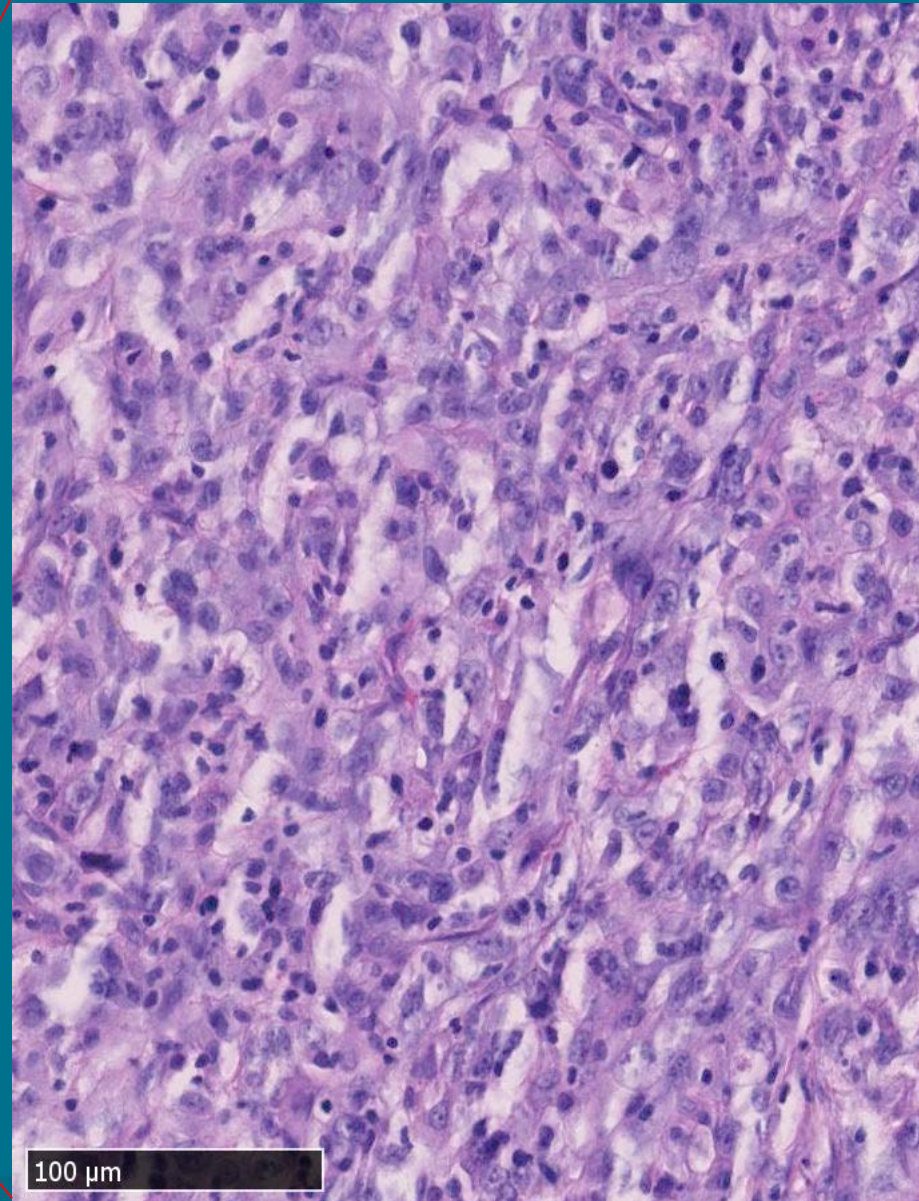
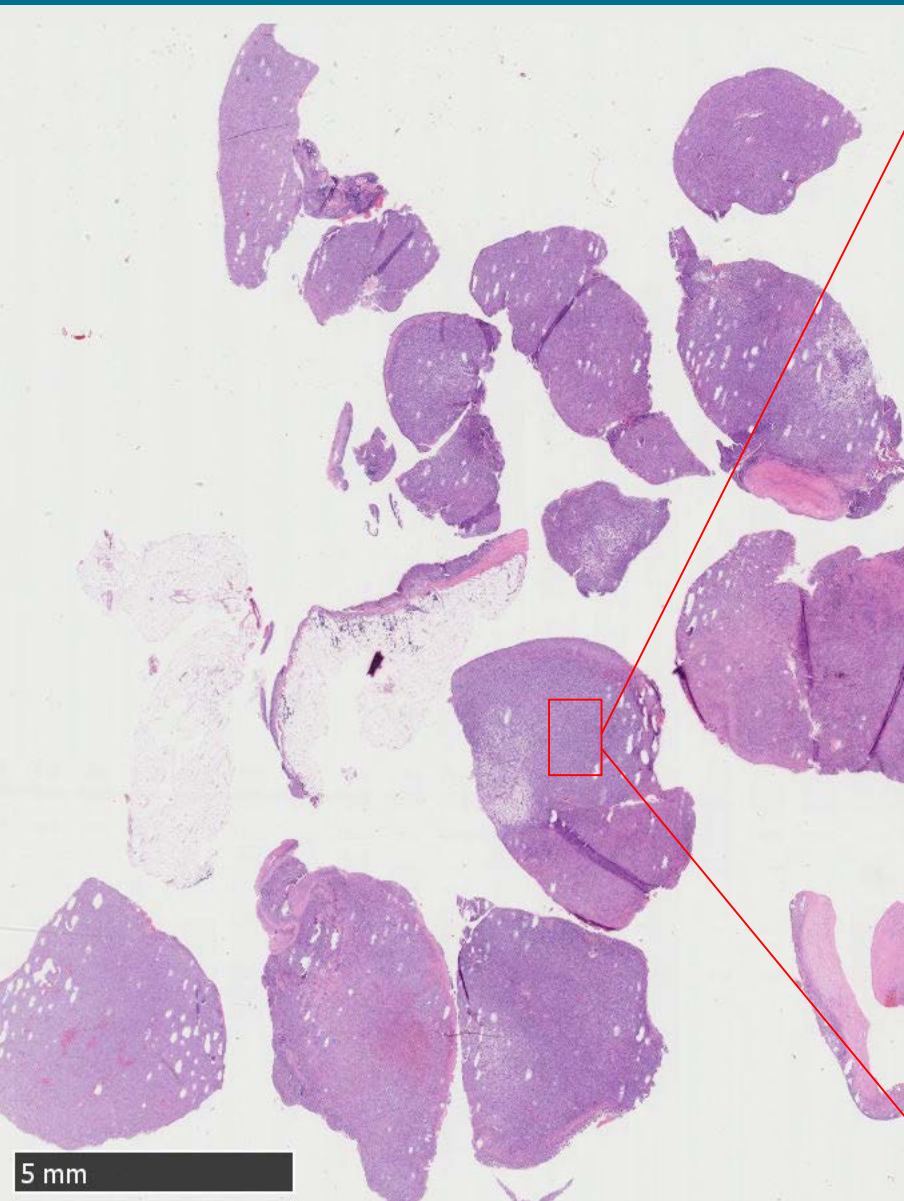
WHO 2004



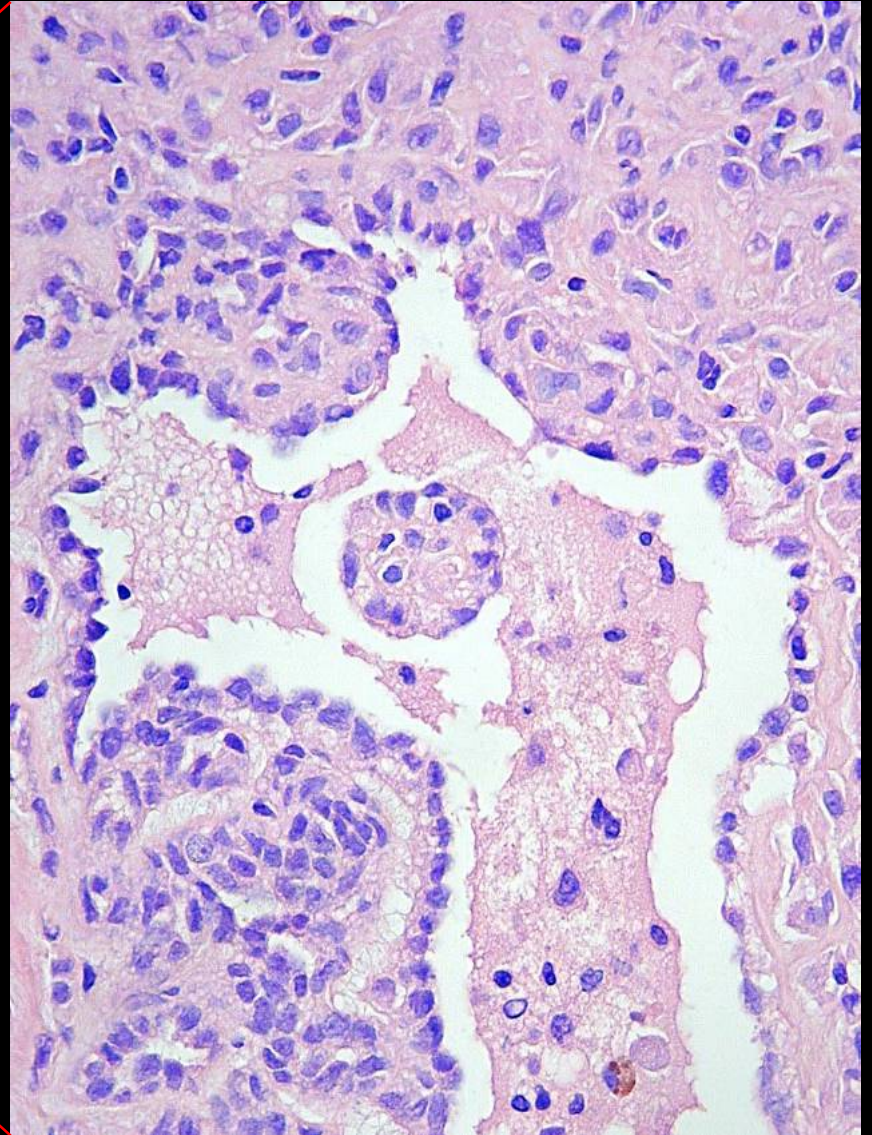
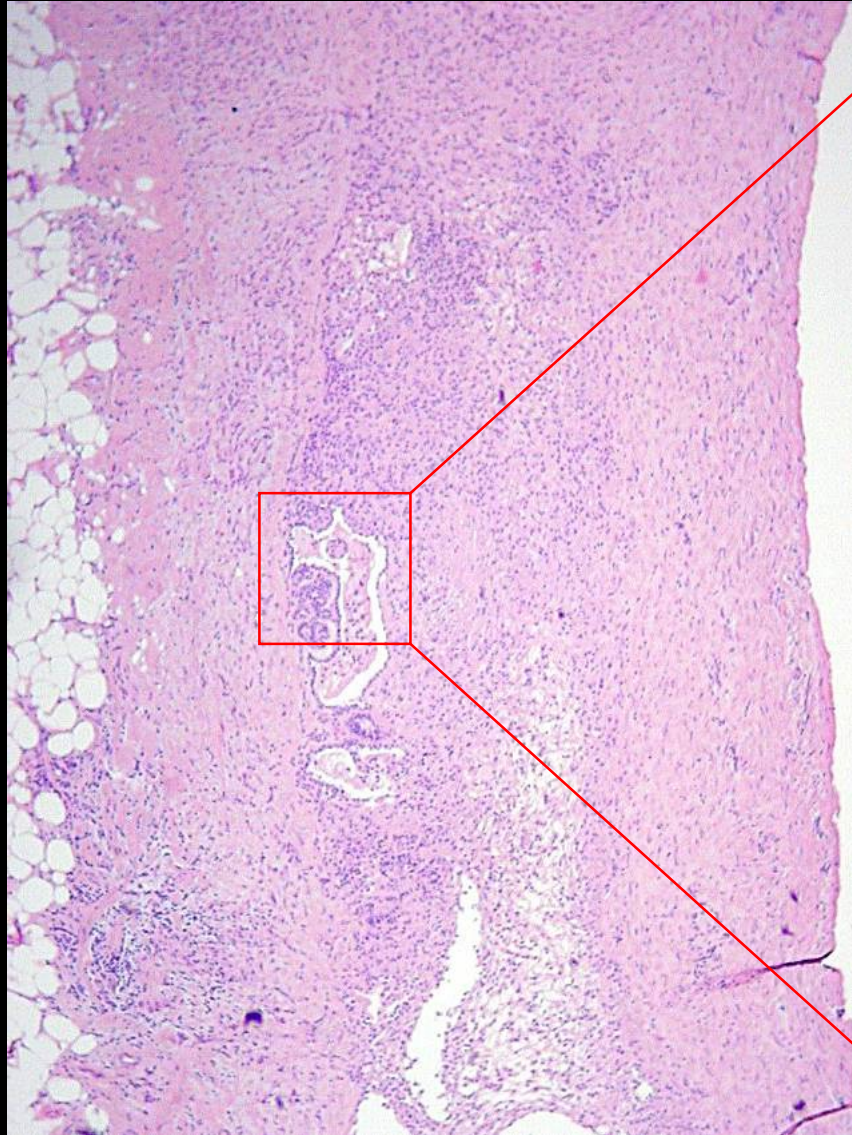
Sarcomatoid mesothelioma WHO 2004



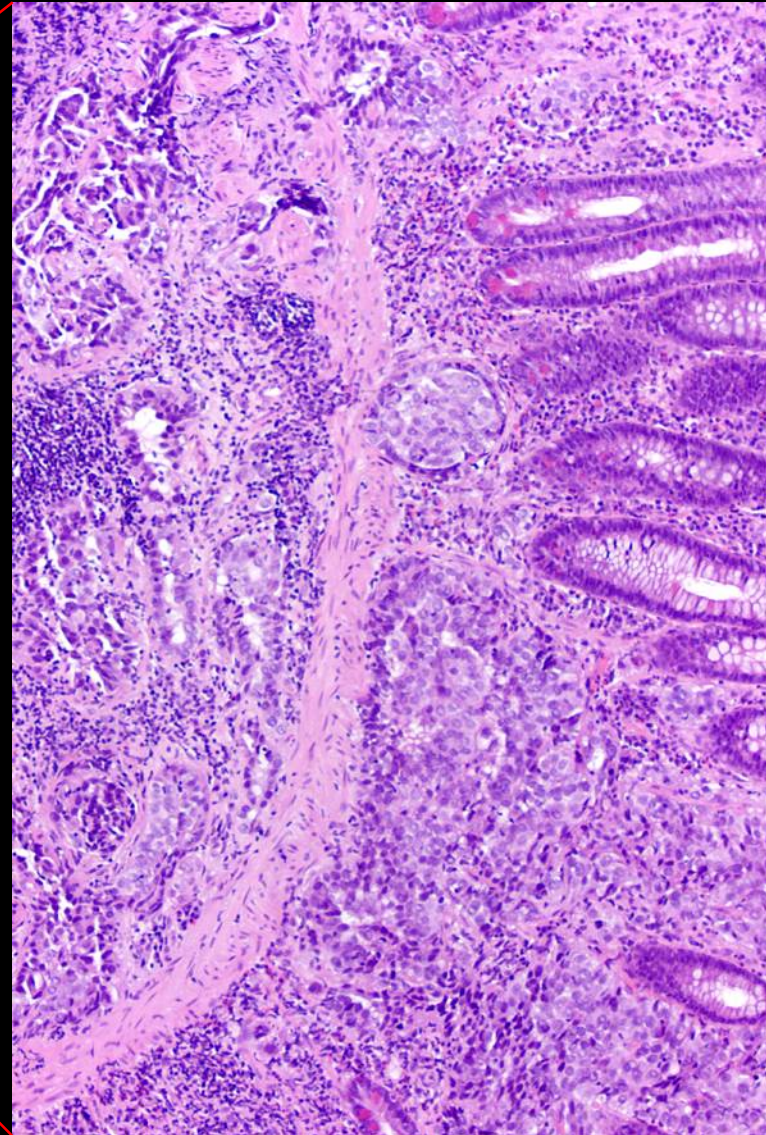
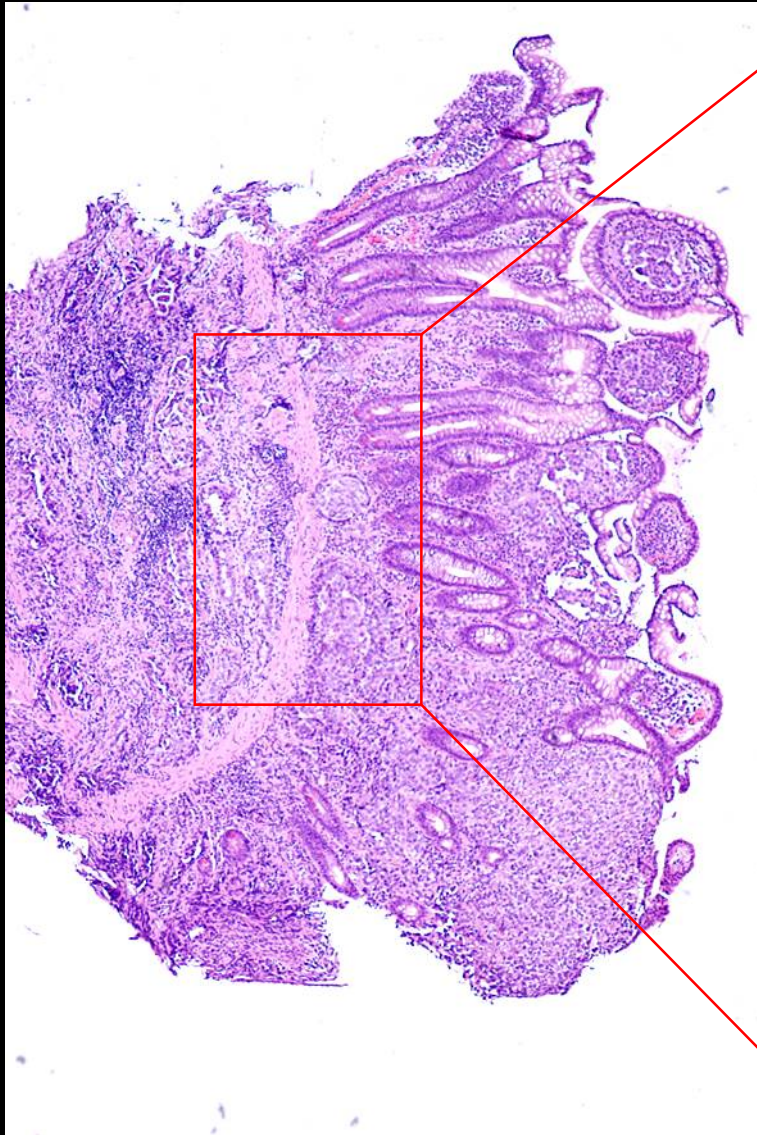
Malignant mesothelioma - Biopsy



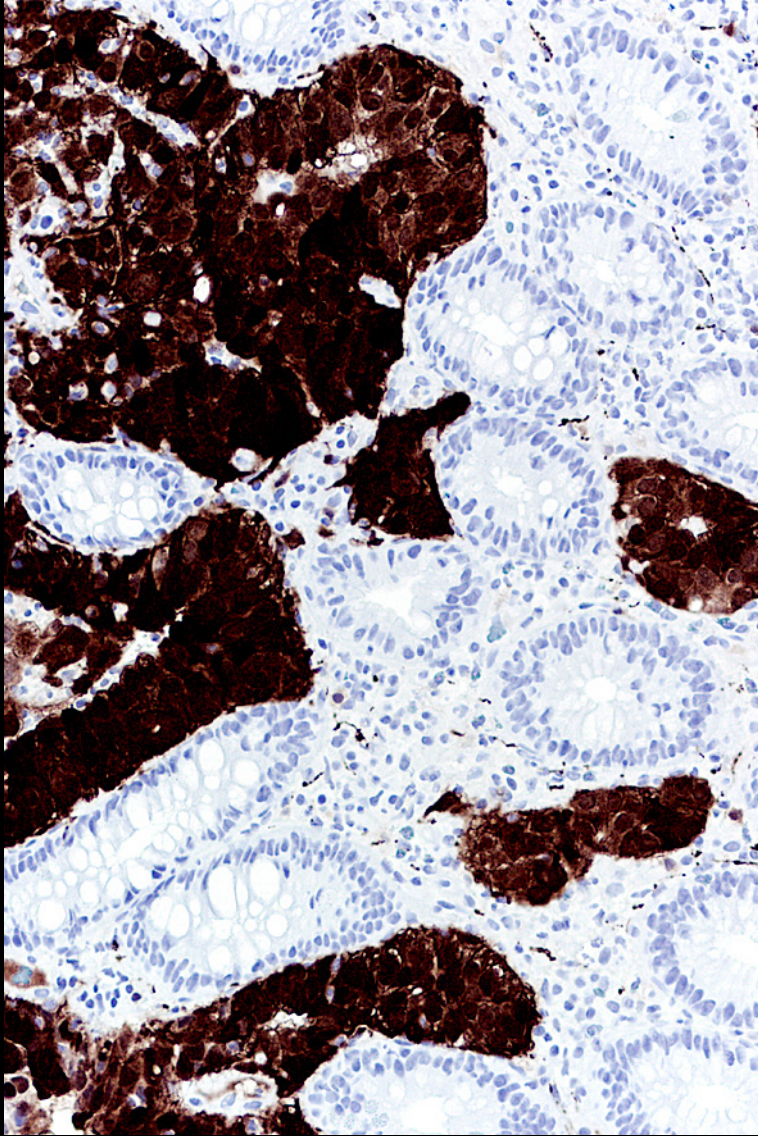
Epithelioid mesothelioma



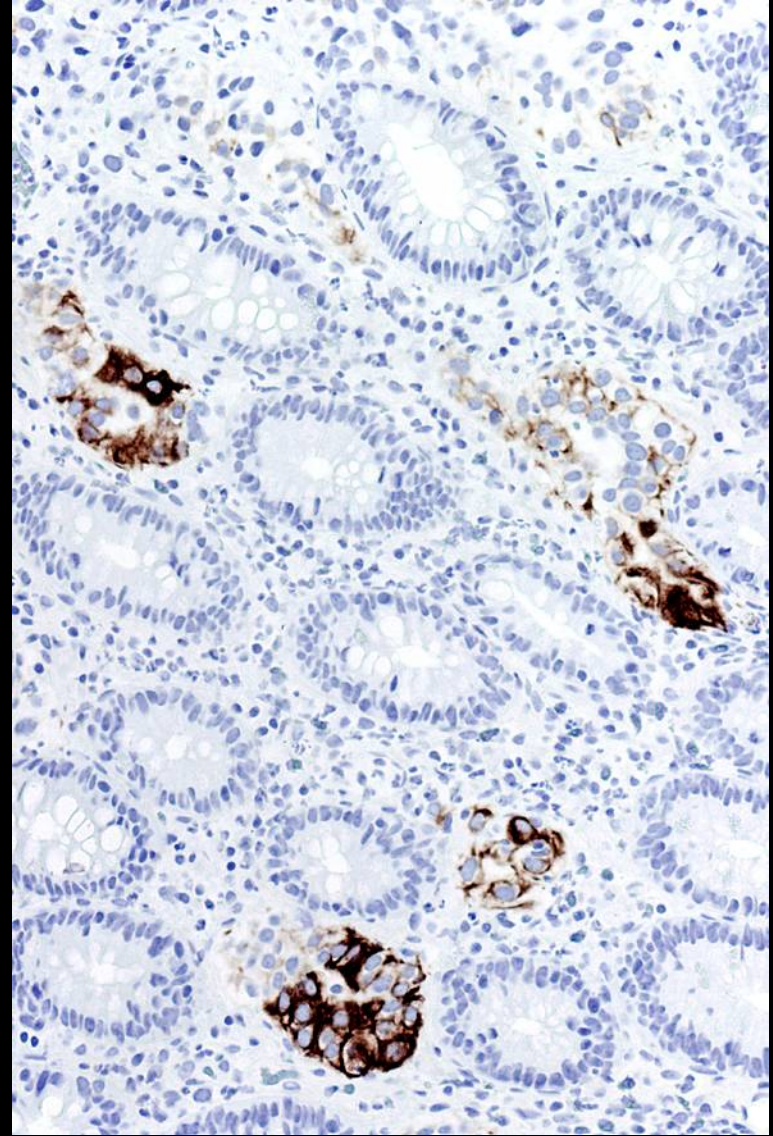
Man 67, stenosing tumor near right flexure



Man 67, stenosing tumor near right flexure



Calretinin



Keratin 5

“Mesothelial markers”

Calretinin

Podoplanin

WT1

Vimentin

CA125

CK5

BAP-1

Malignant mesothelioma vs. lung carcinomas

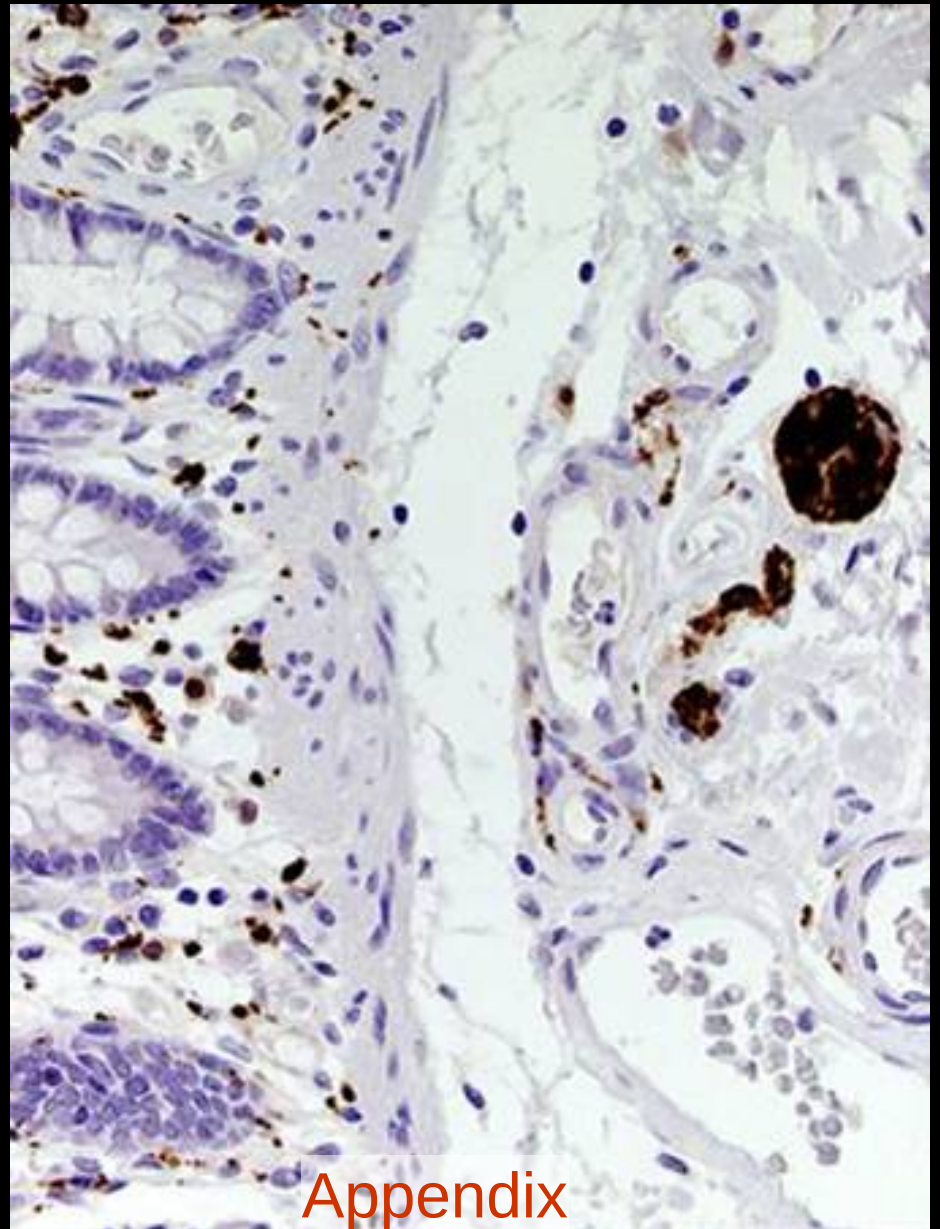
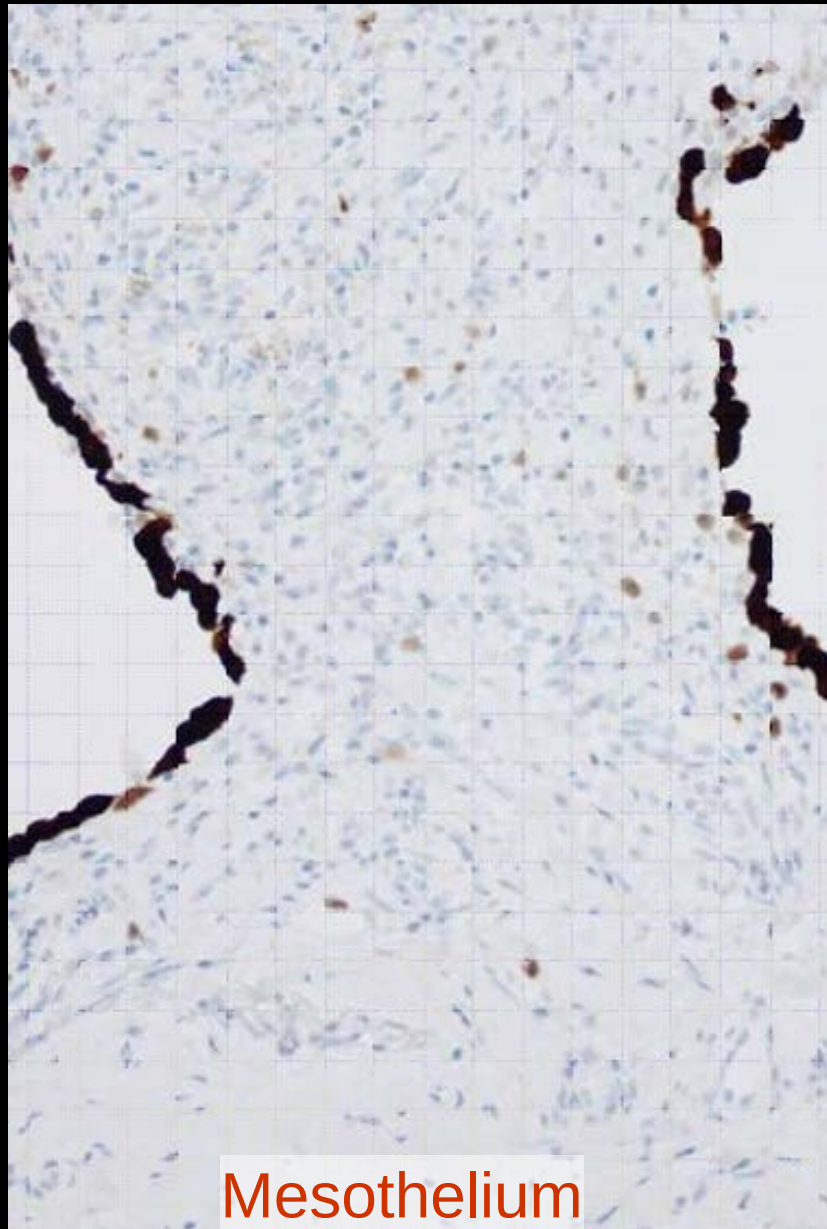
	Calr	Pod	WT1	Vim	CA125	CK5	Ep-CAM	Cld4	NapA	TTF-1	p40
Epithelioid malignant mesothelioma	90	90	90	90	90	80	10 (f)	0	0	0	0
Lung Adenocarcinoma	10 (f)	10 (f)	20?	30	30	5?	95	100	80	80	0
Squamous cell carcinoma	50?	10	0	10?	15	95	95	95	10 (f)	15	95

% positivity
f: focal
?: insuf. data

Calcium-binding protein related to S-100 proteins

- Neurons
- Mesothelial cells
- Steroid producing cells
 - adrenal cortical cells
 - testicular Leydig and Sertoli cells
 - ovarian theca interna cells and surface epithelium
- Neuroendocrine cells
- Breast glands
- . . .

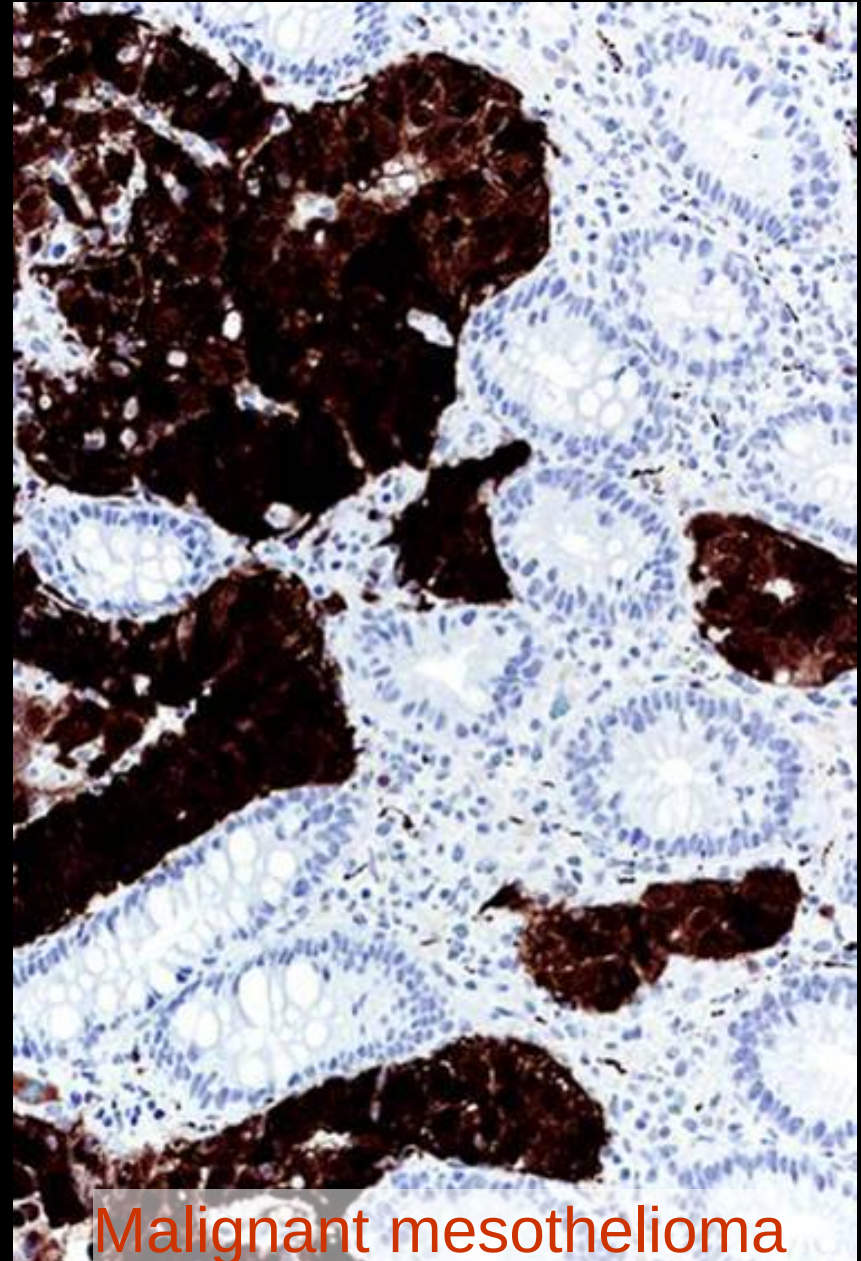
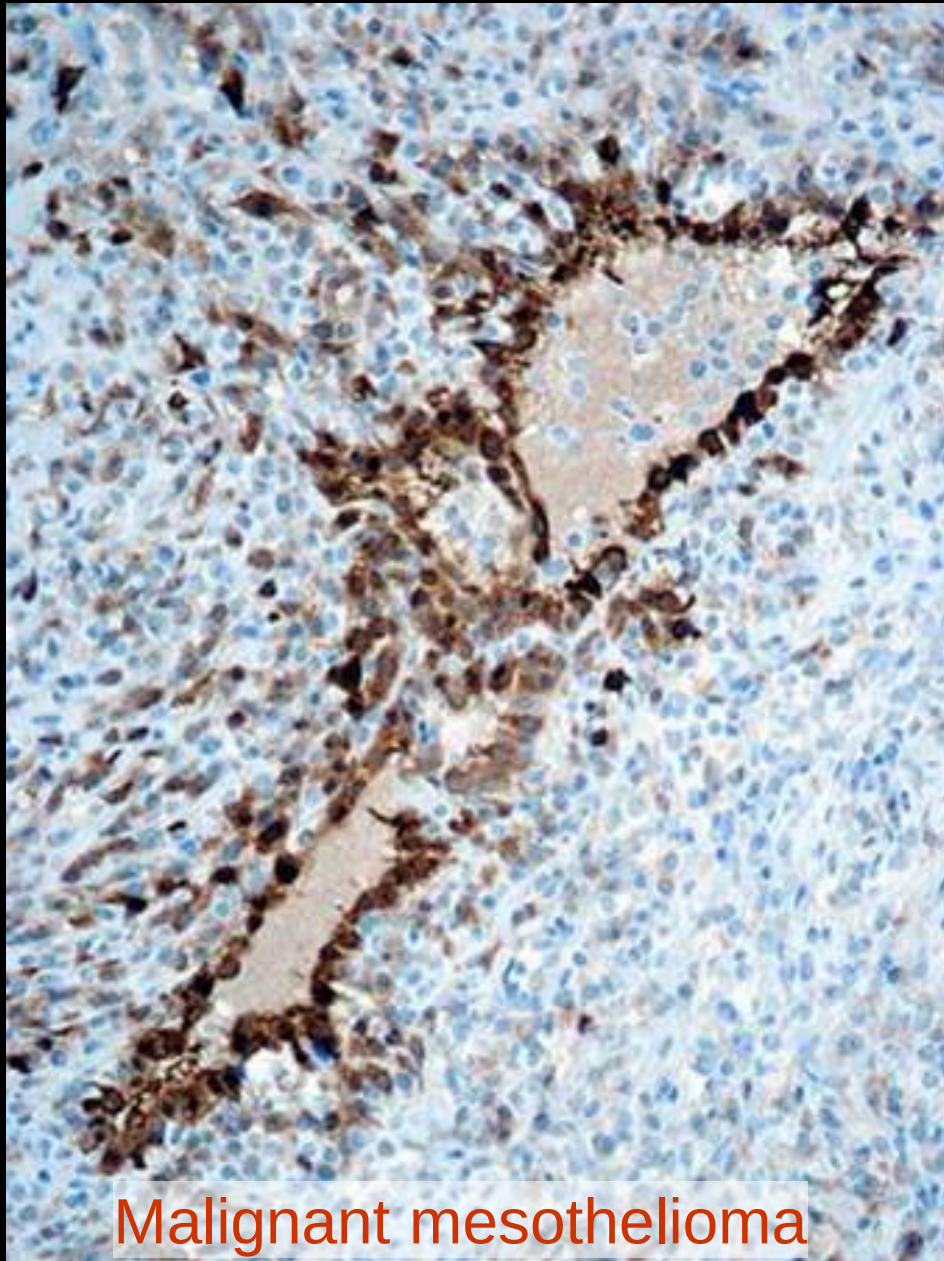
Calretinin



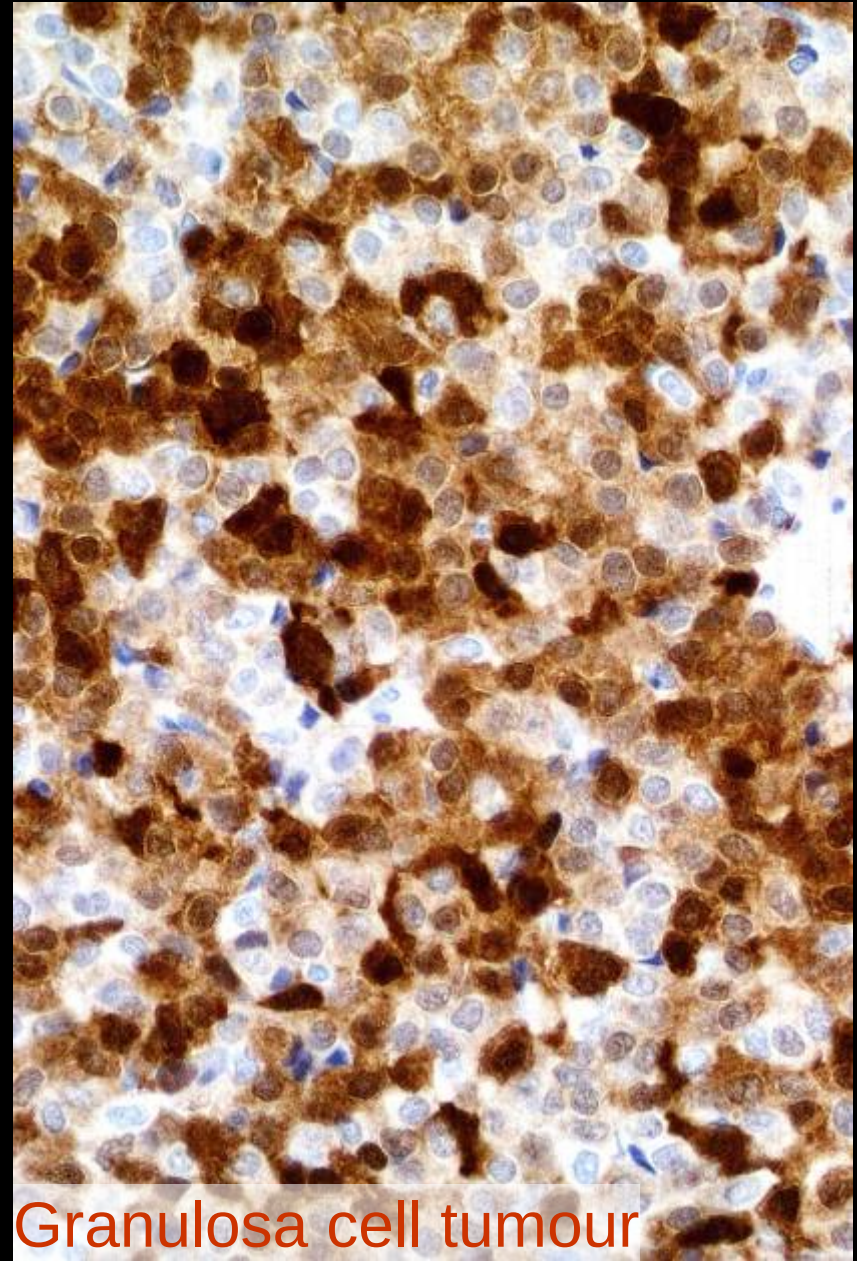
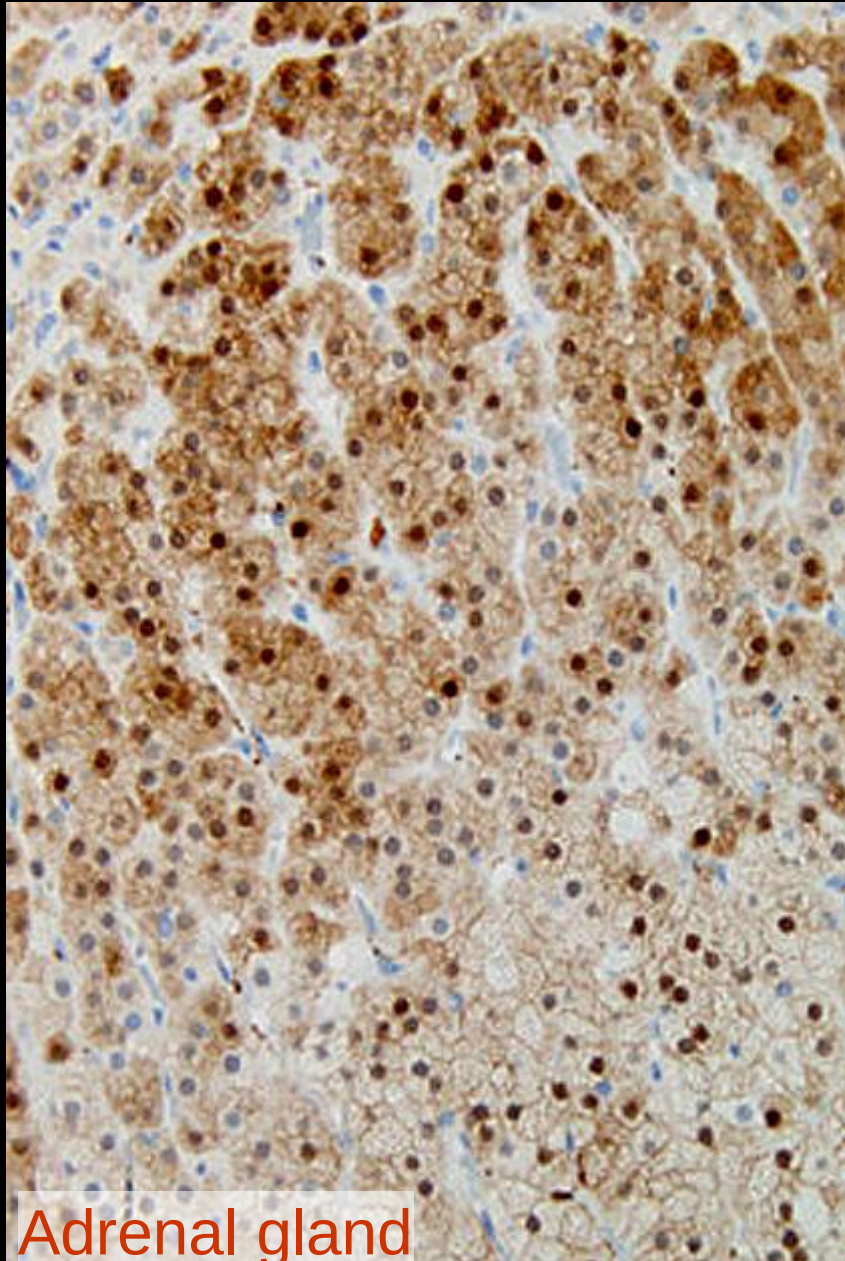
Calretinin

- Malignant mesotheliomas +
- Steroid producing tumours +/-
 - adrenal cortical tumour
 - testicular Leydig and Sertoli cell tumour
 - ovarian thecal tumours
- Adenocarcinomas -(+) (f)
- . . .

Calretinin in neoplasms



Calretinin



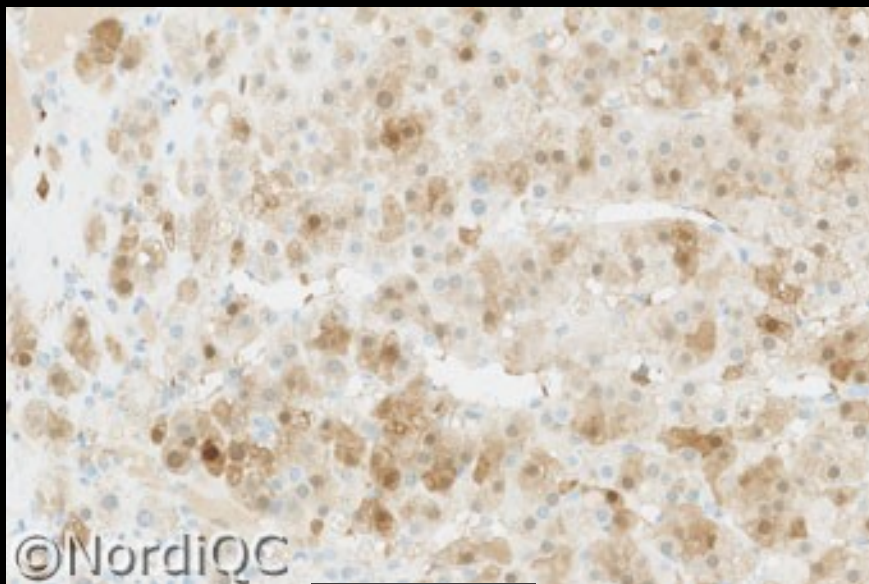
Calretinin – Run 33/2011: 180 labs

Table 1. Abs and assessment marks for CR, run 33

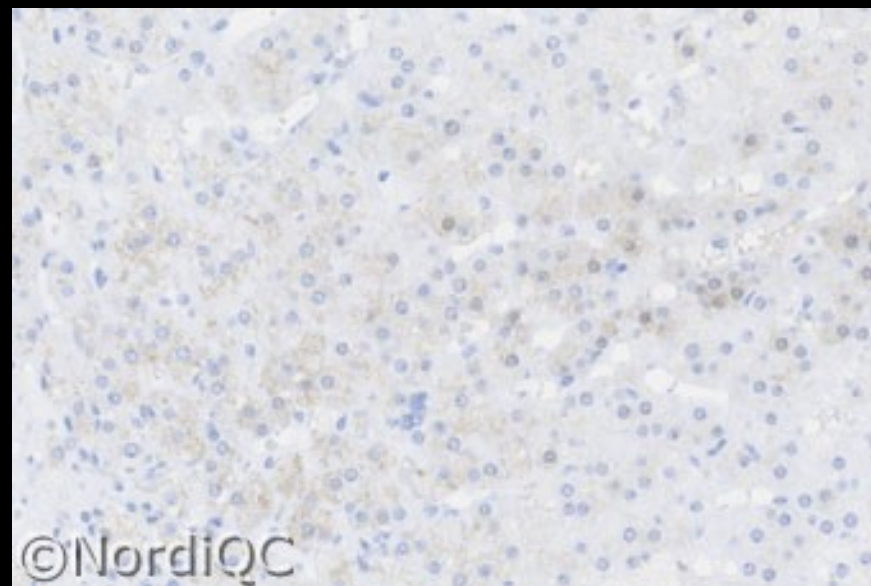
Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. OPS ²
mAb clone 2E7	3	Immunologic	0	2	0	1	-	-
mAb clone 5A5	28	Leica/Novocastra	8	14	10	1	67 %	74 %
	4	Thermo/Neomarkers						
	1	Monosan						
mAb clone DAK-Calret 1	41	Dako	11	17	11	3	67 %	83 %
	1	Thermo/Neomarkers						
rmAb clone SP13	3	Thermo/Neomarkers	0	2	2	2	33 %	
	2	Spring						
	1	Master Diagnostica						
pAb 18-0211	18	Invitrogen/Zymed	10	6	2	0	89 %	100 %
pAb CP092	2	Biocare	0	2	0	0	-	-
pAb E1174	1	Spring	0	1	0	0	-	-
pAb ILM 7696	4	Immunologic	0	2	1	1	-	-
pAb RBK003	1	Zytomed	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone CAL6 PA0346	3	Leica/Novocastra	2	1	0	0	-	-
mAb clone DAK-Calret 1 IS/IR627	26	Dako	7	11	6	2	69 %	78 %
rmAb clone SP65 790-4467	28	Ventana	24	4	0	0	100 %	100 %
pAb 232A-77/78	2	Cell Marque	0	2	0	0	-	-
pAb 760-2700	3	Ventana/Cell Marque	0	3	0	0	-	-
pAb ZA0026	1	Zhongshan	0	1	0	0	-	-
Total	173		62	69	32	10	-	
Proportion			36 %	40 %	18 %	6 %	76 %	

Ventana platform

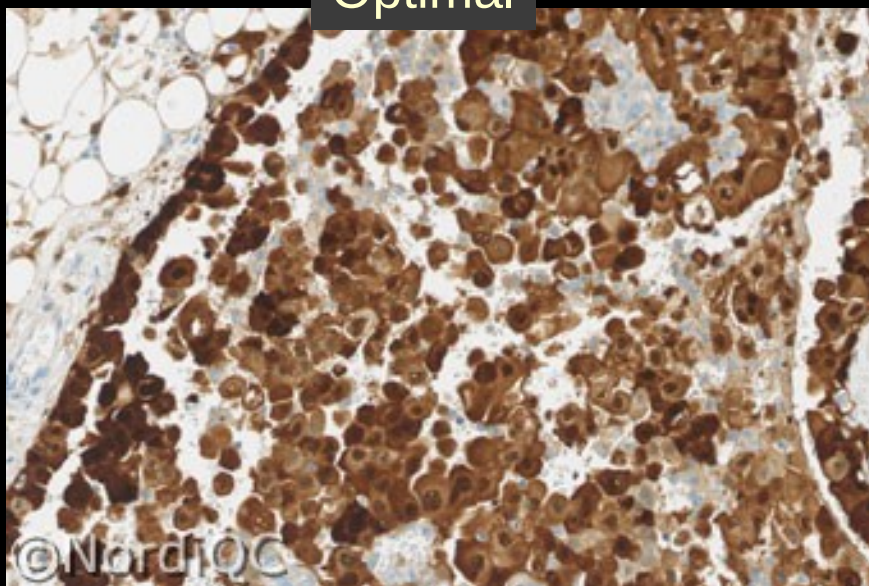
Ventana platform



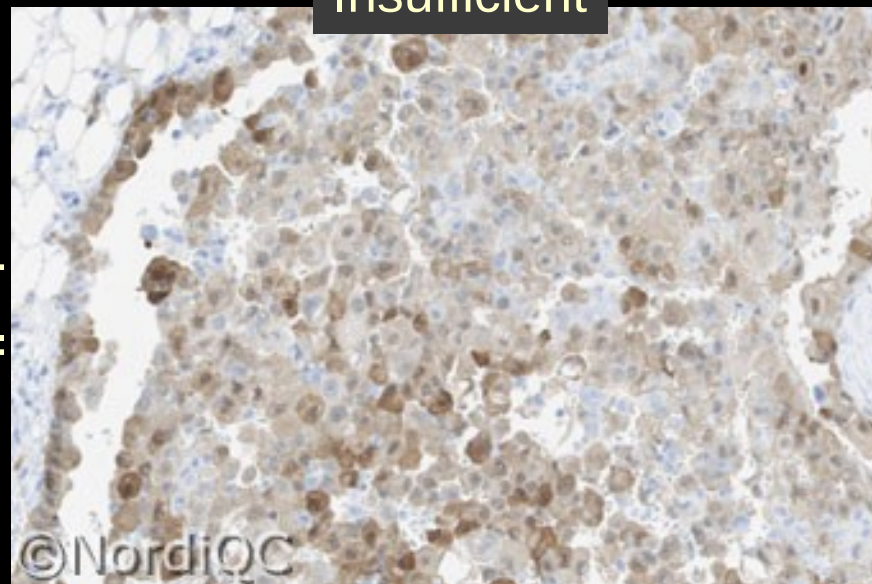
Optimal



Insufficient



mesothelioma



Calretinin

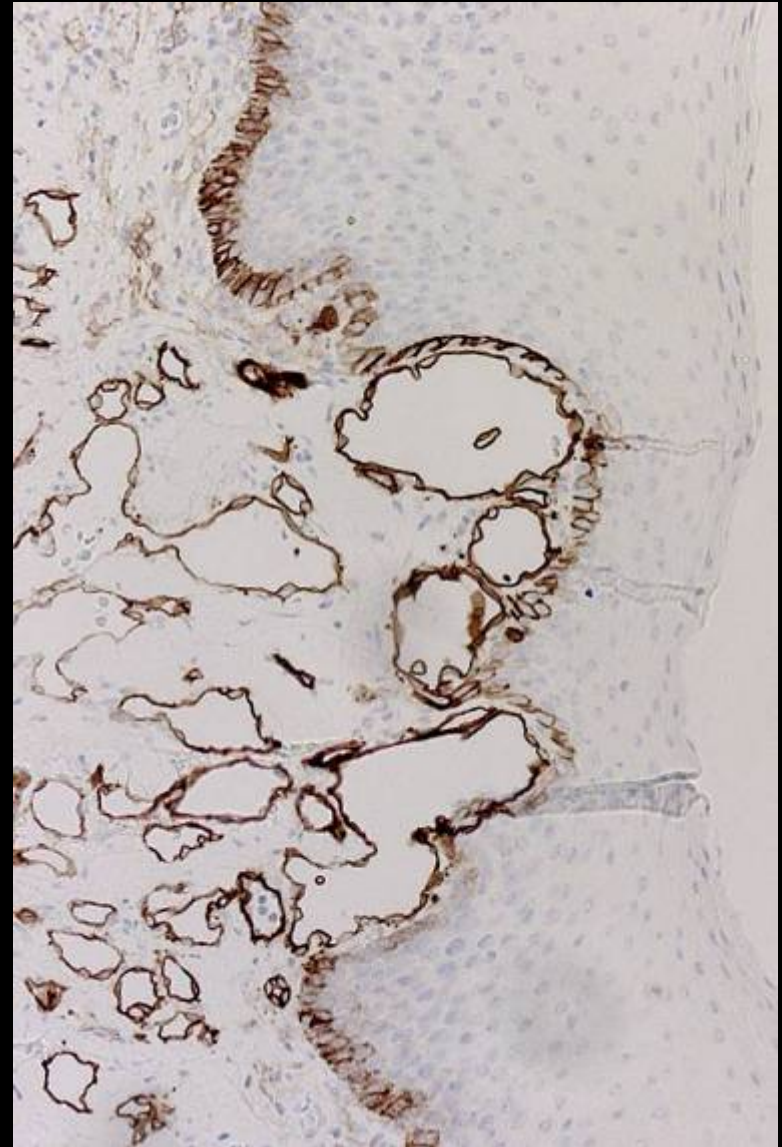
Adenocarcinoma

Optimal

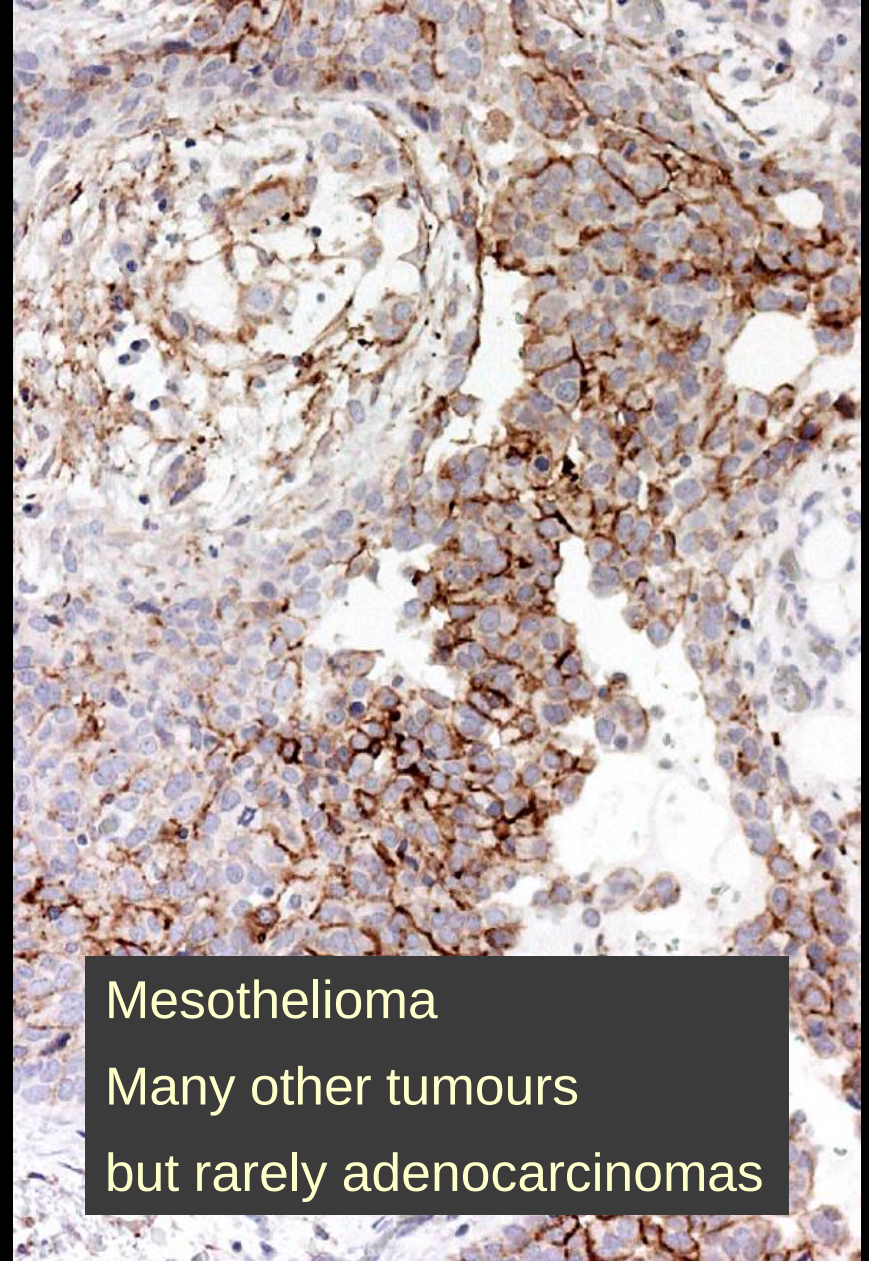
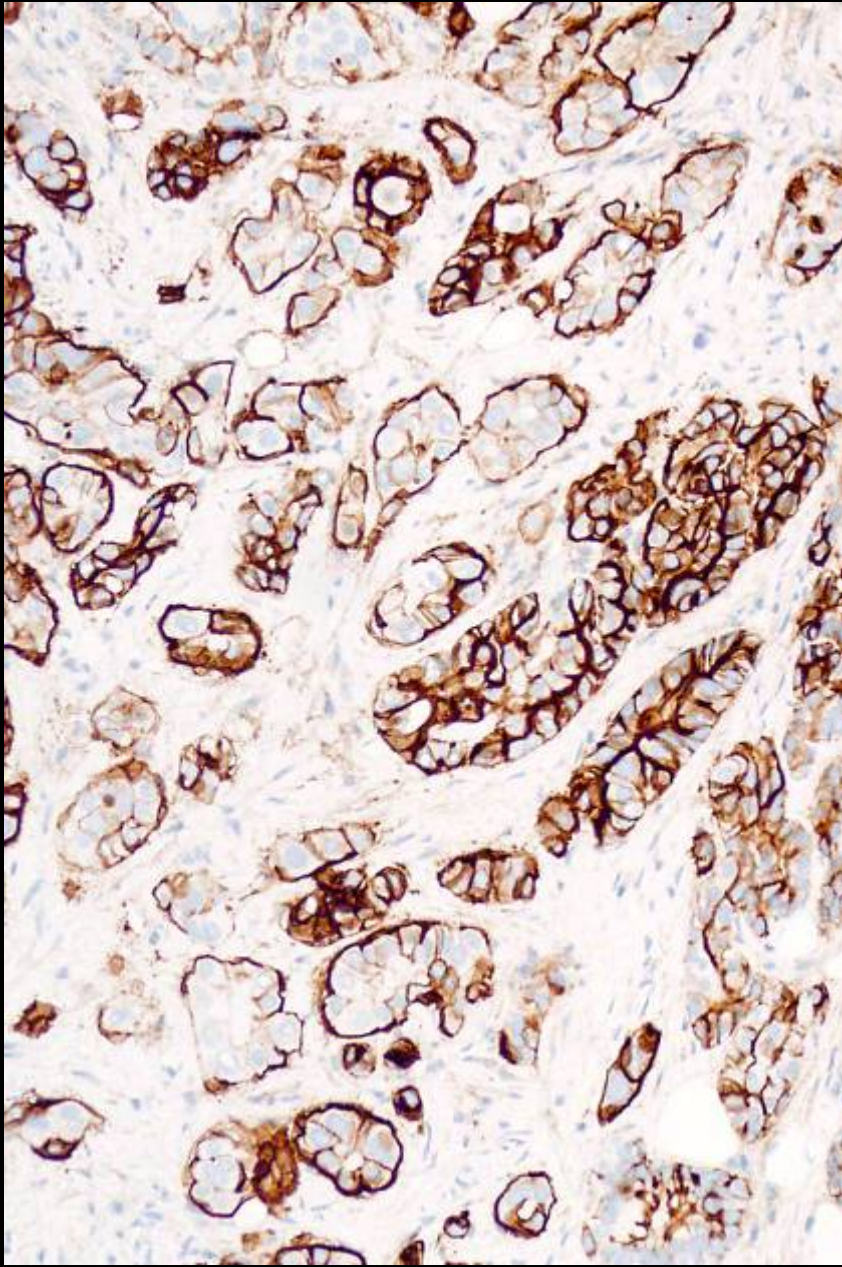
False positive

Podoplanin

- Lymphatic endothelium
- Fibroblasts, osteocytes
- Smooth and striated muscle cells
- Myoepithelial cells, Cajal cells
- Basal squamous epithelial cells
- Gastric crypt c., prostatic basal c.
- Immature Sertoli c. and gonocytes
- Renal glomerular podocytes
- Mesothelium (reactive)
- Follicular dendritic cells
- Some lymphocytes
- Glial/Schwann cells



Podoplanin



Mesothelioma
Many other tumours
but rarely adenocarcinomas

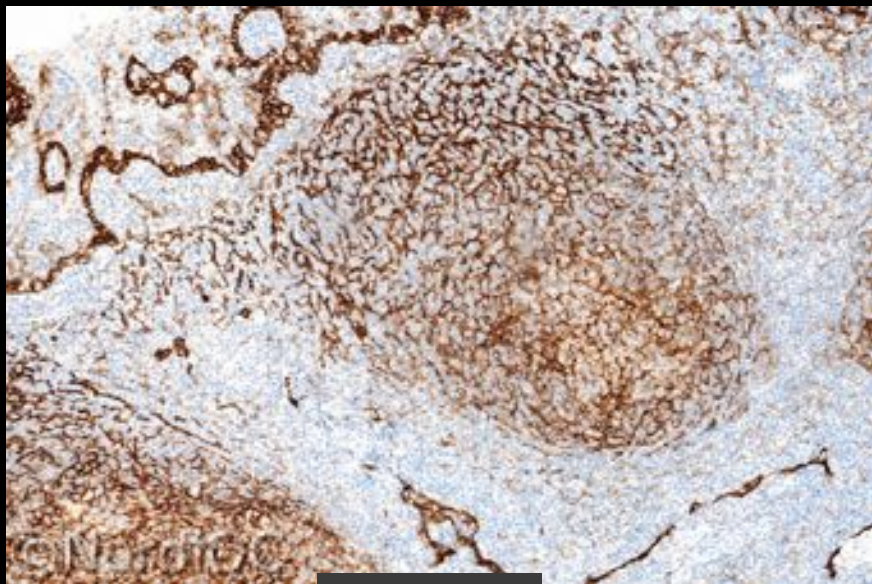
Podoplanin – Run 36/2012: 102 labs

Table 1. Abs and assessment marks for Podop, run 36

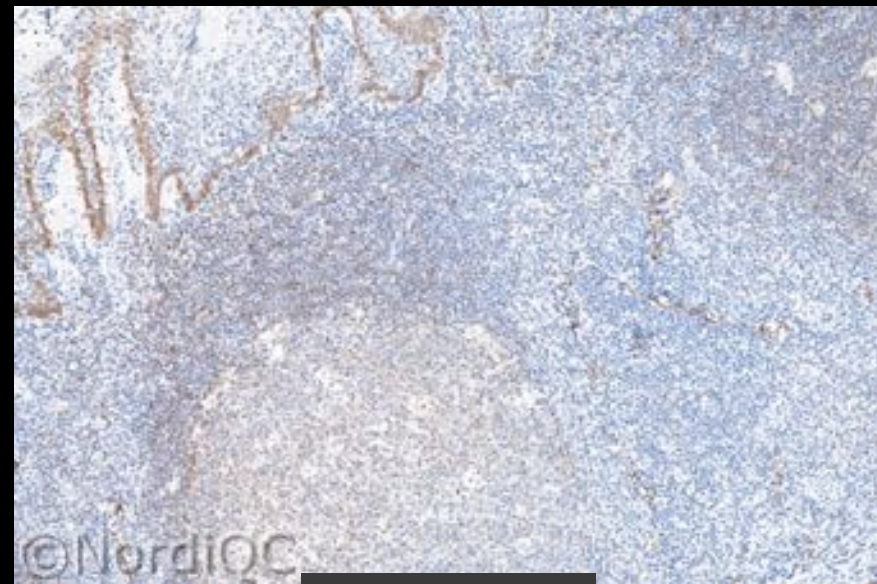
Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. OPS ²
mAb clone D2-40	48 3 2 1 1 1	Dako Signet Biocare Cell Marque Immunologic Zytomed	10	20	22	4	54 %	56 %
mAb clone AB3	1	AngioBio	0	1	0	0	-	-
mAb clone 18H51	1	Acris	0	0	0	1	-	-
rmAb clone EP215	1	Epitomics	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone D2-40 IS/IR072	15	Dako	11	4	0	0	100 %	100 %
mAb clone D2-40 N1607	3	Dako	0	3	0	0	-	-
mAb clone D2-40 760-4395	21	Ventana/Cell Marque	0	8	13	0	38 %	-
mAb clone D2-40 322M-17/18	2	Cell Marque	0	1	1	0	-	-
mAb clone D2-40 MON-RTU1092	1	Monosan	0	1	0	0	-	-
mAb clone D2-40 MAD-000402QD	1	Master Diagnostica	0	1	0	0	-	-
Total	102		21	39	37	5	-	
Proportion			21 %	38 %	36 %	5 %	59 %	

Ventana
platform

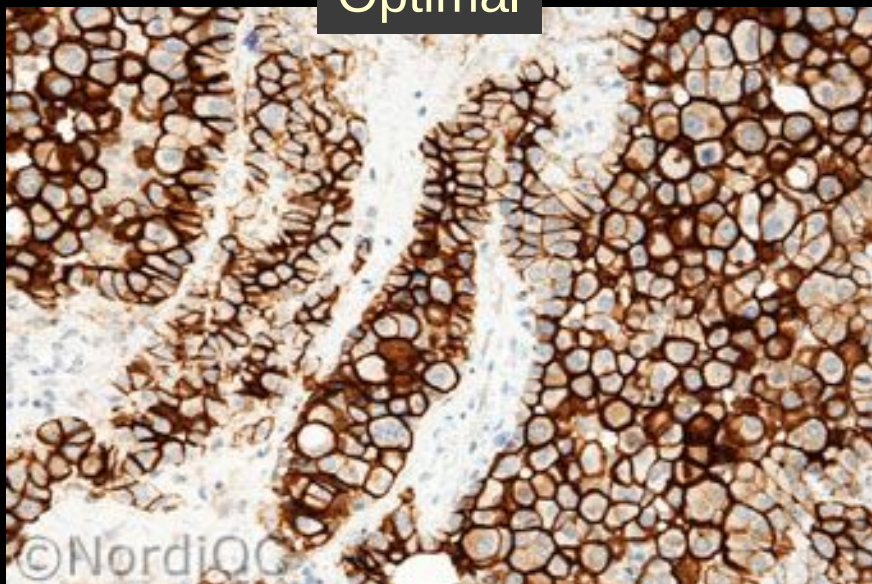
Ventana
platform



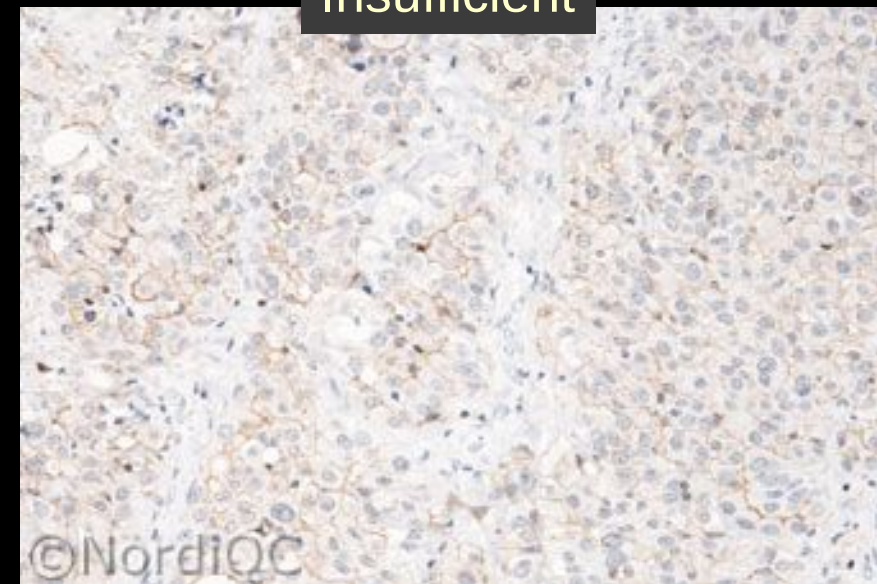
Optimal



Insufficient



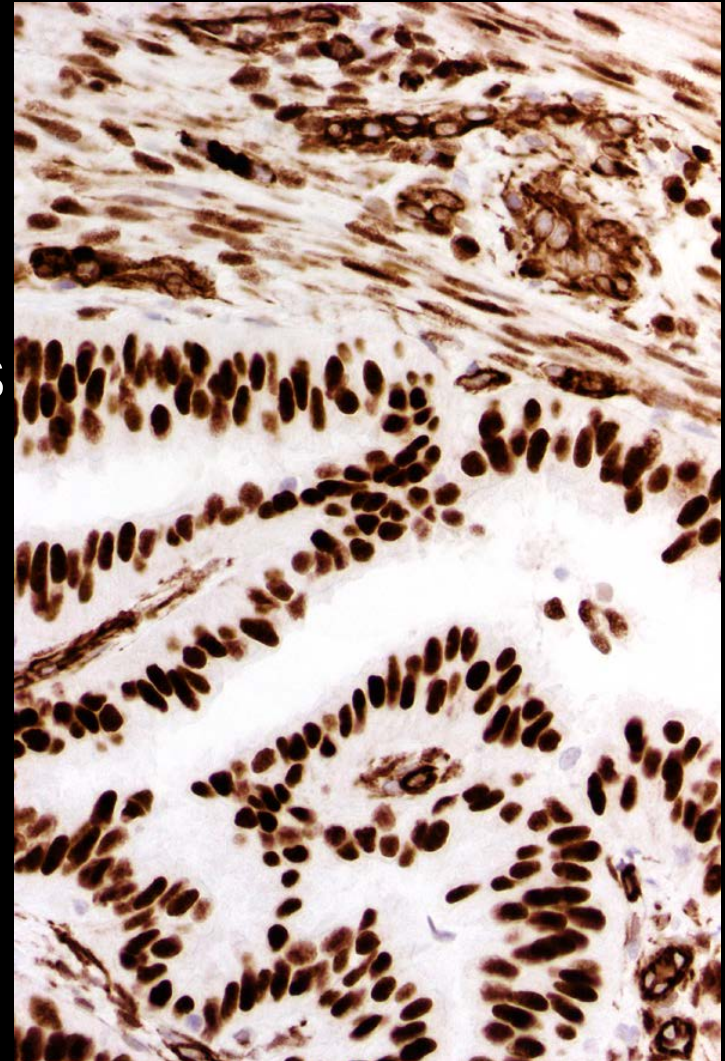
mesothelioma



Wilms' tumour-1 (WT1) protein

Transcription factor for development of the genitourinary system

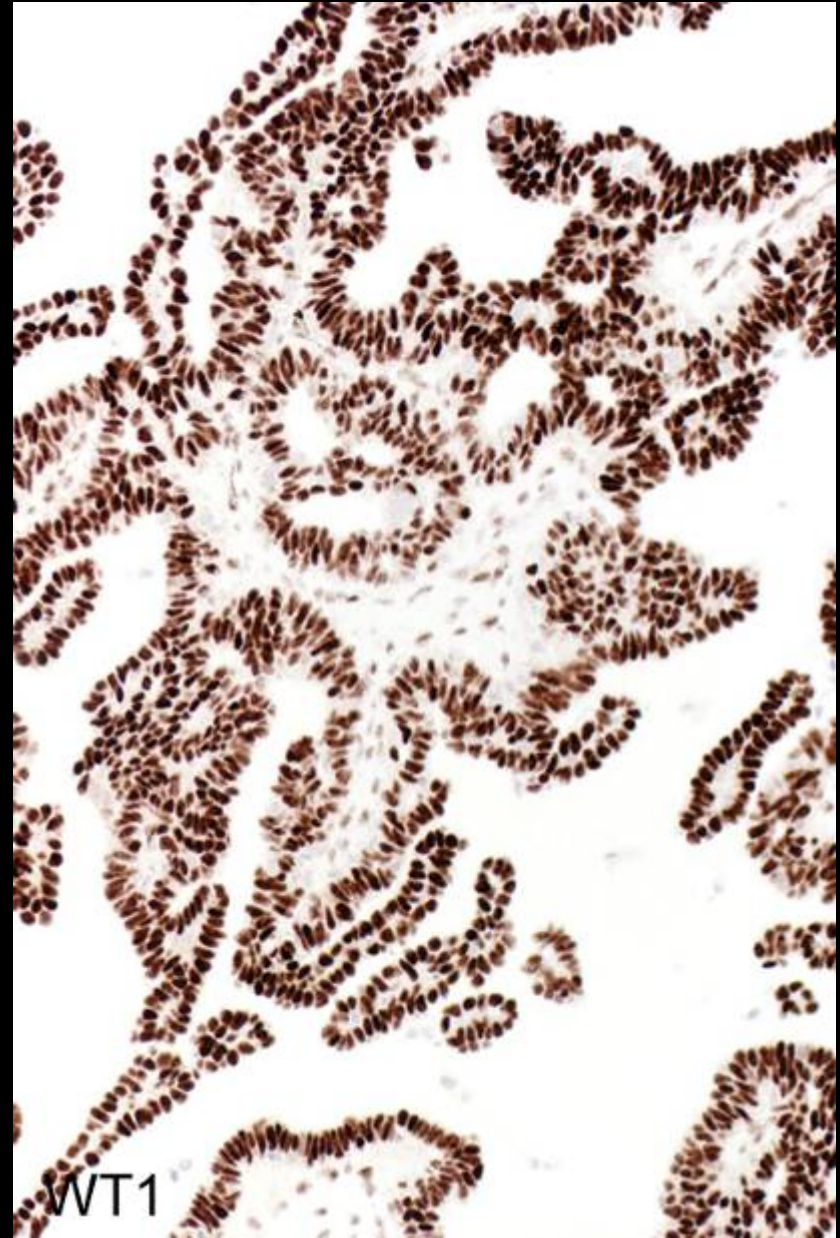
- Mesothelium
- Ovary (surface / inclusion cysts)
- Fallopian tube
- Female genital tract: stromal cells
- Bone marrow stem cells



Fallopian tube

Wilms' tumour-1 (WT1) protein in carcinomas/mesotheliomas

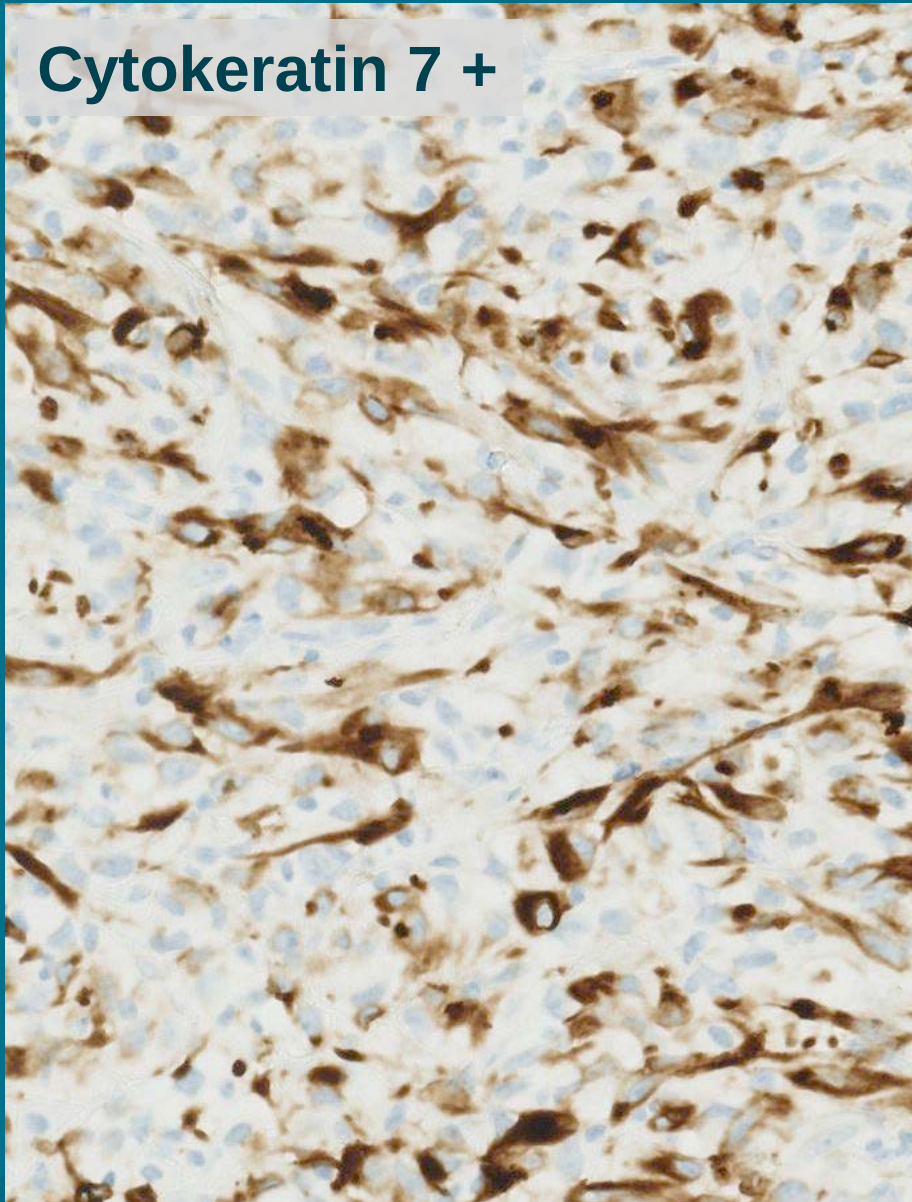
- Serous carcinoma +
- Endometrioid carcinoma +/-
- Other carcinomas -(+)
- Mal. mesothelioma +/-



Serous carcinoma WT1

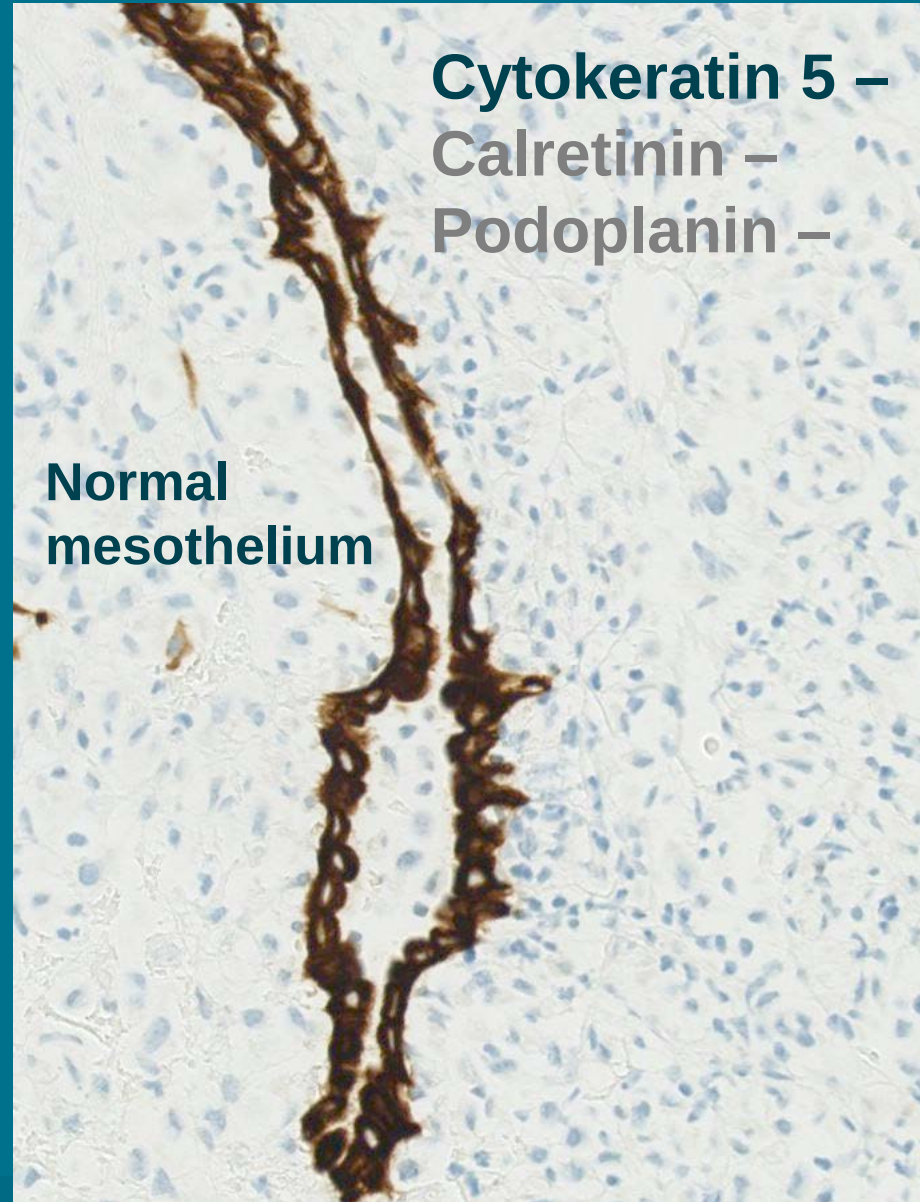
Malignant mesothelioma

Cytokeratin 7 +



Cytokeratin 5 –
Calretinin –
Podoplanin –

**Normal
mesothelium**



Malignant mesothelioma vs. lung carcinomas

	Calr	Pod	WT1	Vim	CA125	CK5	Ep-CAM	Cld4	NapA	TTF-1	p40
Epithelioid malignant mesothelioma	90	90	90	90	90	80	10 (f)	0	0	0	0
Lung Adenocarcinoma	10 (f)	10 (f)	20?	30	30	5?	95	100	80	80	0
Squamous cell carcinoma	50?	10	0	10?	15	95	95	95	10 (f)	15	95

% positivity
f: focal
?: insuf. data

General epithelial markers:

Epithelial cell adhesion molecule (EpCAM)

Claudin 4

EpCAM – Epithelial Cell Adhesion Molecule

“Epithelial specific antigen”: Glycoproteins located on the cell membrane surface (preferentially basolaterally) and in the cytoplasm of :

virtually all epithelial cells with the exception of

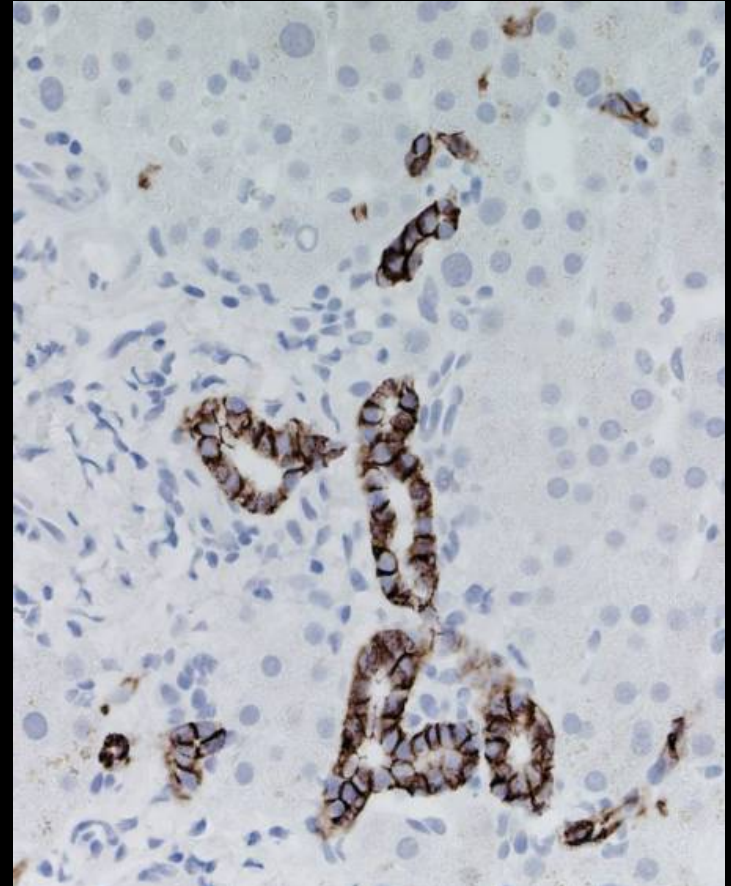
- squamous epithelia (mucosae and reactive focal pos.)
- hepatocytes (pos. in reactive)
- renal proximal tubular cells
- gastric parietal cells
- some endocrine cells

Also negative

- mesothelial cells (pos. in reactive)
- mesenchymal cells
- neural crest cells

(olfactory neurons are pos.)

Liver



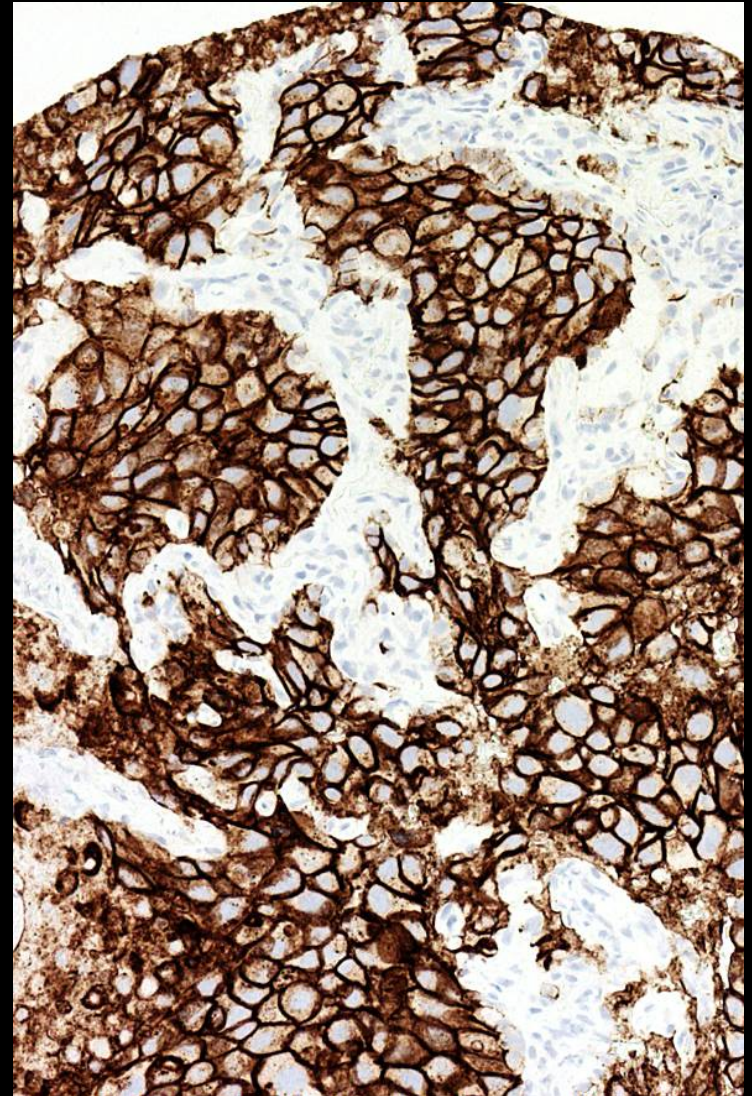
EpCAM

+(-)

- Adenocarcinomas (of most types)
- Neuroendocrine neoplasms

+/-

- Synovial sarcoma
- Brenner tumour
- Desmoplastic small cell tumour
- Olfactory neuroblastoma



Endocrine carcinoma

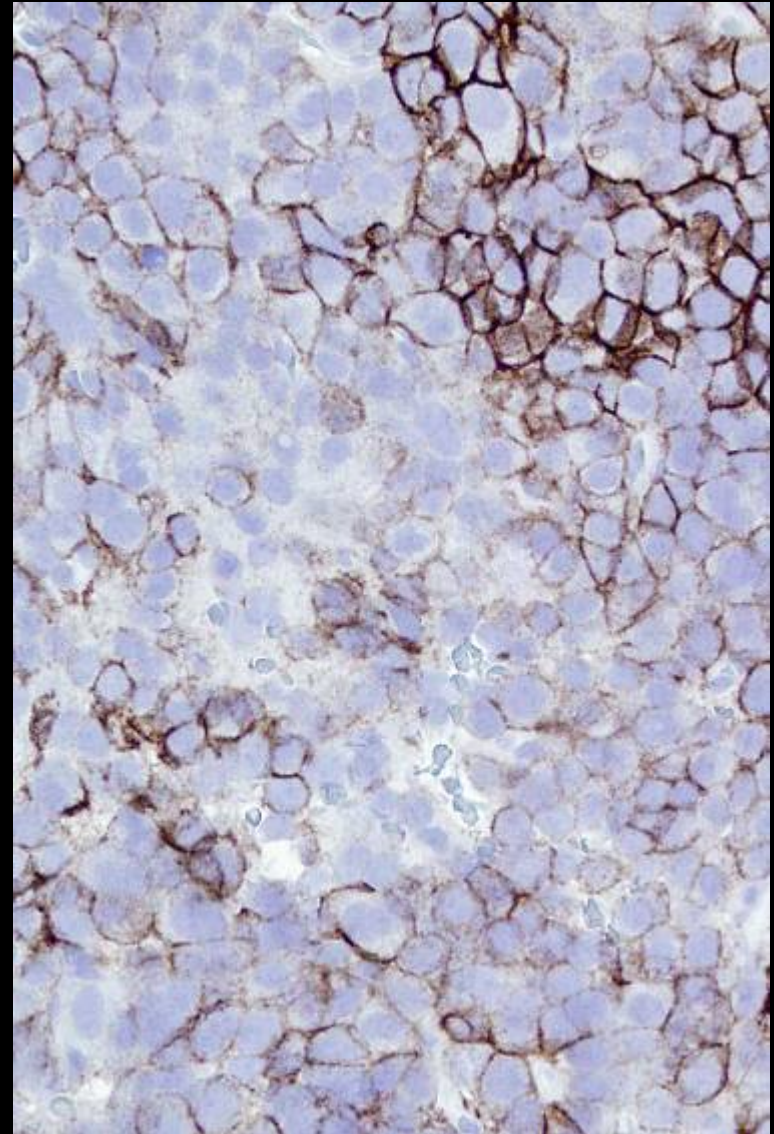
EpCAM

-/+

- Lobular breast carcinoma
- Hepatocellular carcinoma
- Squamous cell carcinoma
- Renal cell carcinoma
- Malignant mesothelioma
- Embryonal carcinoma, seminoma

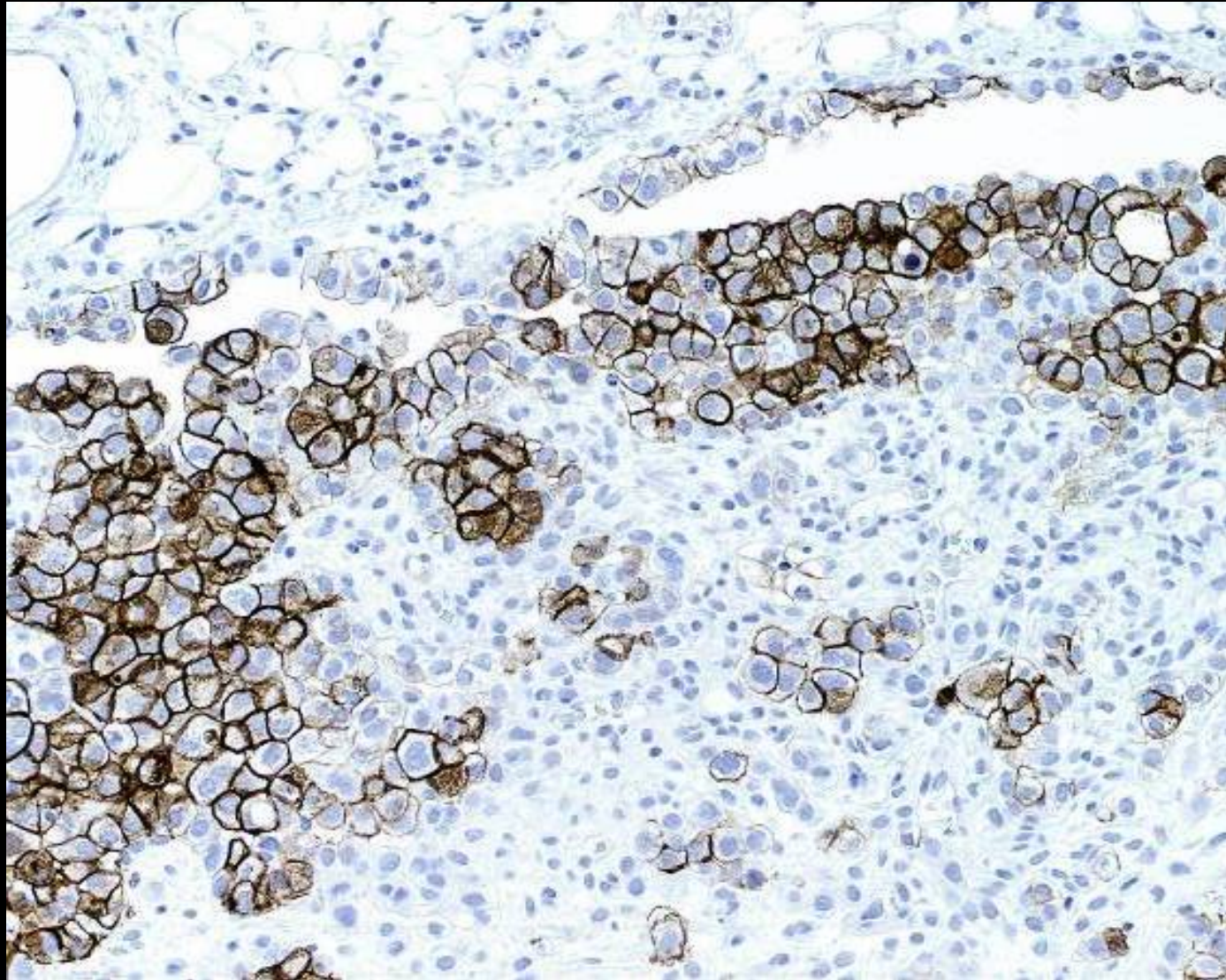
-

- Adrenal cortical carcinoma
- Choroid plexus pap./carcinoma
- Sarcomas (synovial & epithelioid sarc. +)
- Lymphomas



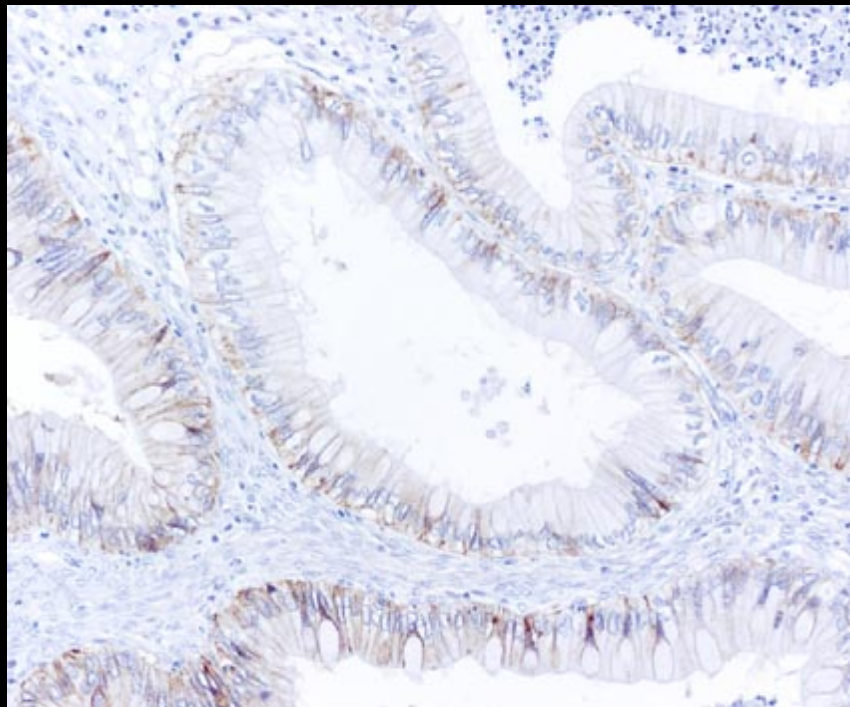
HCC

EpCAM

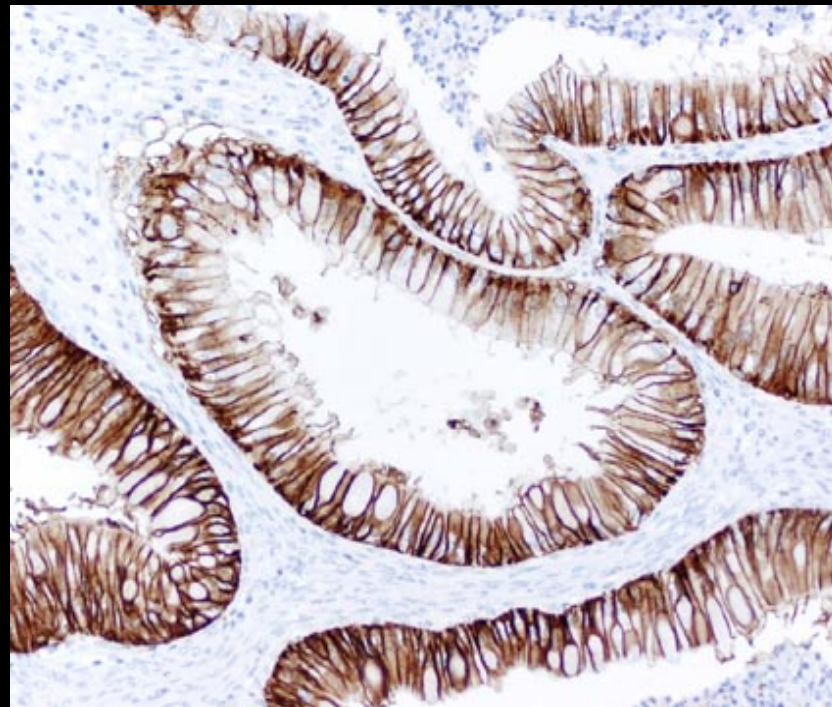


Malignant mesothelioma

Colon adenocarcinoma (Ber-EP4)



Lab A



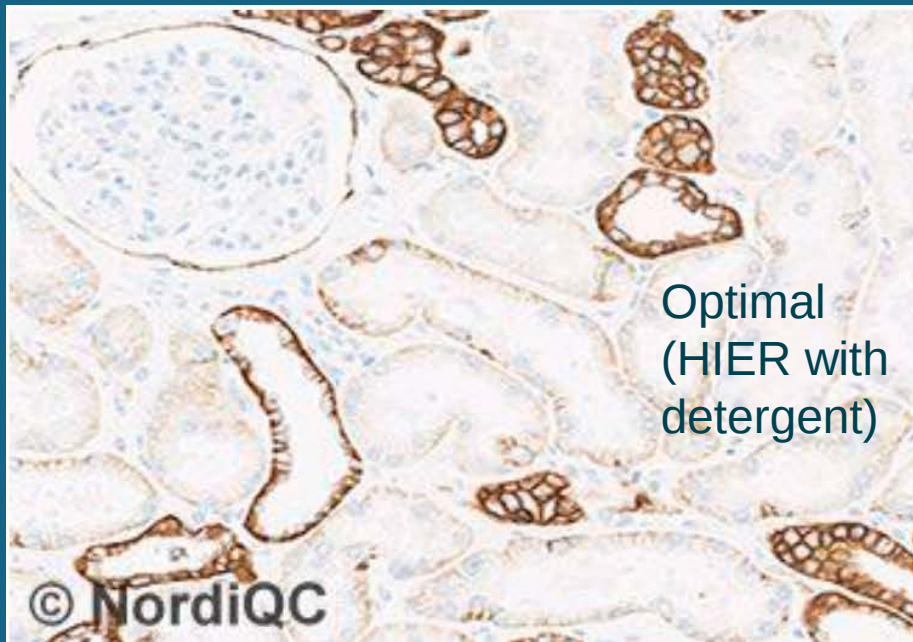
Lab B

Retrieval: TRS pH 6.1 DAKO

Diva2 pH 6.2 Thermo

EpCAM

Ready-To-Use antibodies								
mAb clone Ber-Ep4 760-4383	36	Ventana/Cell Marque	0	6	21	9	17%	-
mAb clone Ber-Ep4 IR/IS637	19	Dako	4	12	1	2	84%	100%
mAb clone Ber-Ep4 GA637	9	Dako	7	1	1	0	89%	100%



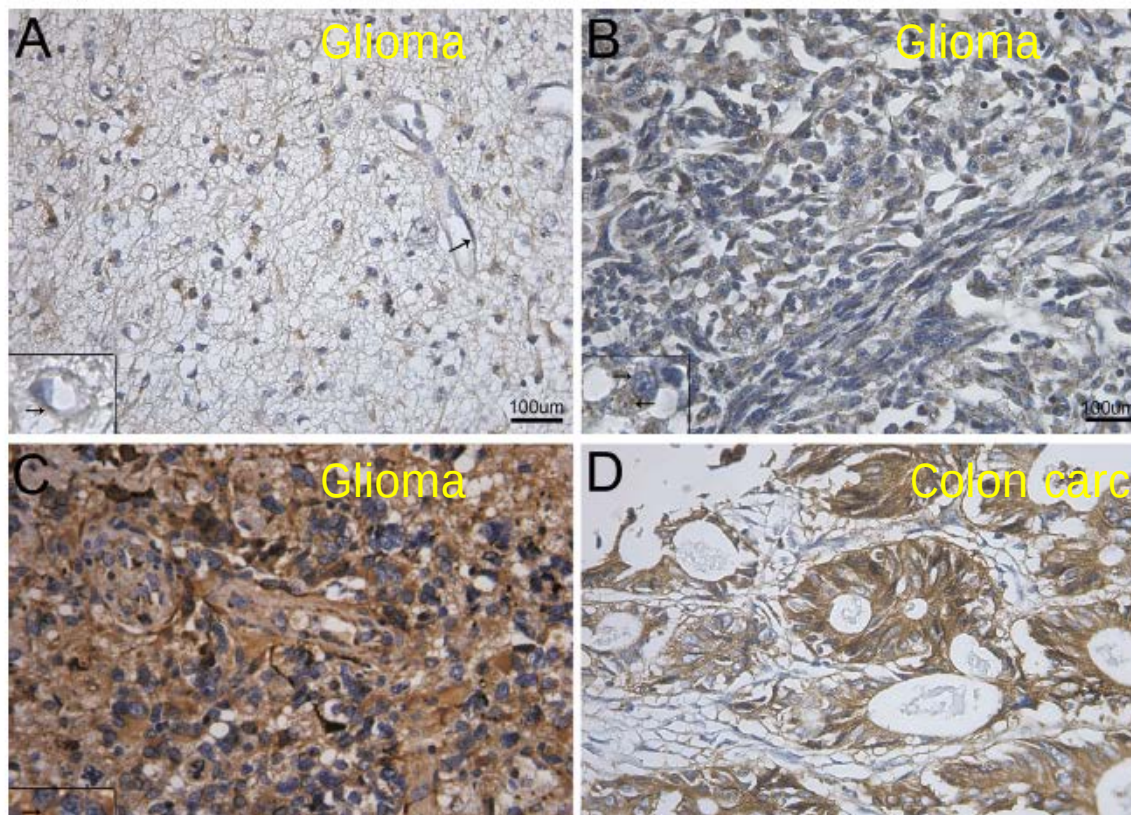
J Neurooncol (2014) 119:39–47
DOI 10.1007/s11060-014-1459-5

1' publication with this finding

LABORATORY INVESTIGATION

The overexpression of Epithelial cell adhesion molecule (EpCAM) in glioma

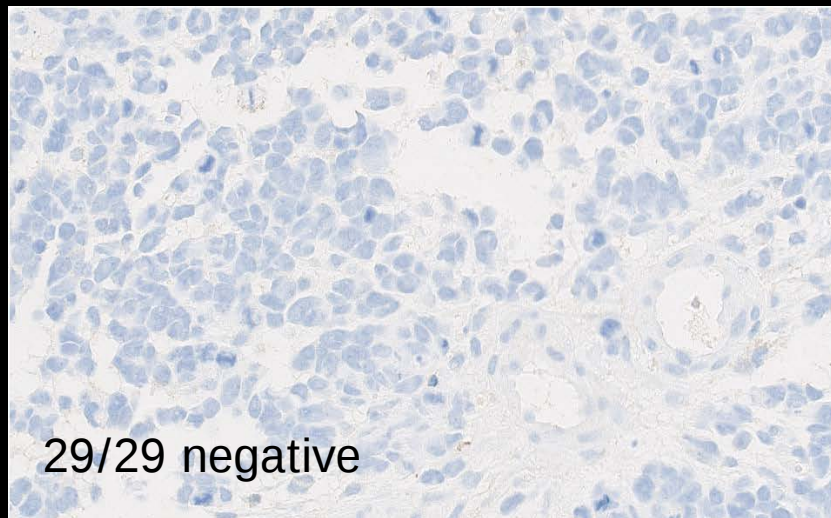
Xin Chen · Wei-Yuan Ma · Shang-Chen Xu · Yu Liang · Yi-Bing Fu ·
Bo Pang · Tao Xin · Hai-Tao Fan · Rui Zhang · Jian-Gang Luo ·
Wen-Qing Kang · Min Wang · Qi Pang



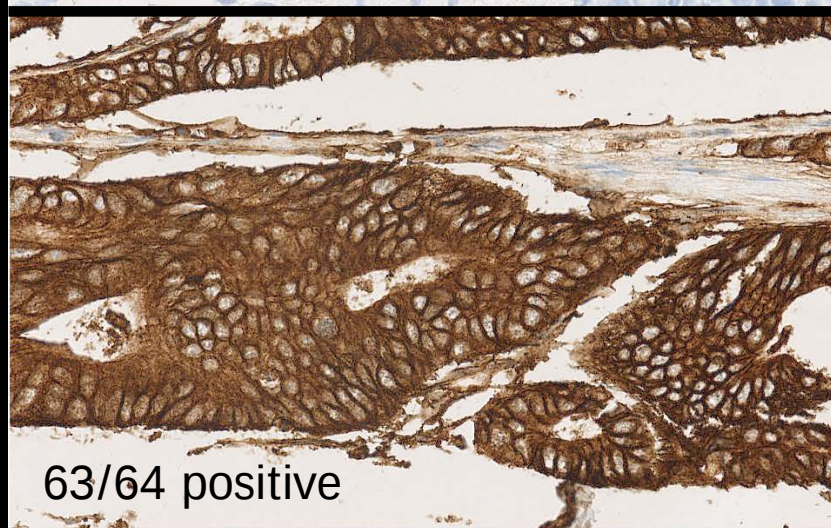
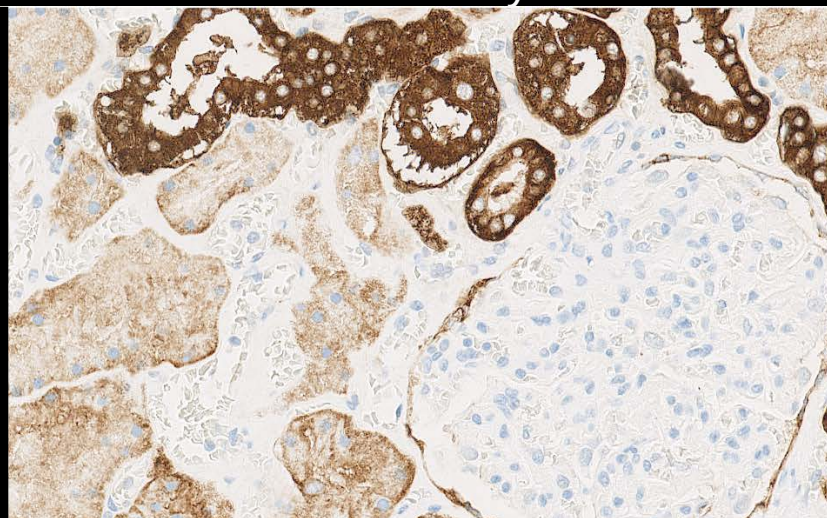
IHC – Biomarker controls

Reference - NordiQC: Ber-EP4: 1:50, HIER TRS pH 6.1, 3-step polymer

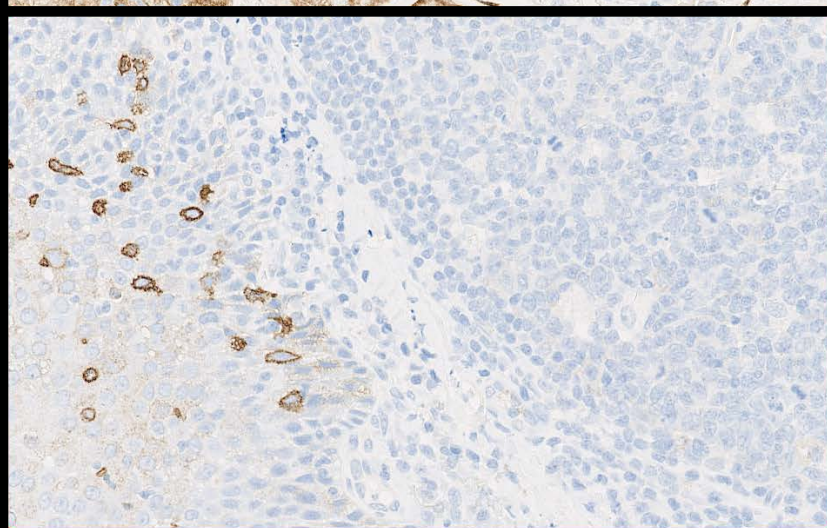
Glioblastoma



Kidney



Colon adenocarc.

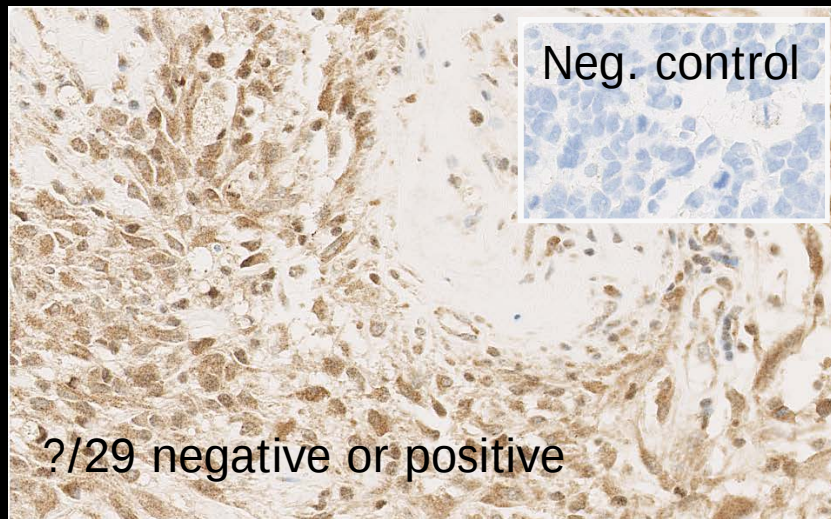


Tonsil

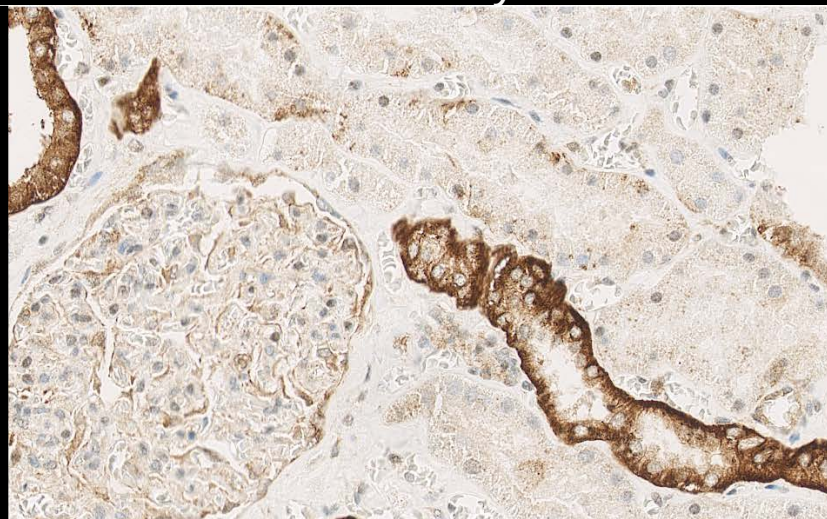
IHC – Biomarker controls

Study protocol: Abcam ab 71916: **1:100**, HIER TRS pH 6.1, 3-step polymer

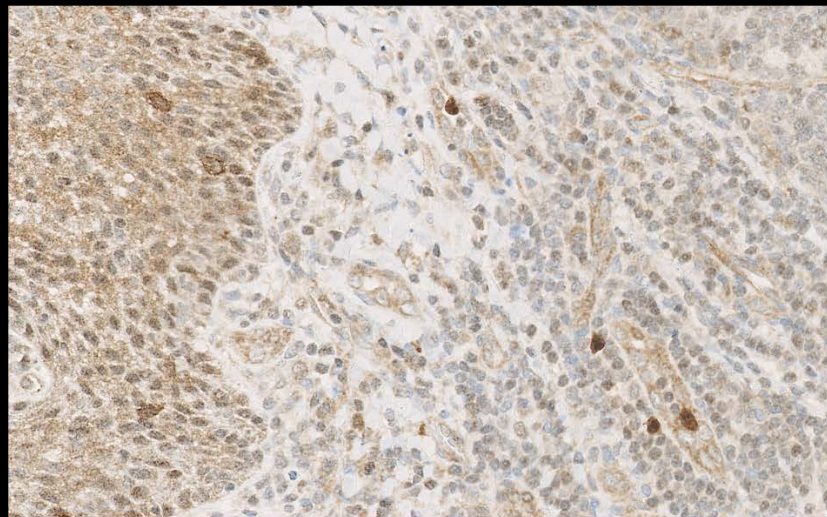
Glioblastoma



Kidney

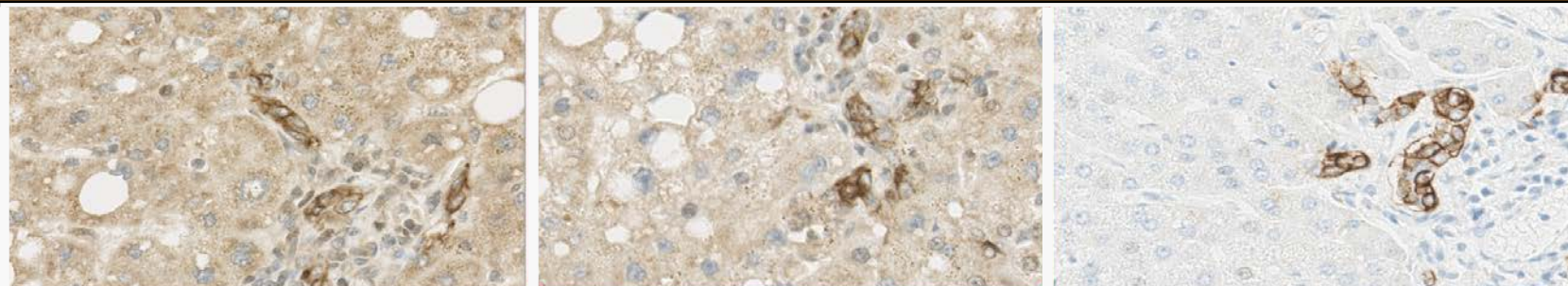


Colon adenocarc.

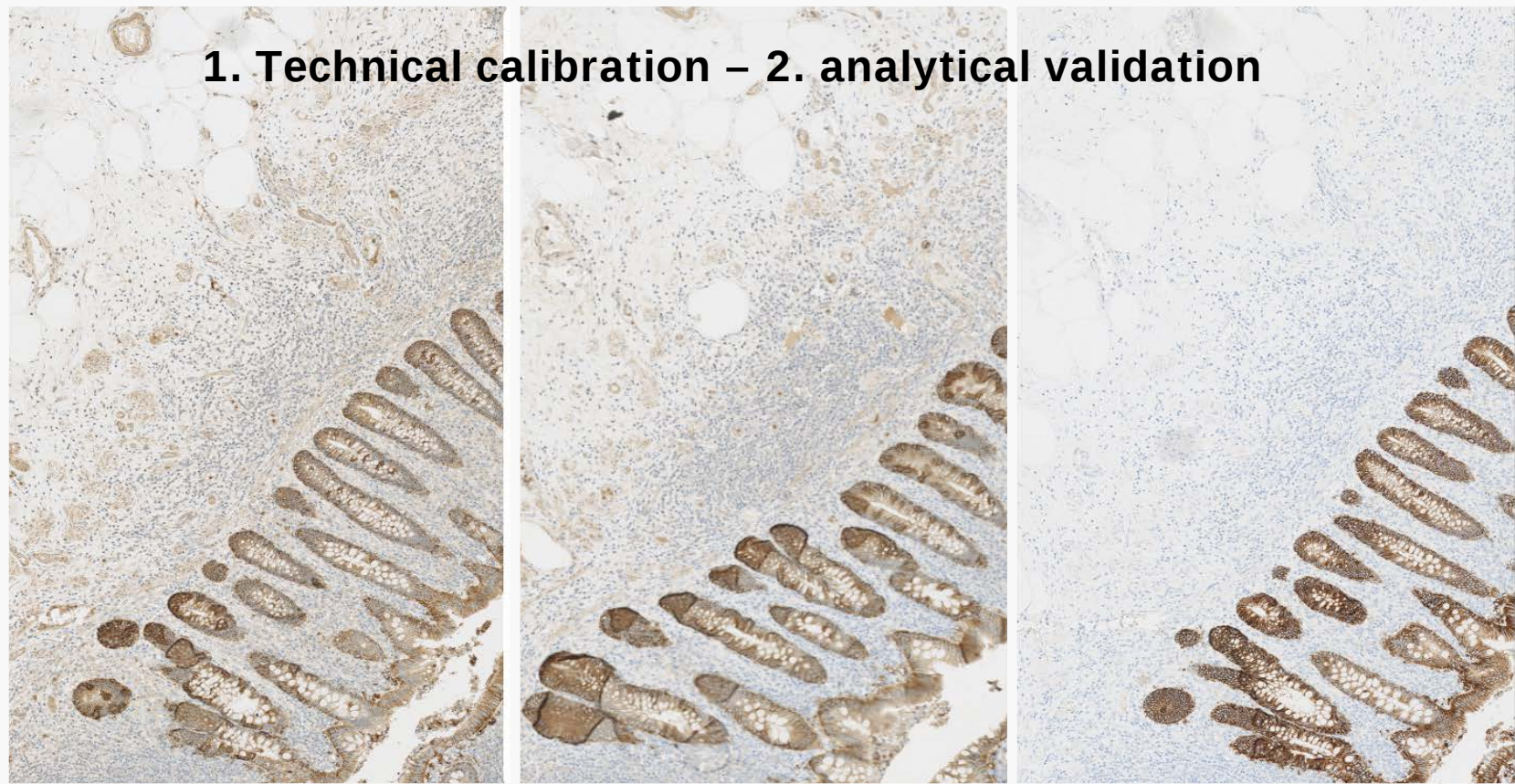


Tonsil

IHC – Biomarker controls



1. Technical calibration – 2. analytical validation



1:100

1:250

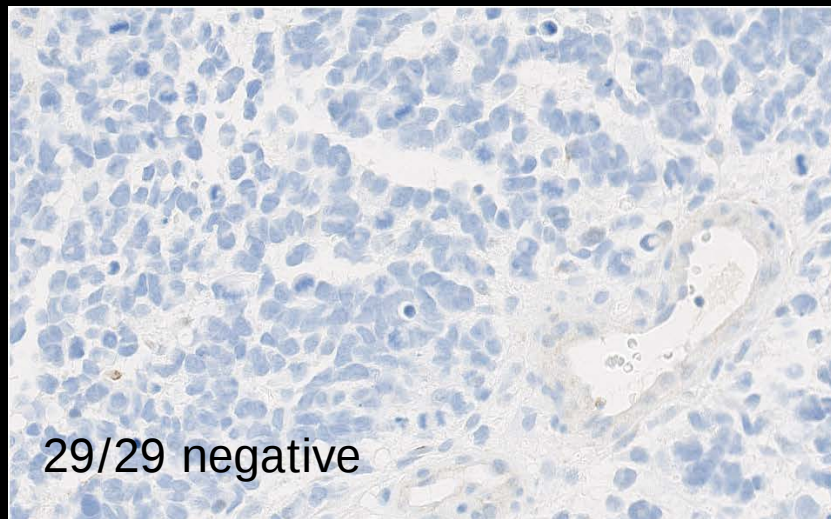
1:600

pAb ab71916 – 20 min. RT – HIER 20 min. Low pH – 3-step pol.

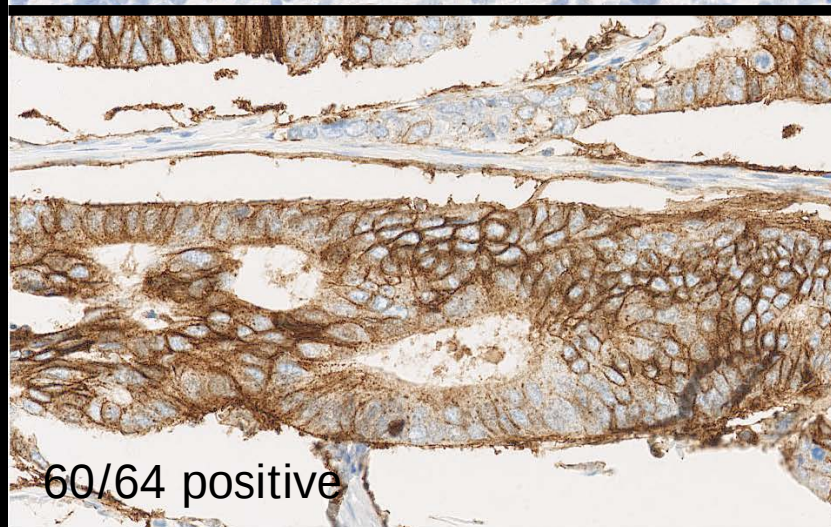
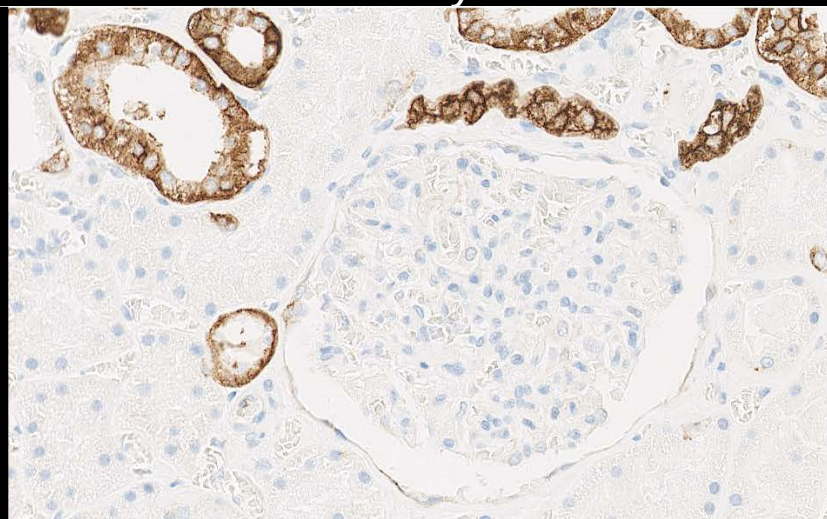
IHC – Biomarker controls

Adjusted protocol: Abcam ab 71916: 1:600, HIER TRS pH 6.1, 3-step polymer

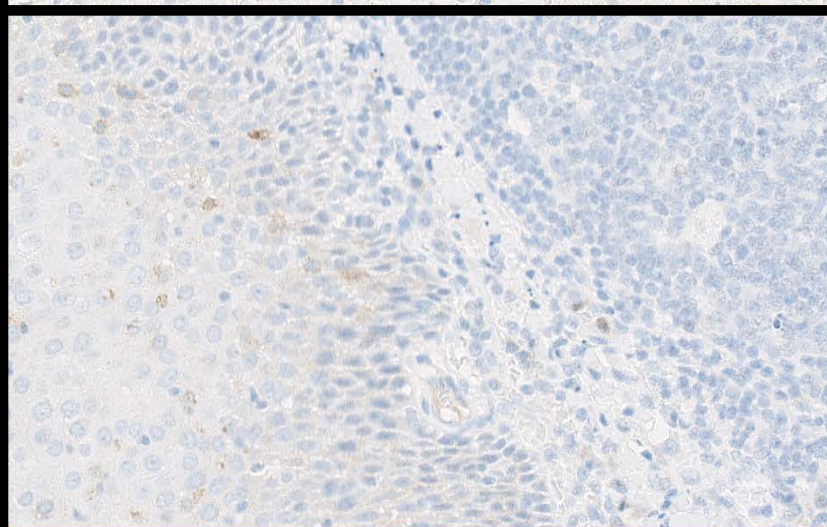
Glioblastoma



Kidney

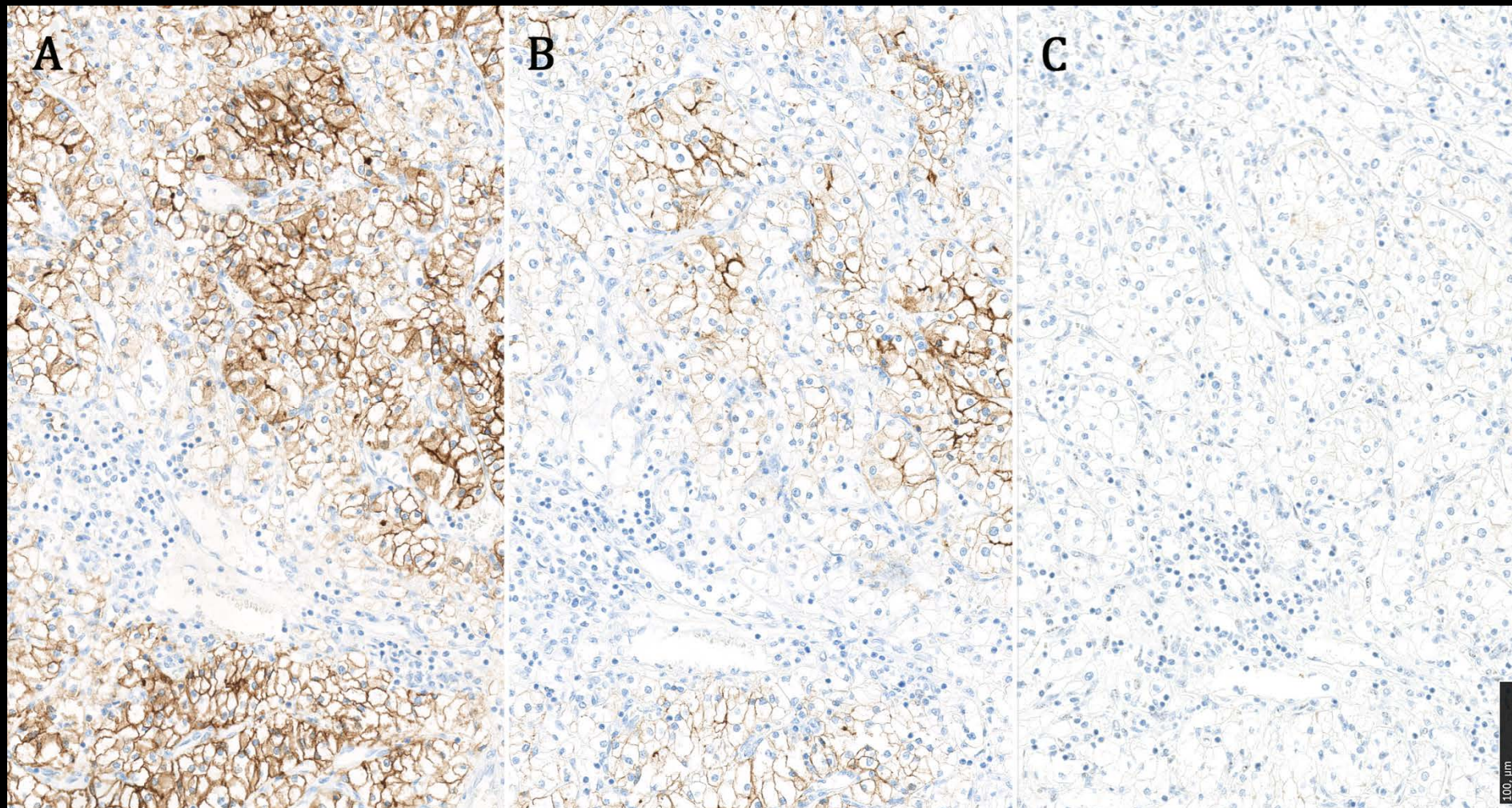


Colon adenocarc.



Tonsil

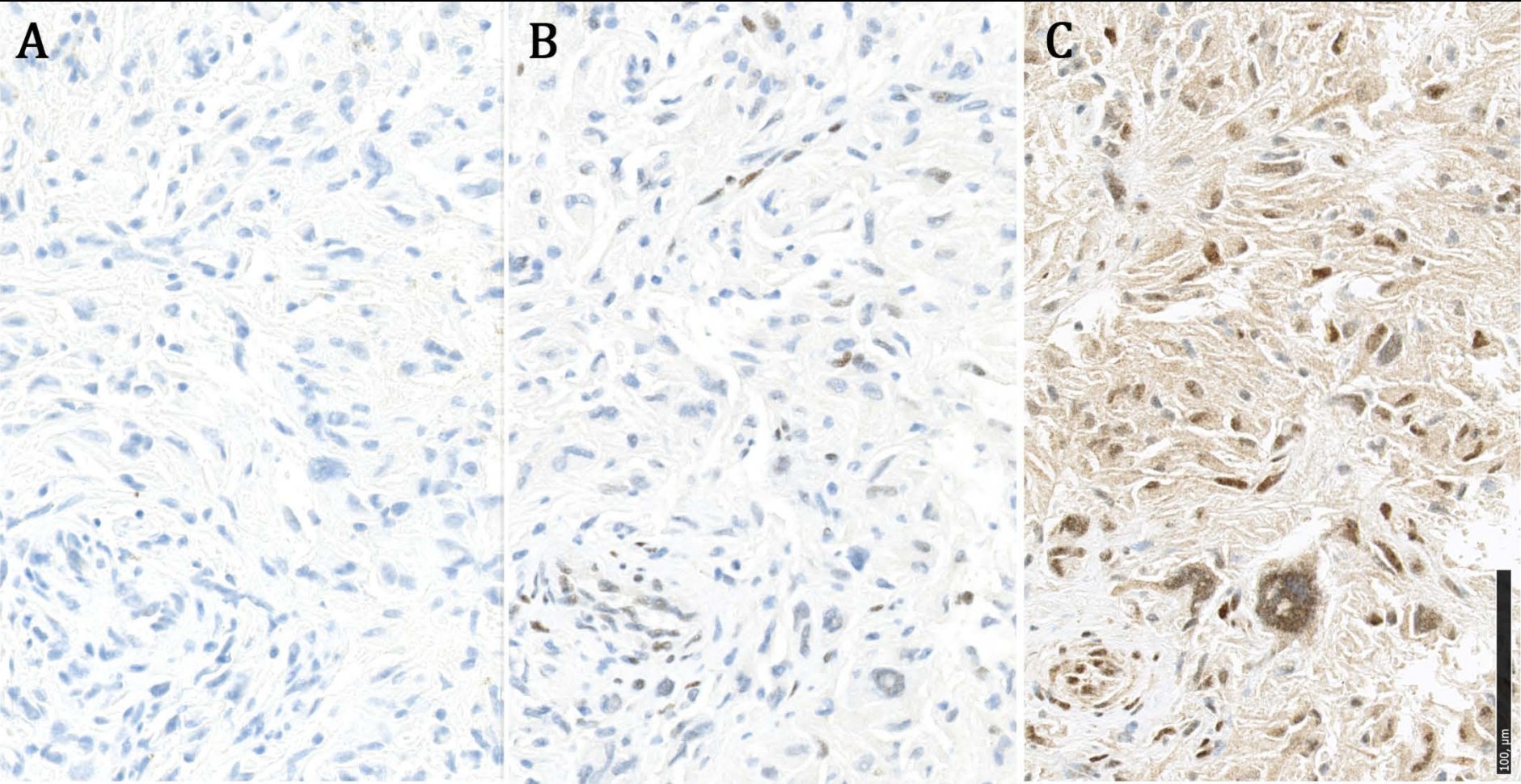
EpCAM antibodies – renal cell carcinoma



SPM491

Ber-EP4

Ab71916



SPM491

Ab71916
optimized

Ab71916
published



With CC1 buffer

Table 1. Antibodies and assessment marks for Ep-CAM, run 45

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 9C4	1	BioLegend	0	0	0	1	-	-
mAb clone BS14	2	Nordic Biosite	2	0	0	0	-	-
mAb clone C-10	1	Santa Cruz Biotech	0	0	1	0	-	-
mAb clone Ber-Ep4	77	Dako					31%	89%
	2	Diagnostic BioSystems	9	16	38	18		
	2	Thermo/NeoMarkers						
mAb clone MOC-31	19	Dako					63%	100%
	3	Leica/Novocastra	9	6	6	3		
	1	Cell Marque						
	1	Monosan						
mAb clone VU-1D9	3	Novocastra					75%	75%
	3	Thermo/LabVision	3	3	2	0		
	1	Merck Millipore						
	1	Thermo/Pierce						
mAb clone E144	1	Abcam	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone Ber-Ep4 760-4383	36	Ventana/Cell Marque	0	6	21	9	17%	-
mAb clone Ber-Ep4 IR/IS637	19	Dako	4	12	1	2	84%	100%
mAb clone Ber-Ep4 GA637	9	Dako	7	1	1	0	89%	100%

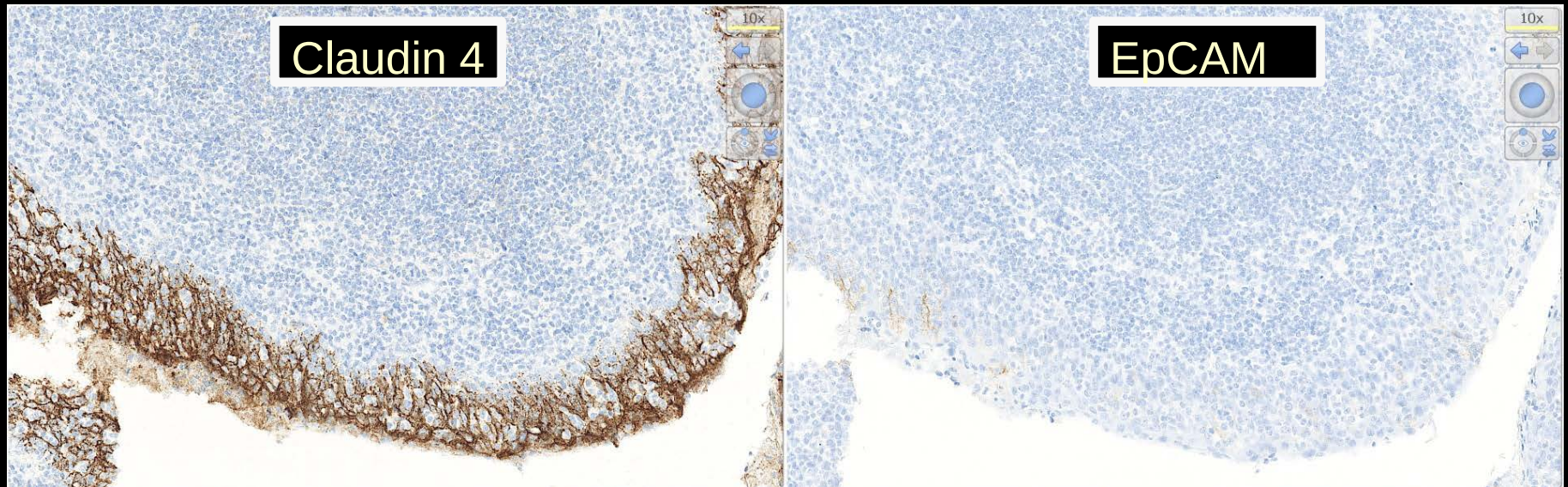
Claudin 4

Integral membrane protein, which belongs to the claudin family.

The protein is a component of **tight junction** strands and may play a role in internal organ development and function.

Highly sensitive for epithelial neoplasms

Not found in mesothelioma



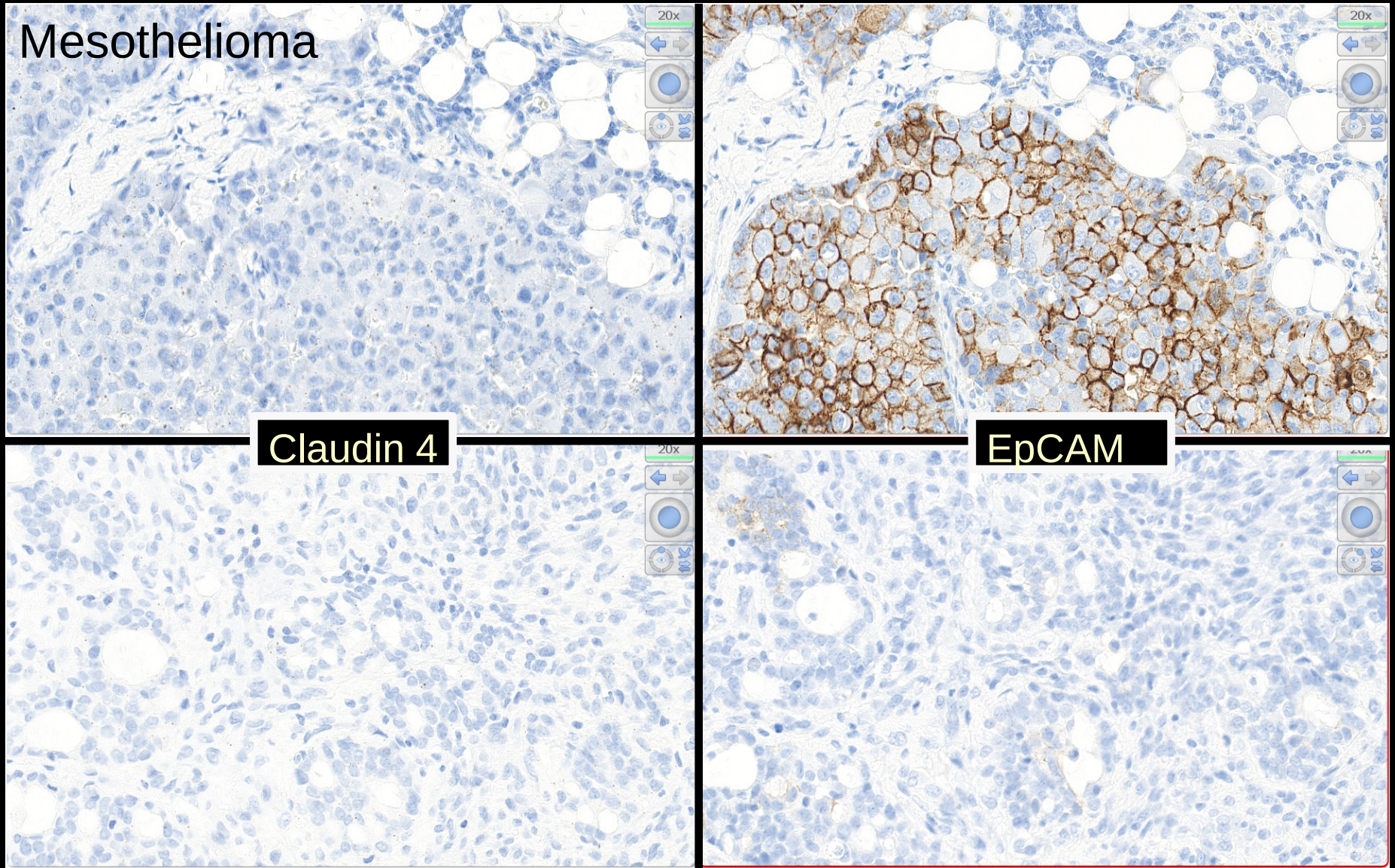
Tonsil

Claudin 4 vs. EpCAM

Mesothelioma

Claudin 4

EpCAM



“Lung markers”

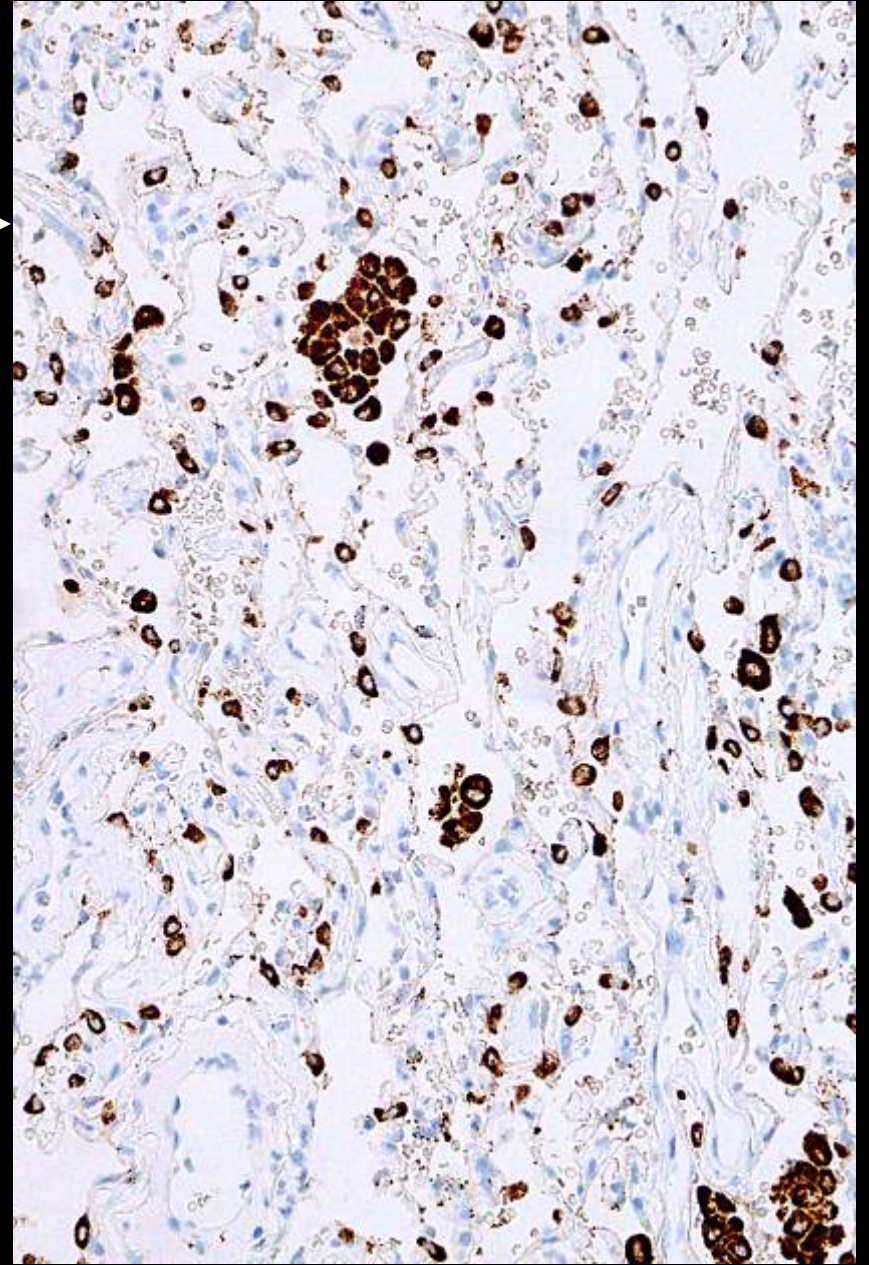
Napsin

TTF-1

Napsin A

Aspartic proteinase

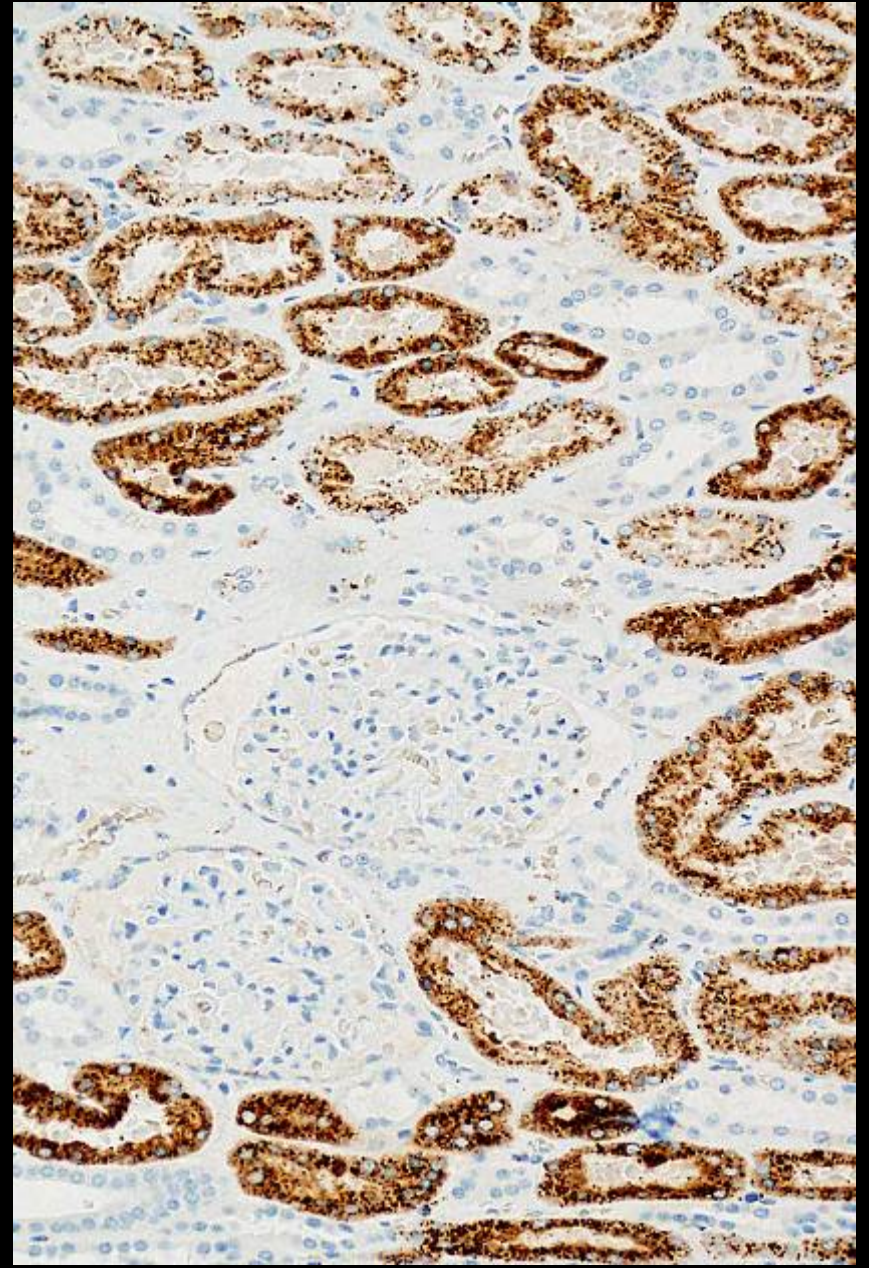
- Type II pneumocytes →
- Proximal and convoluted renal tubules
- (Pancreatic acini and ducts)



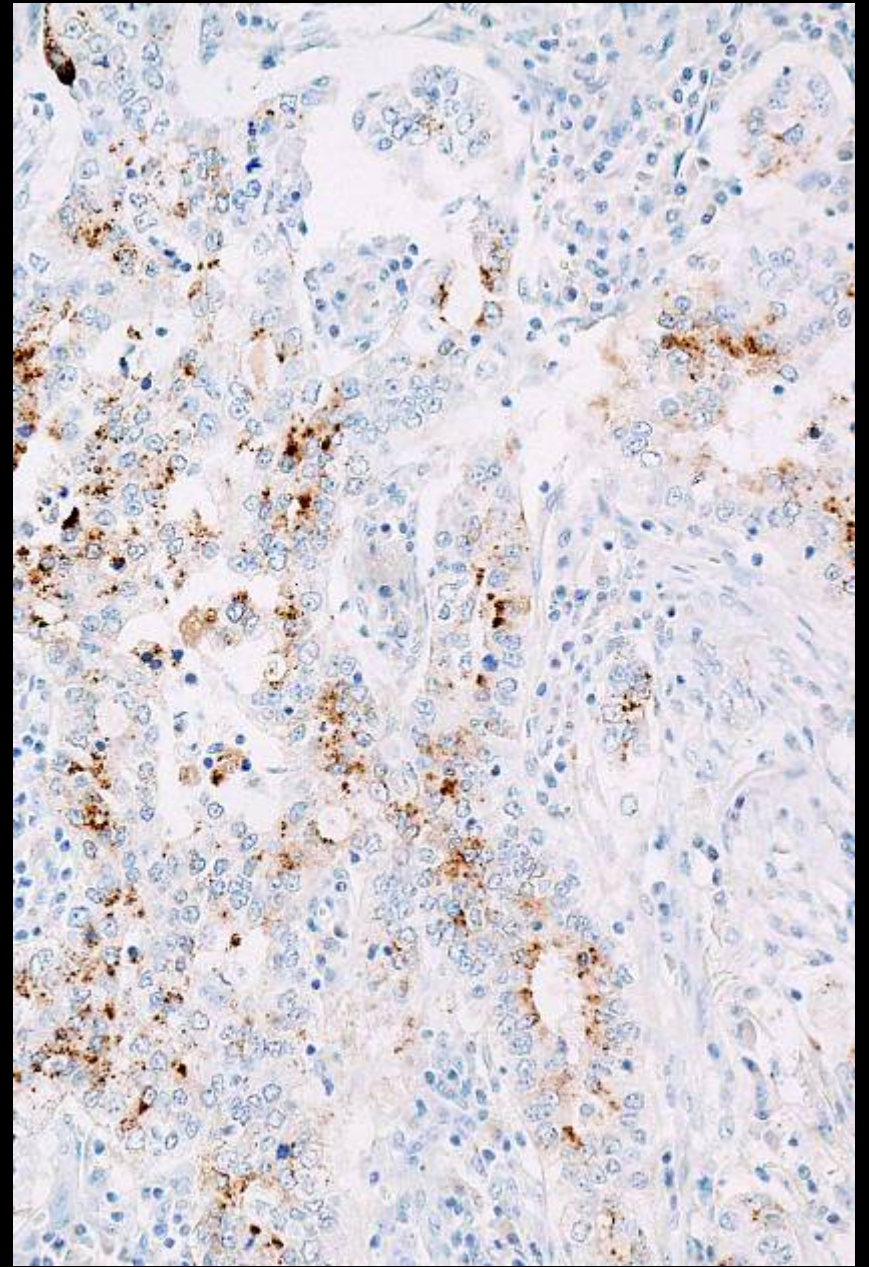
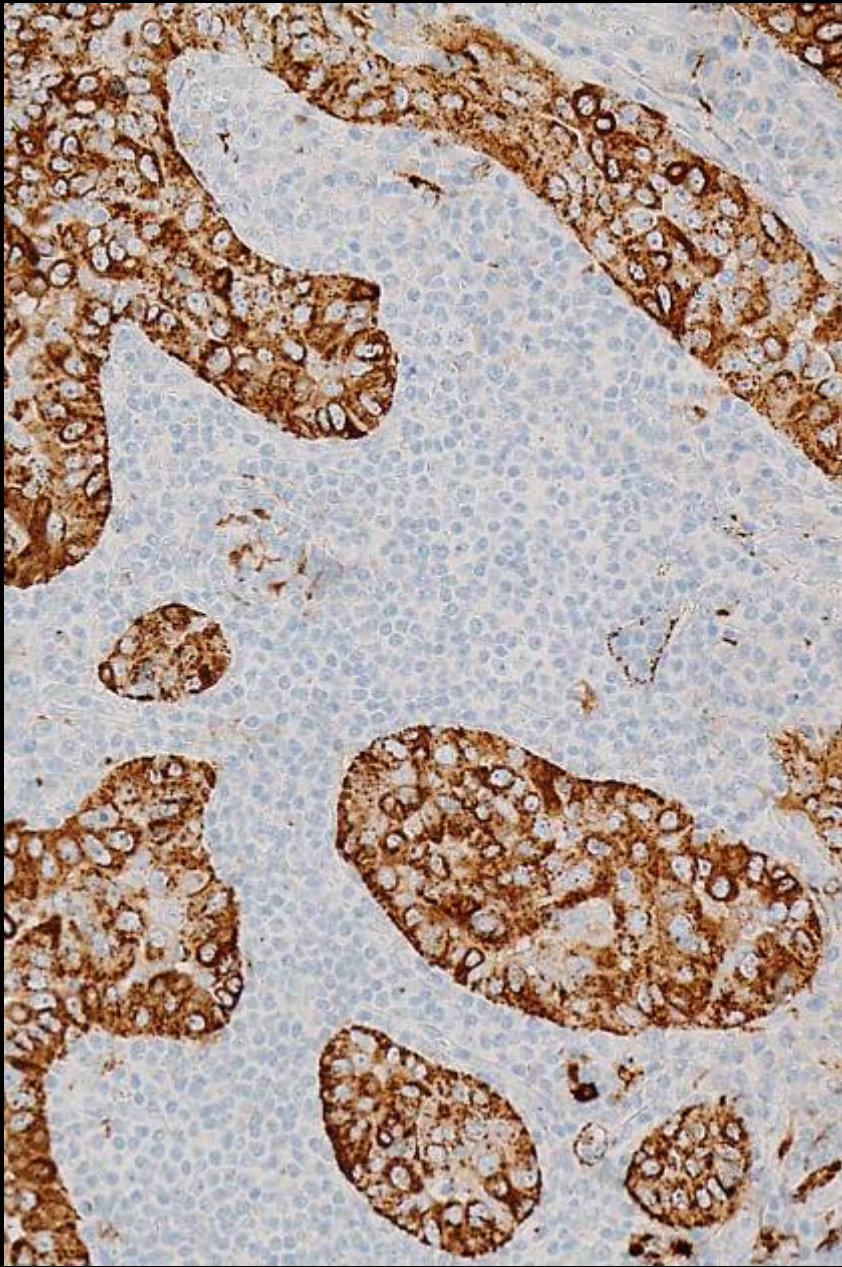
Napsin A

Aspartic proteinase

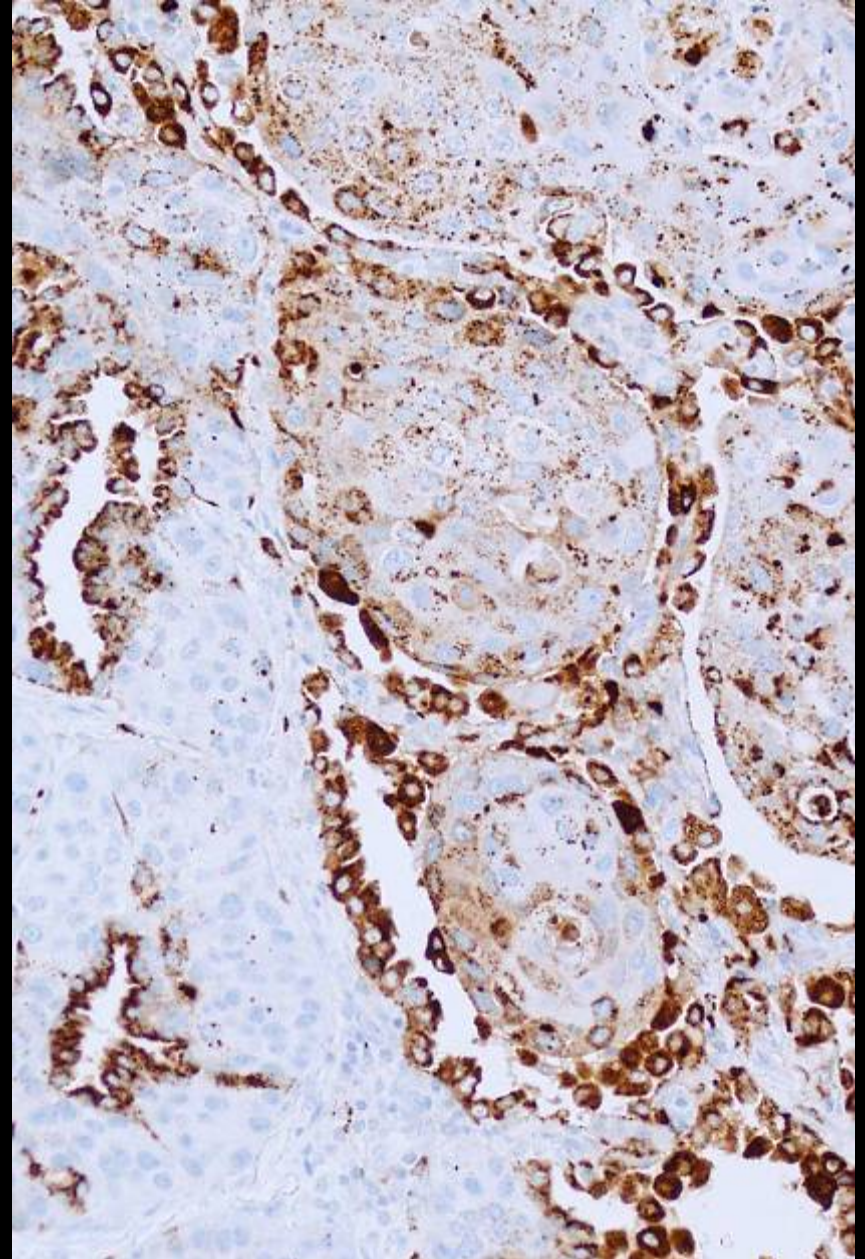
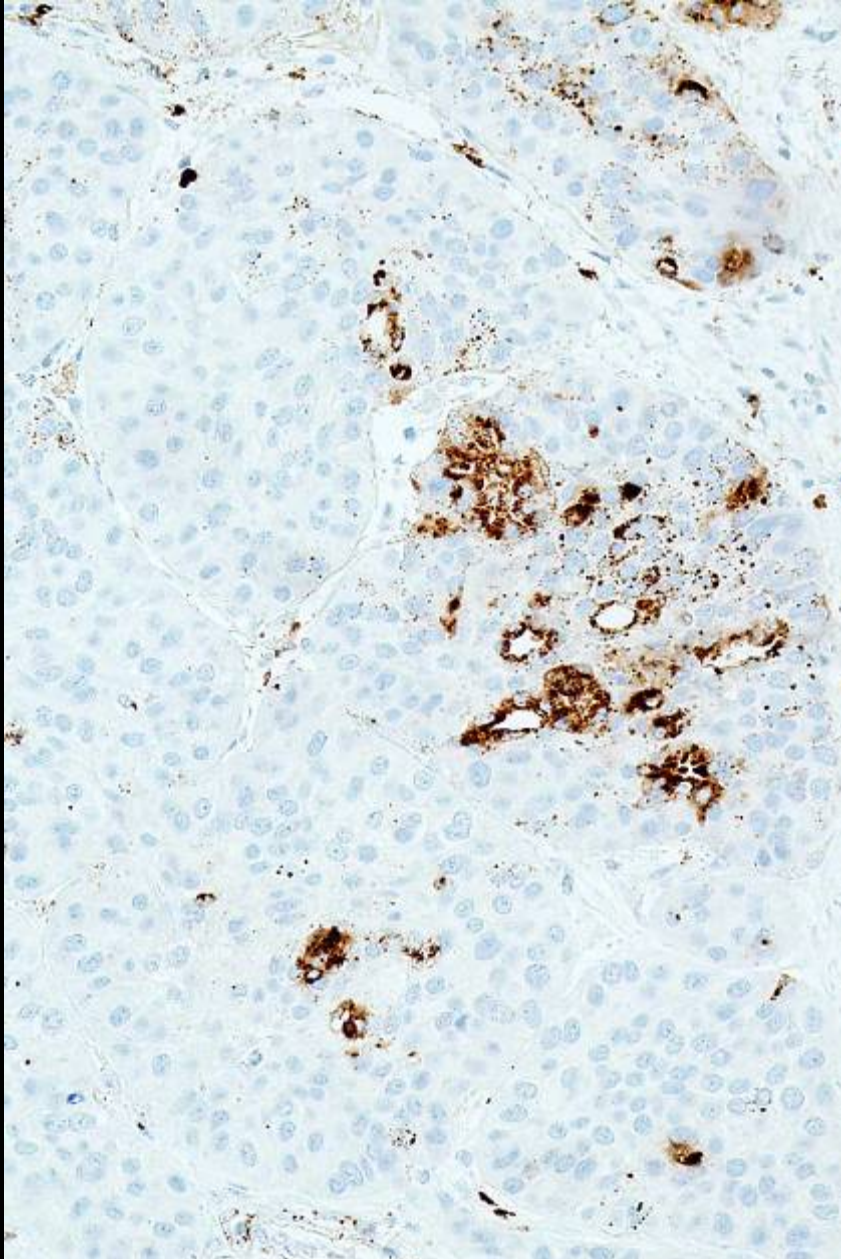
- Type II pneumocytes
- Proximal and convoluted renal tubules
- (Pancreatic acini and ducts)



Napsin A: lung adenocarcinoma ~ 80%



Napsin A: Lung squamous carcinoma 0 ~ 30%



Napsin A: Renal cell carcinoma 0 ~ 70%

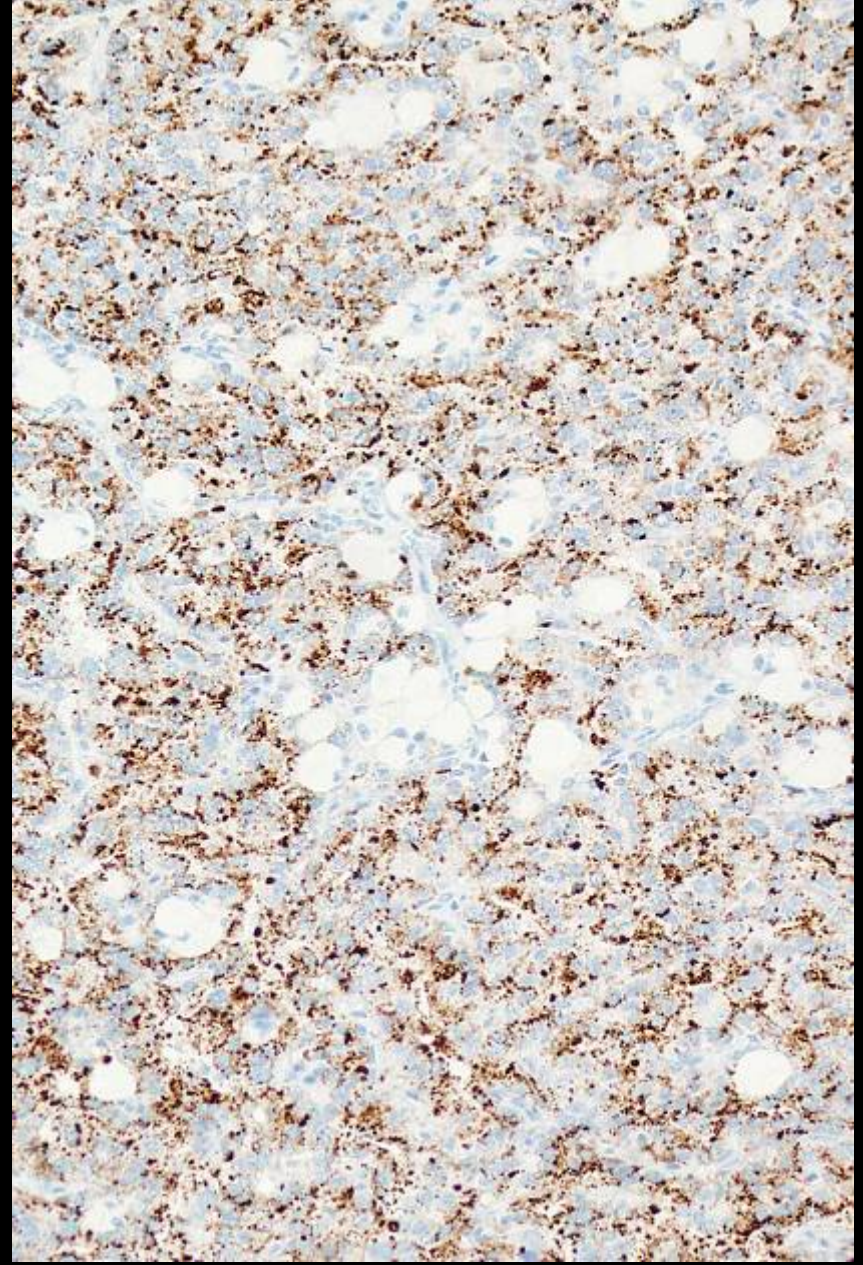
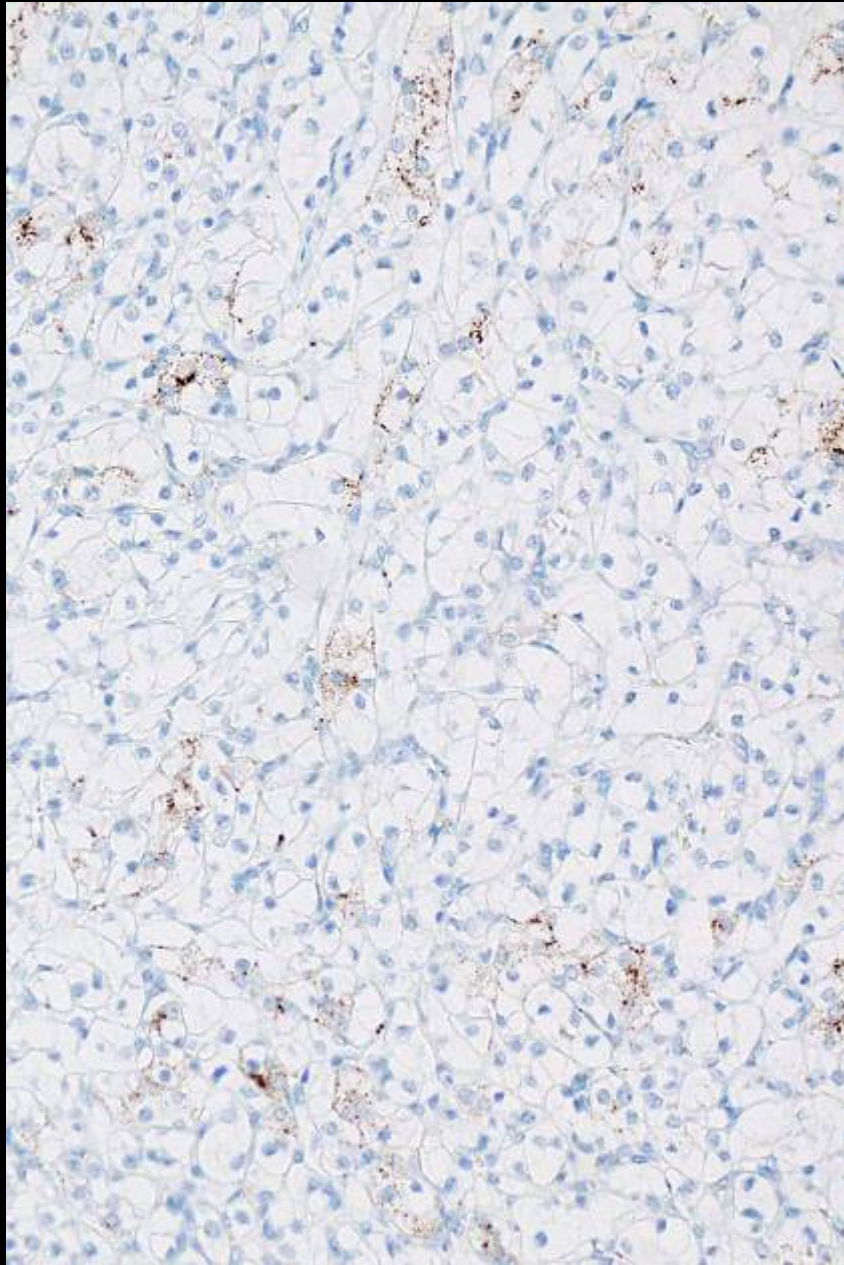
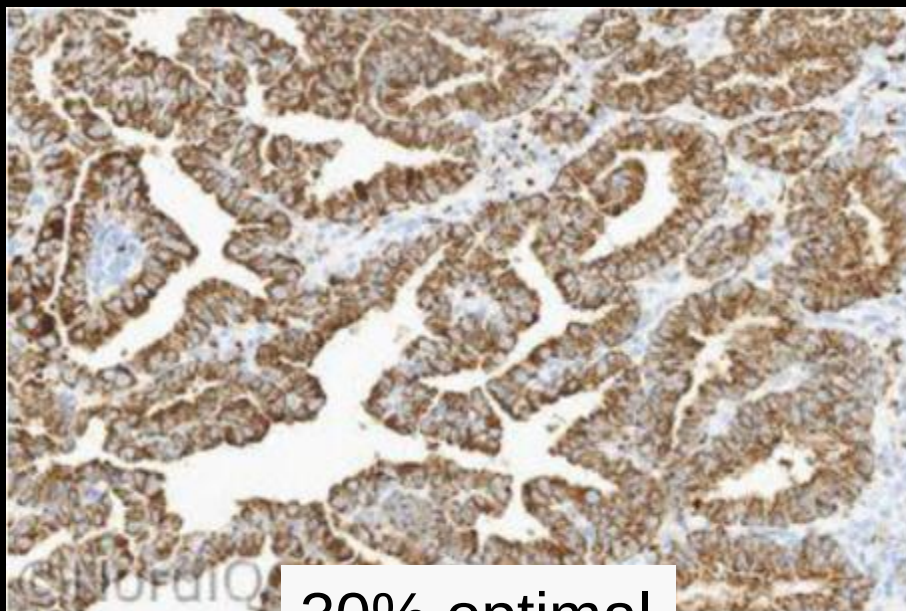
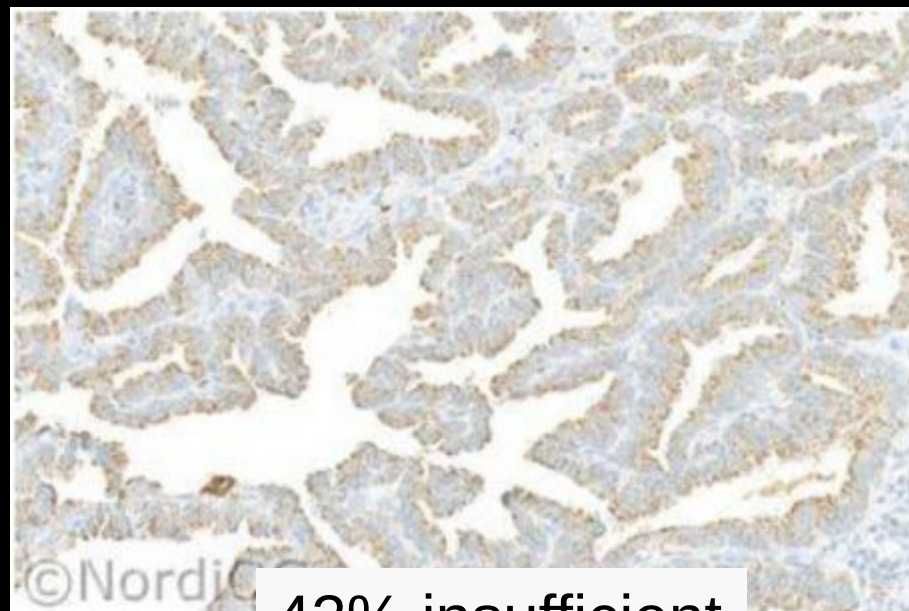


Table 1. Antibodies and assessment marks for Napsin A, run 44

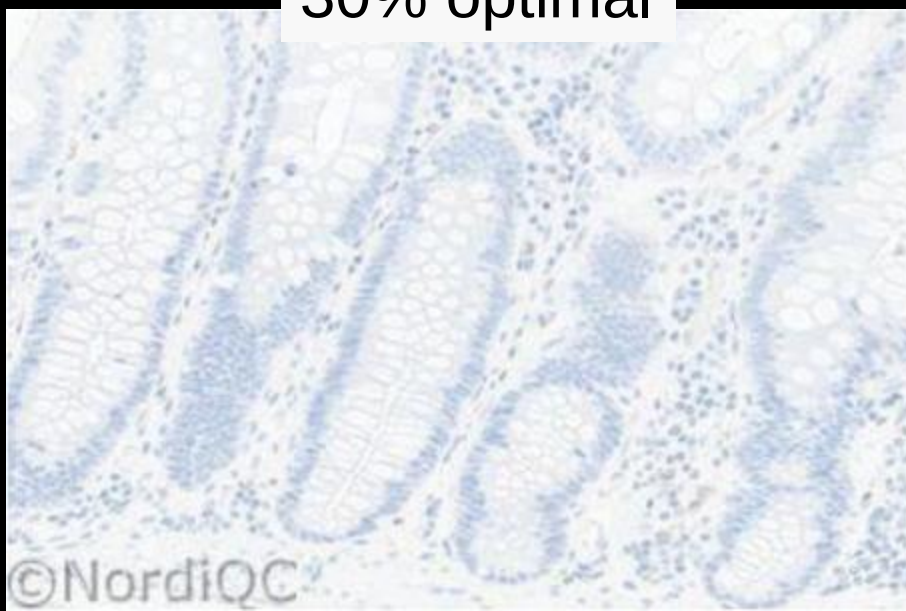
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone IP64	86	Leica/Novocastra	39	39	6	2	91%	92%
mAb clone MRQ-60	8	Cell Marque	3	4	1	0	88%	100%
mAb, clone TMU-Ad02	4	Biocare	1	2	4	0	43%	-
	3	IBL						
rmAb clone KCG1.1	2	Zytomed						
	2	Diagnostic Biosystems						
	1	Abcam	1	5	0	0	100%	-
	1	Acris						
rmAb clone BC15	1	Zytomed	1	0	0	0	-	-
mAb, clone BS10	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone EPR6252	1	Abcam	1	0	0	0	-	-
pAb 352A-7x	8	Cell Marque	0	1	1	6	13%	-
Ready-To-Use antibodies								
mAb clone MRQ-60 760-4867	18	Ventana/Cell Marque	1	16	1	0	84%	-
mAb clone MRQ-60 352M-98	3	Cell Marque	0	3	0	0	-	-
mAb clone MRQ-60 MAD-000633QD	3	Master Diagnostica	0	3	0	0	-	-
rmAb clone BC15 API 3043	1	Biocare	0	0	1	0	-	-
mAb clone IP64 AM701-5M	1	BioGenex	0	0	1	0	-	-
mAb clone IP64 ZM-0473	1	ZSGB-BIO	0	1	0	0	-	-
rmAb clone EP205 352R-18	1	Cell Marque	1	0	0	0	-	-
mAb clone MX015 MAB-0704	1	Maixin	0	1	0	0	-	-
pAb 760-4446	12	Ventana/Cell Marque	0	1	0	11	8%	-
pAb PPM428DS	1	Biocare	0	0	0	1	-	-
pAb MP-394-DS6	1	Menapath	0	0	0	1	-	-
pAb RAB-0639	1	Maxim	0	1	0	0	-	-
Total	162		49	77	15	21	-	
Proportion			30%	48%	9%	13%	78%	



30% optimal



42% insufficient



Thyroid transcription factor-1

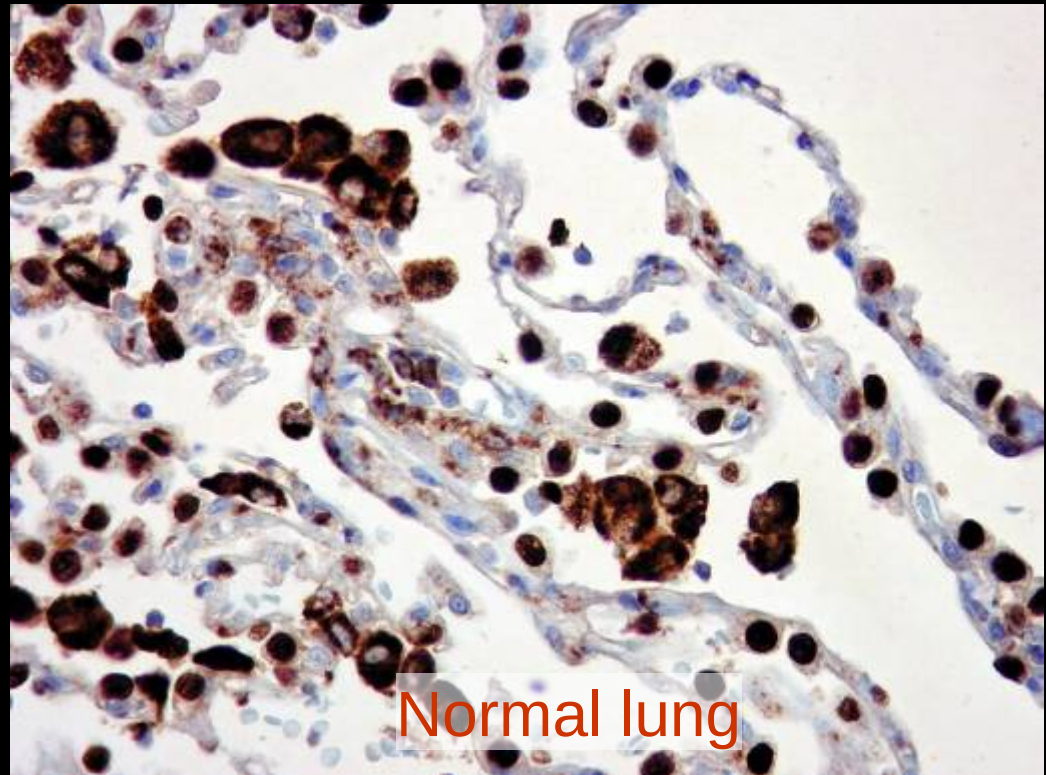
Thyroid gland:

regulating thyroglobulin, thyroperoxidase and thyrotropin receptor

Lung:

regulating surfactant proteins and Clara cell secretory protein

- Thyroid follicles and C-cells
- Lung pneumocytes II and Clara cells
- Brain
- Pituitary
- Parathyroid



Thyroid transcription factor-1

	% pos.:	SPT24	8G7G3/1
Lung adenocarcinoma		80	70
- large cell carcinoma		50	40
- small cell carcinoma		90	90
- carcinoid		60	20
- squamous cell carcinoma		15	0
Non-lung small cell carcinoma		20-40	?
Thyroid carc. (non-anaplastic)		100	100
Non-lung carcinomas		0 - 5	0 - 5
Non-lung carcinoids		~ 0	~ 0



TTF-1 pos. neoplasms **outside** lung – reported

Neuroendocrine neoplasms

Small cell carcinoma

GI tract, prostate, bladder, liver, uterine cervix, breast

Large cell neuroendocrine carcinoma:

prostate, uterine cervix

Neuroendocrine tumor/ carcinoid: GI tract

Merkel cell carcinoma of skin

Non-neuroendocrine adenocarcinoma

Gastric, Colorectal, Breast, Primar peritoneal,

Endocervical, Endometrial, Ovarian,

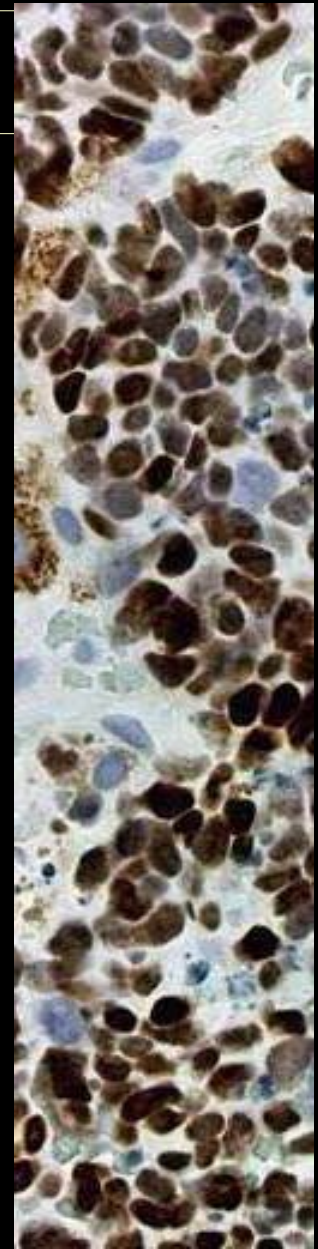
Nasopharyngeal papillary

Others

Synovial sarcoma

Melanoma

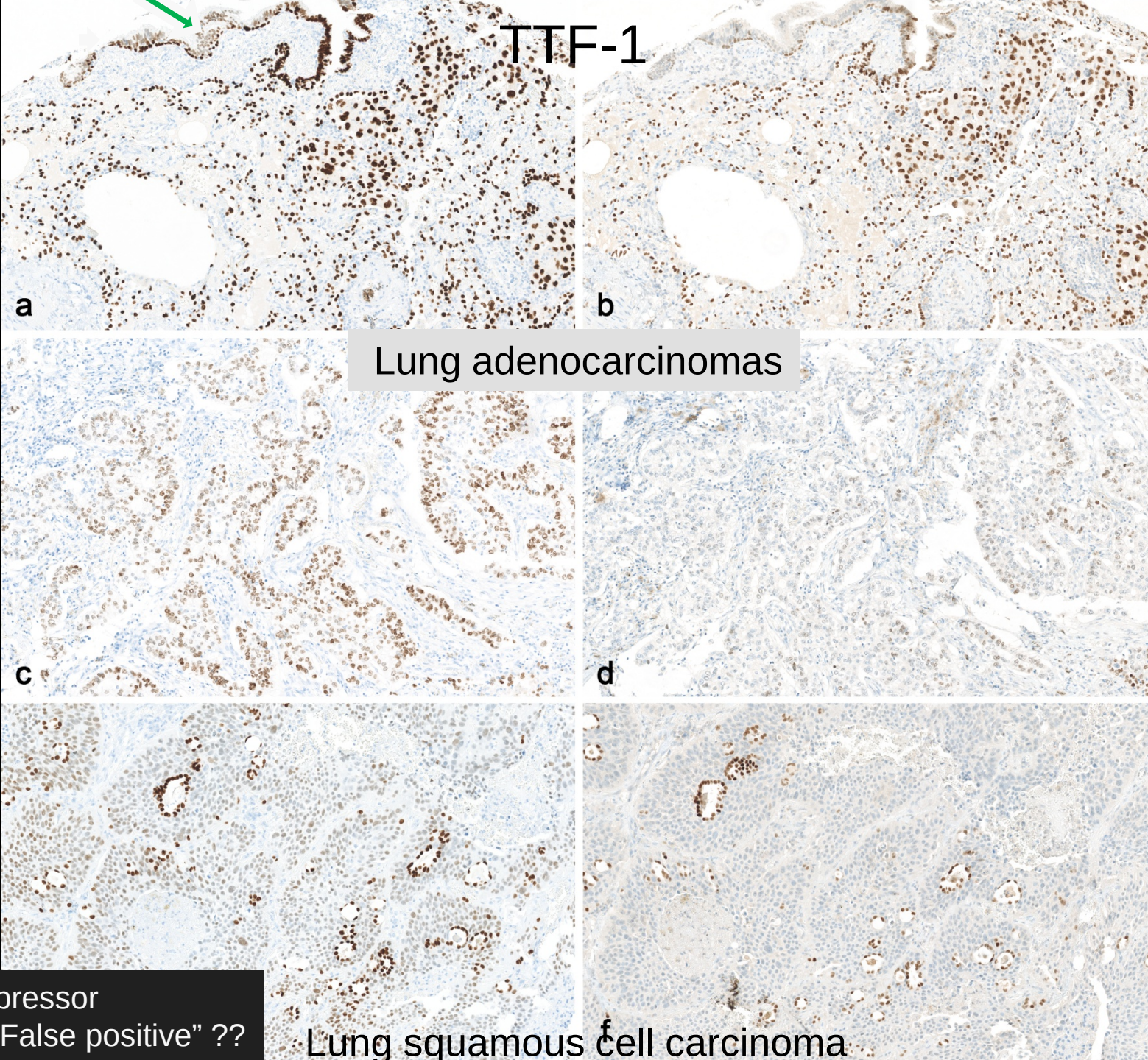
Brain tumors



8G7G3/1, MX011

TTF-1

SPT24, SP141



Lung adenocarcinomas

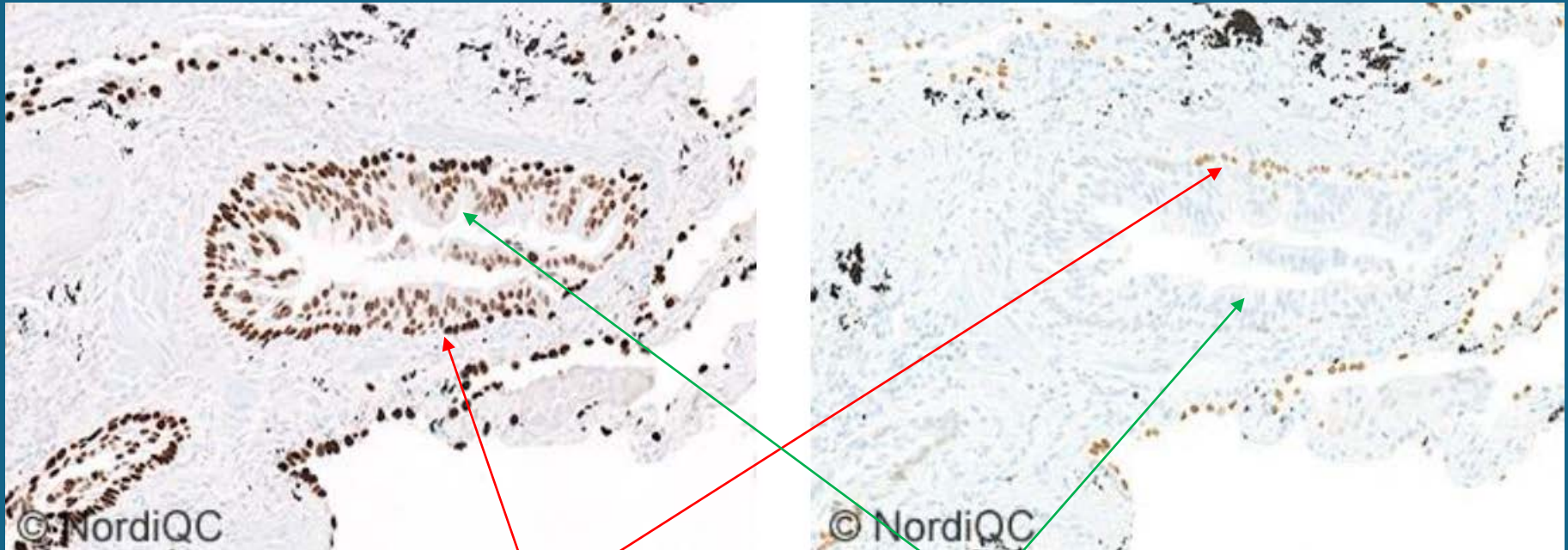
Lung squamous cell carcinoma

Low expressor
WHO: "False positive" ??

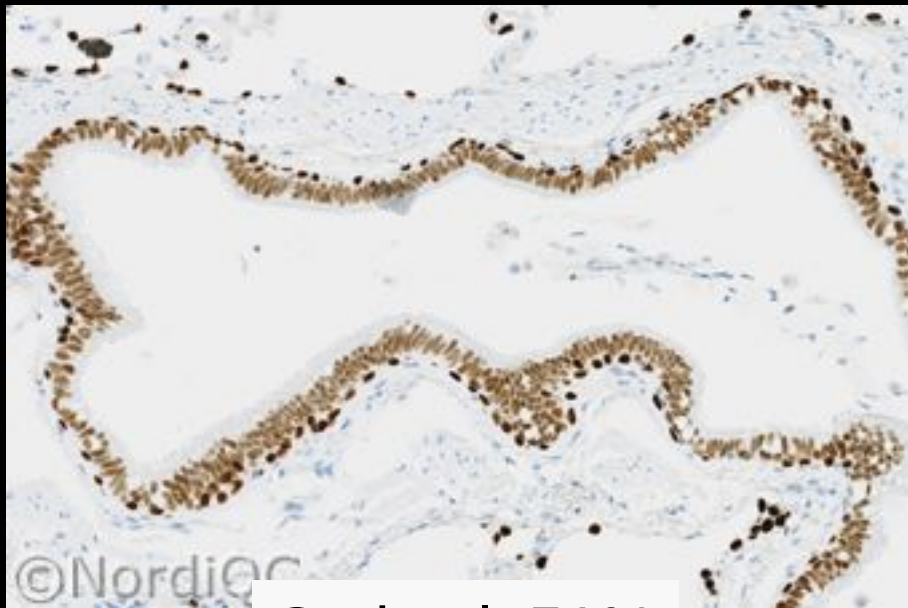
Table 1. Antibodies and assessment marks for TTF1, run 46

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 8G7G3/1	15 3 6 1	Dako/Agilent Thermo/NeoMarkers Cell Marque Zeta Corp.	0	0	14	11	0%	-
mAb clone SPT24	120 9 5 2	Leica/Novocastra Monosan Immunologic BioCare	76	43	14	3	88%	89%
rmAb clone EP229	1	Cell Marque	1	0	0	0	-	-
rmAb clone SP141	1	Spring Bioscience	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone 8G7G3/1 790-4398	16	Ventana/Roche	0	1	7	8	7%	-
mAb clone 8G7G3/1 IR056	31	Dako/Agilent	0	1	24	6	3%	-
rmAb clone SP141 790-4756	50	Ventana/Roche	30	17	3	0	94%	94%
mAb clone SPT24 PA0364	8	Leica/Novocastra	7	1	0	0	100%	100%

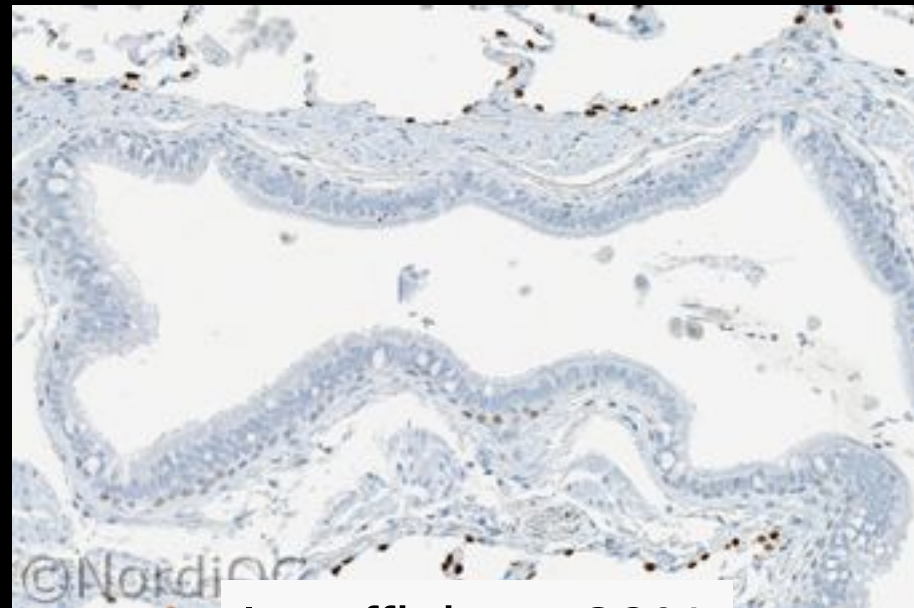
TTF-1 – run 46, 272 labs



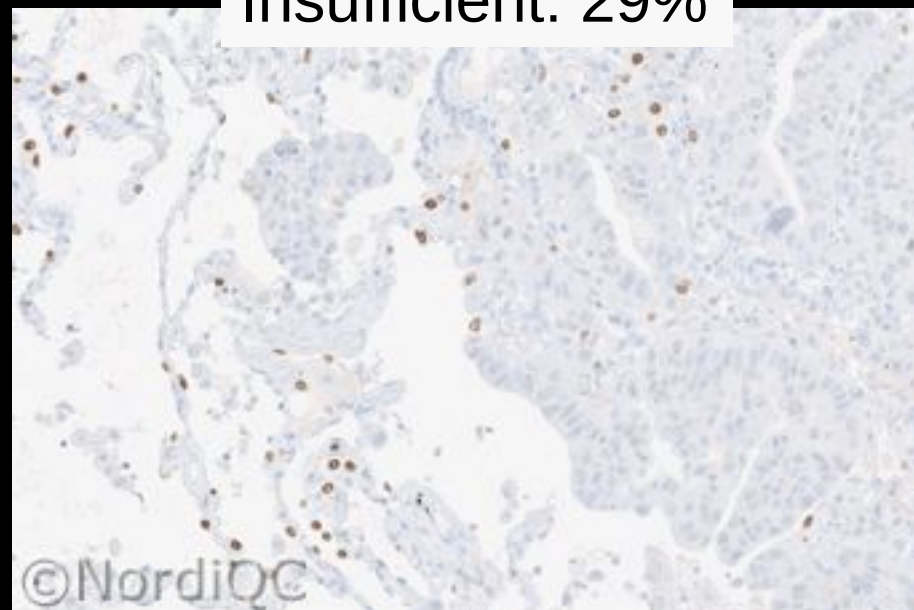
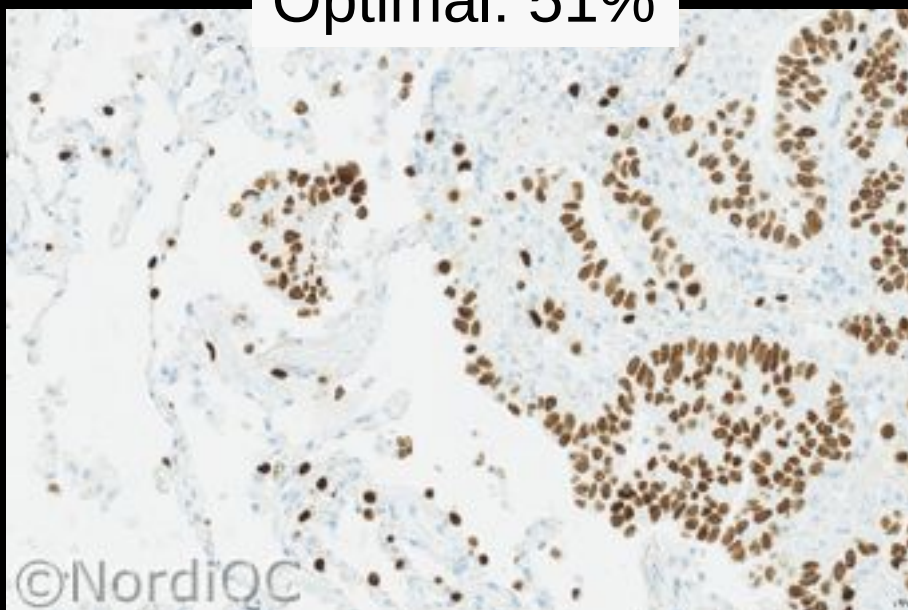
High expressor Low expressor



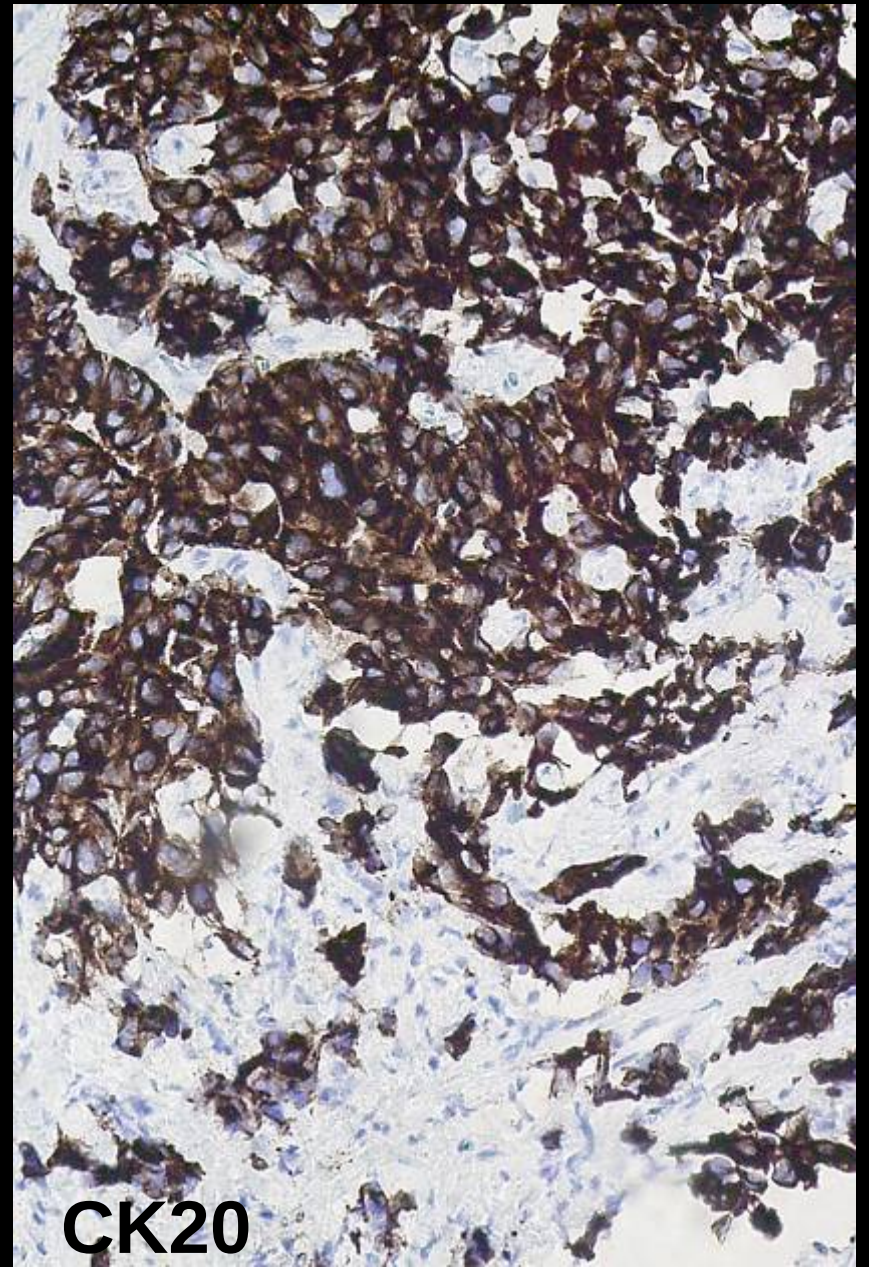
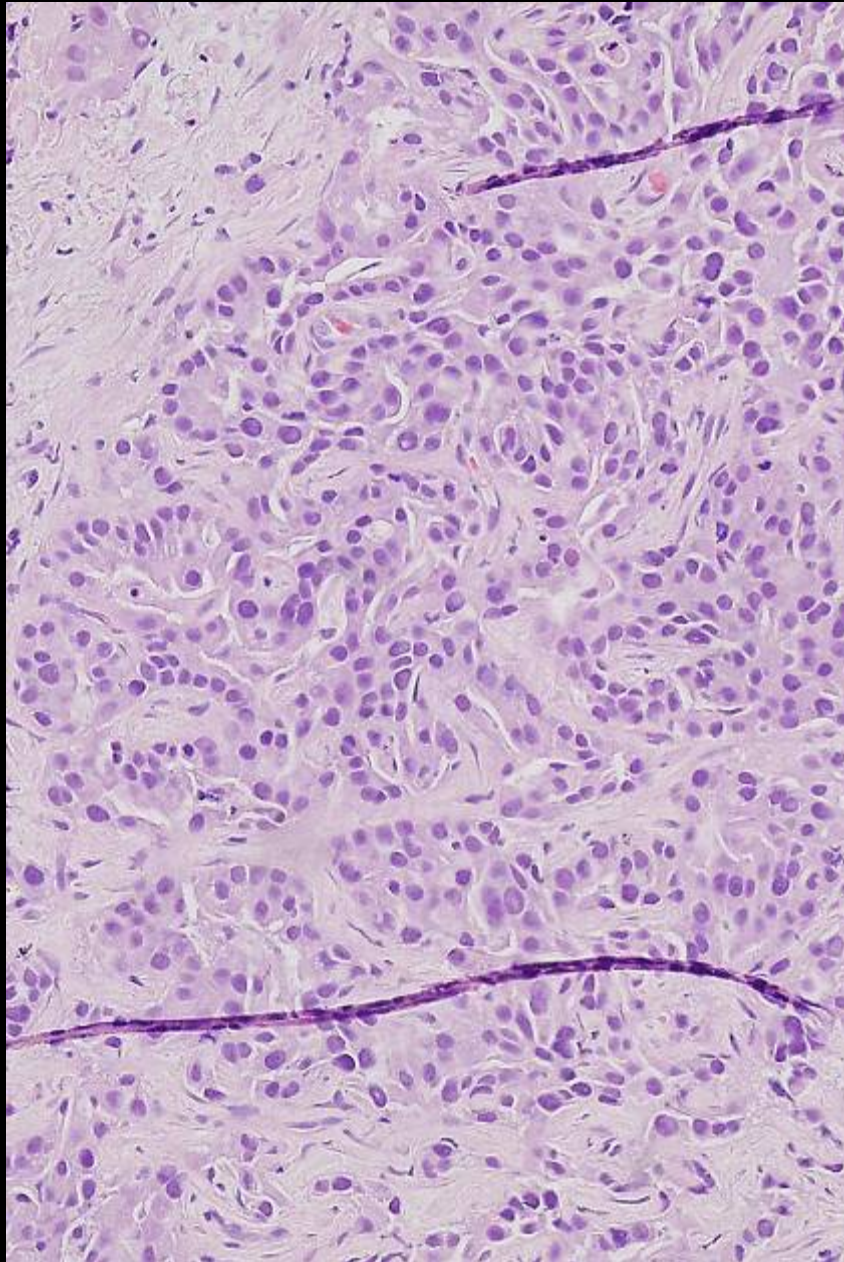
Optimal: 51%



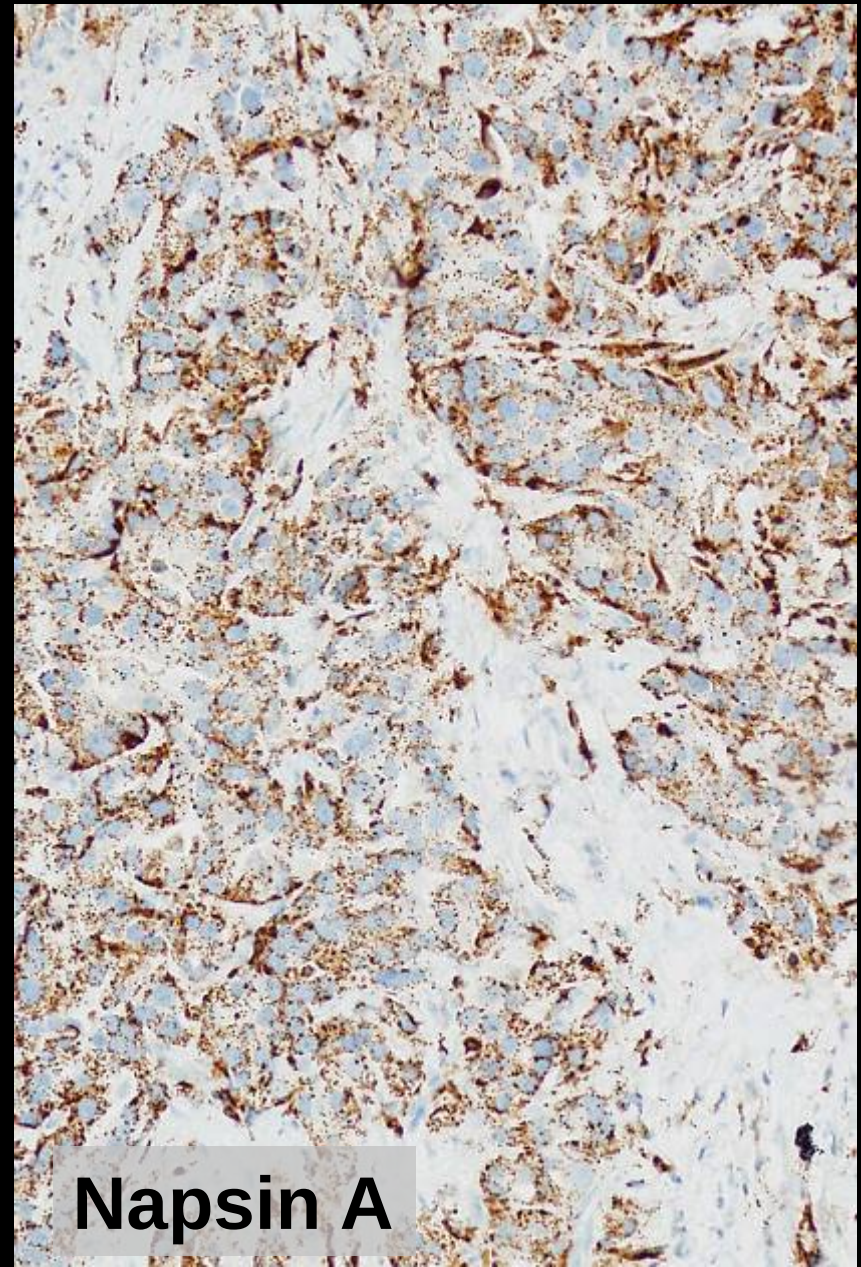
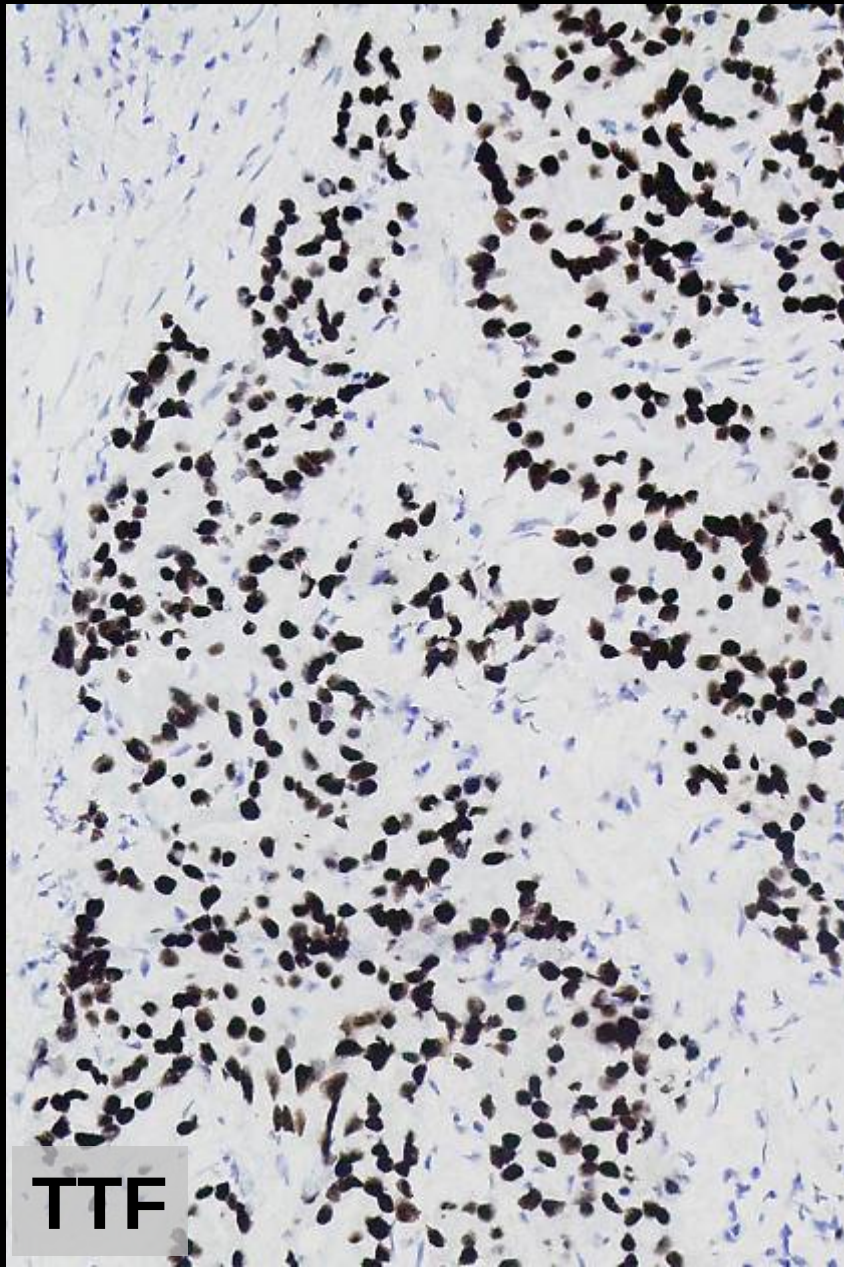
Insufficient: 29%



♂ 49 – Liver adenocarcinoma of unknown primary



♂ 49 – Liver adenocarcinoma of unknown primary: Lung



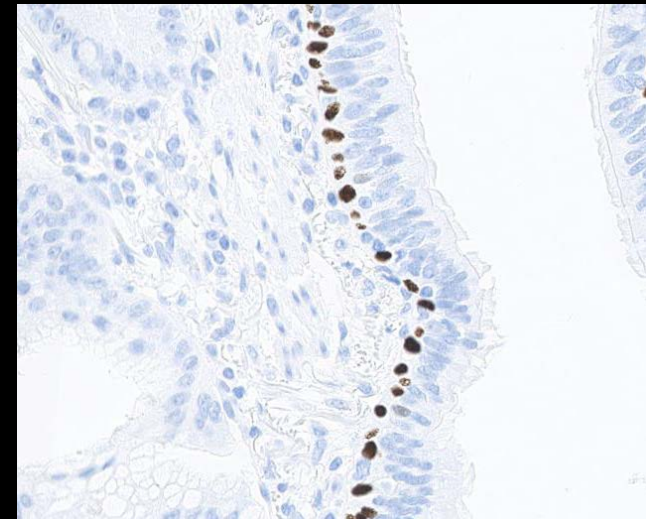
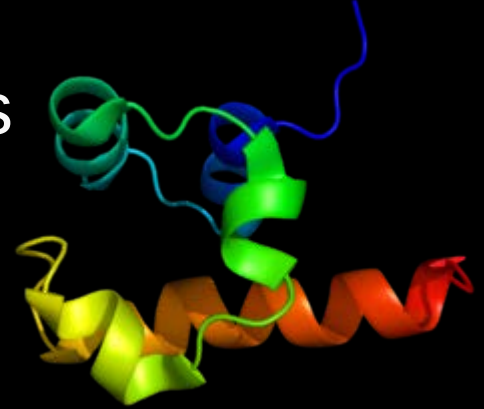
“Squamous cell markers”

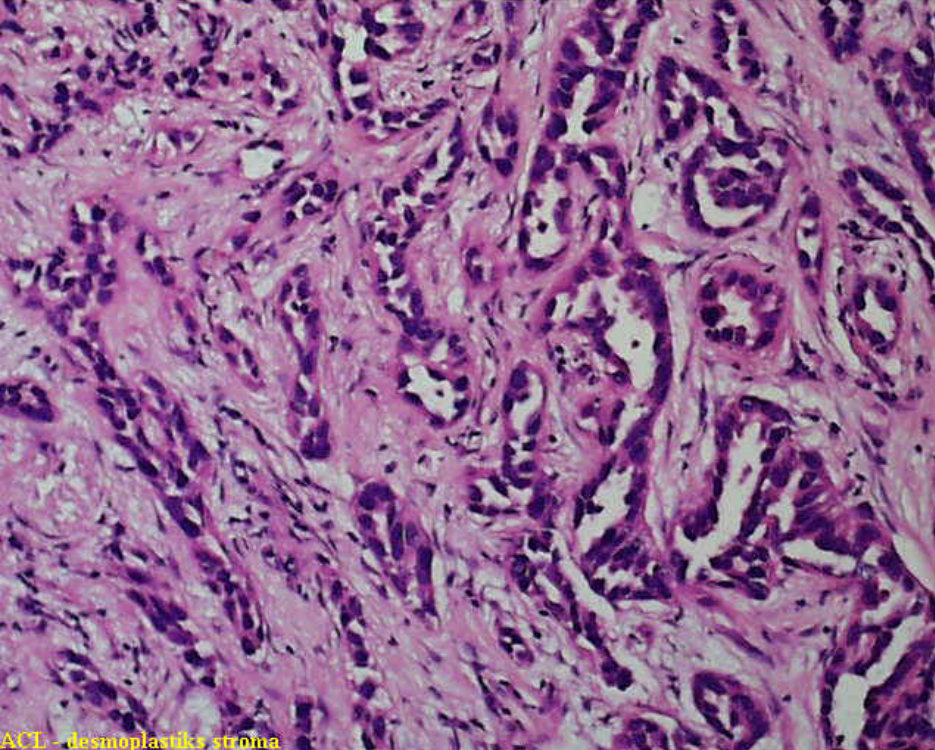
p63/p40

p16

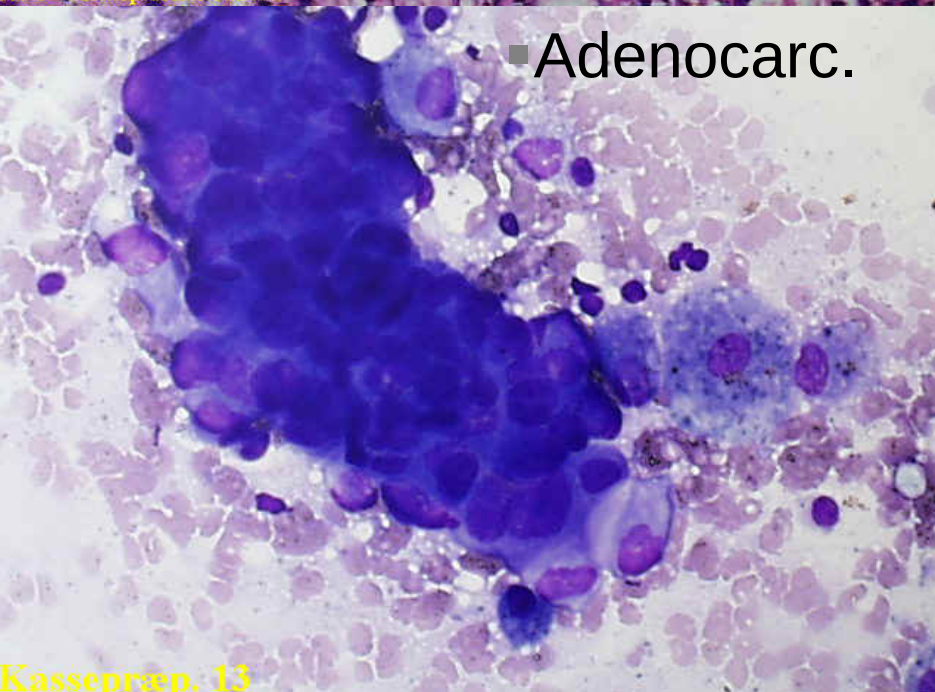
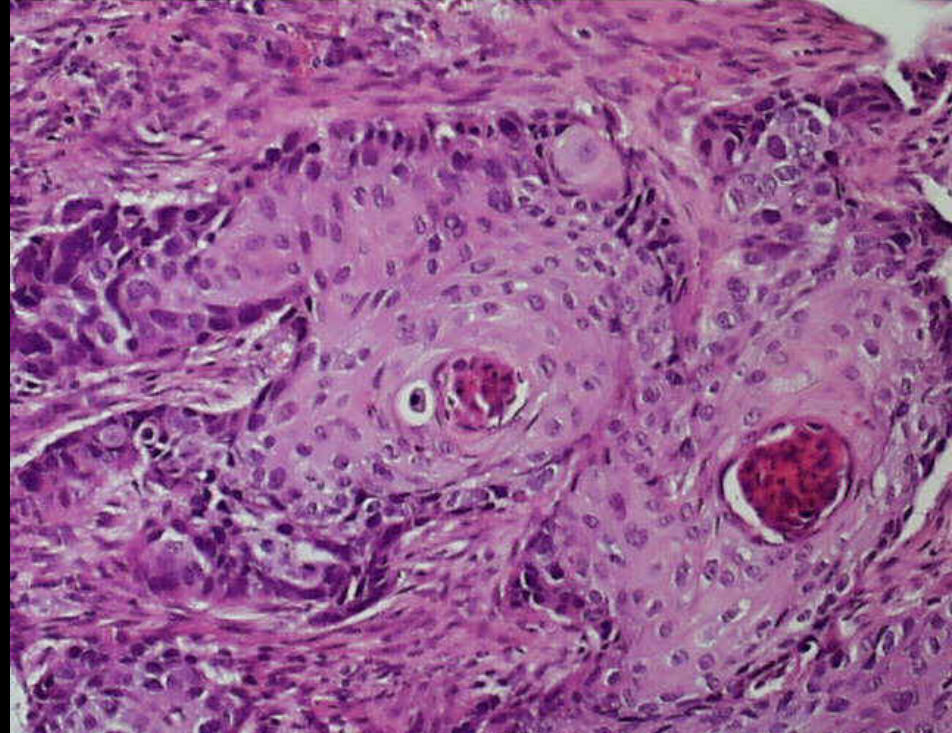
HMW-CKs

- Nuclear proteins - transcription factors
- Belongs to the p53 gene family
- Plays a critical role in the growth and development of many epithelia
- Confined to
 - squamous epithelia
 - urothelium
 - basal cells/myoepithelial cells
 - lung, breast, sweat glands, salivary glands, and prostate

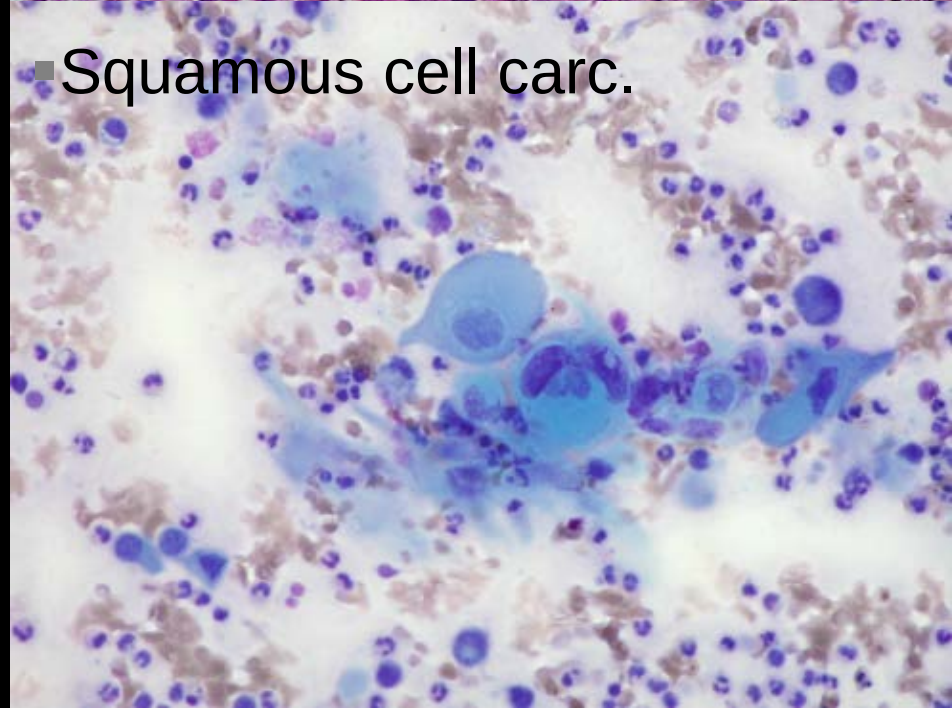




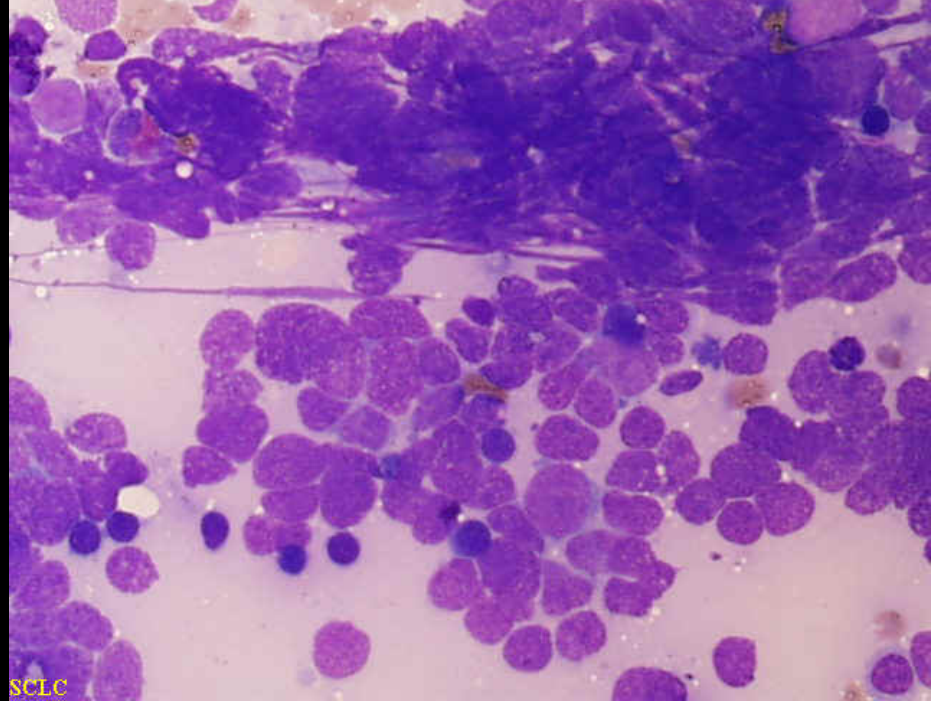
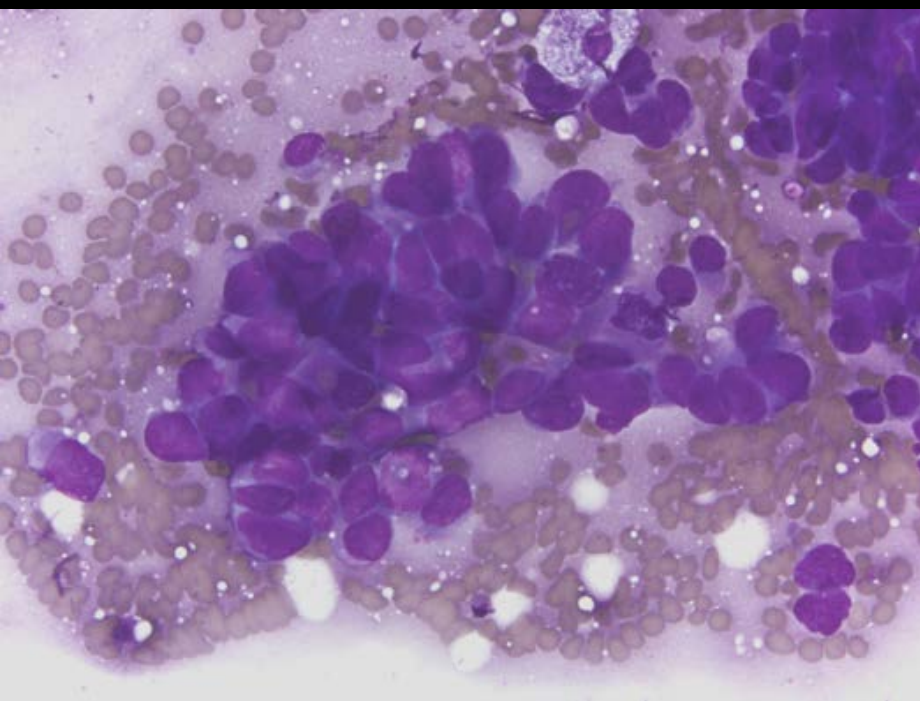
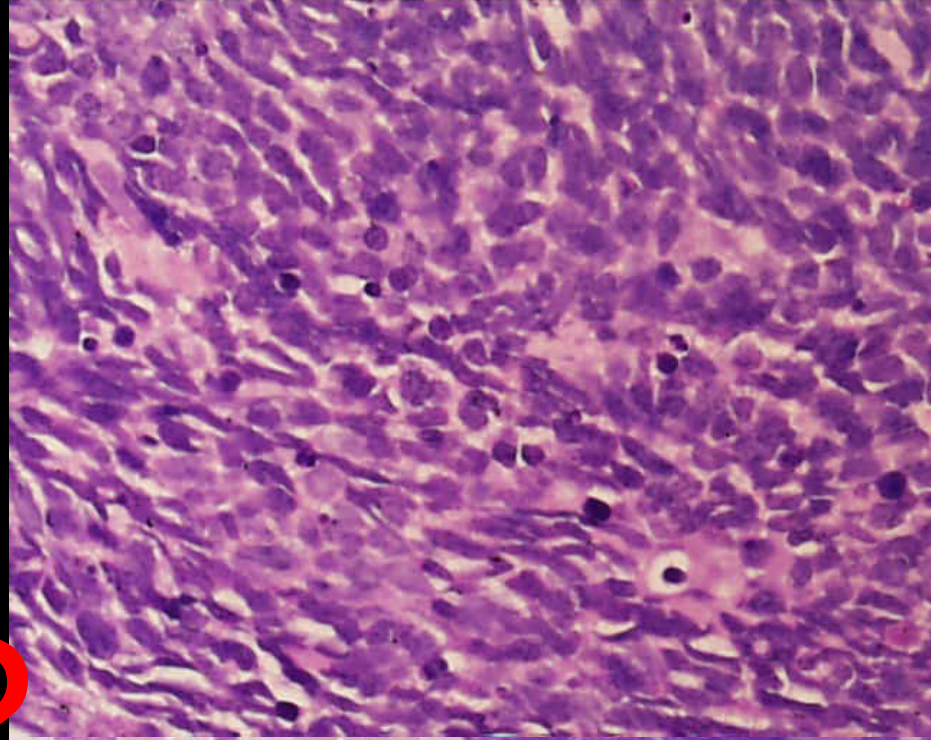
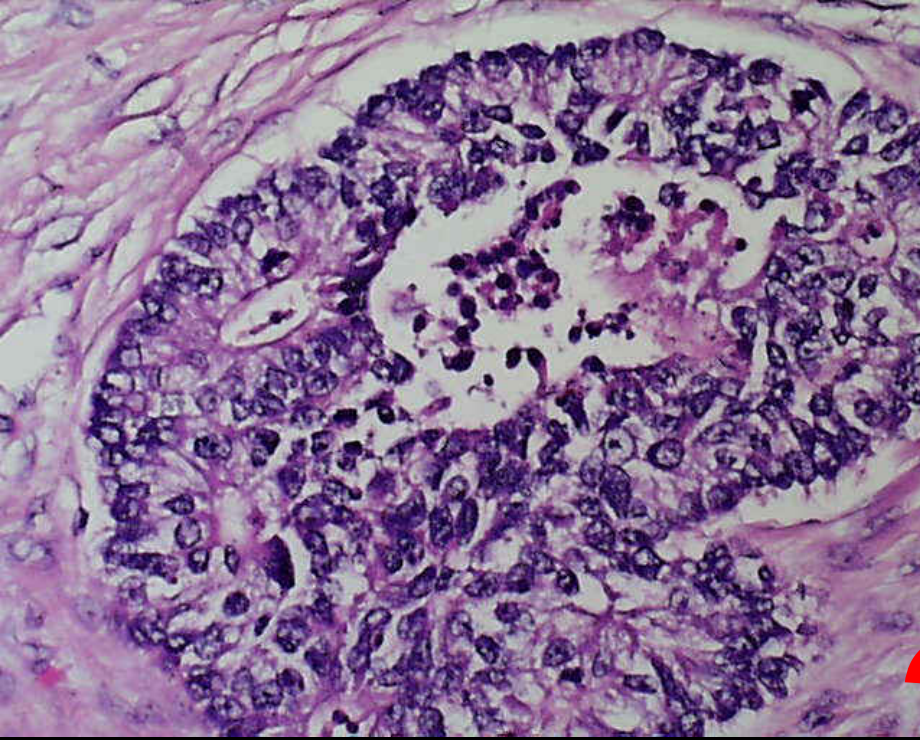
ACL - desmoplastik stroma



■ Adenocarc.



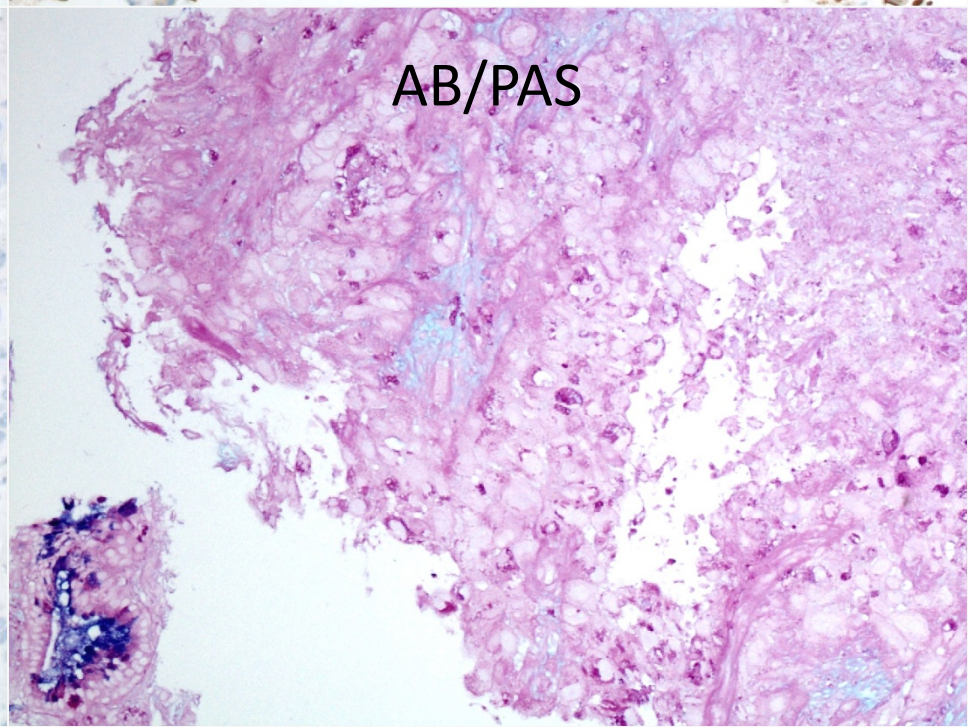
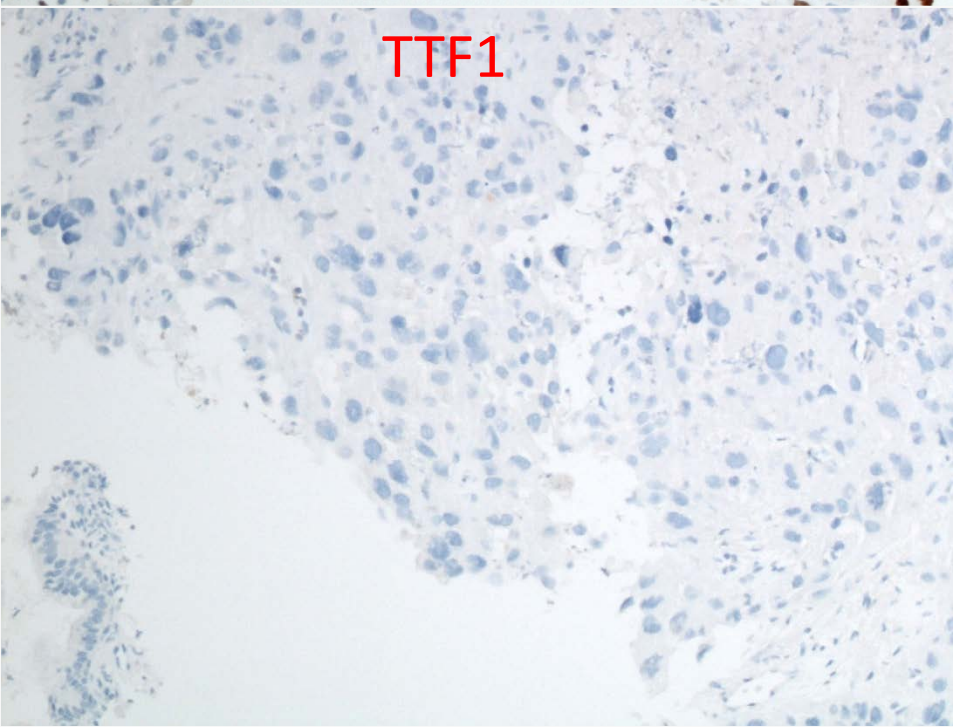
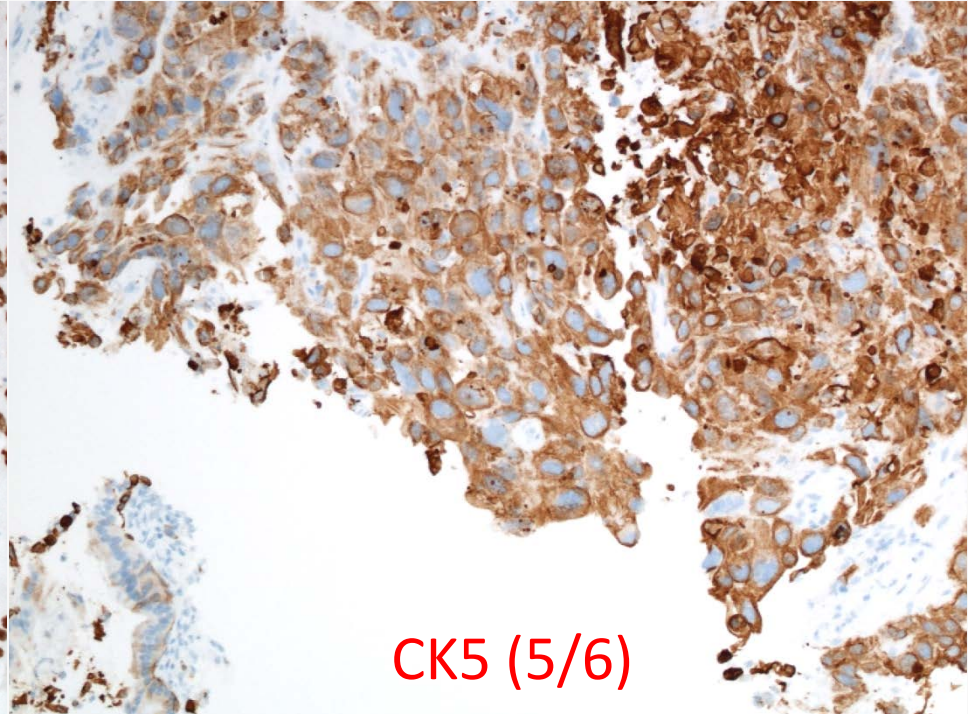
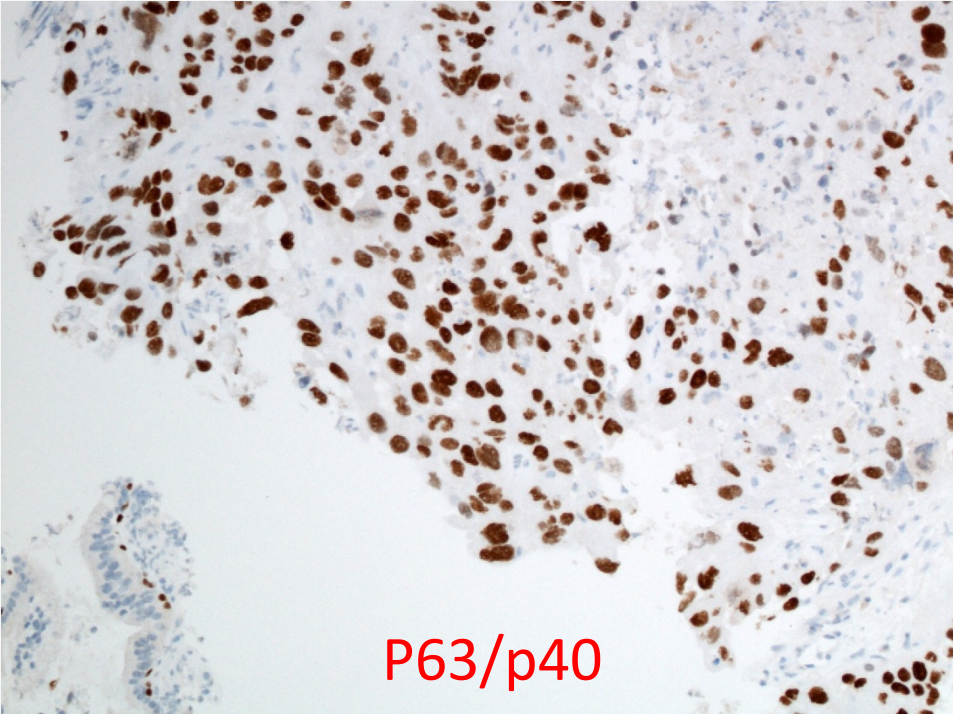
■ Squamous cell carc.

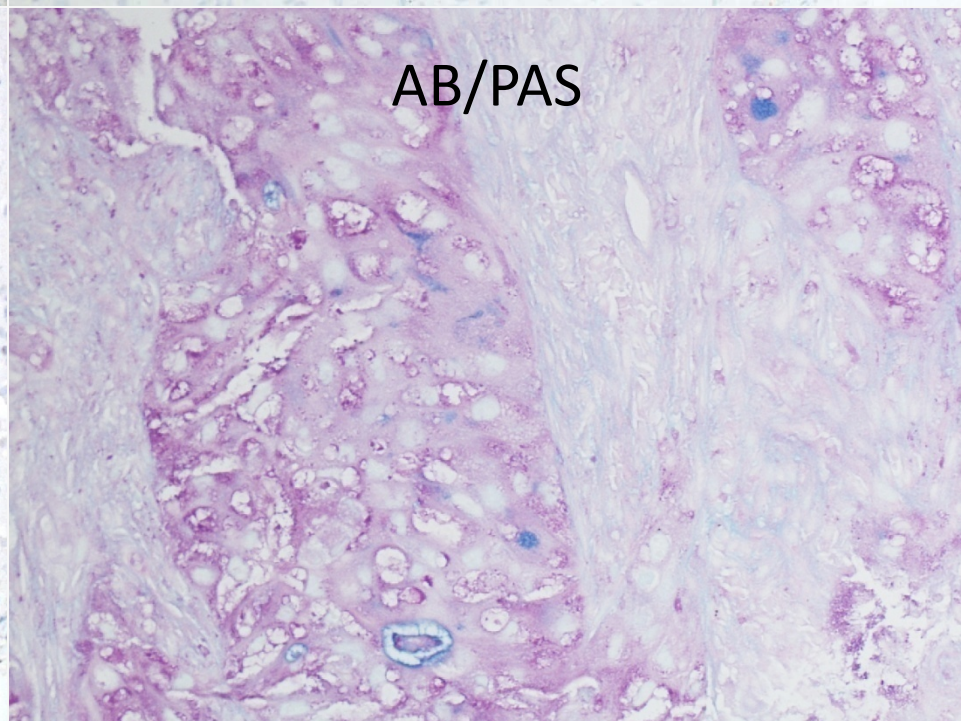
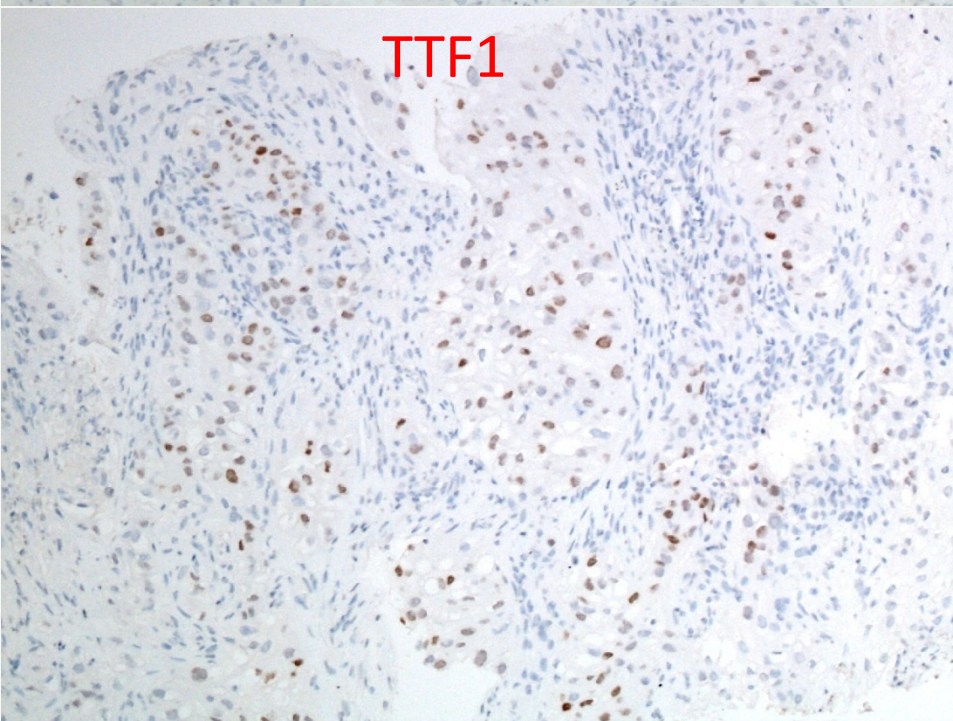
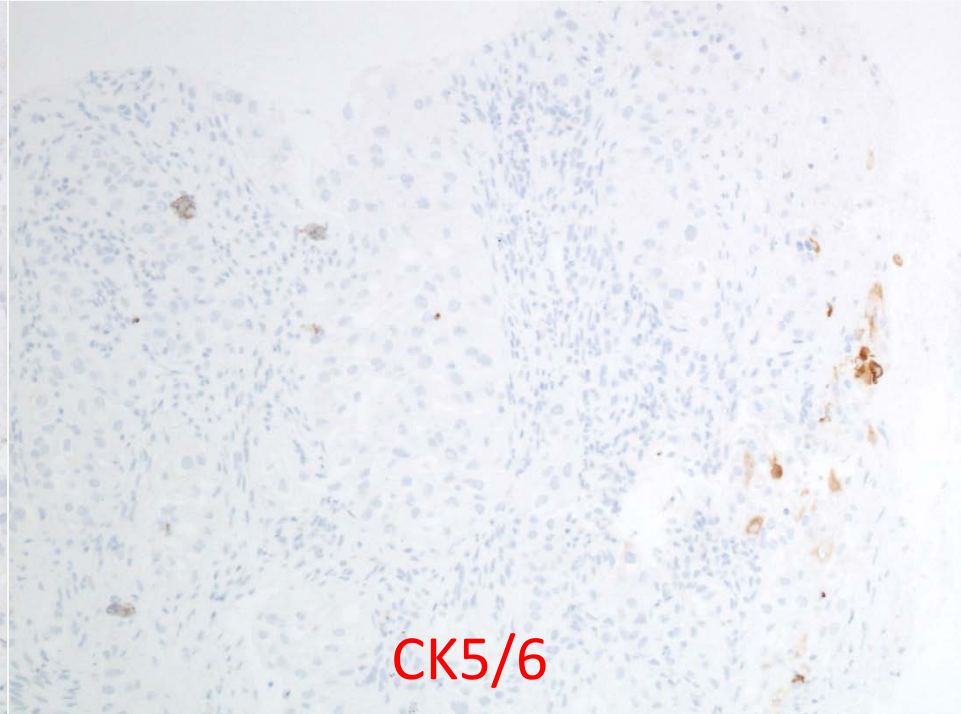
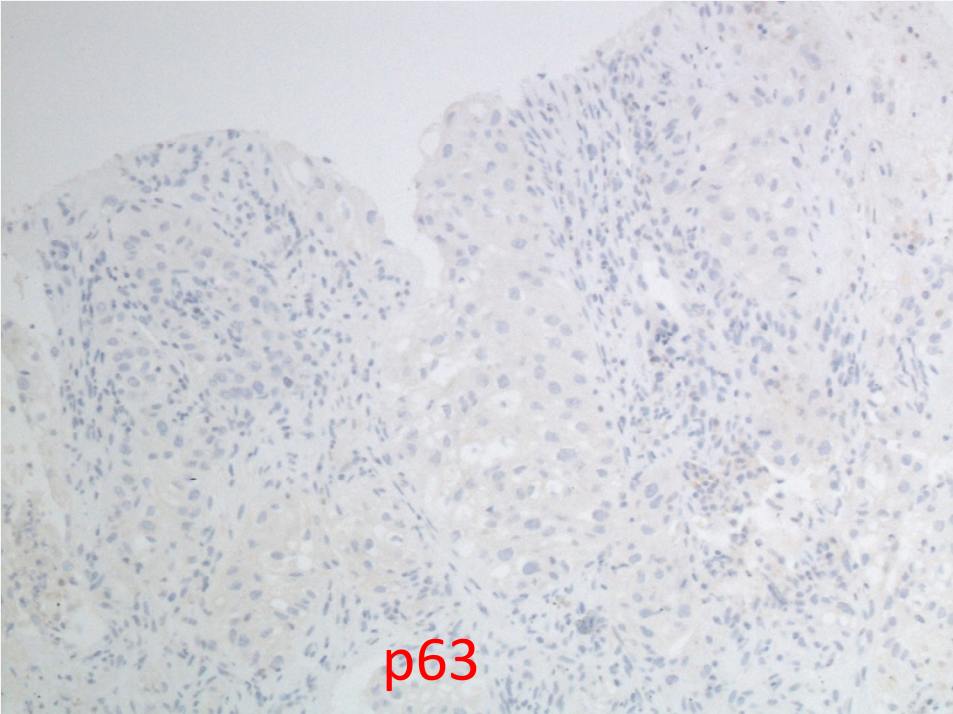


SCLC

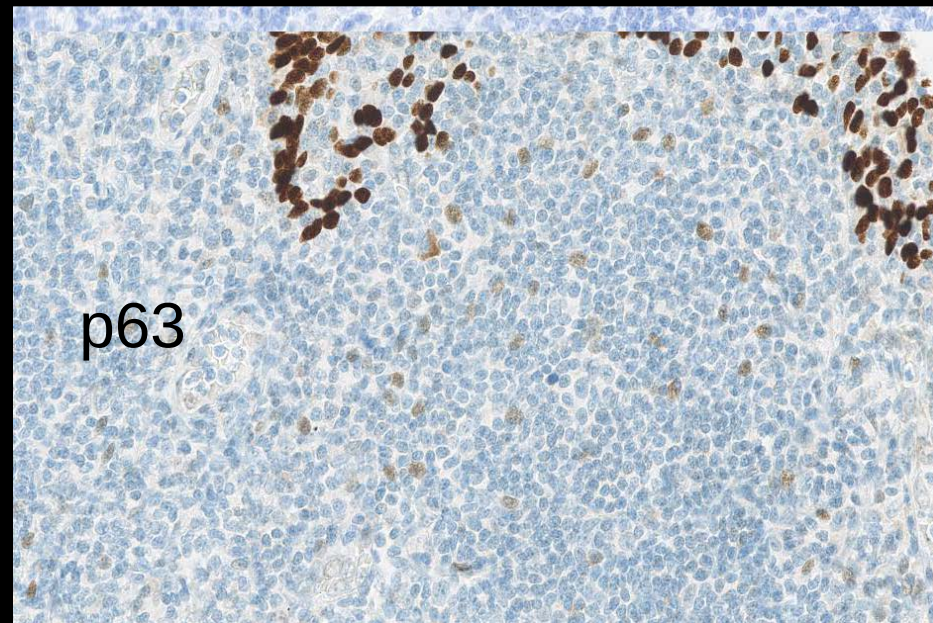
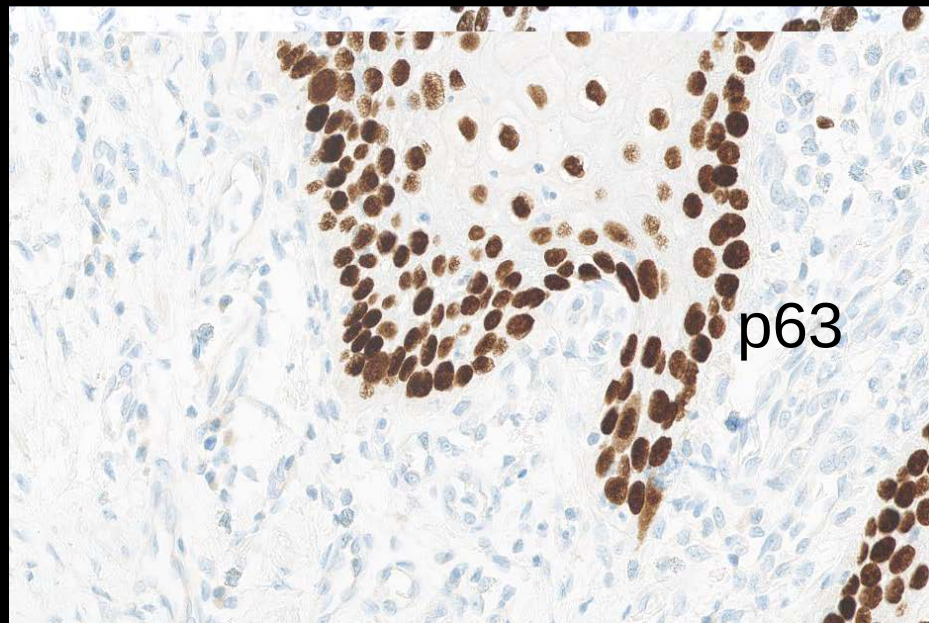
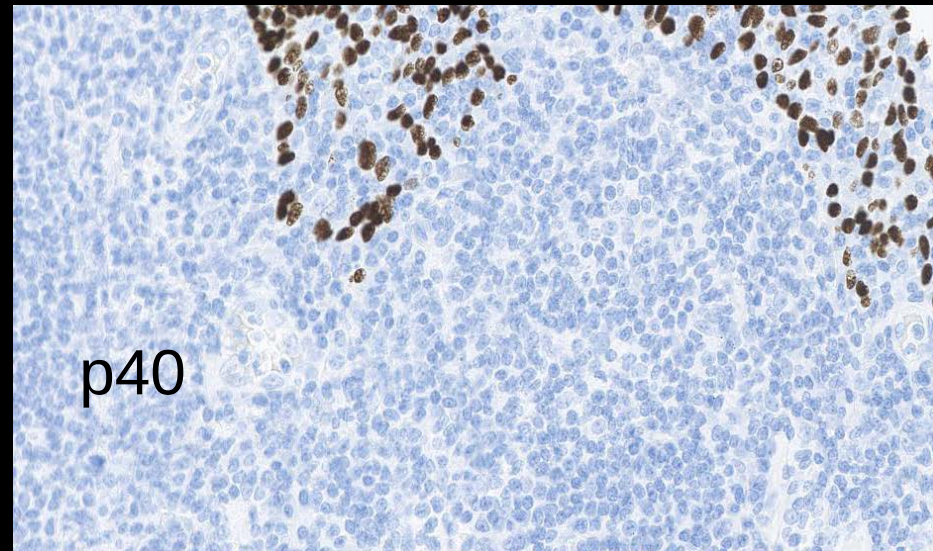
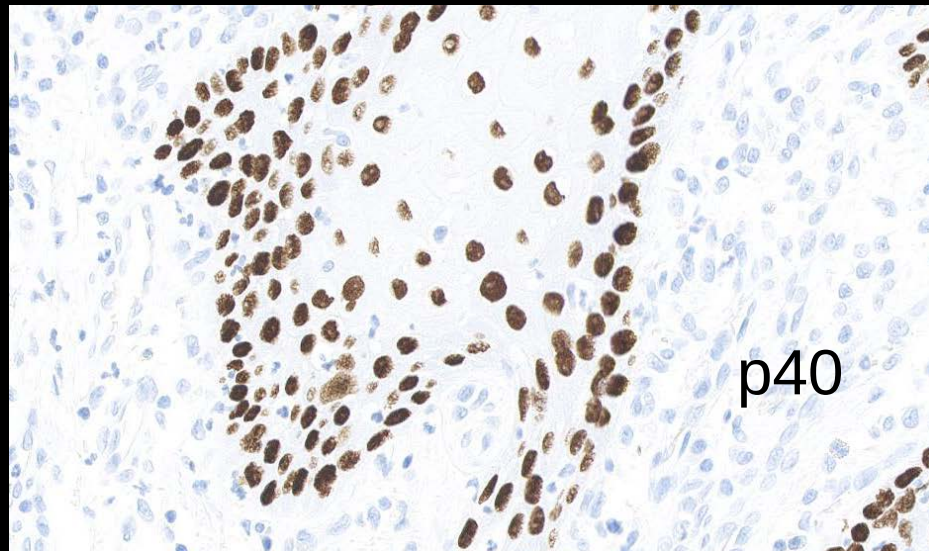
Lung cancer panel

	Adenocarcinoma	Squamous cell carcinoma
TTF1	+/-	-(+)
Napsin	+/-	-(+)
CK5	-(+)	+(-)
p63/p40	-(+) / -	+(-)
PAS/AB	+/-	-(+)

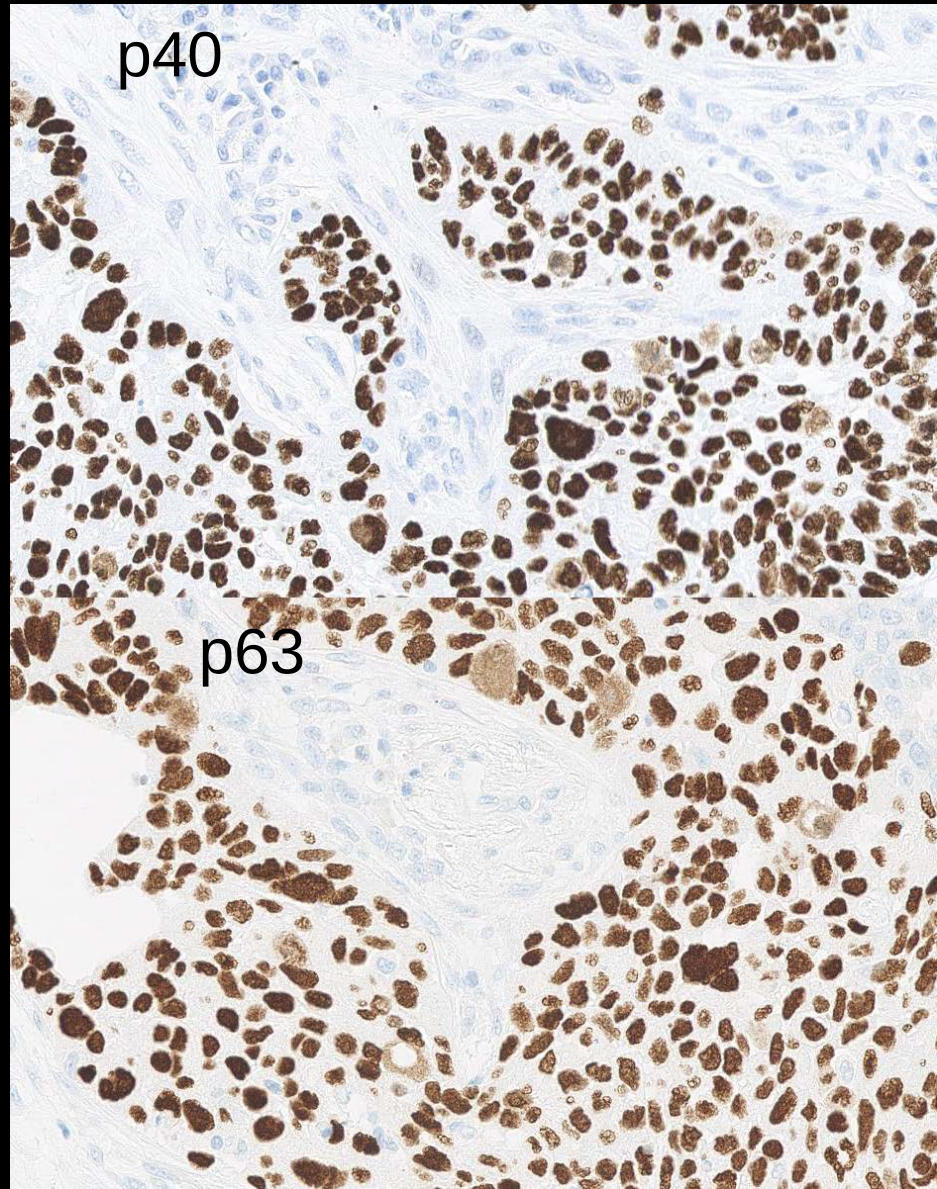




Tonsil – p63/p40



Squamous cell carcinoma



Diffuse large B-cell lymphoma

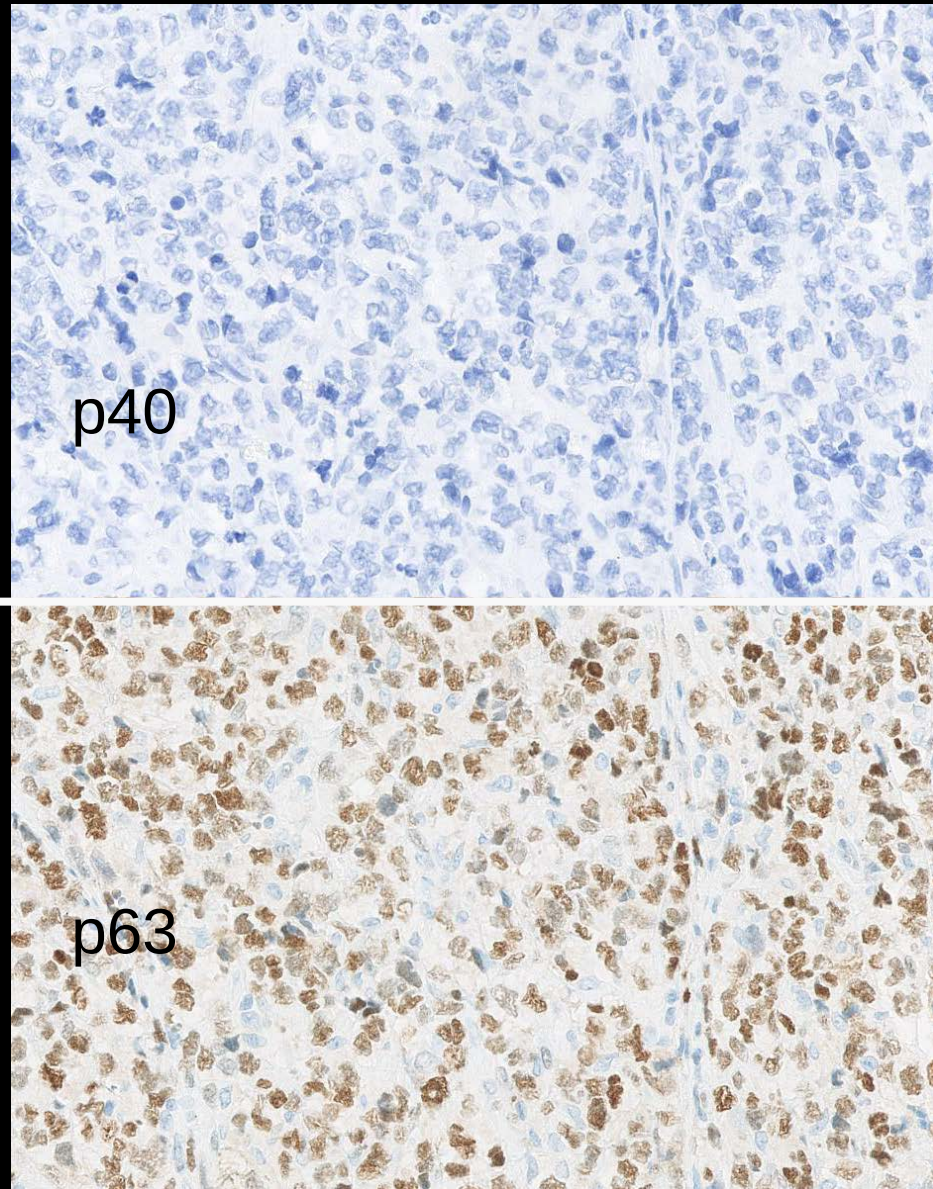
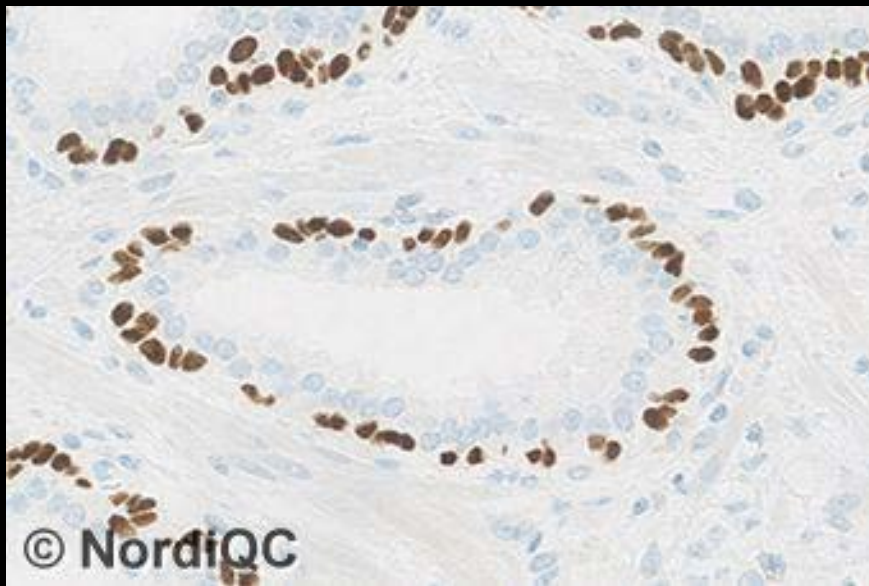
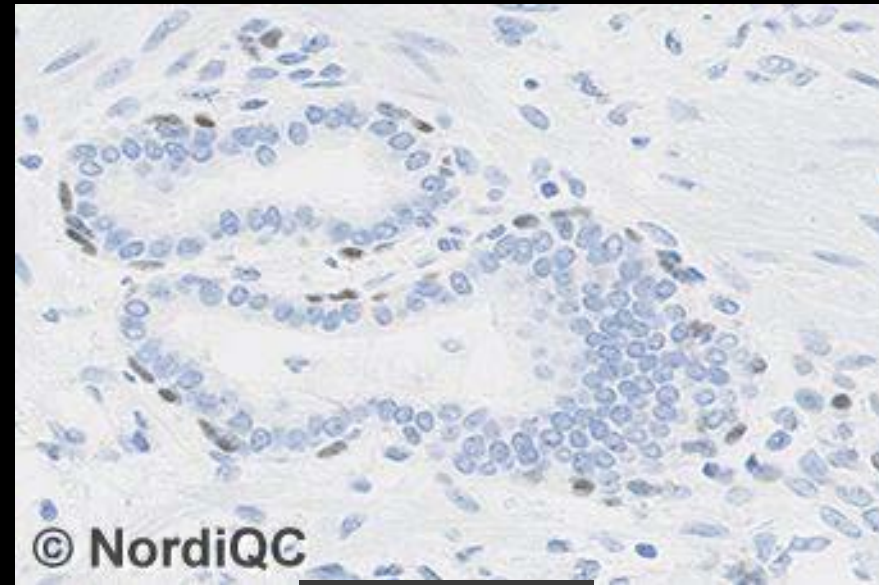


Table 1. Antibodies and assessment marks for p40, run 44

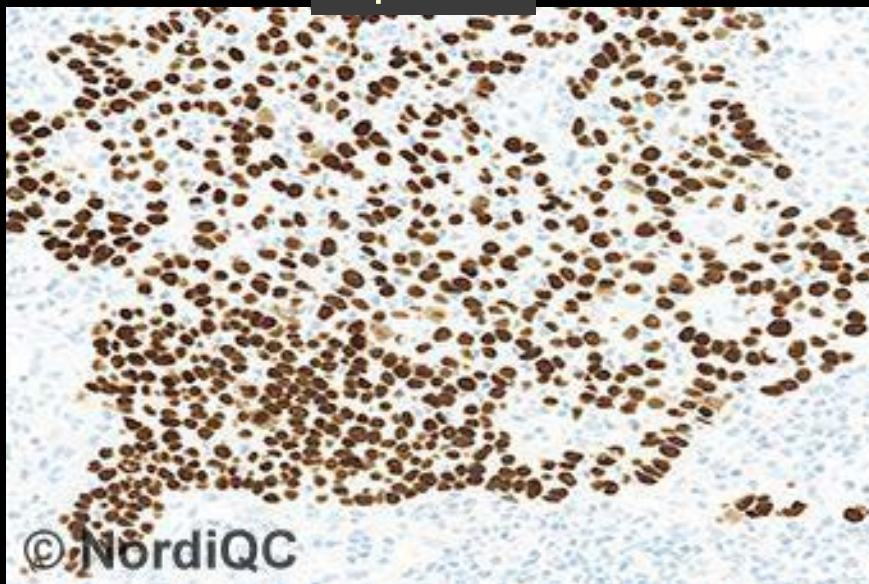
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone BC28	49	Biocare						
	3	Zytomed	26	17	10	0	81%	86%
	1	Nordic Biosite						
rmAb clone ZR8	12	Immunologic						
	1	Zeta Corporation	3	6	4	0	69%	80%
pAb AC13030	16	Biocare	0	3	11	2	19%	-
pAb PC373	9	Calbiochem, Merck	0	2	7	0	22%	-
pAb RP163	5	Diagnostic Biosystems						
	1	Medac diagnostica	0	2	4	1	29%	-
	1	ITK DIAGNOSTICS BV						
pAb ab99513*	4	Abcam	0	0	4	0	-	-
pAb PDR 055	1	Diagnostic Biosystems						
	1	ITK DIAGNOSTICS BV	0	1	0	1	-	-
pAb RBK054	2	Zytomed	0	1	1	0	-	-
pAb ab166857	1	Abcam	0	0	0	1	-	-
pAb ab167612	1	Abcam	0	0	0	1	-	-
pAb BP4206	1	ID Labs	0	1	0	0	-	-
pAb ILP-3726	1	Immunologic	0	0	1	0	-	-
pAb Unknown	1	Unknown	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone BC28	6	Biocare	3	2	1	0	83%	100%
API 3066								
mAb clone BC28		Ventana	1	1	0	0	-	-
790-4950								
pAb API 3030		Biocare	0	2	1	0	-	-
pAb RAB-066		Maixin	0	1	2	0	-	-
pAb PDR 055	2	ITK DIAGNOSTICS BV	0	0	2	0	-	-
mAb MAD-000623QD	2	Master Diagnostica	0	0	2	0	-	-
Total	129		33	39	51	6	-	
Proportion			26%	30%	40%	4%	56%	



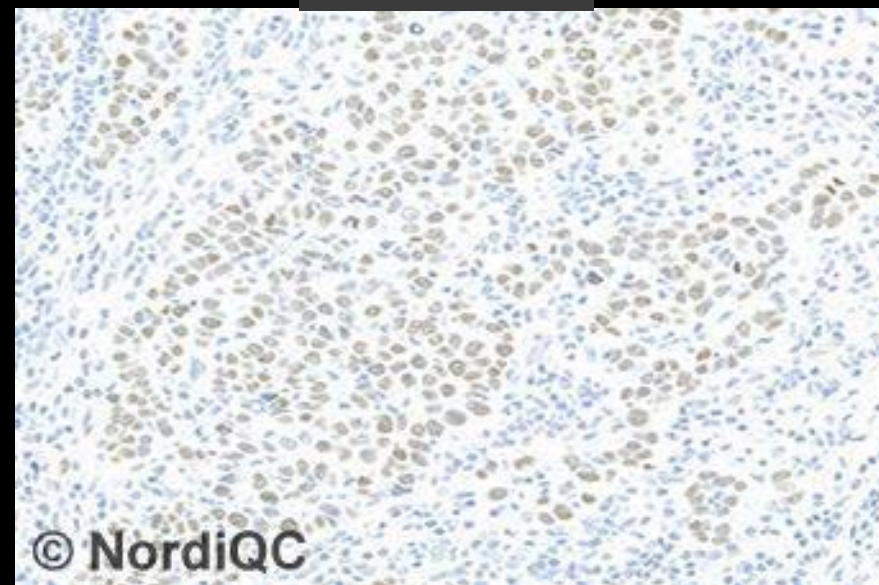
Optimal



Insufficient



squamous cell carc.



International Symposium on Immunohistochemistry

January 4th - 7th, 2018

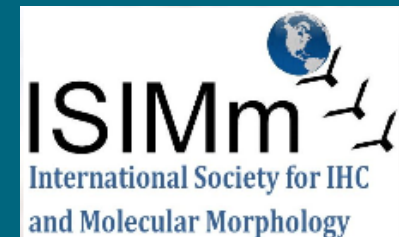
Hosted by Dept. of Histopathology, Tata Medical Center, Kolkata, India

In collaboration with NordiQC, Alborg, Denmark and ISIMM, California, USA



Diagnostic IHC in lung and pleura pathology

Thank you
for your attention



Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark