

The unknown primary tumour: IHC for diagnostic use

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Unknown primary tumour

- Tumour appearing in metastatic setting without a known primary tumour
- Important to correctly classify since tumour type determines surgical and oncological treatment
- Diagnostic approach for unknown primary tumour can be used in most diagnostic settings

Unknown primary tumour

- Differences in prognosis
- Differences in treatments
 - Lymphomas (malign or “benign”)
 - Carcinomas
 - Germ cell tumours
 - Sarcomas
- Pathology tests can guide diagnostic proces
- Risk of hereditary or occupational cancer

Unknown primary tumour

Ideally we want to know

- Type of cancer
- Primary or metastasis
- Site of origin



Immunohistochemical panels !

Based on

- Most likely diagnosis (clinical information, morphology)
- Relevant other diagnosis
- Rare but important diagnosis

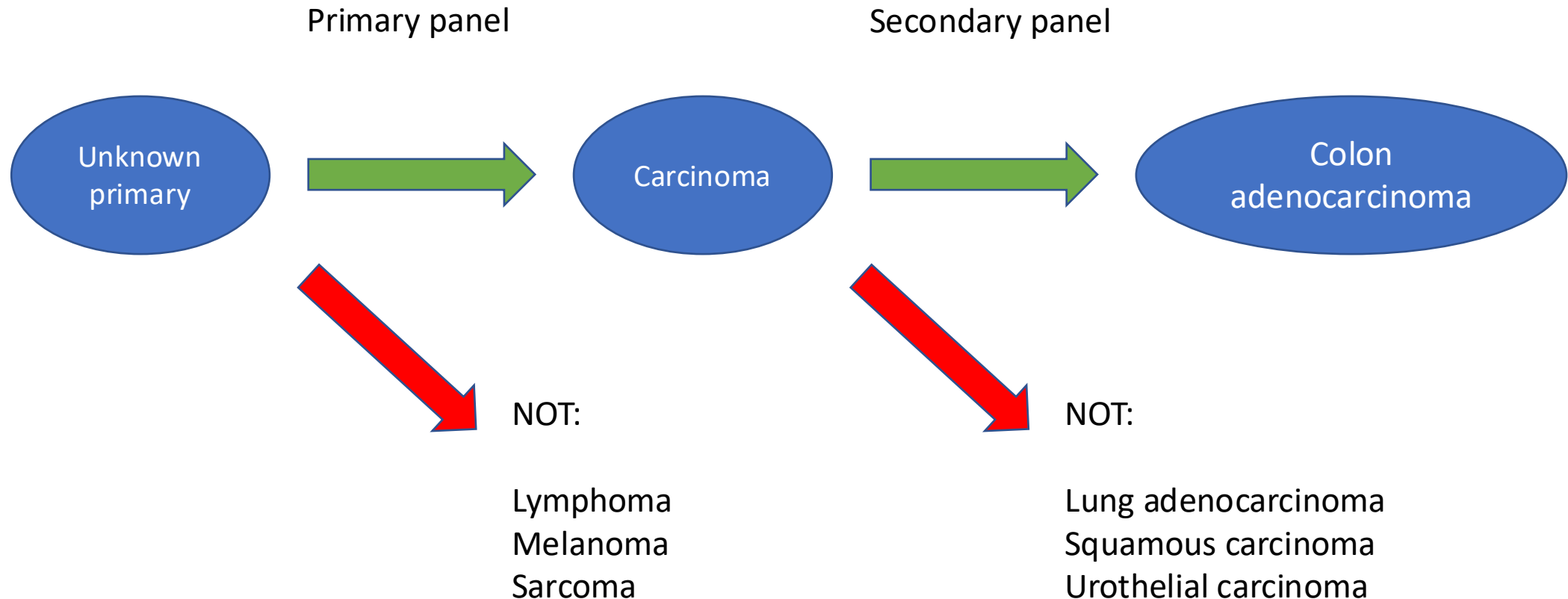
Selection of IHC antibodies for diagnostic algorithm



IHC panels for unknown primary tumour

- Primary panel
 - Which overall tumour type
- Secondary cytokeratin panel
 - Information on site of origin
- Secondary organ specific panel
 - Confirmation (or rejection) of diagnosis with organ specific marker
- Hematology panel

IHC panels for unknown primary tumour



Unknown primary tumour

Bad for the patient – fascinating for the pathologist!

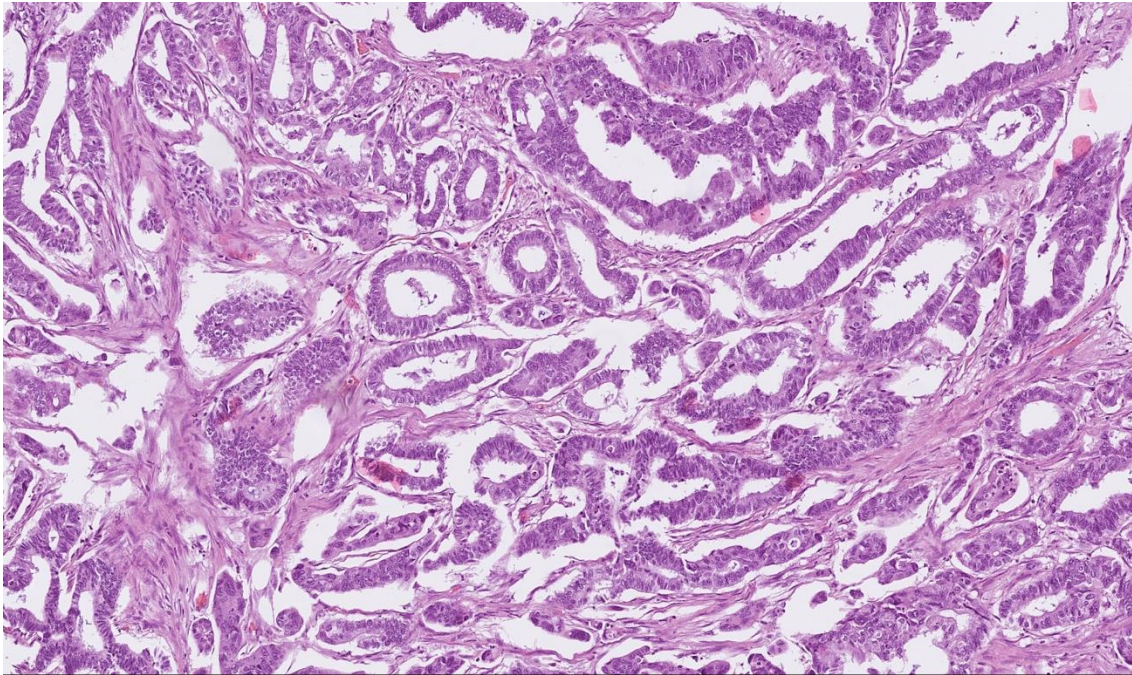
- Diagnostic challenge
- Integration of knowledge
- Brings together morphology and IHC
- Rare and unusual tumors
- Intellectual rewarding



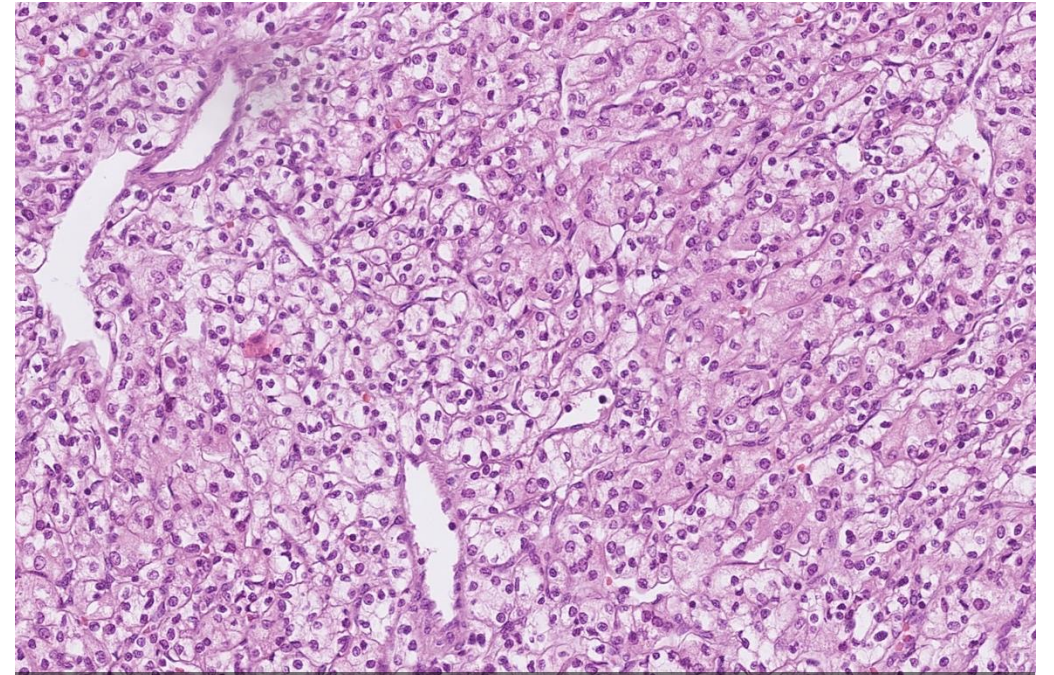
IHC classification of unknown primary tumour

- Adenocarcinomas (80-90%)
 - Lung, breast, prostate, colon, ovary, pancreas
- Squamous cell carcinoma (5-10%)
 - Lung, esophagus, cervix, head and neck
- Undifferentiated neoplasms (5-10%)
 - Carcinomas, melanomas,
 - Lymphomas
 - Sarcomas
 - Germ cells tumours

Easy cases – no IHC?!

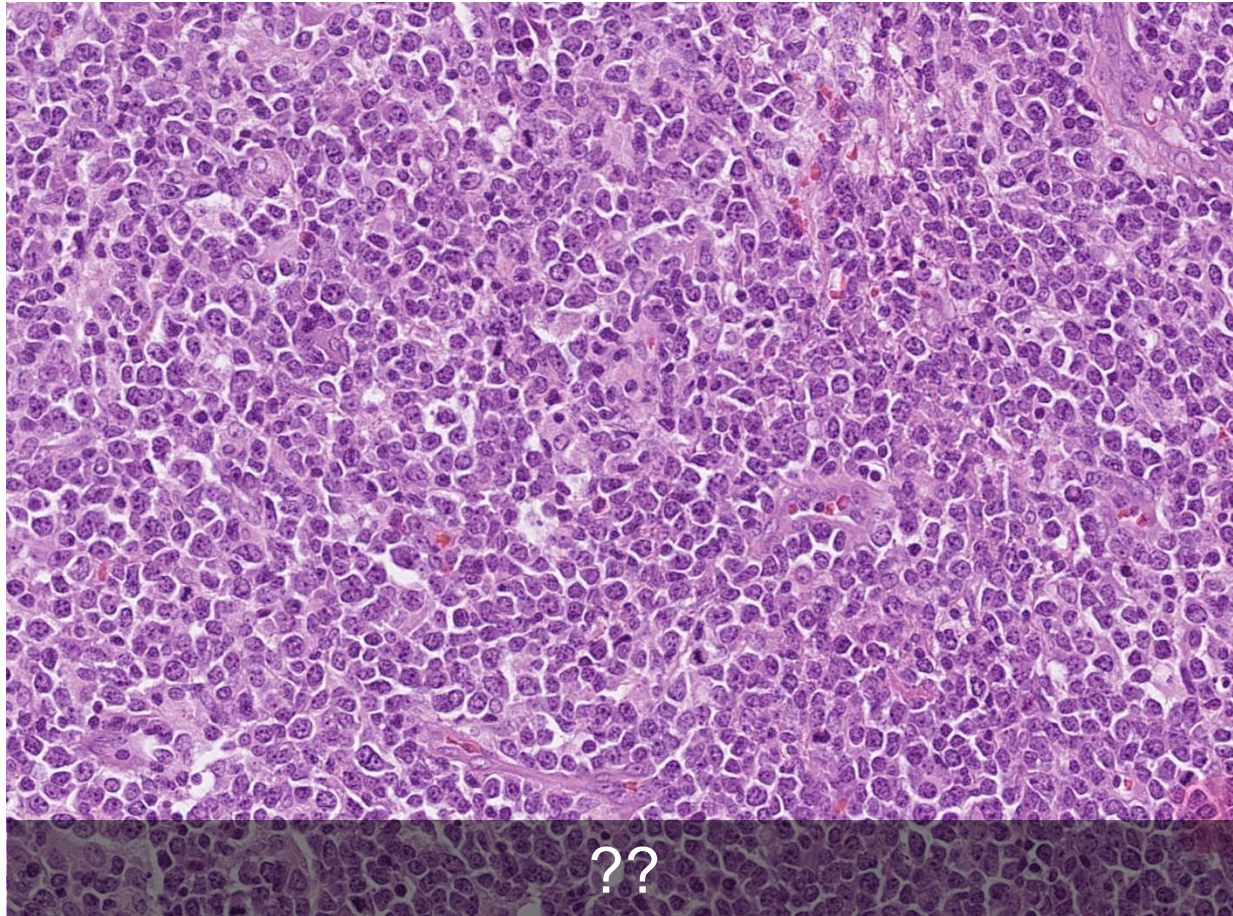


Colon adenocarcinoma



Renal clear cell carcinoma

Undifferentiated cases



Primary panel

Unknown primary tumour

Primary panel

+	~ 100% pos.
(+)	> 90%
+/-	~ 50-90%
-/+	~ 10-50%
(-)	< 10%
-	~ 0%

Neoplasms	CD45	Pan cytokeratin	S100	Vimentin
Haematopoietic / lymphoid	(+)	(-)	(-)	(+)
Epithelial	-	(+)	-/+	-/+
Mesothelial	-	+	-	+
Mesenchymal	-	(-)	(-)	+
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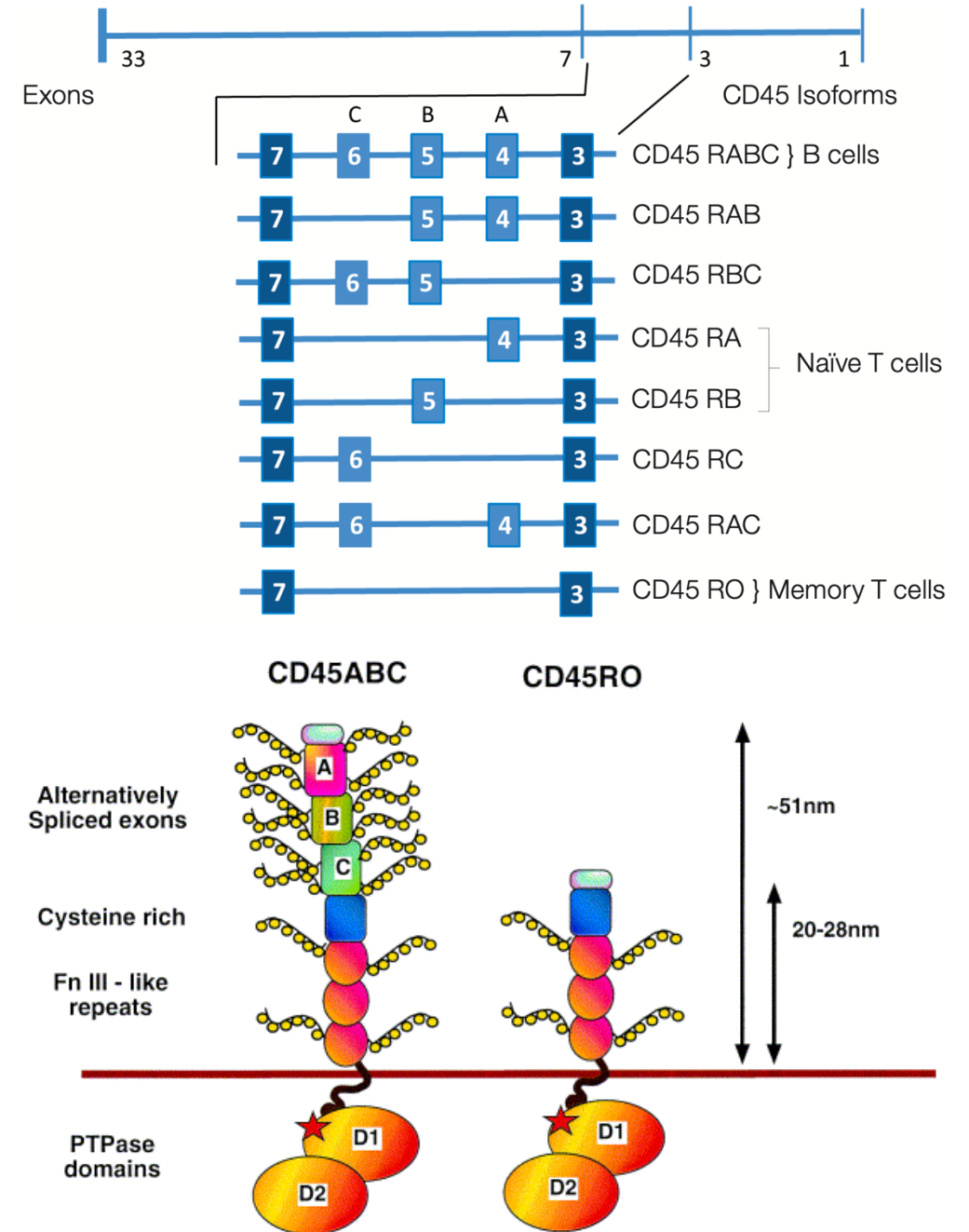
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CD45 – Leucocyte common antigen

- Protein tyrosin phosphatase, receptor type C (PTPRC)
- Previously called Leucocyte common antigen (LCA)
- Key role in activation and differentiation of T- and B-cells
- Expressed on all hematopoietic cells
- Exception: Plasma cells, erythrocytes and megakaryocytes

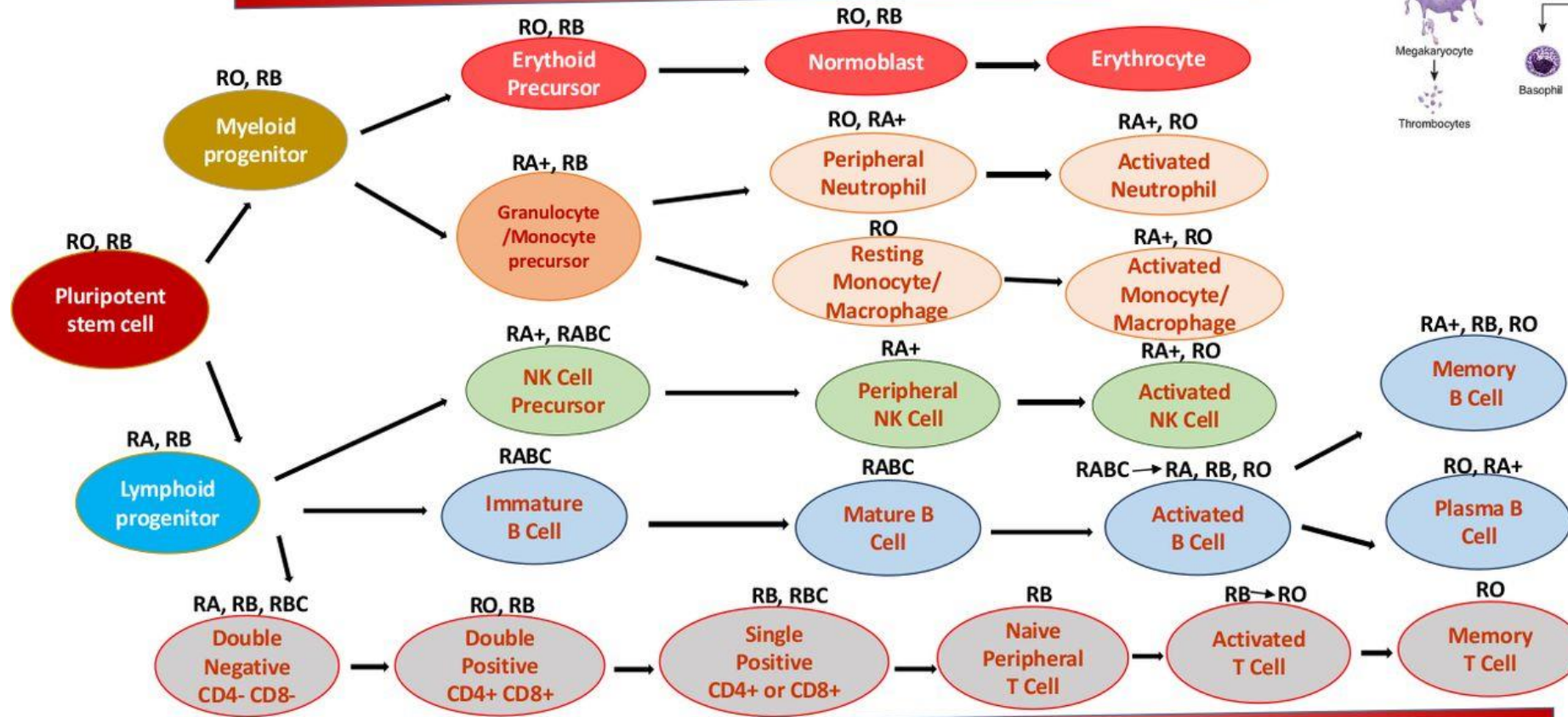
CD45 - isoforms

- Alternatively spliced variance in extracellular components
- Six human isoforms identified:
 - RABC, RAB, RBC, RA, RB, R0
- Isoforms expressed on different subsets of lymphocytes

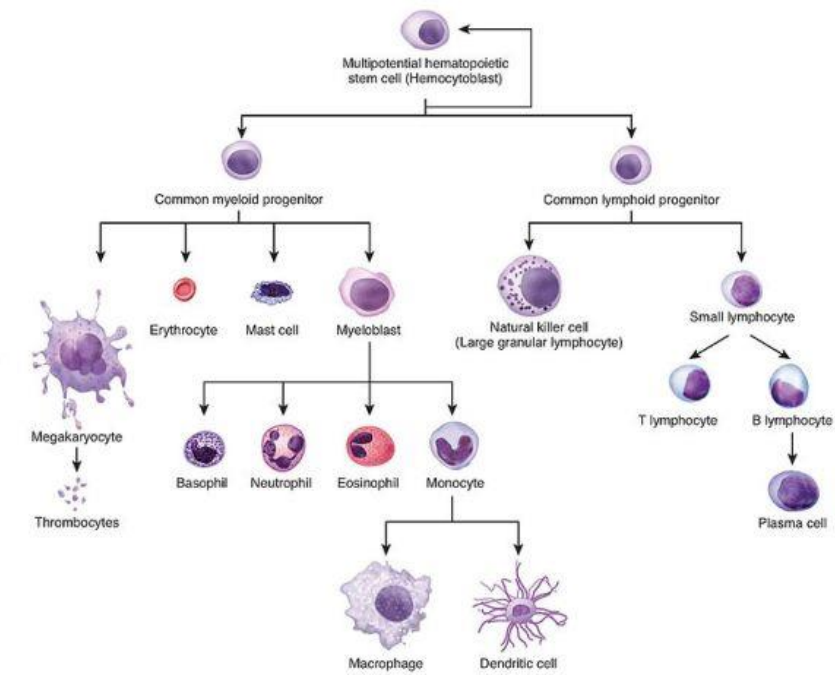


CD45 - isoforms

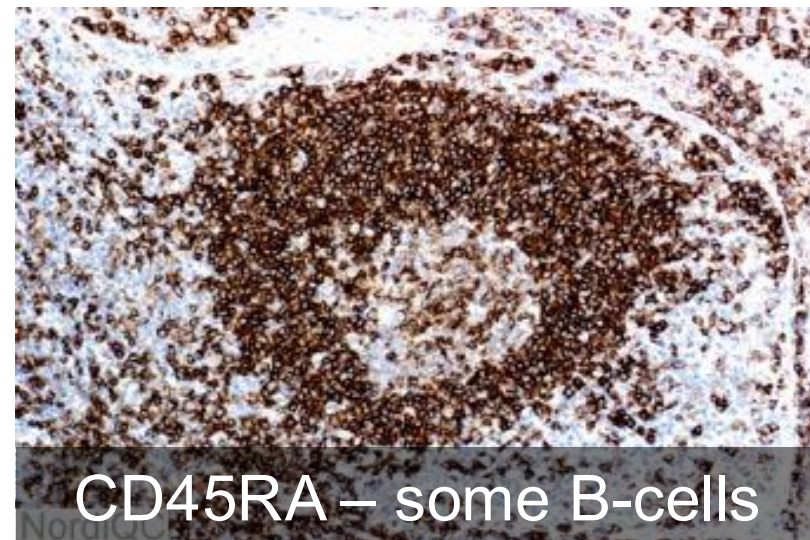
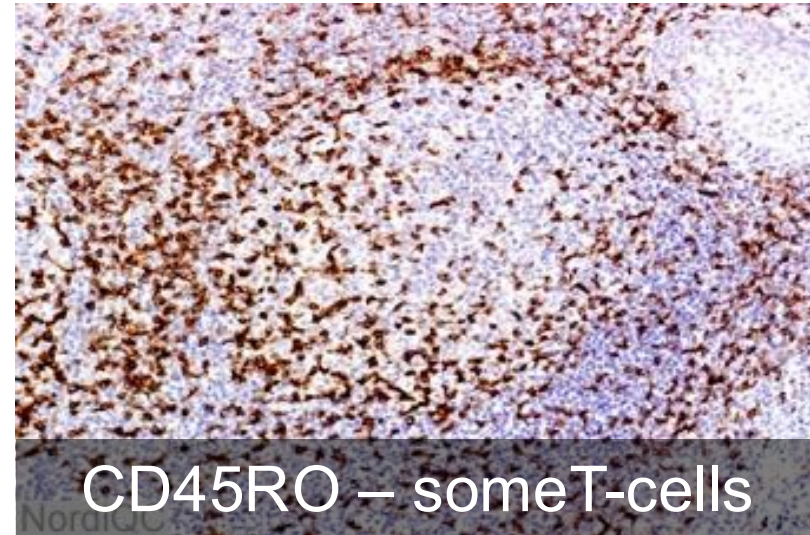
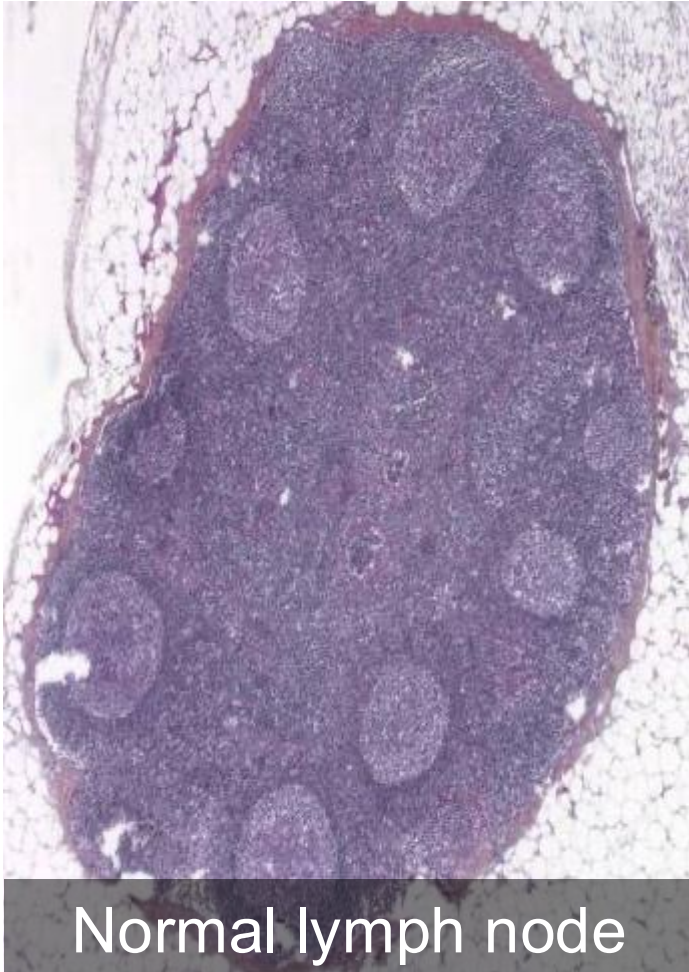
Erythroid cell lineage CD45 isoforms surface expression



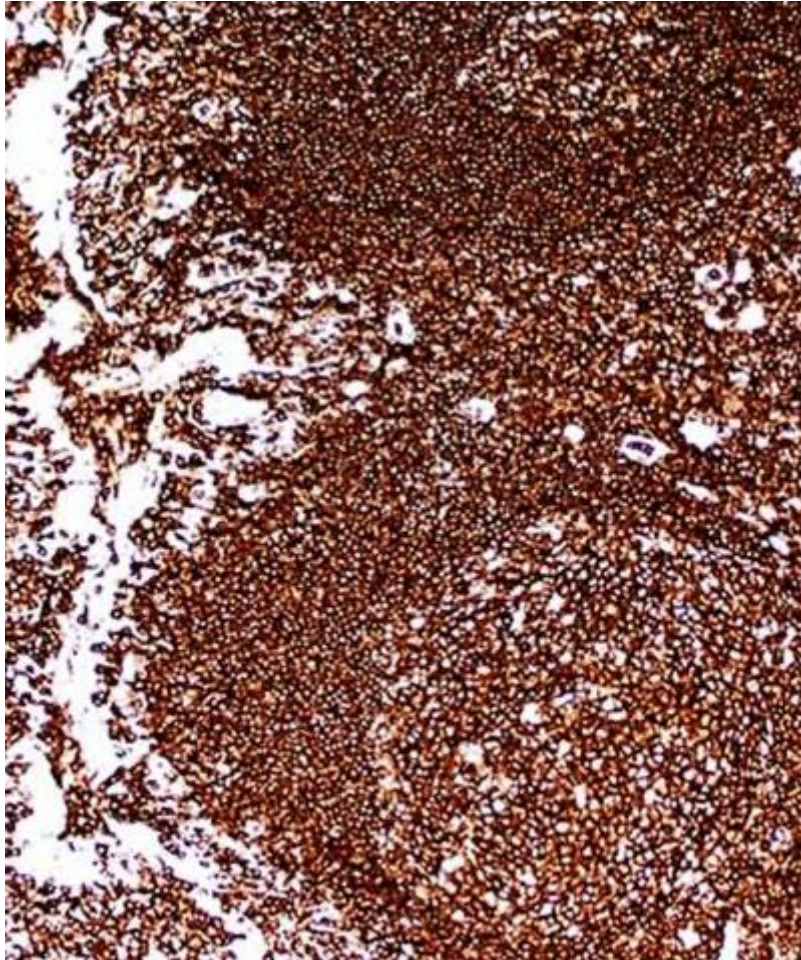
Myeloid and lymphoid cell lineage CD45 isoforms surface expression



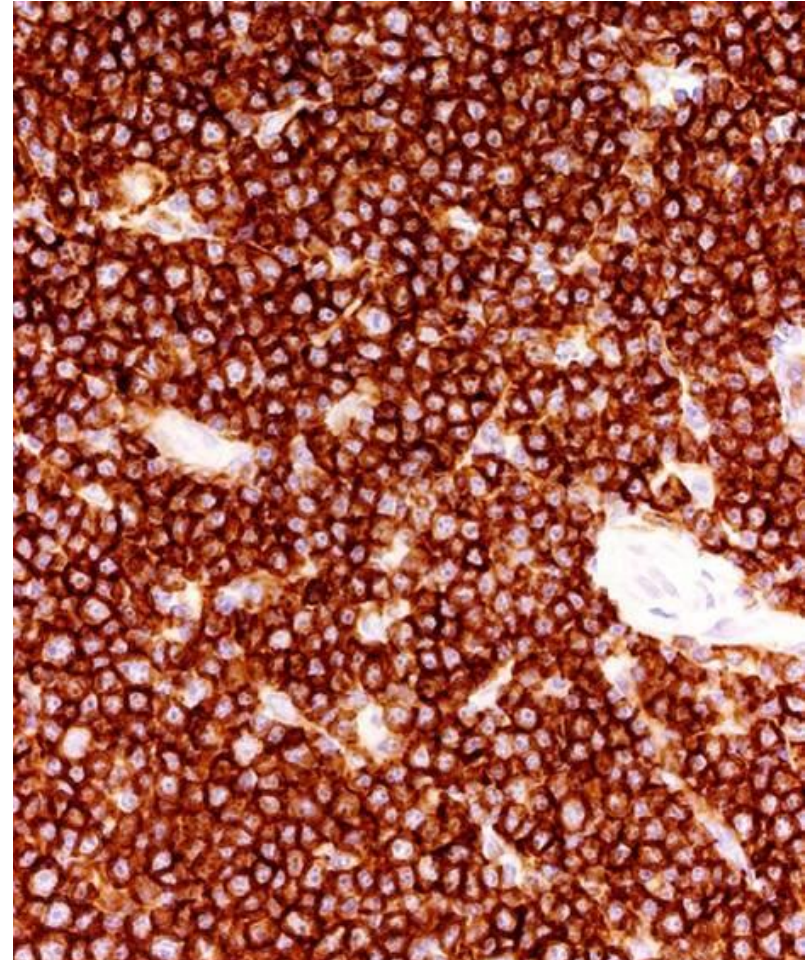
CD45 - isoforms



CD45

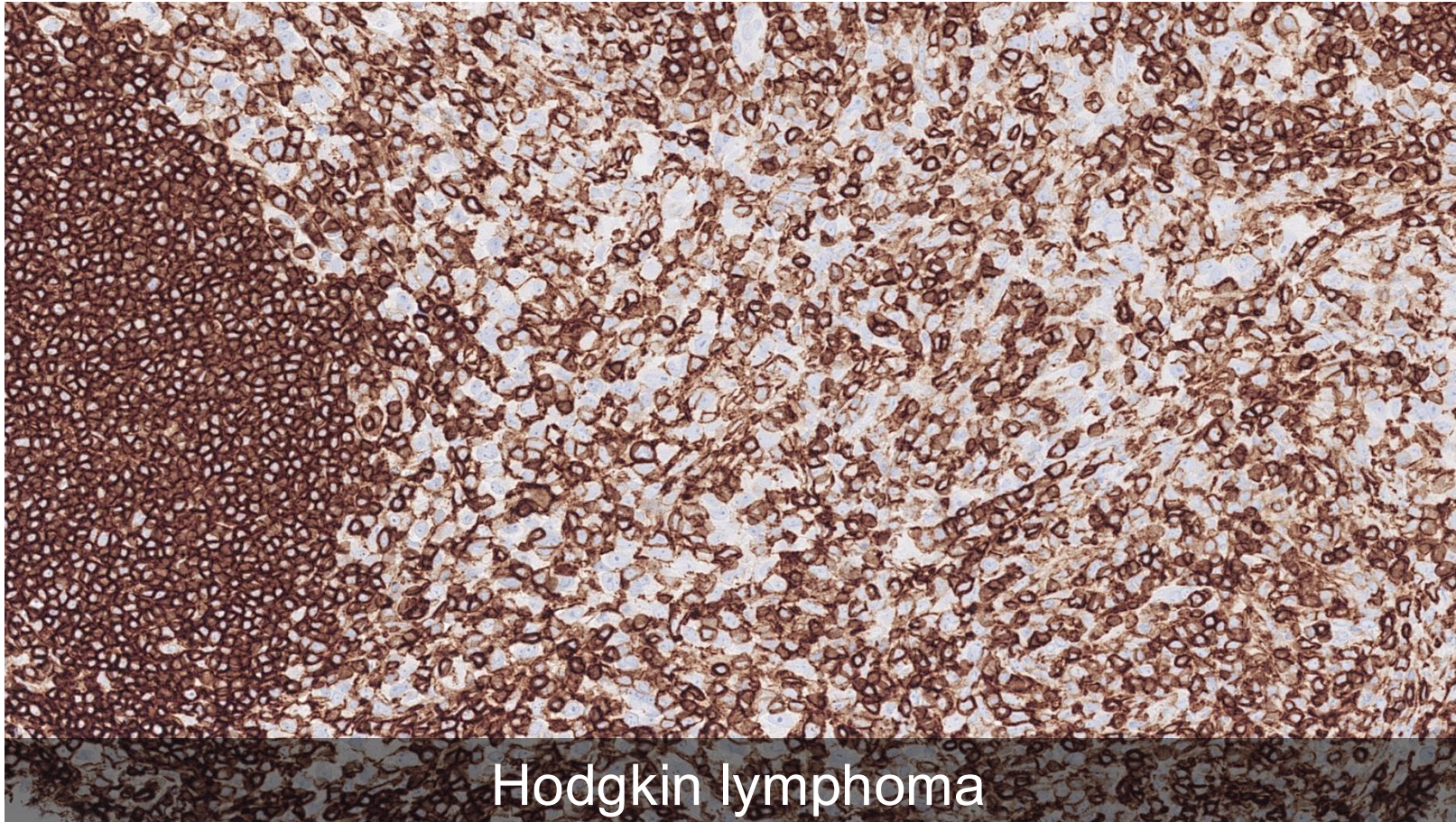


Normal lymph node

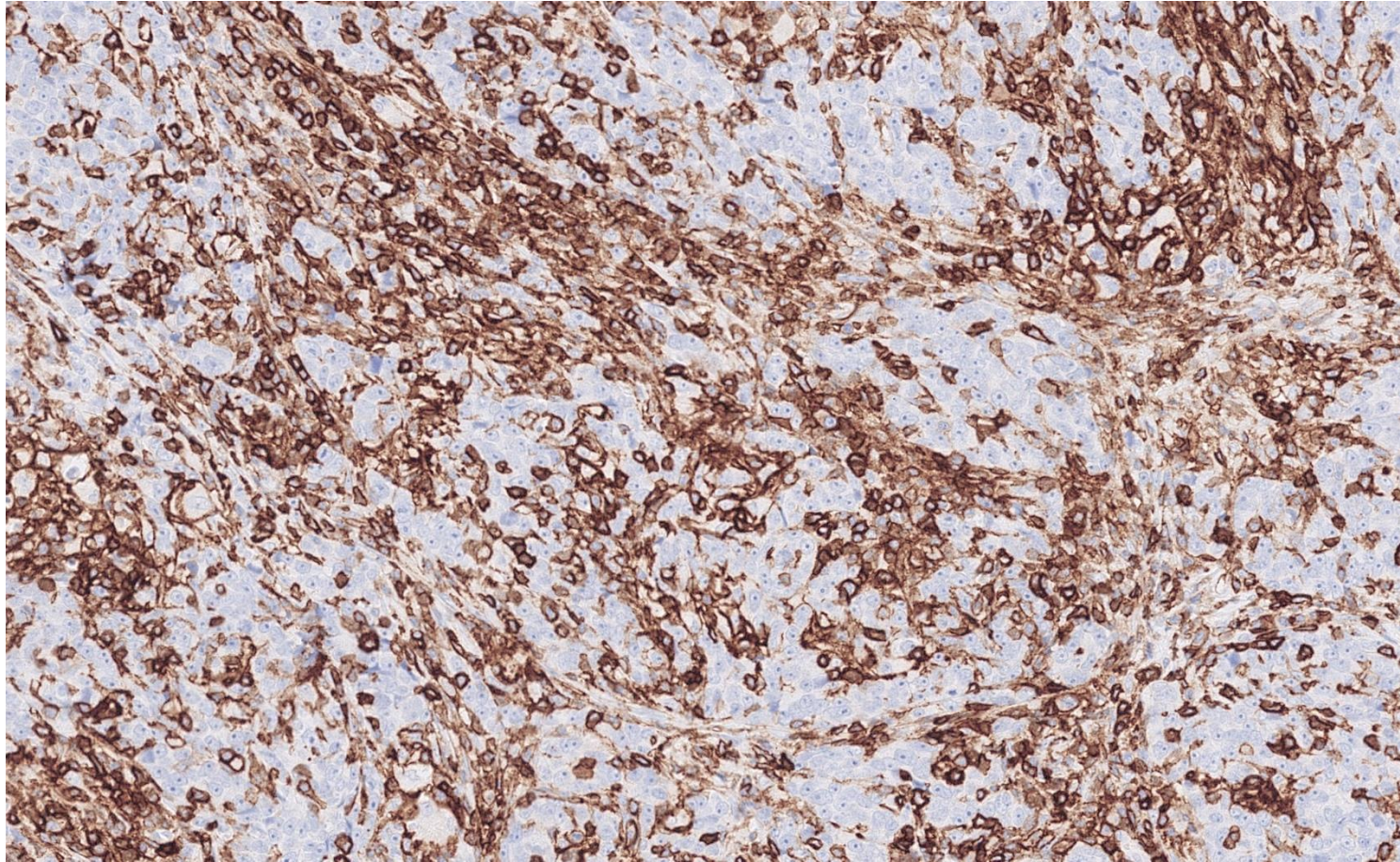


Malignant lymphoma

CD45 – negative in some lymphomas



CD45 – positive carcinoma?



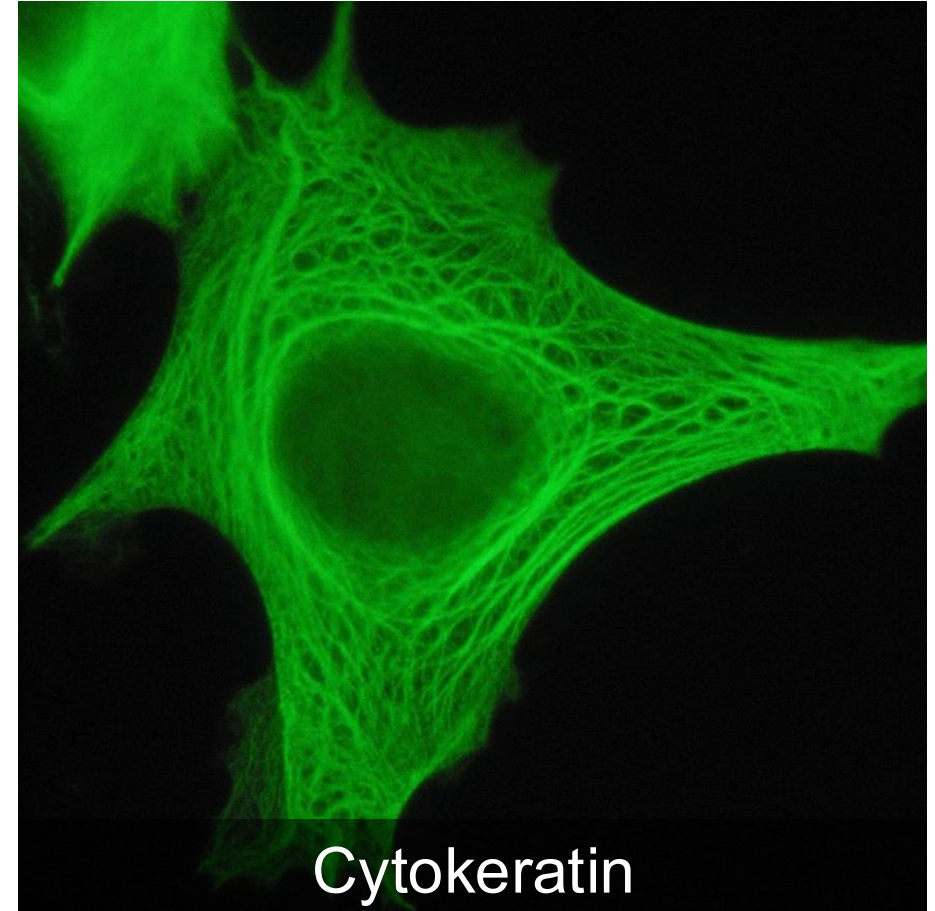
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Melanoma	-	-	+	+
Germ cell	-	-/+	-/+	+

Pan cytokeratin

- Group of intermediary filaments
- Part of the cytoskeleton in epithelial cells
- Important for mechanical strength of cells and cellular functions
- Many different types:
 - Group I: acidic
 - Group II: basic or neutral
- Paired expression
- Different cytokeratins seen in different tissues



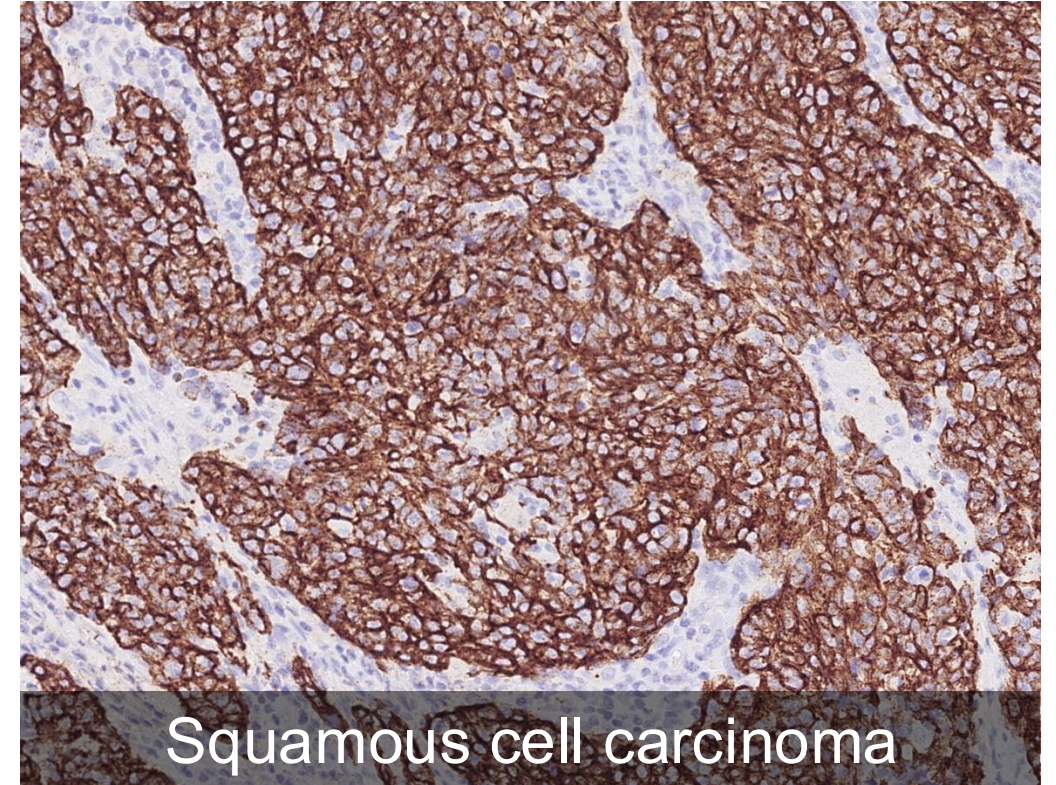
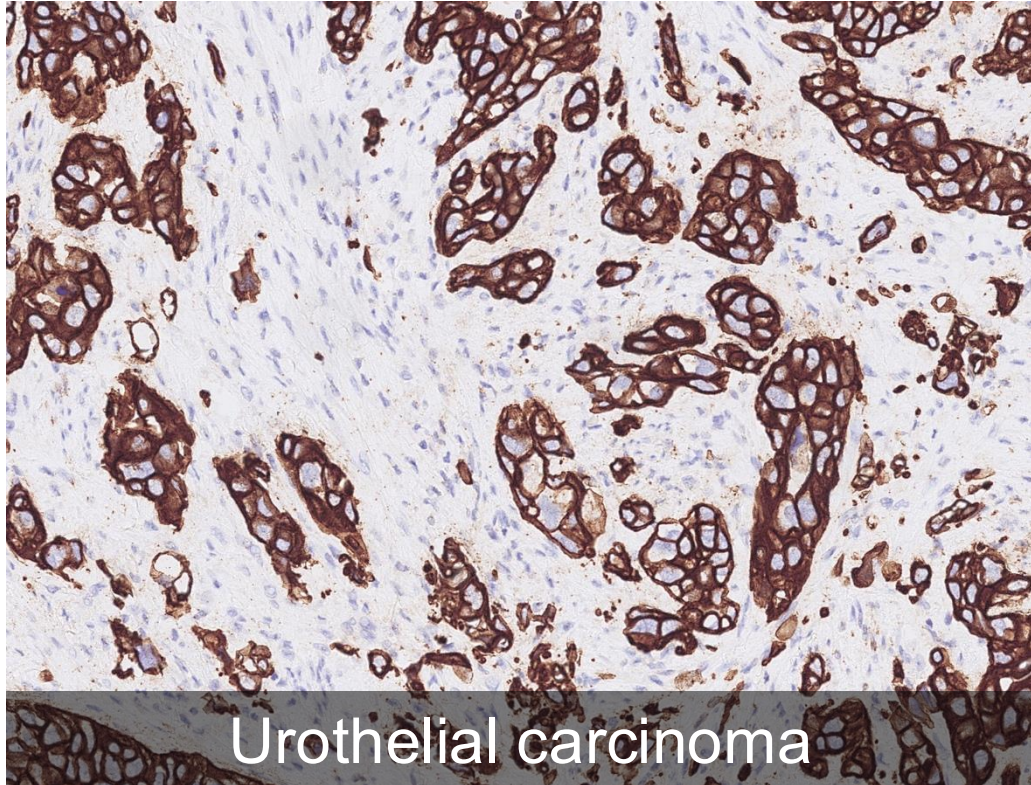
Neutral/Basic (B, class II)	1	4		5				7		8
Acidic (A, class I)	10		13		14	17	19		20	18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (tonsil, mucosa)	-	-	-	+++	+++	(+)	(++)	(+)	-	(+)
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermediary. / basal cells	-	(+)	+++	(+++)	-	(++)	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+)	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (Merkel, thyroid)	-	-	-	-	-	-	(+++)	(++)	(+++)	+++
Smooth muscle (vasc., myom.), myofibrobl., sm.ves.endothelia	-	-	-	-	-	-	+	(++)	-	++

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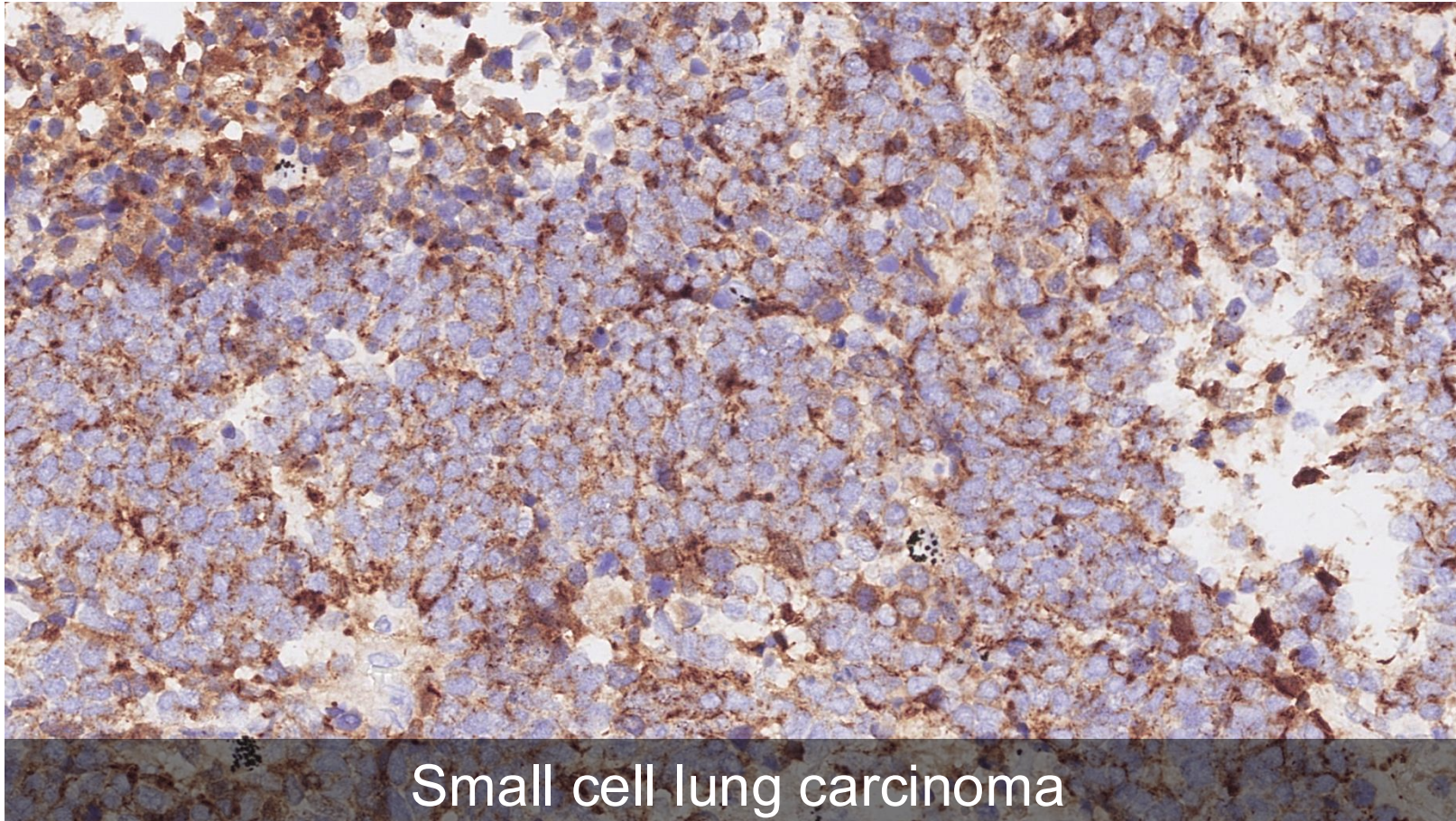
Pan cytokeratin



Pan cytokeratin - micrometastasis



Pancytokeratin

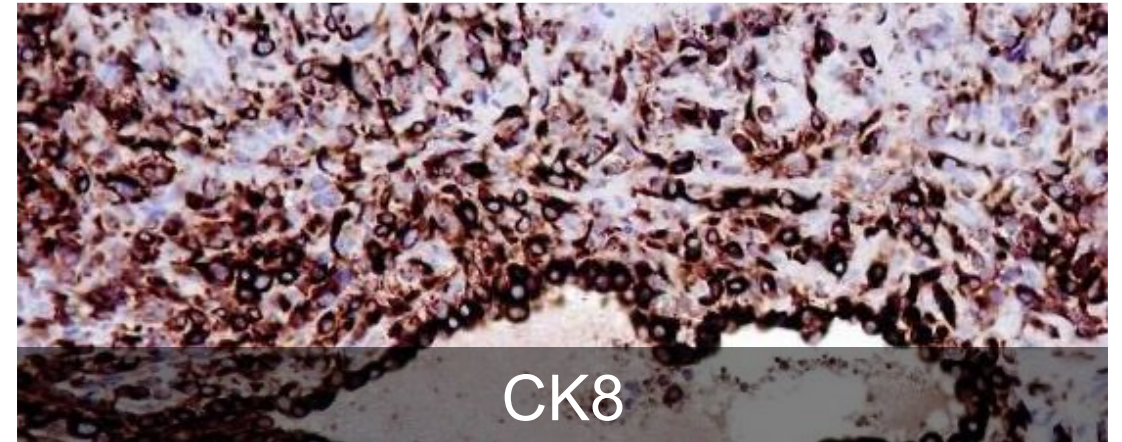
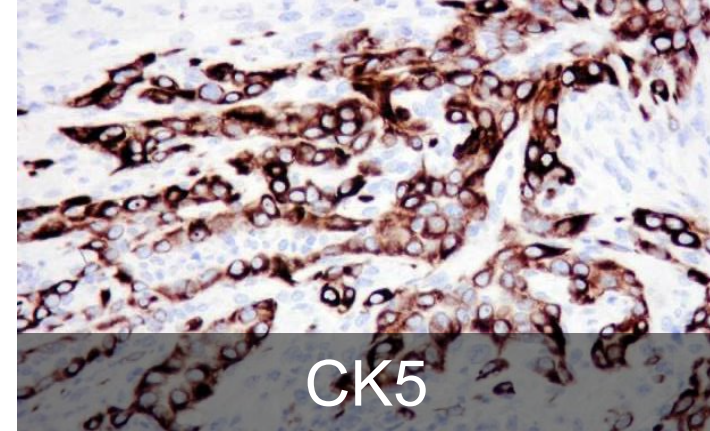
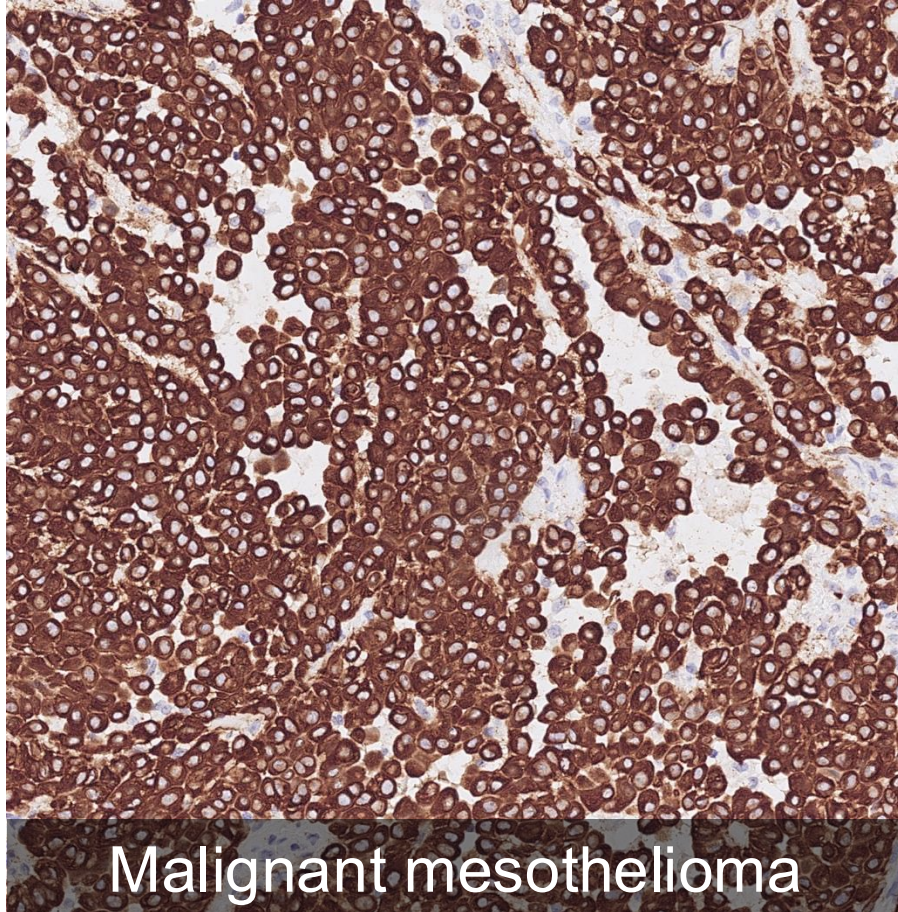


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Pan cytokeratin - mesothelioma

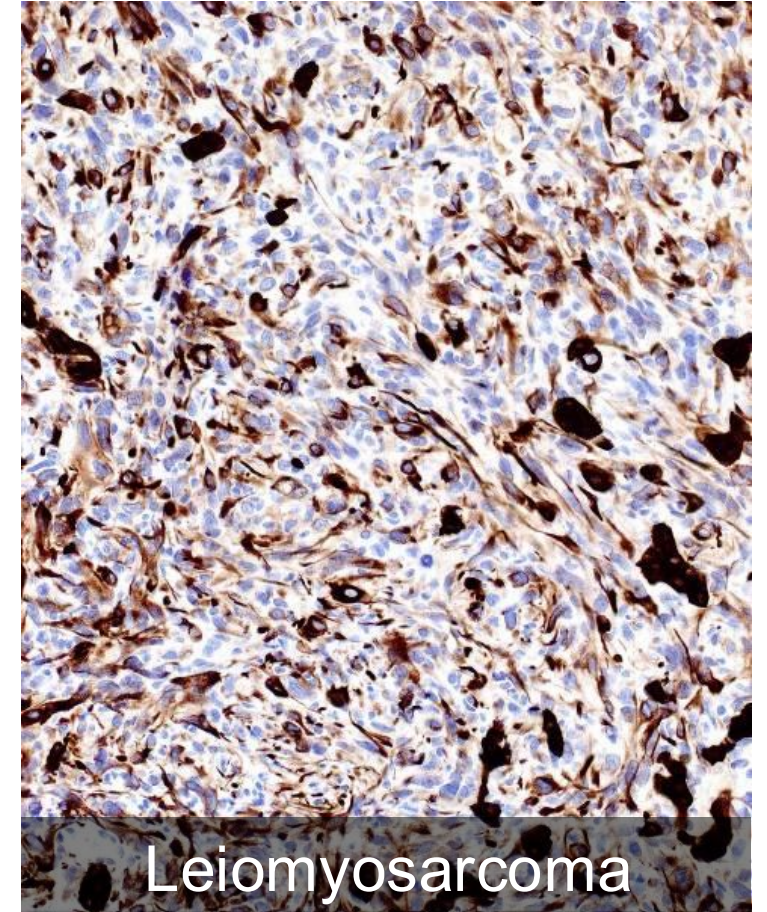
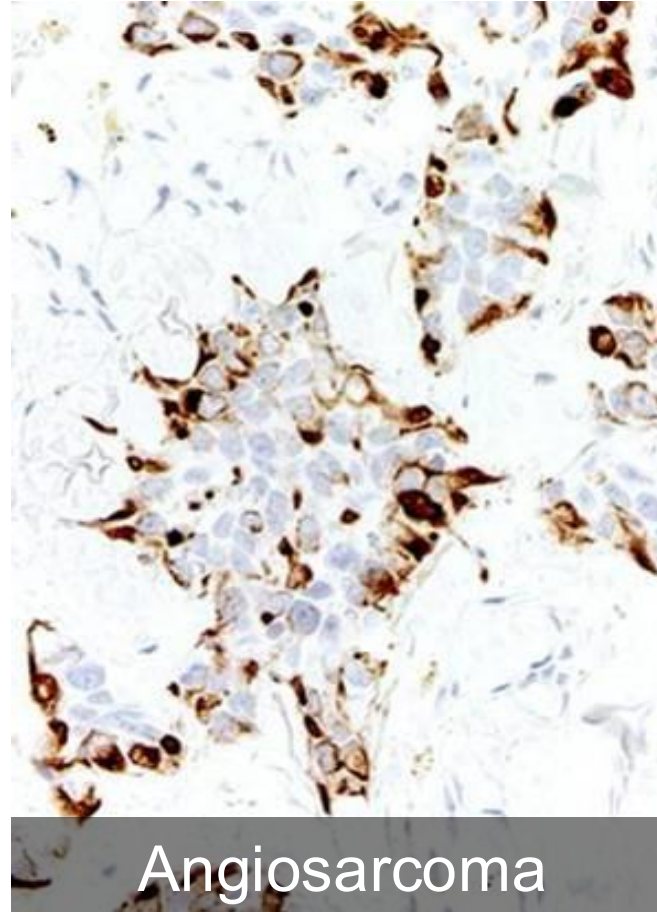
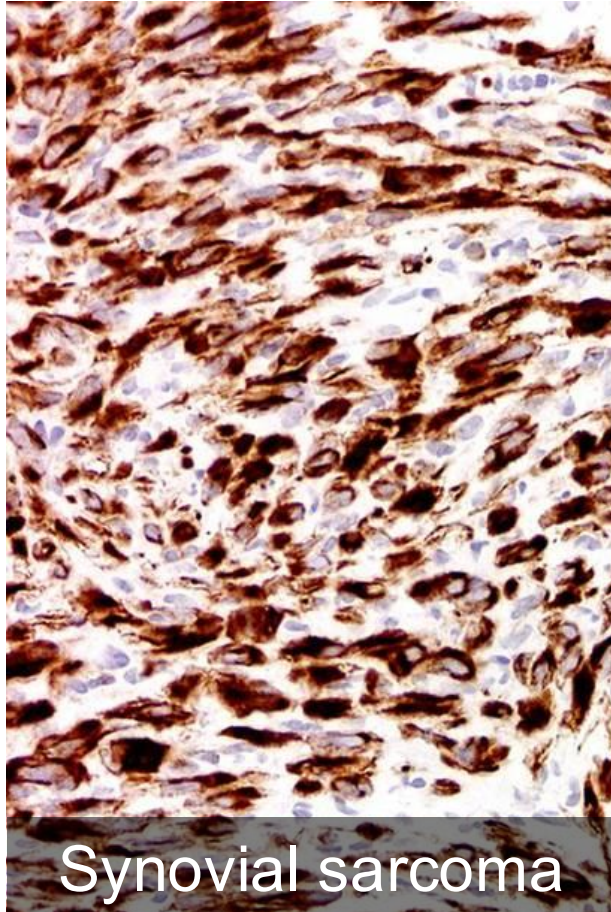


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Pan cytokeratin – mesenchymal neoplasias



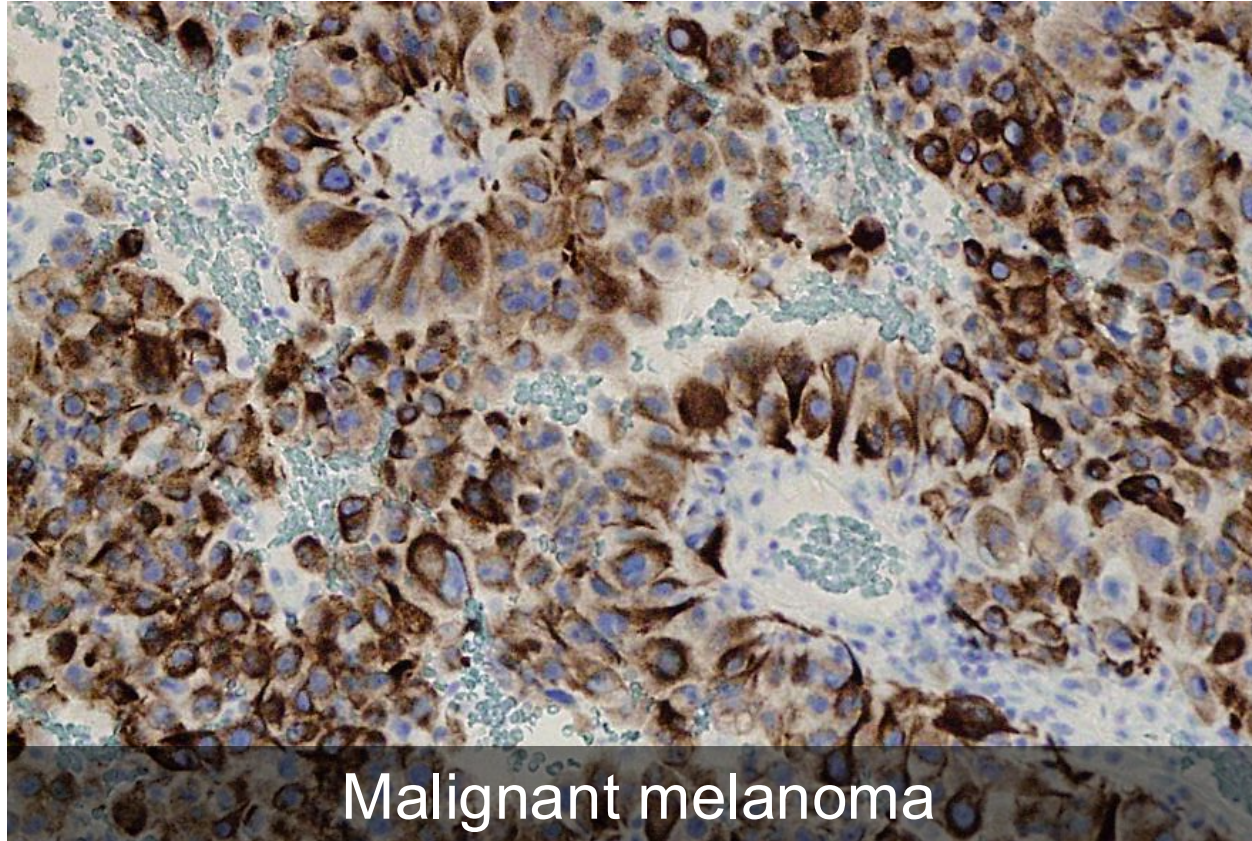
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Pan cytokeratin – melanoma

very rare positive reaction



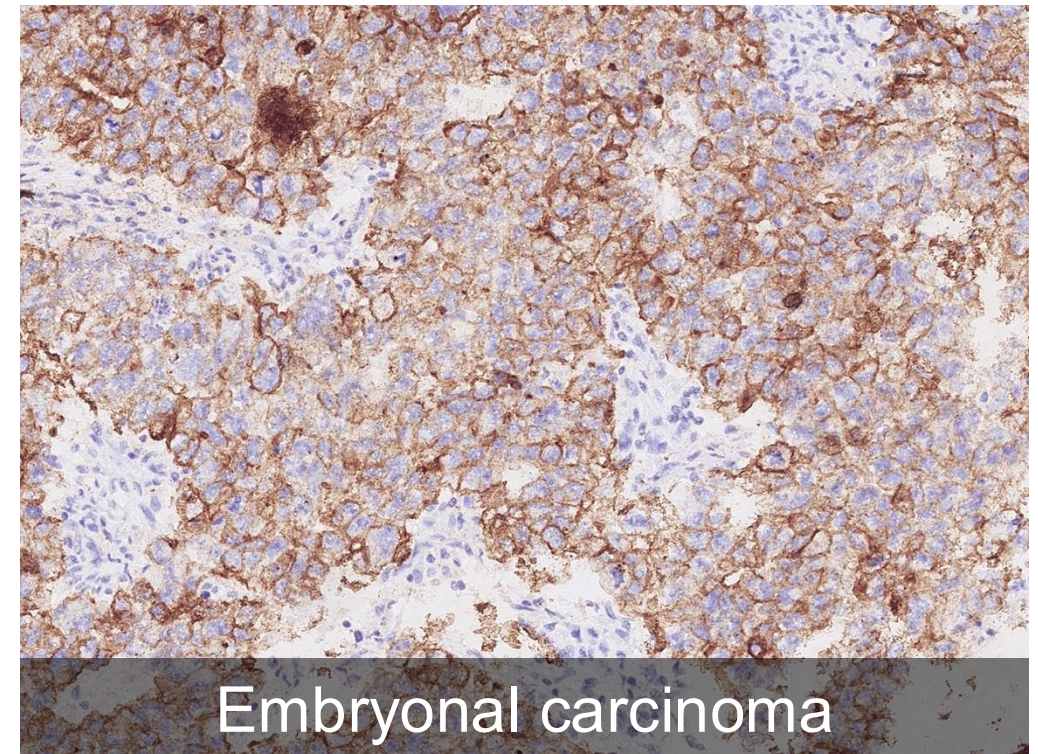
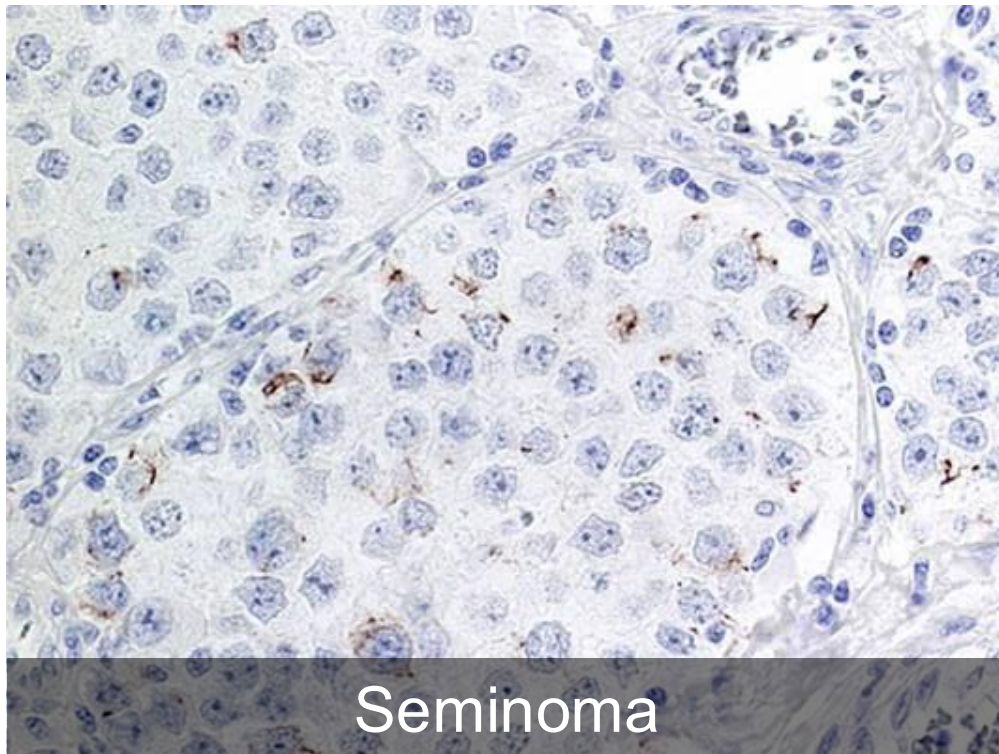
Malignant melanoma

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Germ cell	-	-/+	-/+	+

Pan cytokeratin – germ cell neoplasia



Pan cytokeratin – antibody clones

Table 4.6 Comparison of the specificity of various keratin antibody clones

Which screening keratin should I use?																			
Clone	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AE1/AE3	X	X	X	X	X	X		X		X				X	X	X			X
MAK-6								X						X	X	X		X	X
MNF116					X	X		X									X		
CAM5.2							X	X											
KL1	X	X			X	X	X	X			X			X		X	X	X	
34βE12	X				X					X				X					
• AE1/AE3-based assays are in widest clinical use • CAM5.2 only recognizes low and 34βE12 only recognizes high-molecular-weight keratins, so these should not be used in isolation • It is not the number of keratins, per se, but rather the affinity that matters (e.g., CAM5.2 may be more sensitive than AE1/AE3 in HCC/RCC) • Stratified epithelia: K1-6, 9–17 • Simple epithelia: K7, 8, 18, 19, 20																			

Ordóñez NG. *Hum Pathol.* 2013 Jul;44(7):1195–215

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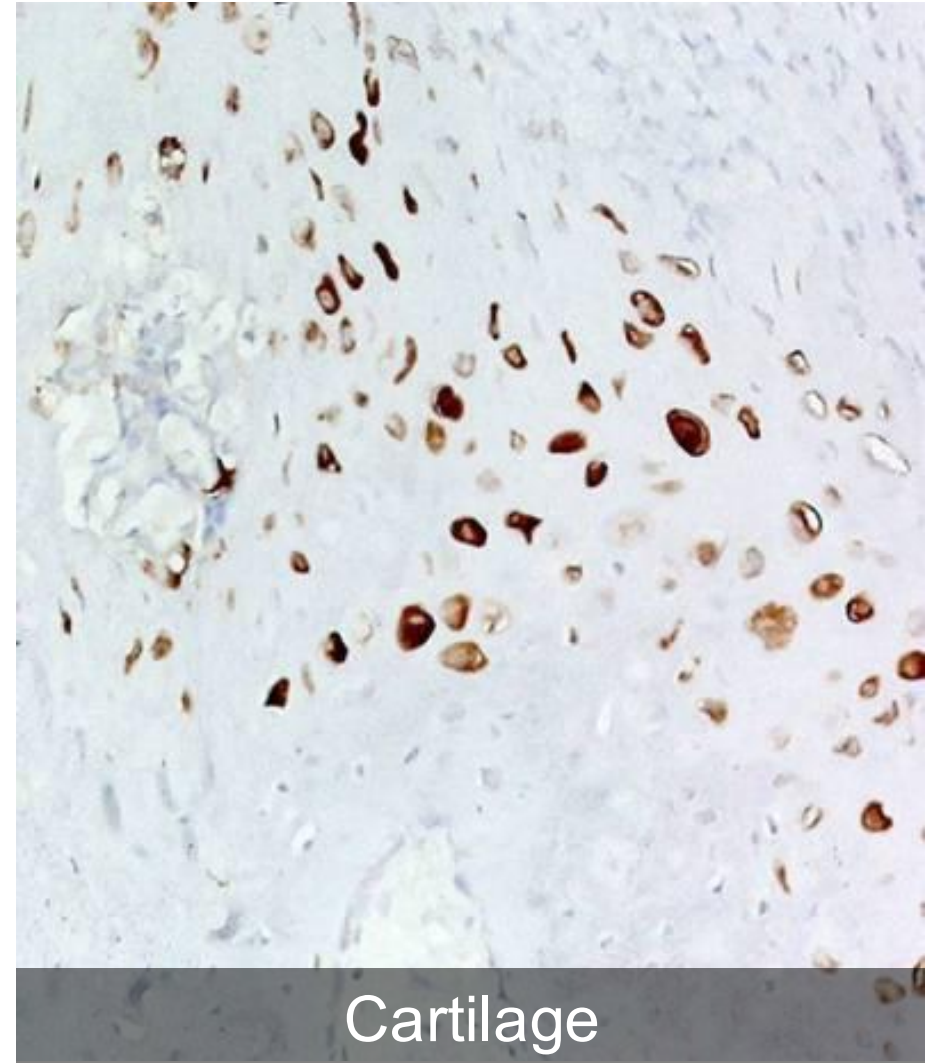
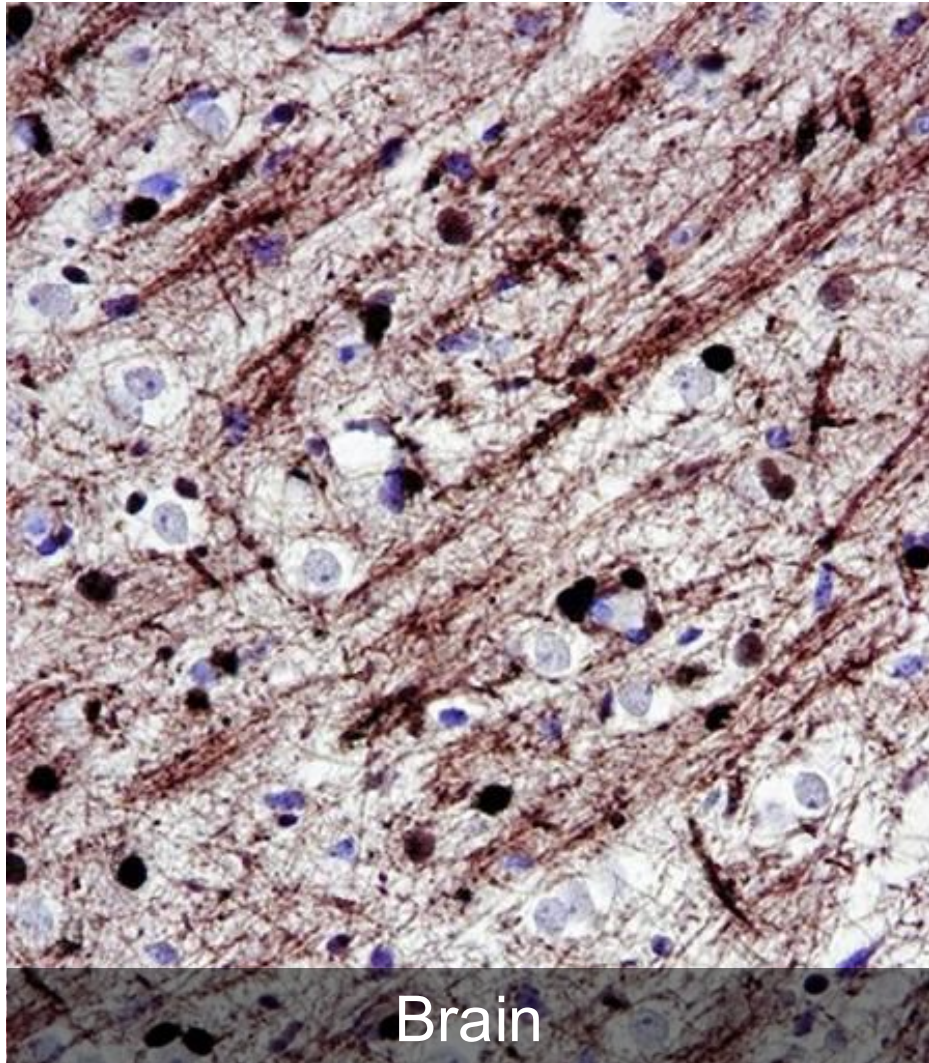
S100

- Family of low molecular weight calcium binding molecules
- Located in nuclei, cytoplasm and cell membranes
- At least 21 different types exist (S100A1, S100A2... & S100B, S100P)
- S100B expressed in
 - Glial cells in brain
 - Melanocytes
 - Fat cells
 - Langerhans cell
 - Myoepithelial cells

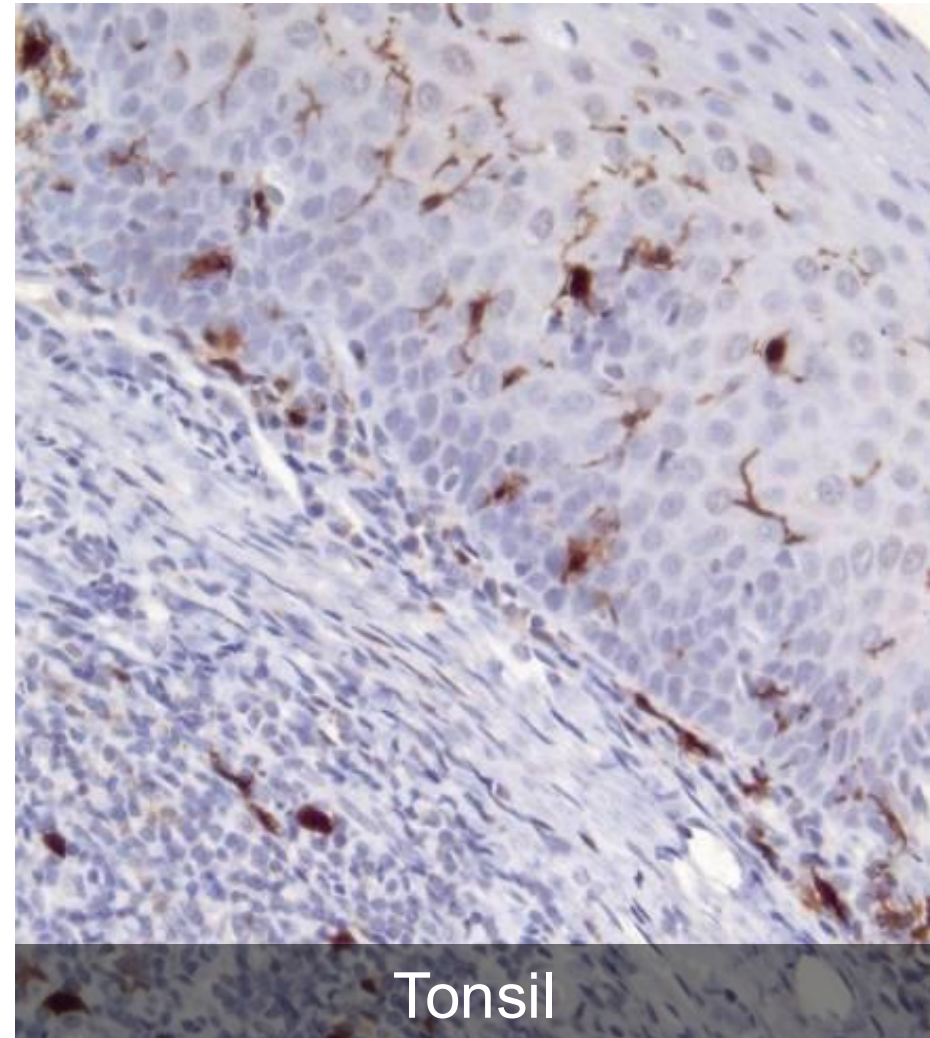
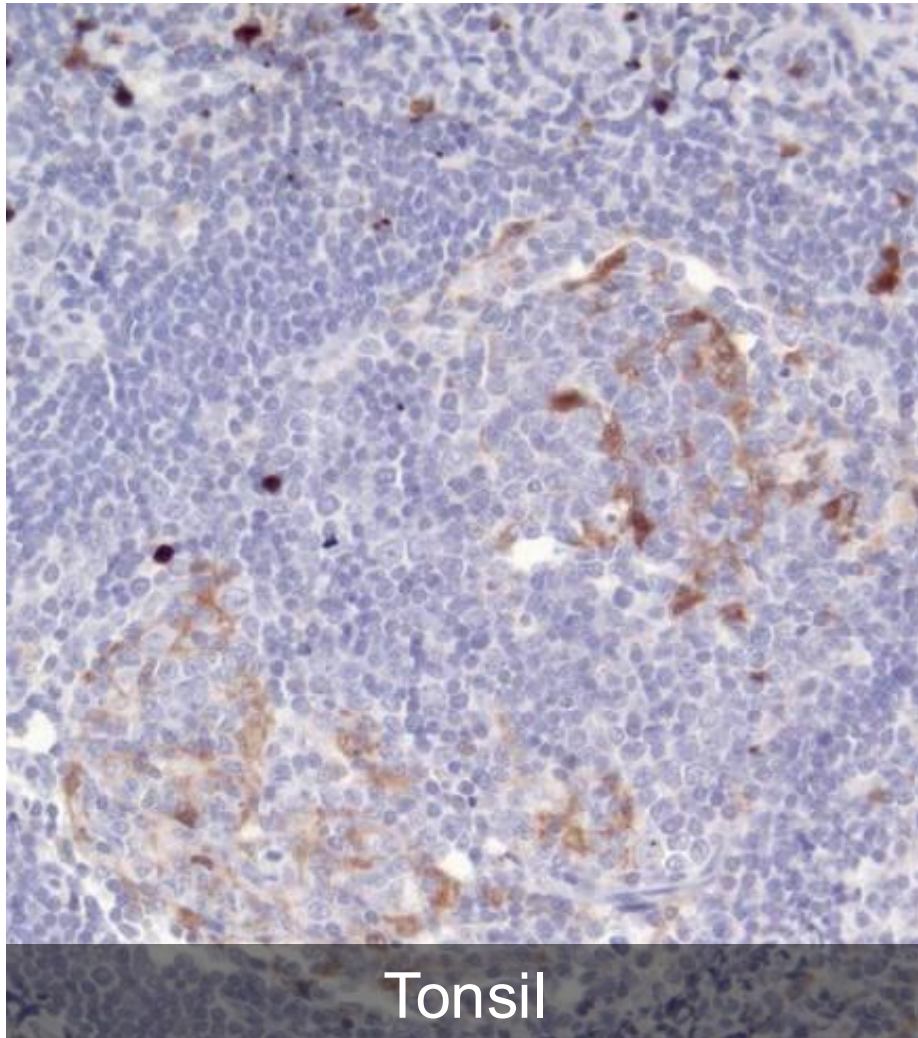
S100

- Expressed in malignant melanoma
- Also seen in
 - Schwannomas
 - Neurofibromas
 - Chondromas
 - Liposarcomas

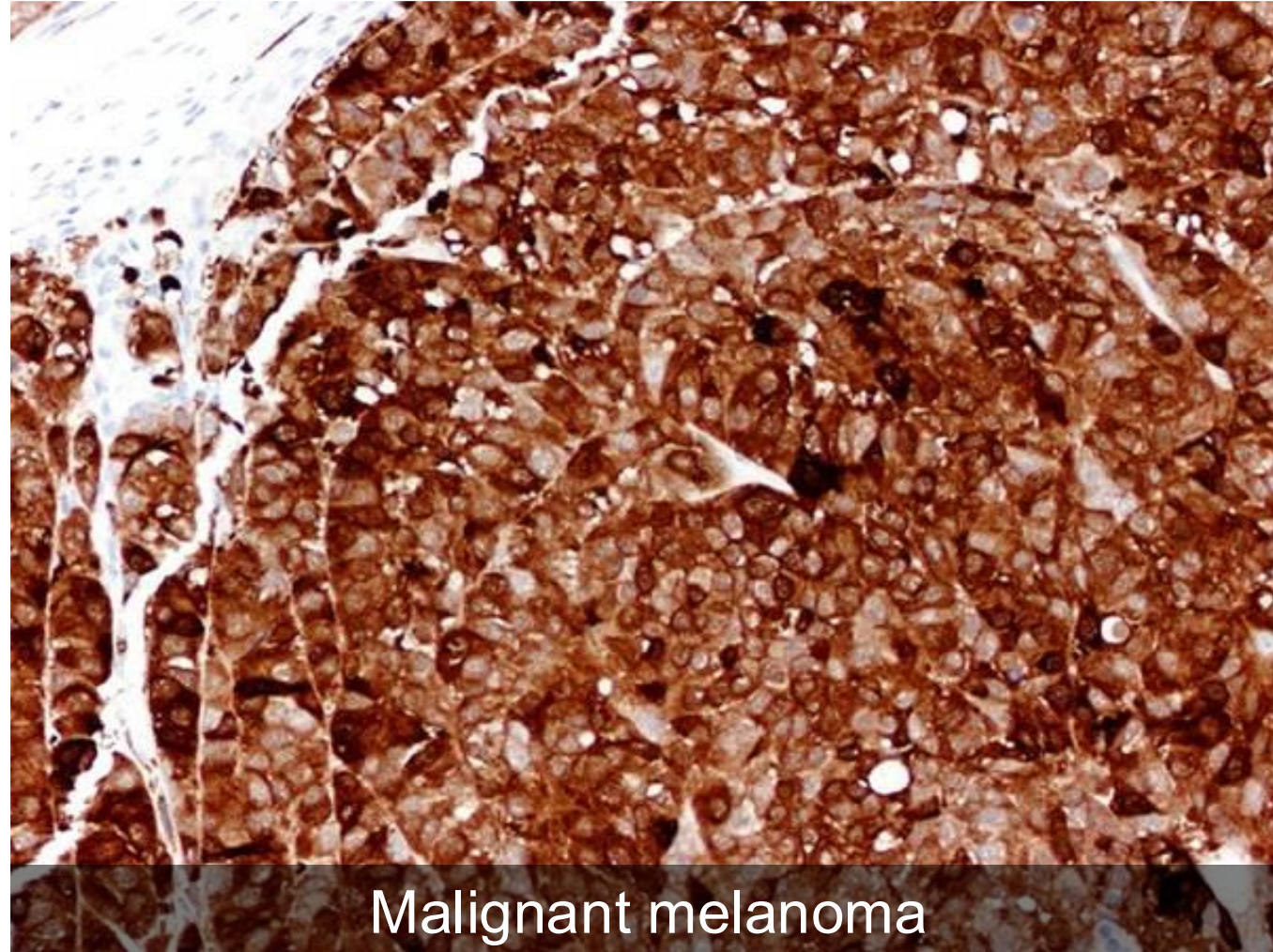
S100



S100



S100



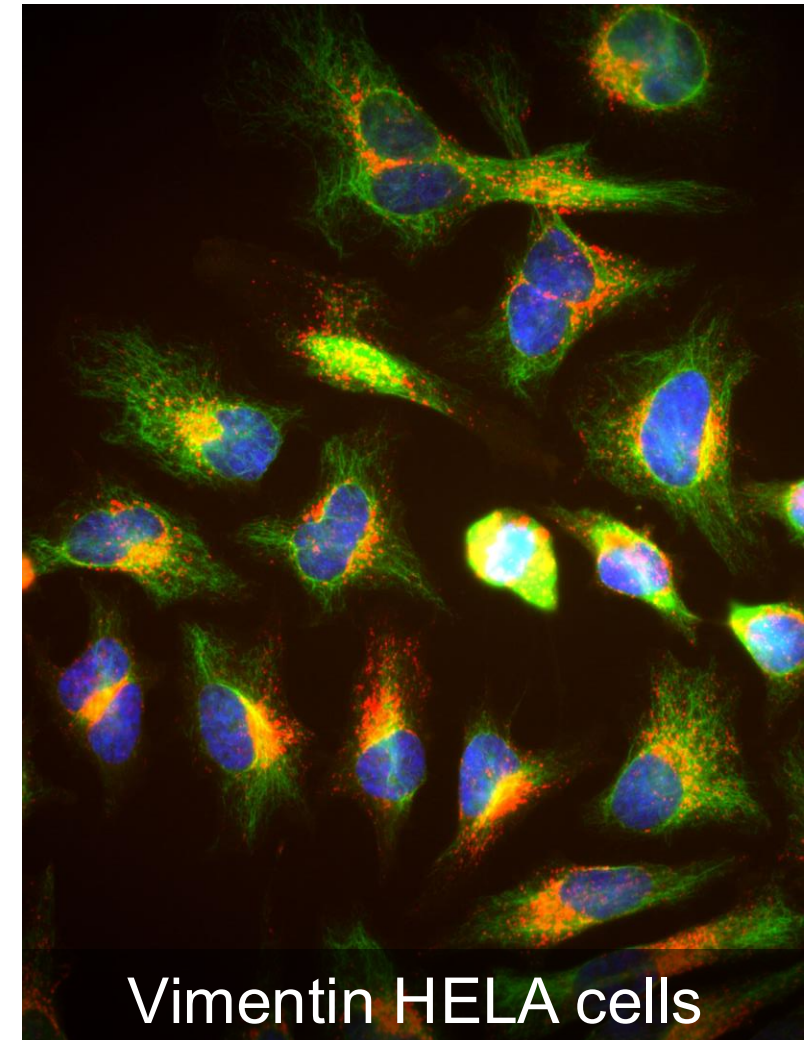
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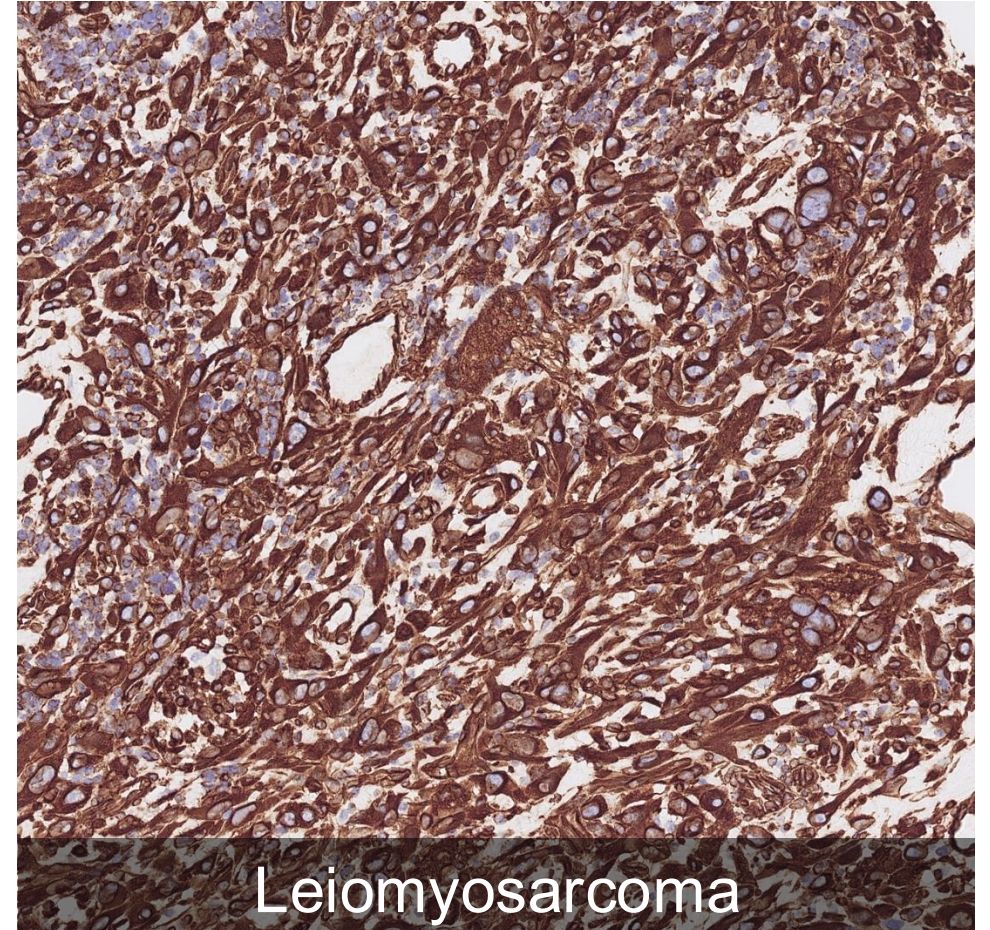
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Vimentin

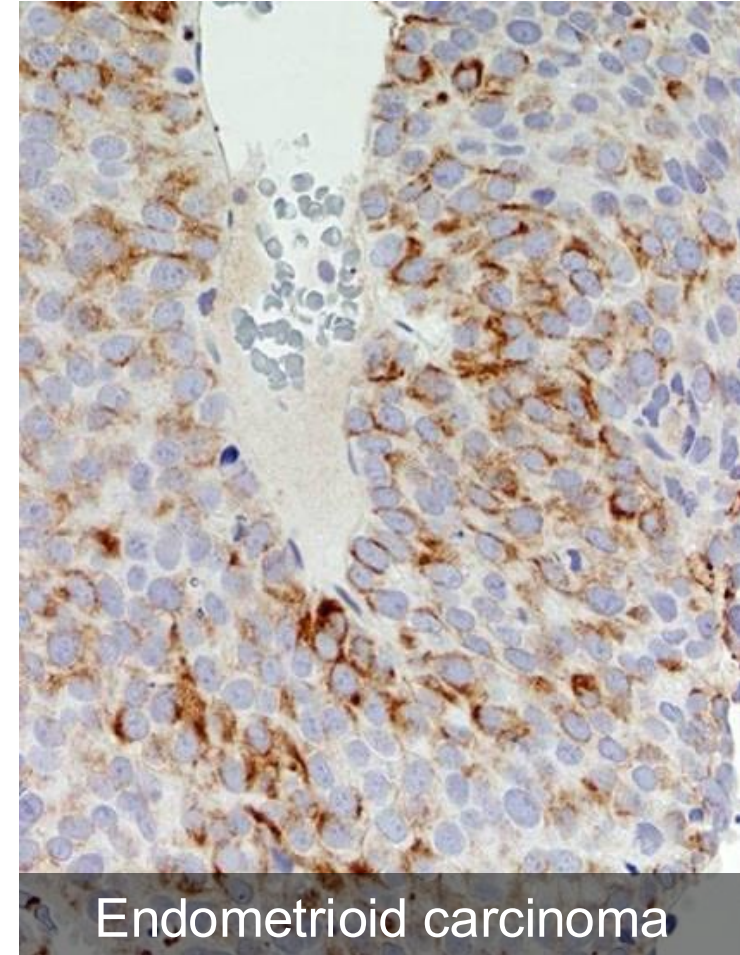
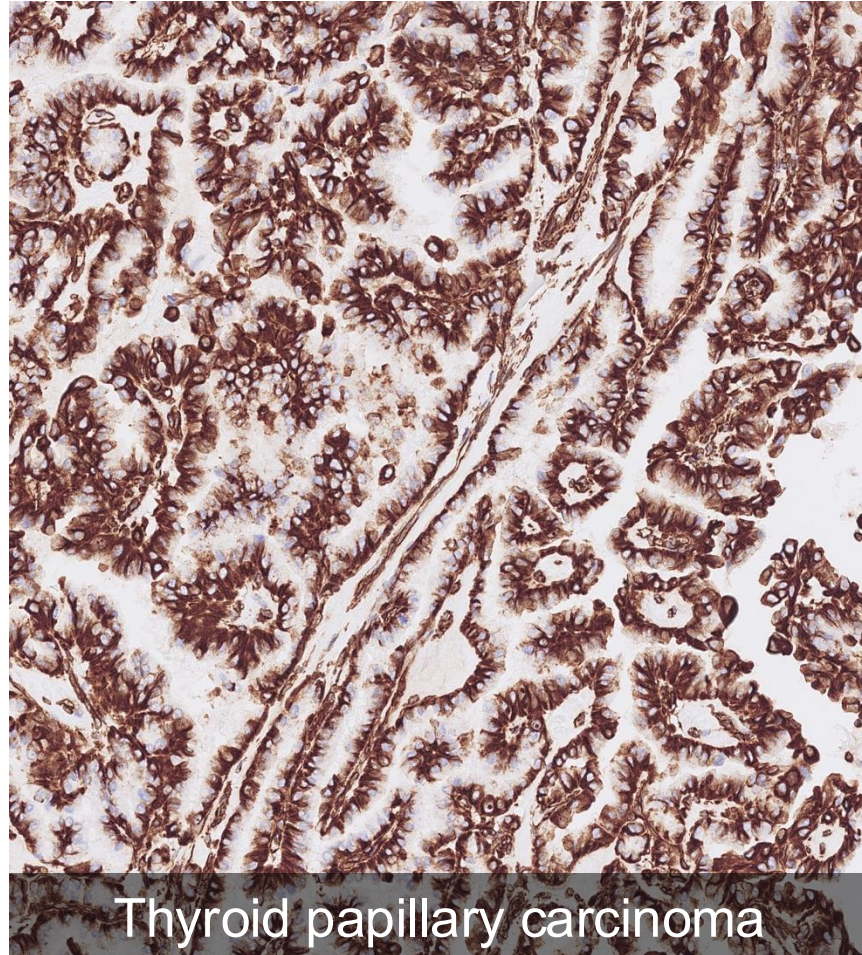
- Cytoplasmic intermediate filament
- Cytoskeleton, support and positioning of organelles
- Expressed in all mesenchymal cells
- Co-expressed with cytokeratins in some epithelia
- Co-expressed in mesothelium



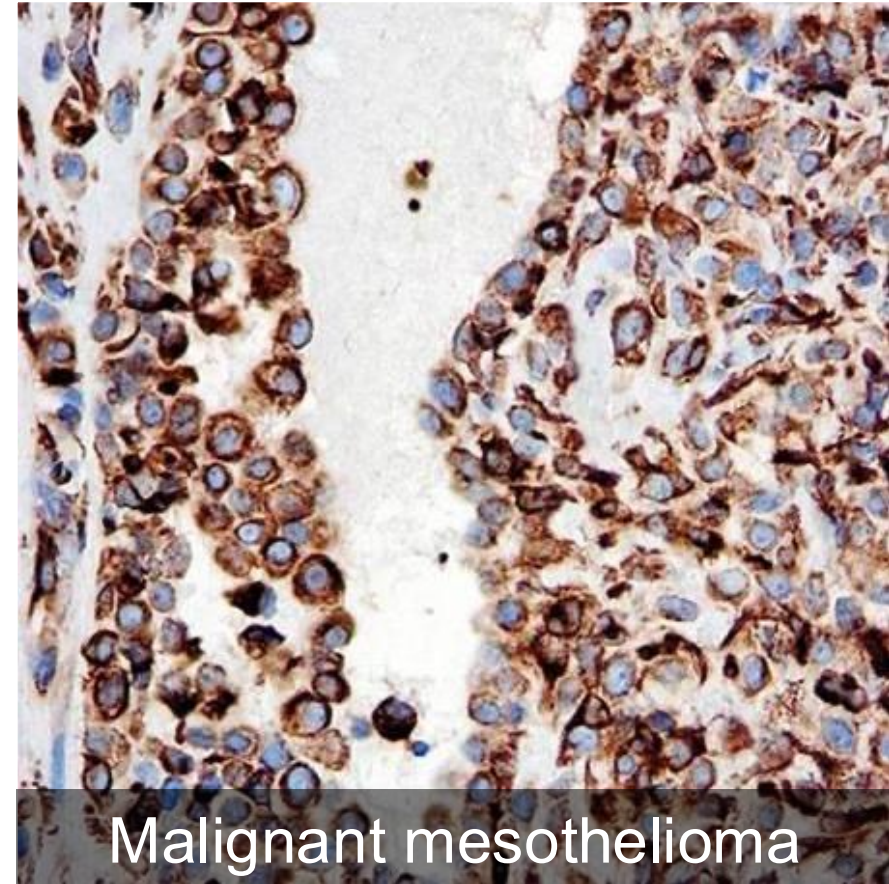
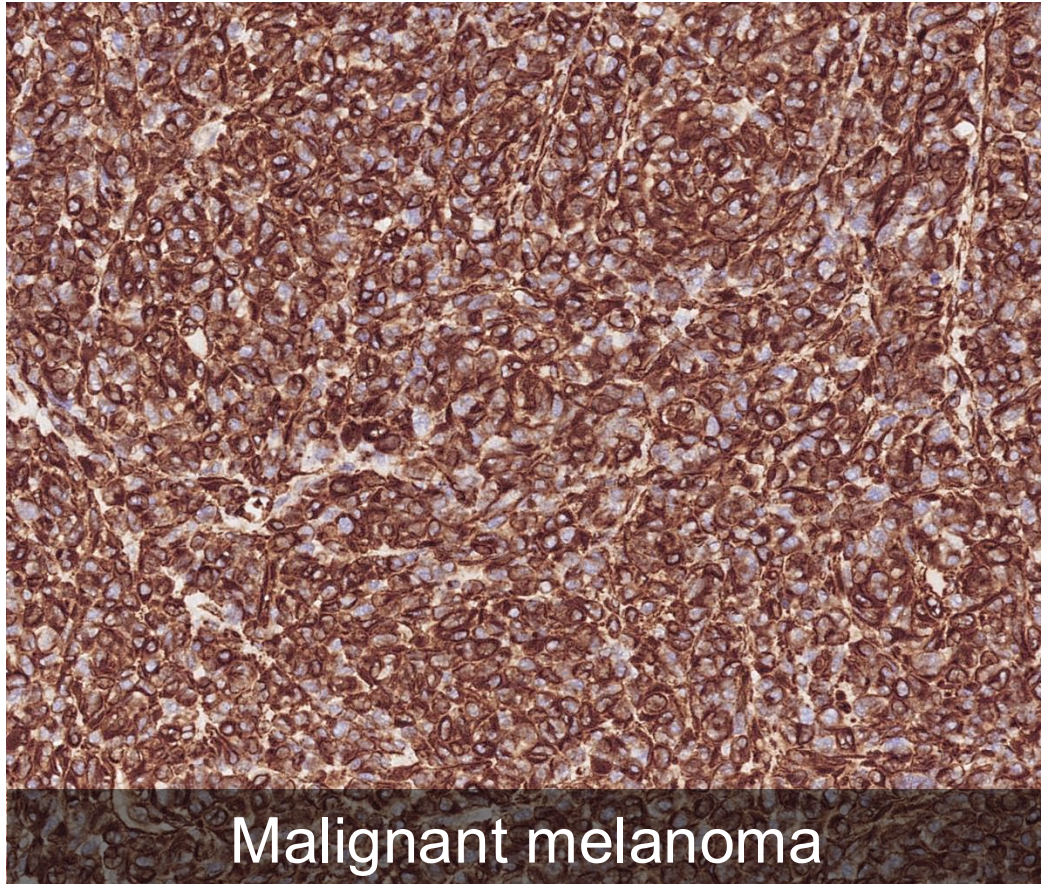
Vimentin – stromal tumours



Vimentin - carcinomas



Vimentin – other neoplasia

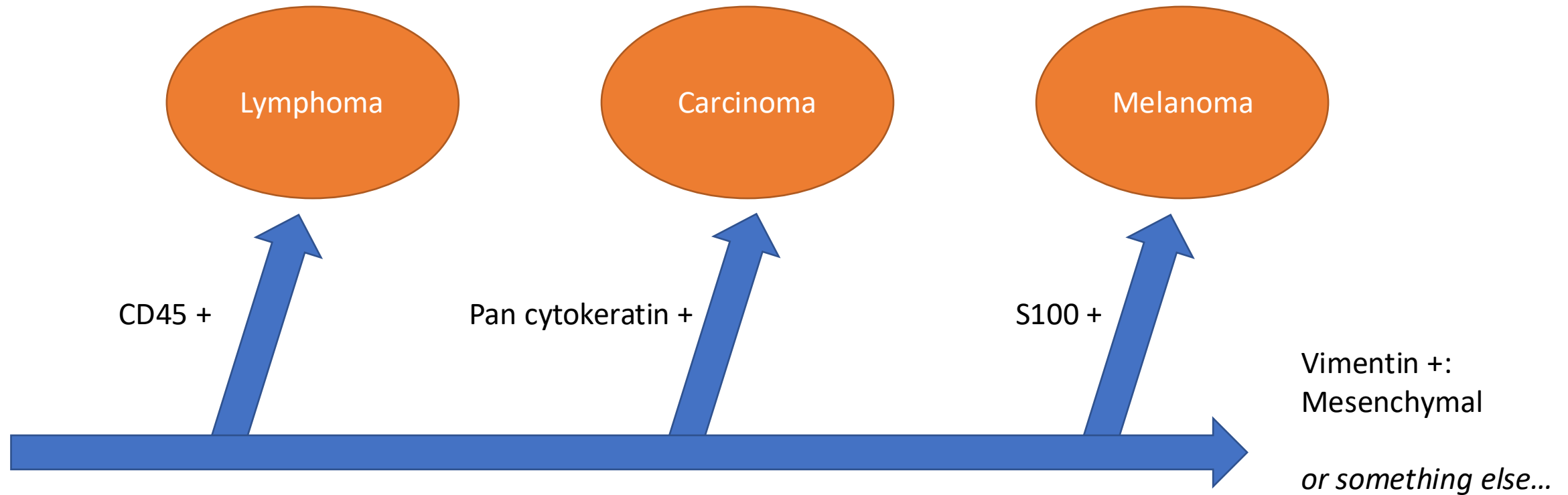


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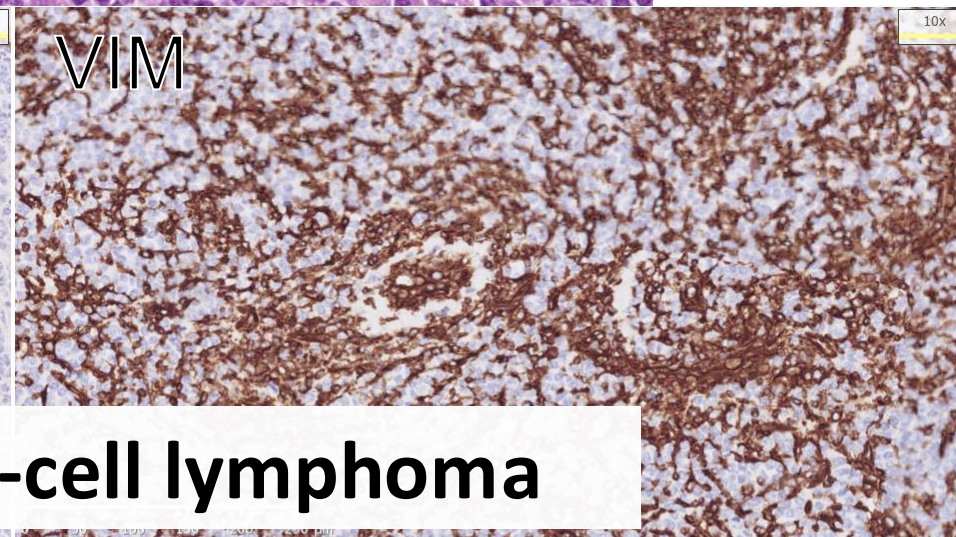
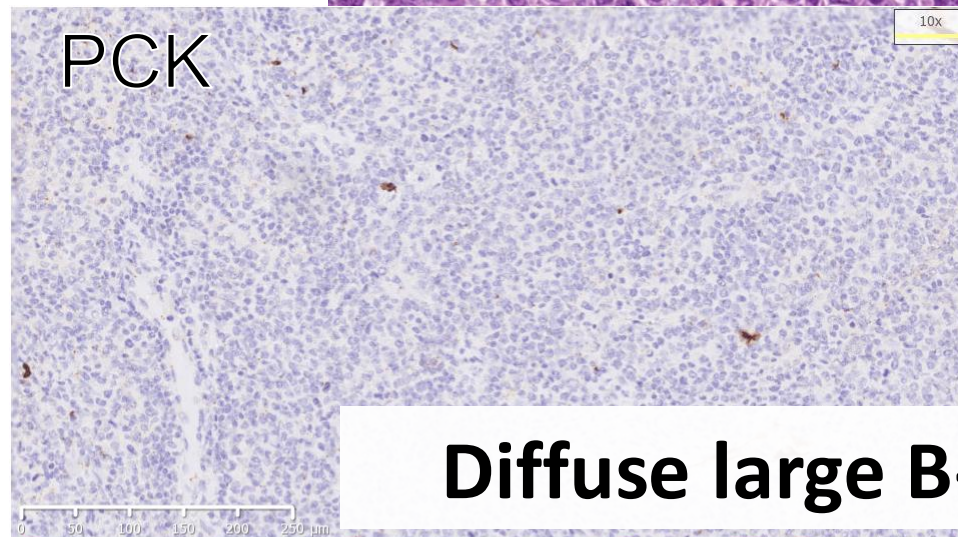
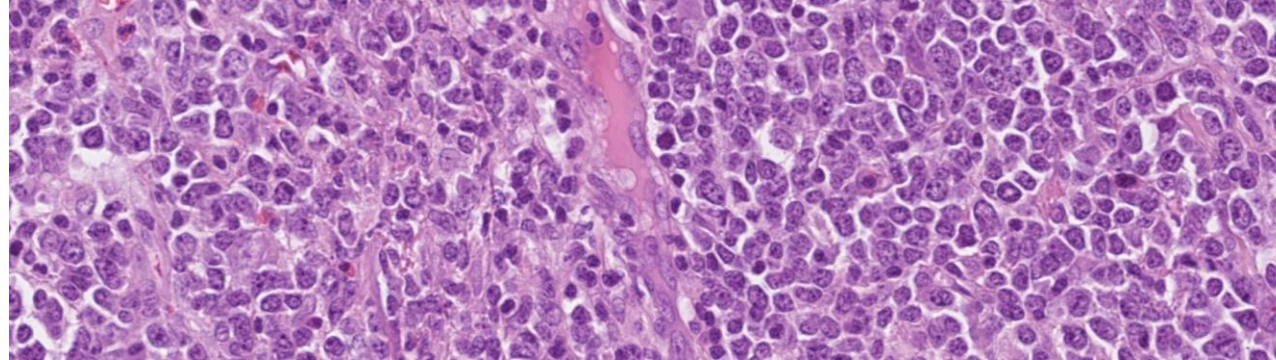
Primary panel in action



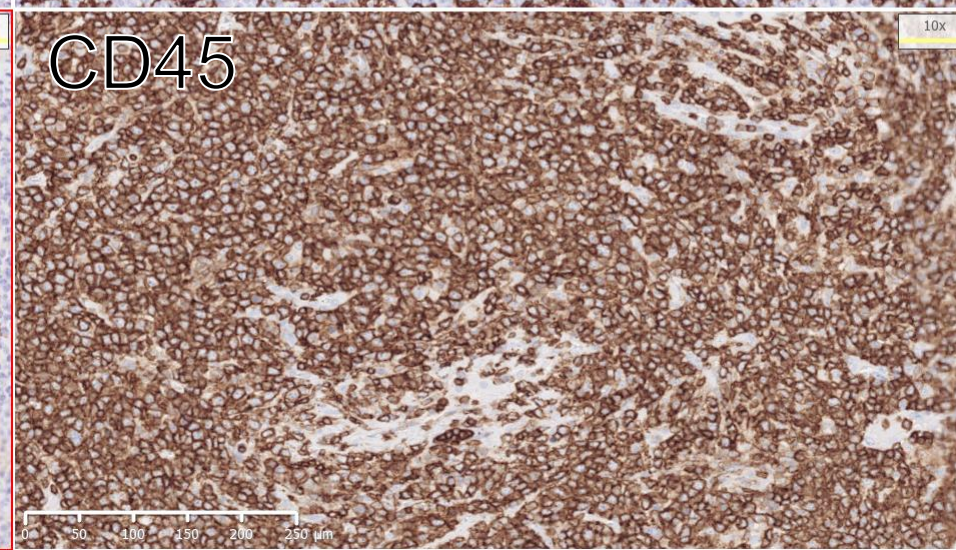
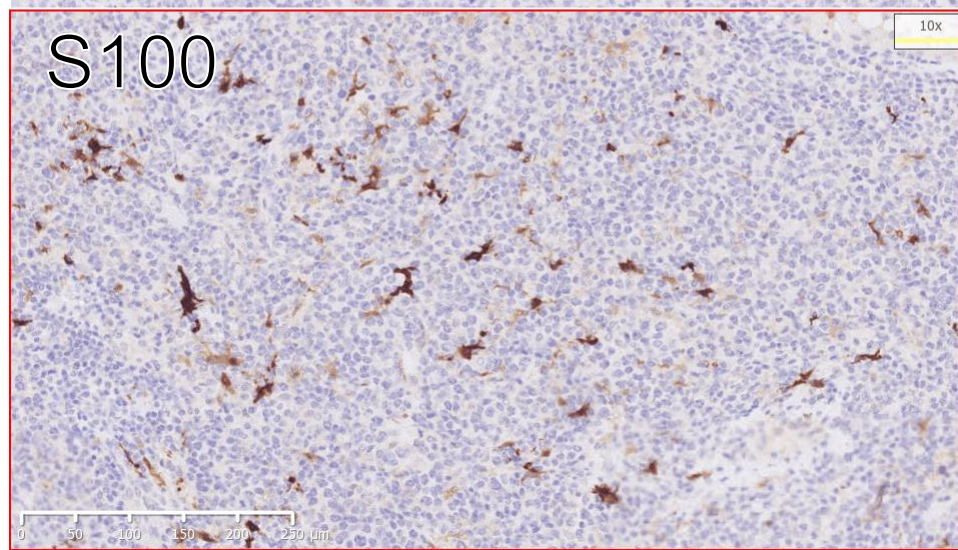
Undifferentiated cases

- 56 year old female
- History of smoking
- Heavy drinker
- Previous cancer
 - Cervix squamous
- Enlarged lymph node on neck





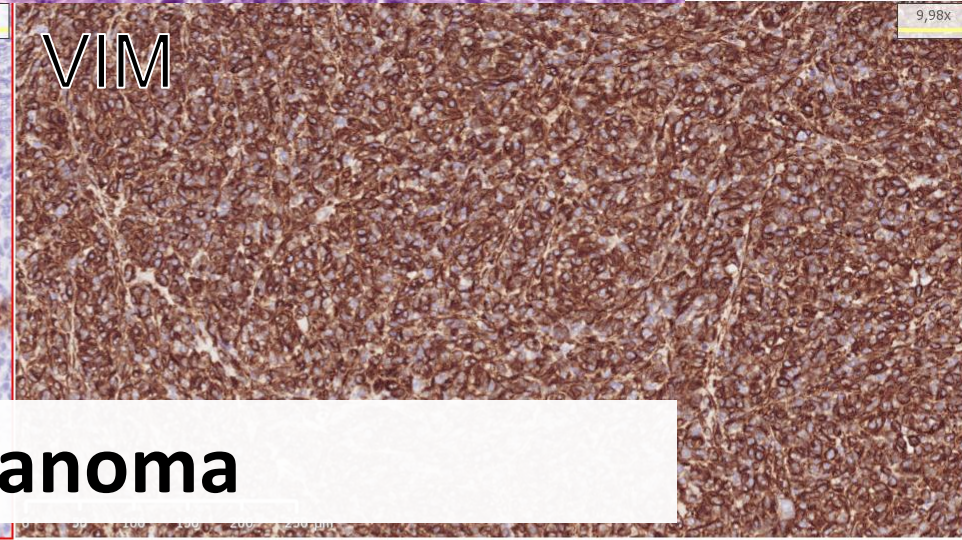
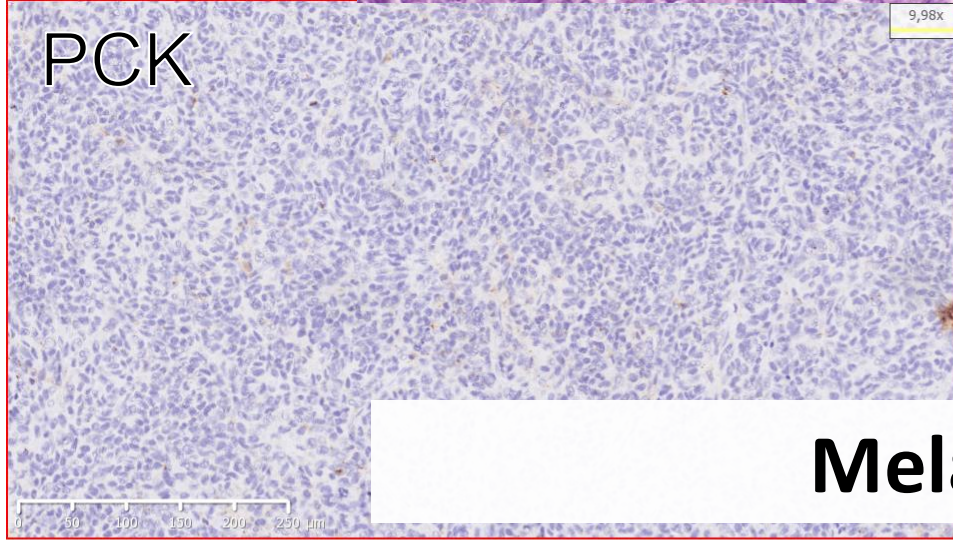
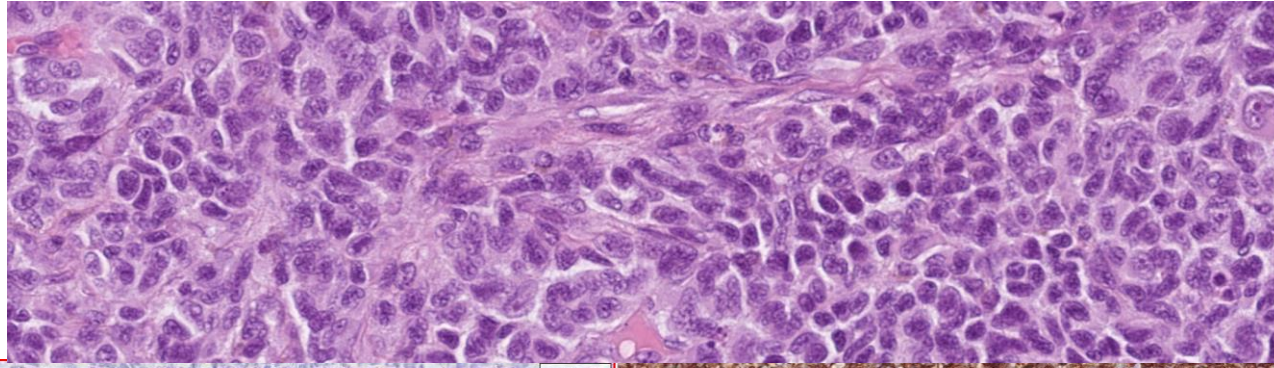
Diffuse large B-cell lymphoma



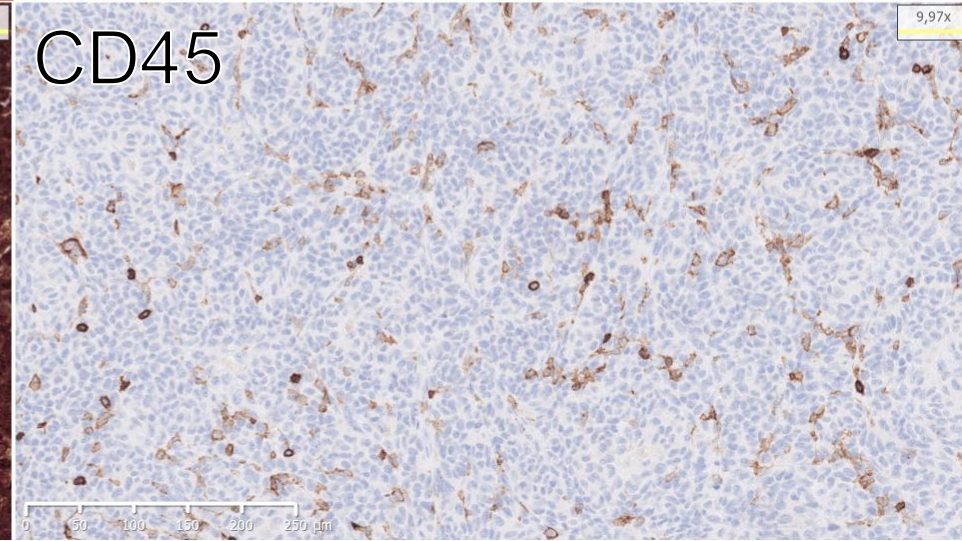
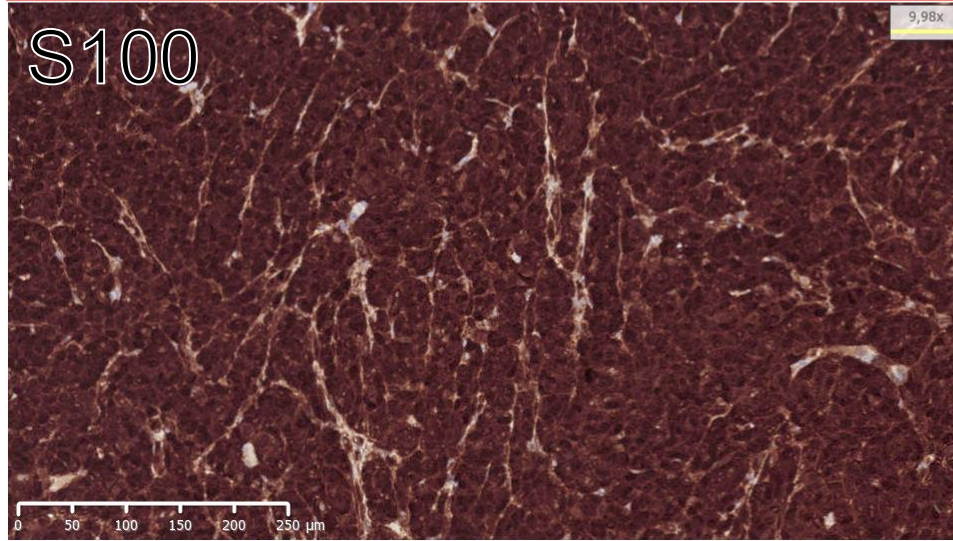
Undifferentiated cases

- 49 year old male
- History of smoking
- Previous cancer
 - Seminoma of testis
- Enlarged lymph node in groin





Melanoma



Cytokeratin panels

Unknown primary tumour

IHC panels for unknown primary tumour

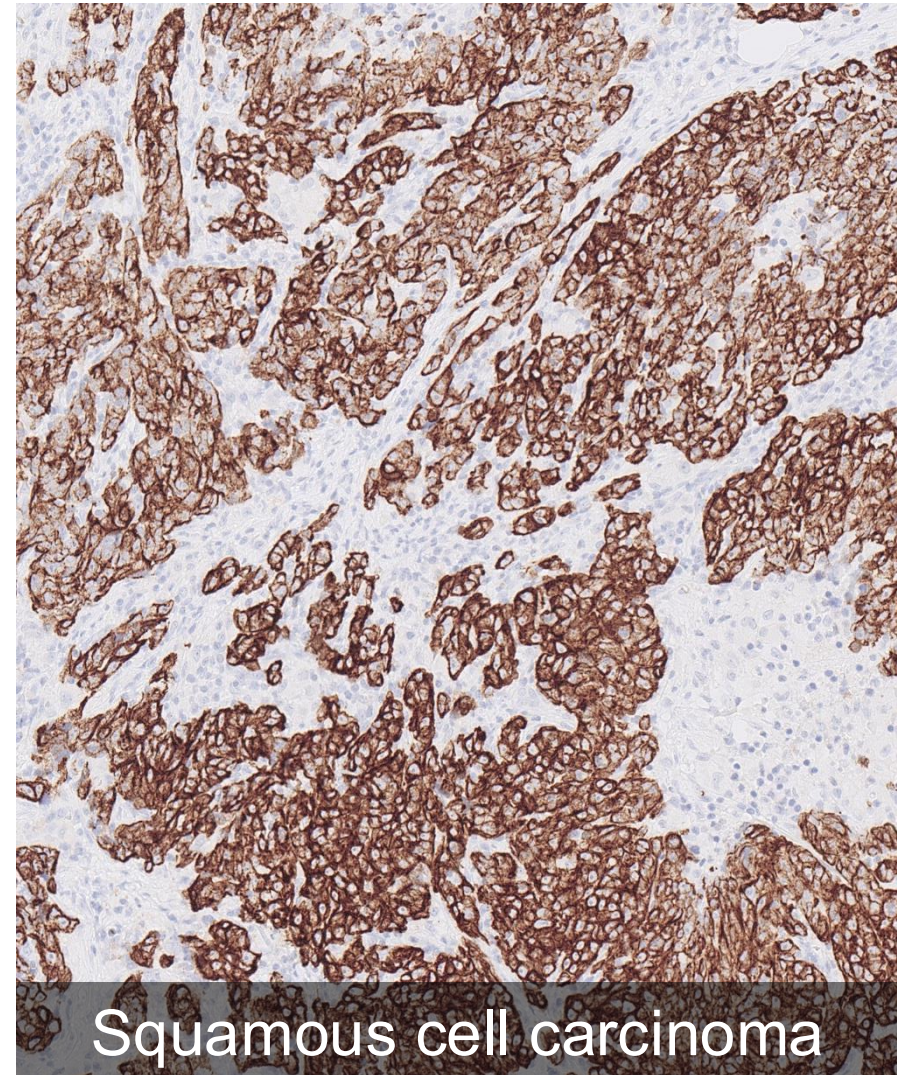
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Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (tonsil, mucosa)	-	-	-	+++	+++	(+)	(++)	(+)	-	(+)
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermediary. / basal cells	-	(+)	+++	(+++)	-	(++)	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+)	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (Merkel, thyroid)	-	-	-	-	-	-	(+++)	(++)	(+++)	+++
Smooth muscle (vasc., myom.), myofibrobl., sm.ves.endothelia	-	-	-	-	-	-	+	(++)	-	++

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Urothelial cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epithelia (bronch., breast, prost.)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma										
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

CK5

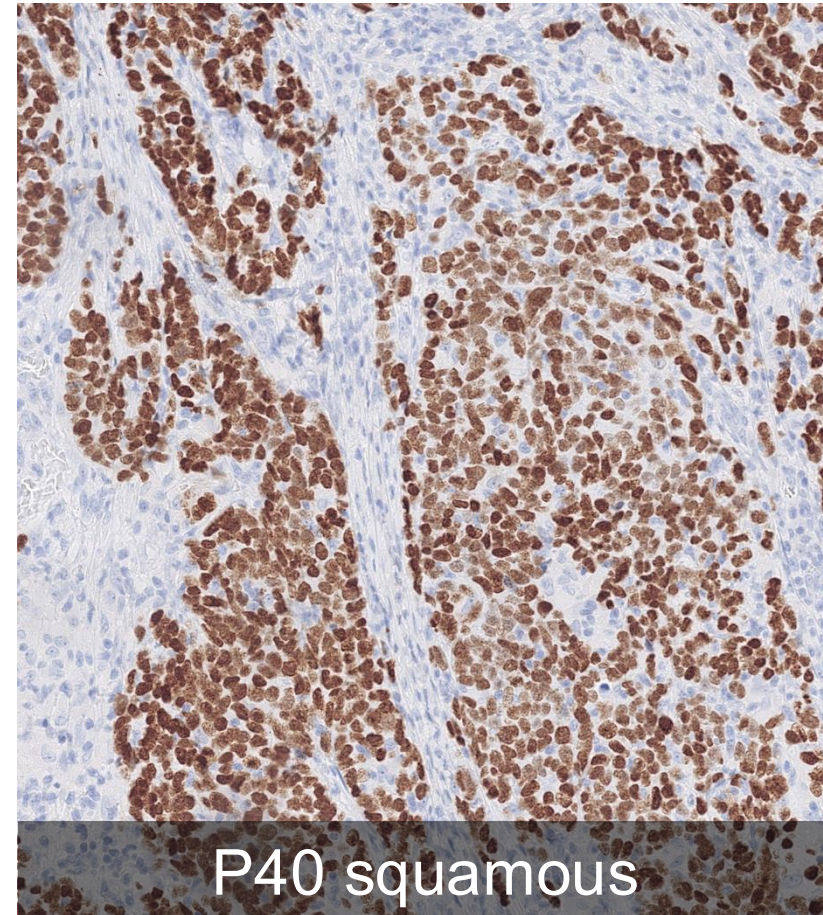
- Indicative of squamous differentiation
- Strongly positive in squamous cell carcinoma
- Strongly positive in mesothelioma
- Often focally positive in urothelial carcinoma



Squamous cell carcinoma

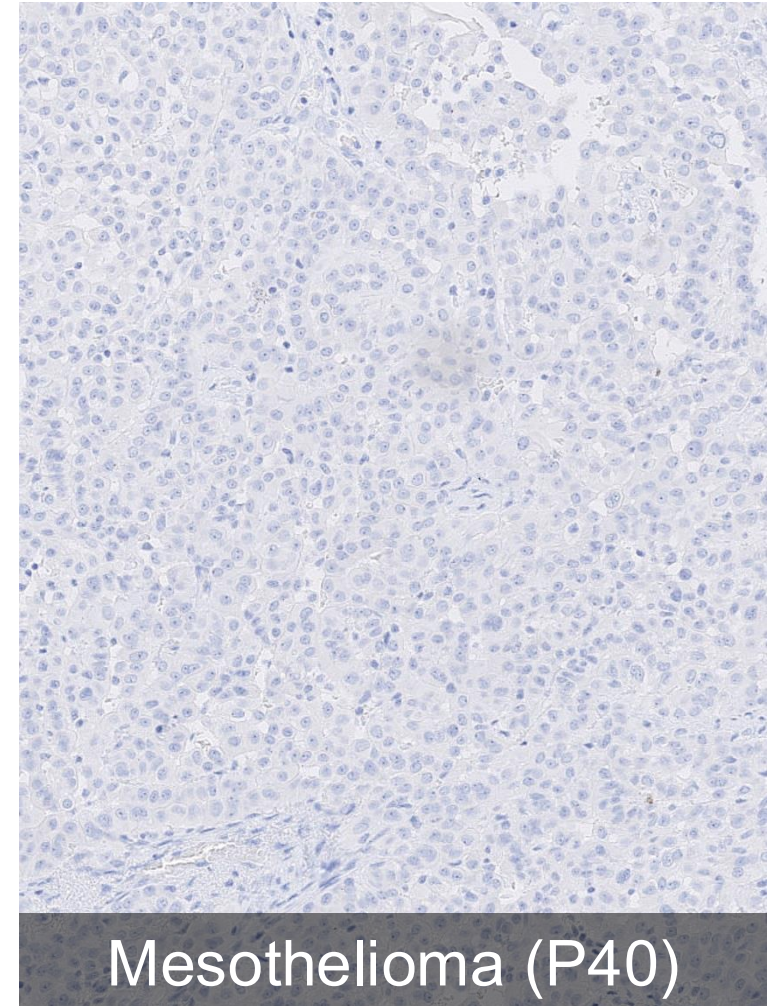
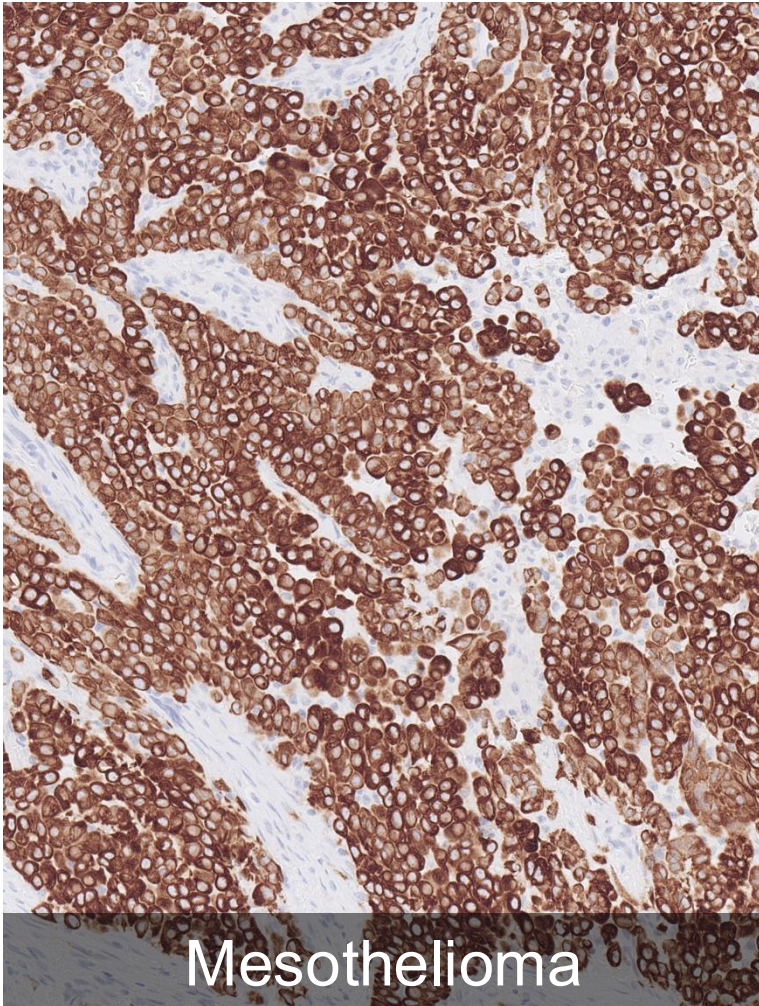
Confirm squamous cell carcinoma

- Use p40!
- Nuclear transcription factor
- Limited to squamous cell carcinomas (and urothelial carcinomas)

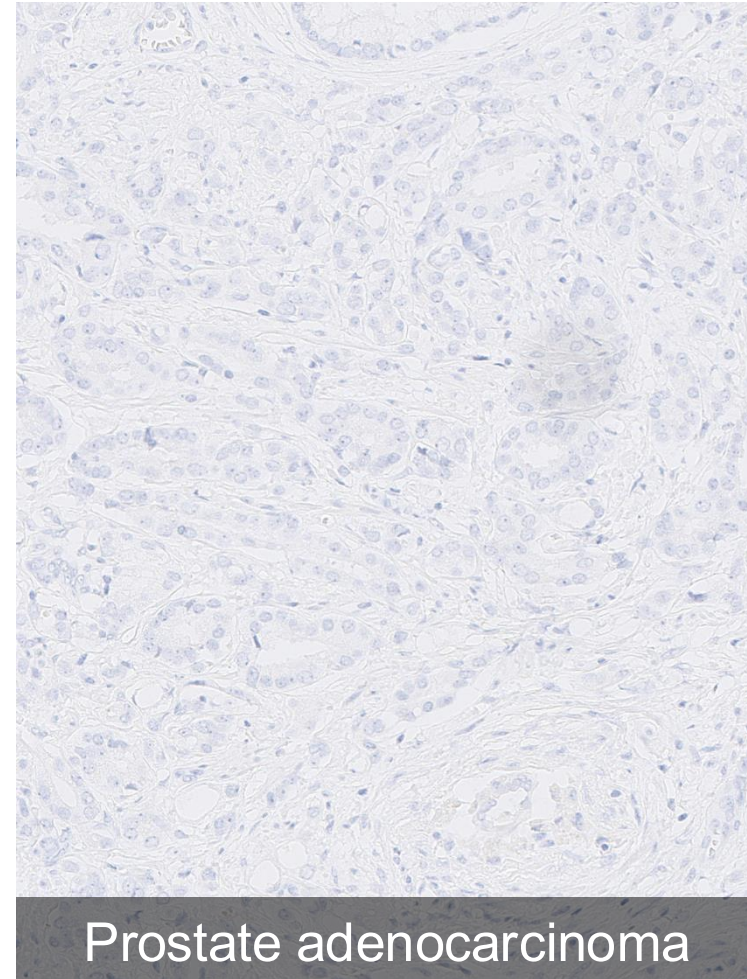
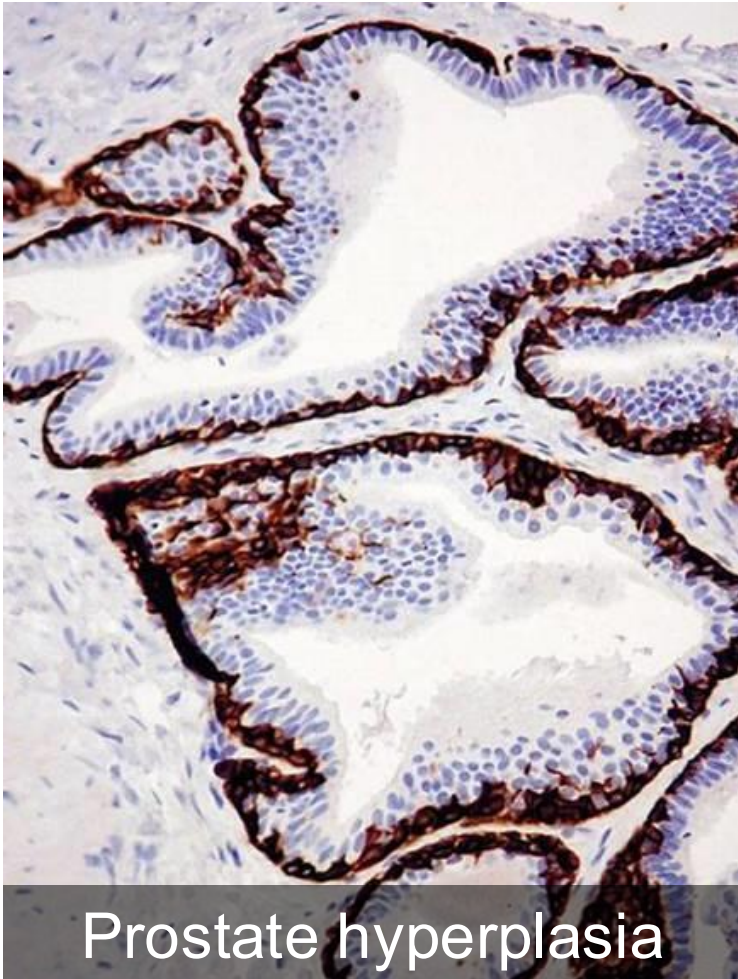


P40 squamous

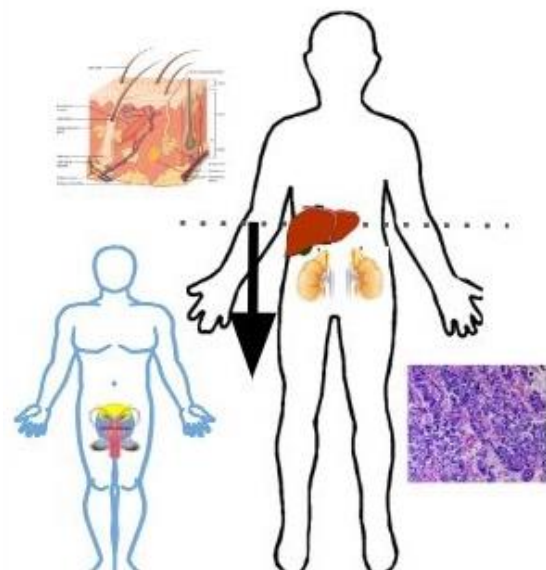
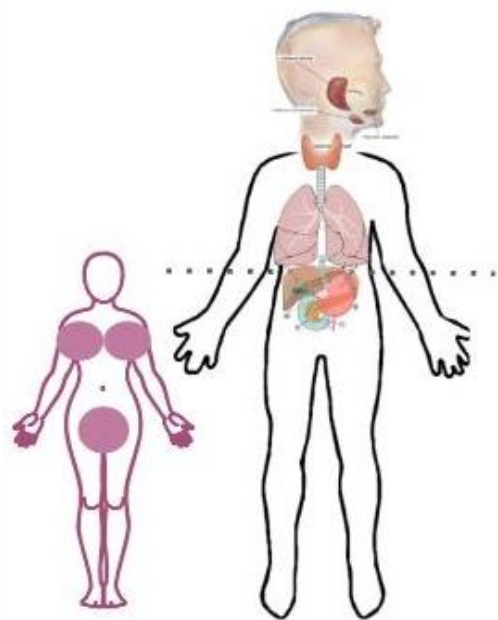
CK5 in mesothelioma



CK5 – other uses

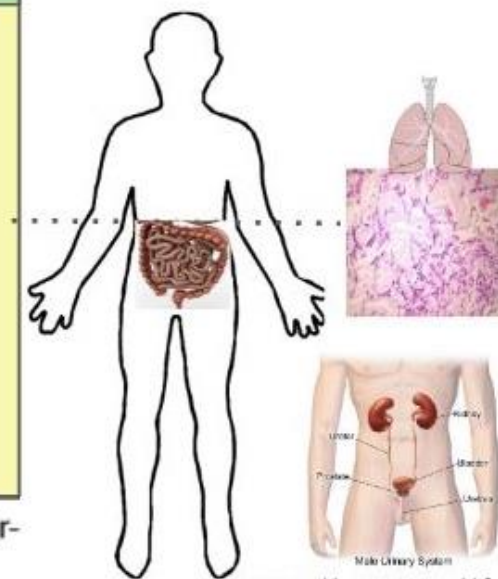
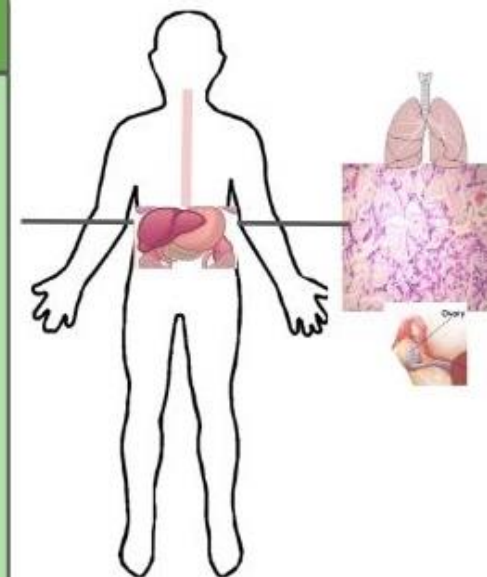


Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epithelia (bronch., breast, prost.)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma										
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

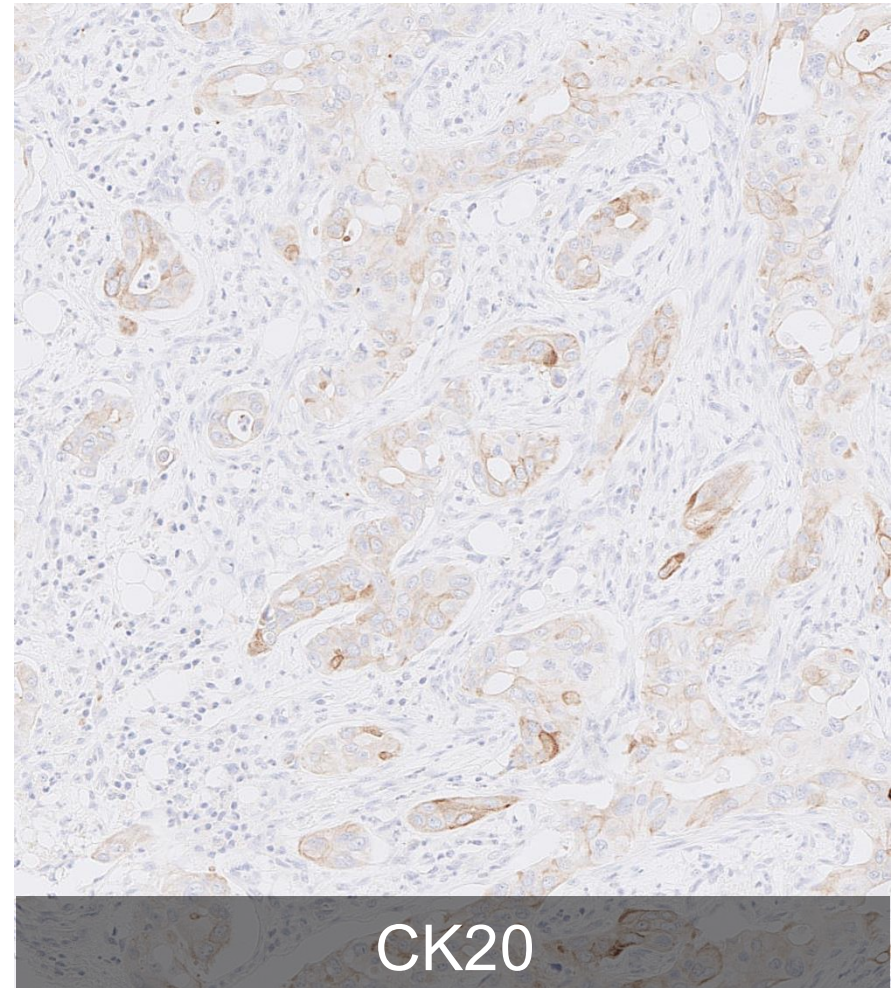
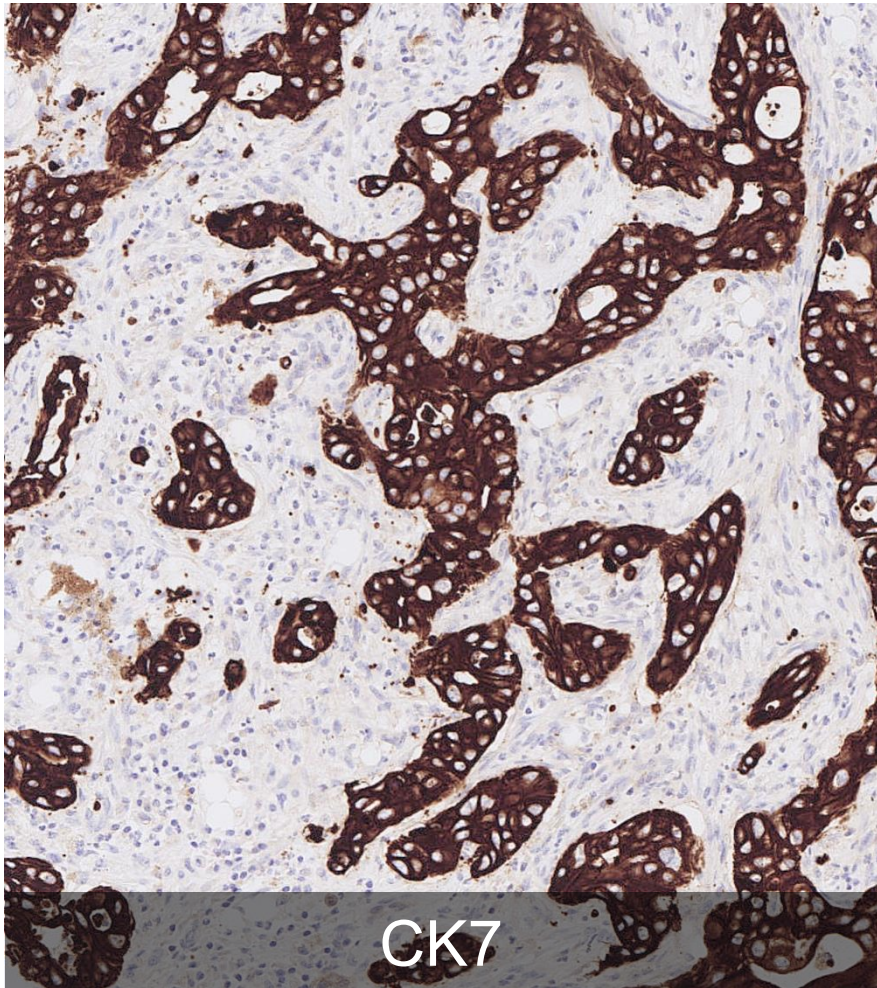


	CK20-	CK20+
CK7+	• Lung • Breast • Upper GI ADC • Pancreatic/biliary ADC • Endometrial/endocervical ADC • Thyroid • Thymic CA • Salivary gland duct CA • Hepatocellular CA, fibrolamellar type • Ovarian serous CA • Anal duct CA • Mesothelioma	• Urothelial CA • Esophagus ADC • Gastric ADC • Small bowel ADC • Mucinous ADC of lung • Ovarian mucinous CA • Pancreaticobiliary ADC • Cholangiocarcinoma
CK7-	• Hepatocellular CA • Clear cell renal cell carcinoma • Adrenal cortical CA • Prostate ADC • Small cell carcinoma • Squamous cell CA • Germ cell tumors • Neuroendocrine neoplasm • Medullary CA of the colon	• Colorectal ADC • Small bowel ADC • Bladder ADC • Merkel cell carcinoma • Appendiceal ADC • Mucinous ADC of lung • Papillary renal cell carcinoma, type II

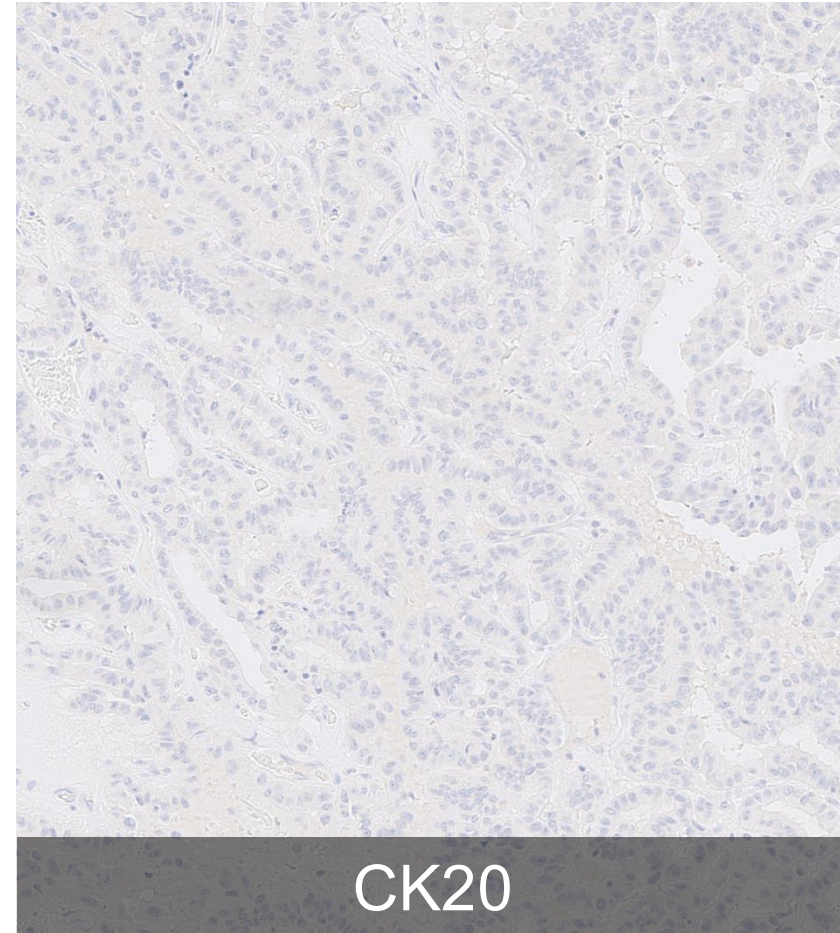
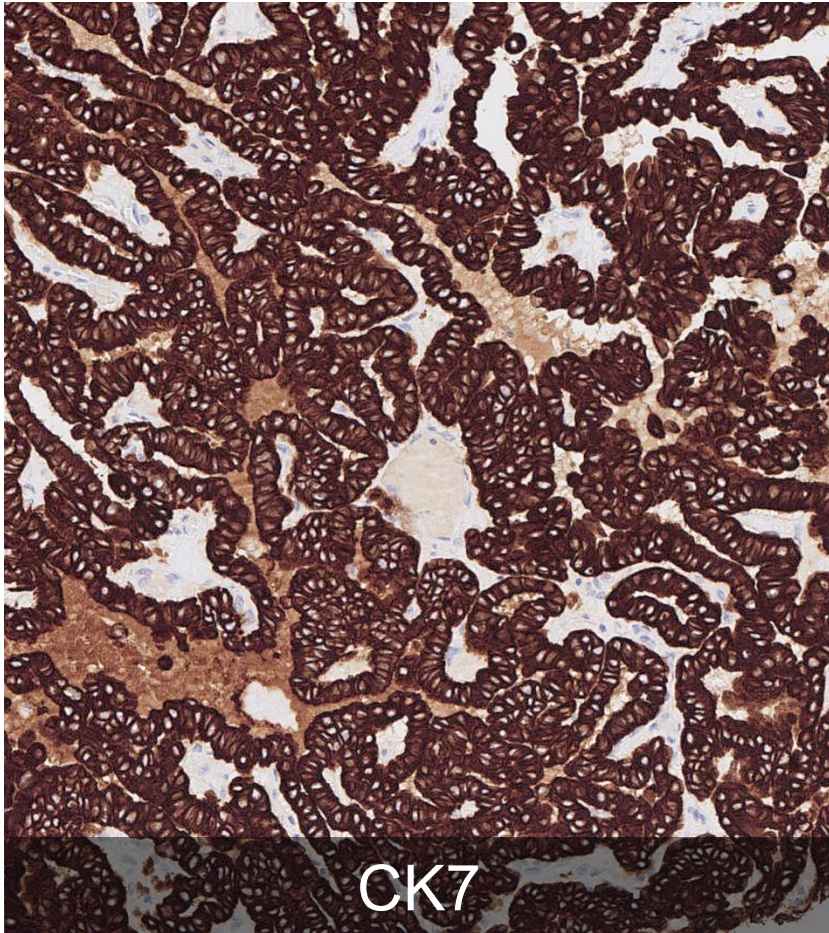
Abbreviations: ADC, adenocarcinoma; CA, carcinoma; CK, cytokeratin; GI, gastrointestinal.



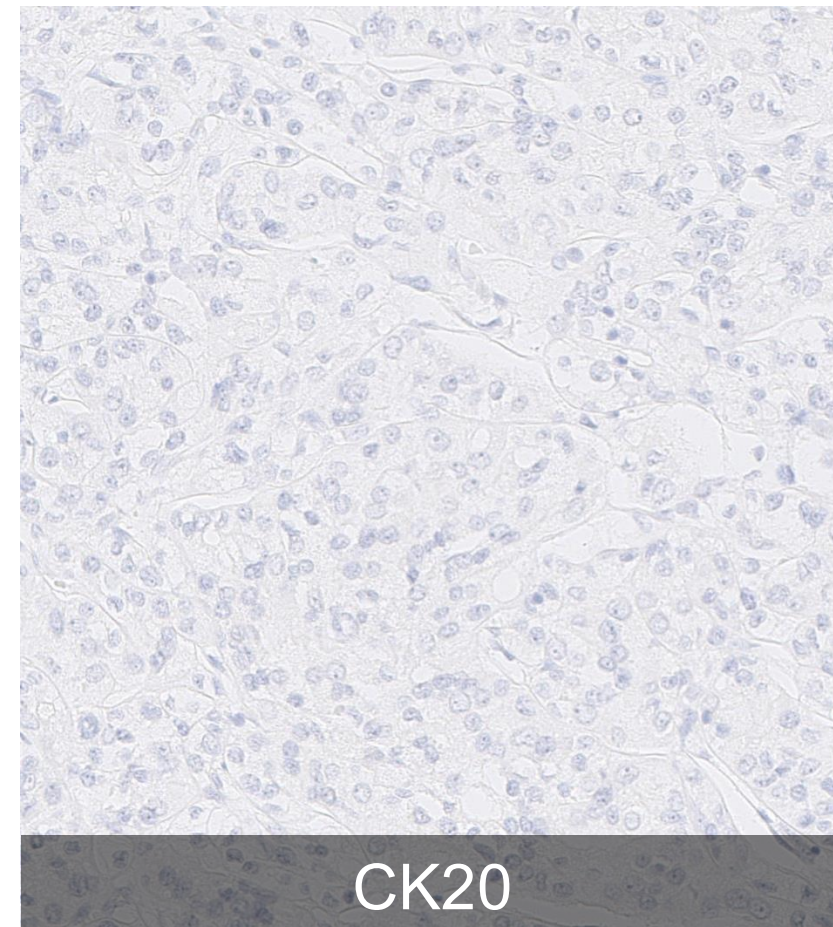
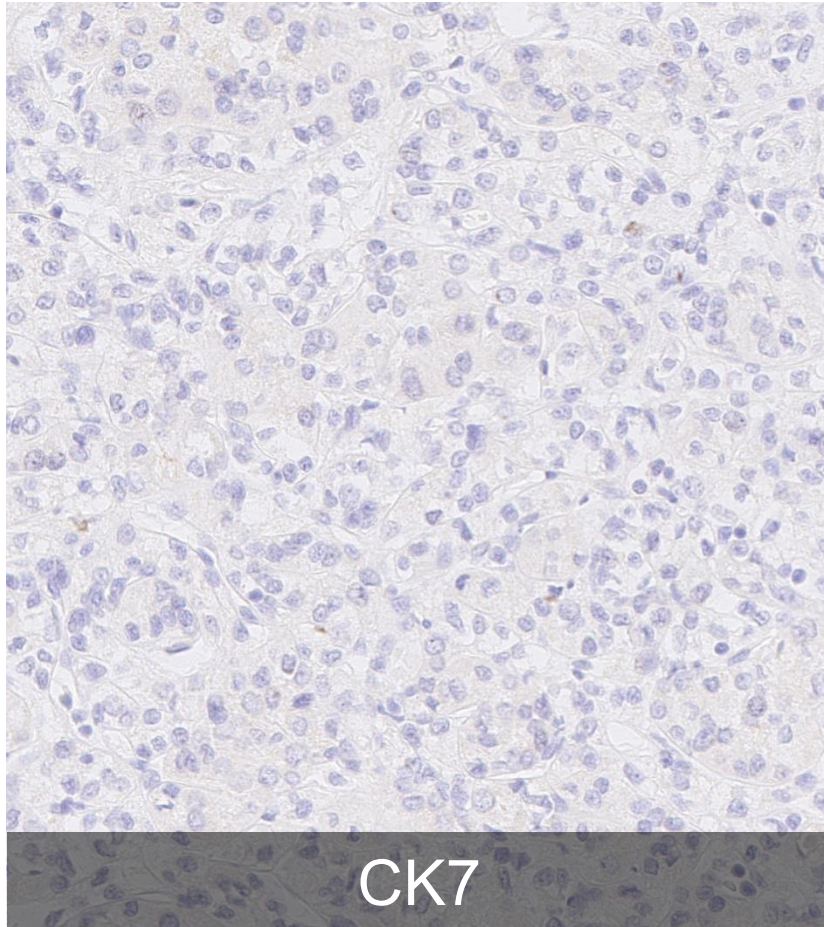
Pancreas adenocarcinoma



Thyroid carcinoma



Renal clear cell carcinoma

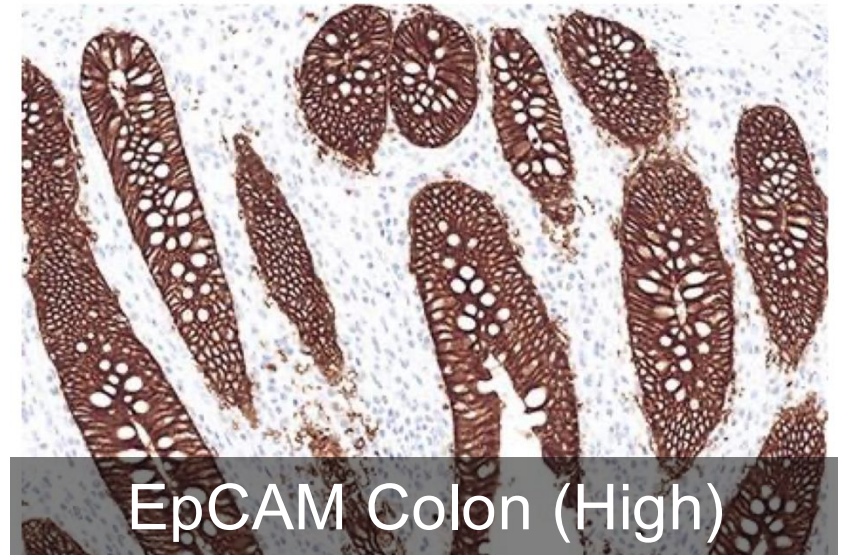


Other epithelial markers

Unknown primary tumour

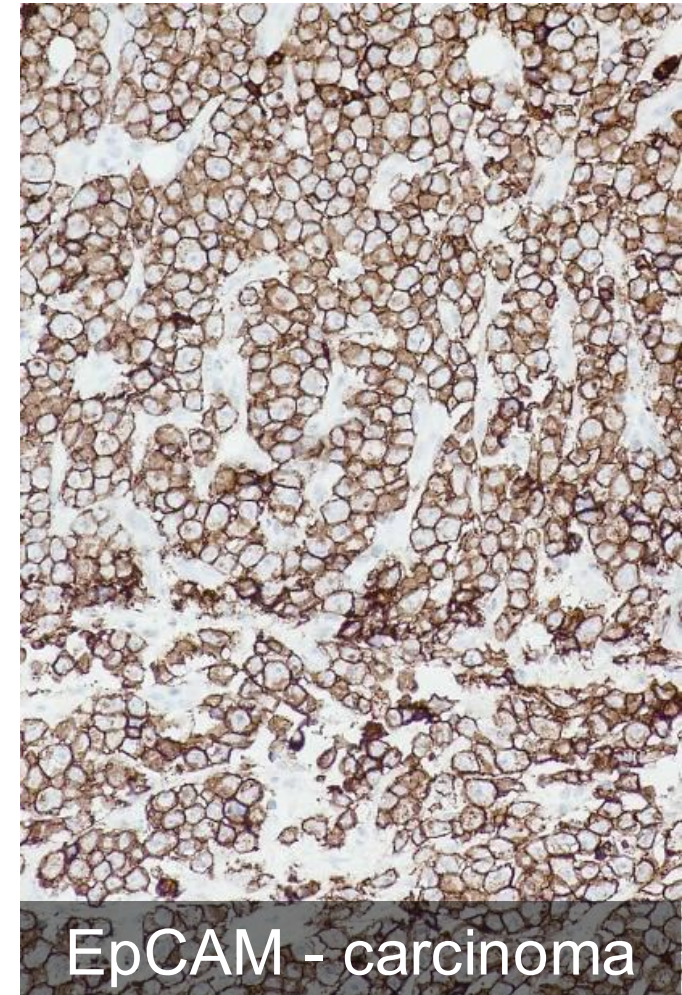
EpCAM

- Epithelial Cell Adhesion Molecule
- Transmembrane adhesion molecule
- Broad spectrum epithelial marker
- Negative in
 - Mesothelium (may be positive in reactive)
 - Superficial layers of squamous epithelium



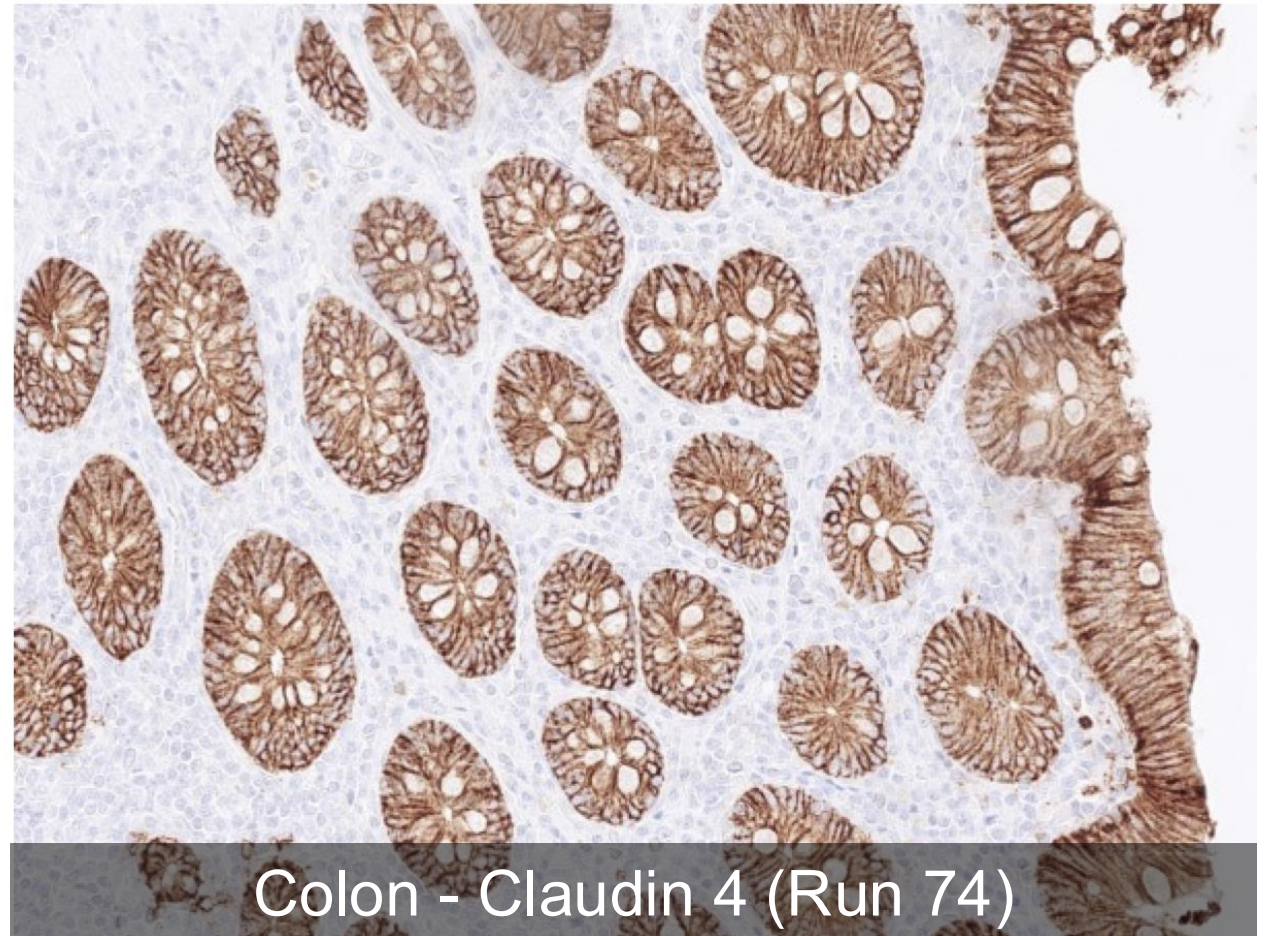
EpCAM

- Expressed in most carcinomas – especially adenocarcinomas
- Negative in mesothelioma (most)
- Focal or negative in (skin) squamous carcinomas



Claudin 4

- Tight junction protein
- Most epithelial cells and carcinomas
- Negative in mesotheliomas



Colon - Claudin 4 (Run 74)

Tissue specific panels

Unknown primary tumour

Tissue specific panels

- Large number of somewhat tissue specific markers
- Several markers for each potential diagnosis are optimal
 - Increases chance of correct diagnosis in tumours with aberrant staining
- Should be combined in panels based on each specific case
- Negative stains also contain important information

Tissue specific markers

Breast:

Gata3
Estrogen receptor
TRPS1
SOX10

Lung:

TTF
Napsin

Gastrointestinal:

CDX2
Cadherin 17
SATB2

Kidney:
PAX8

Prostate:

NKX3.1

Melanoma:

SOX10
MLA

Mesothelioma:

Calretinin
Podoplanin

Bladder:

GATA3
Uroplakin II

Germ cell:

OCT3/4
CD30
SALL4

Female genitals:

PAX8
WT1
ER

Pancreas:

SMAD4
Gata3

Neuroendocrine:

SYP
CGA
INSM1

Thyroid:

TTF
PAX8

Tissue “specific” markers

Breast:

Gata3

Estrogen receptor

TRPS1

SOX10

Lung:

TTF

Napsin

Gastrointestinal:

CDX2

Cadherin 17

SATB2

Kidney:

PAX8

Prostate:

NKX3.1

Bladder:

GATA3

Uroplakin II

Melanoma:

SOX10

MLA

Mesothelioma:

Calretinin

Podoplanin

Neuroendocrine:

SYP

CGA

INSM1

Germ cell:

OCT3/4

CD30

SALL4

Female genitals:

PAX8

WT1

ER

Pancreas:

SMAD4

Gata3

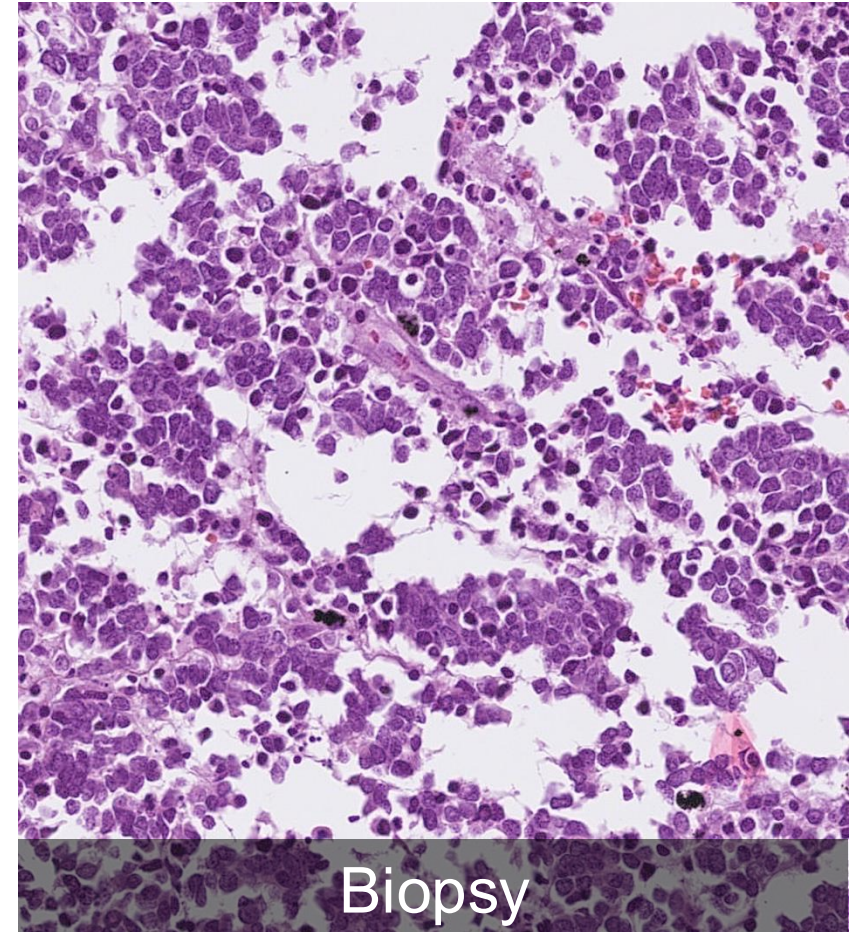
Thyroid:

TTF

PAX8

Case

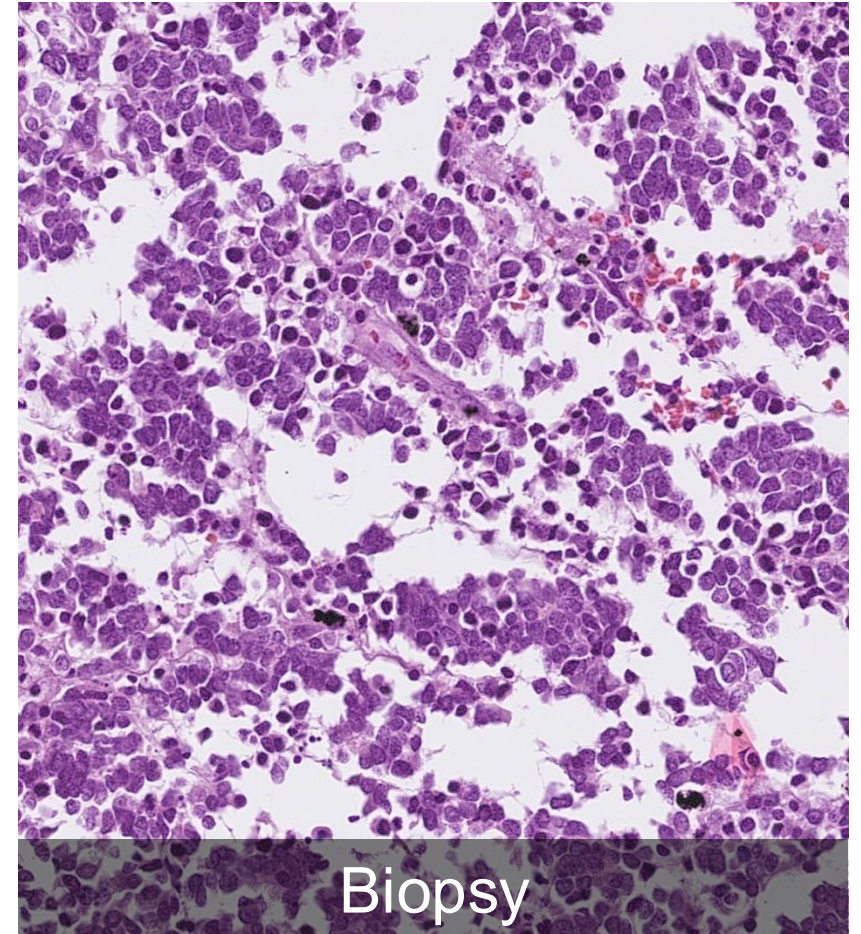
- 67 y.o. female
- Heavy smoker
- Several tumours in both lungs
- Pleural plaques and exposed to asbestos
- Large lymph nodes in mediastinum
- Previous ovarian serous carcinoma



Case

- Carcinoma
- Lung cancer ?
 - Adeno? Squamous? Small cell (neuroendocrine)?
- Relapse ovarian serous carcinoma ?
- Mesothelioma ?

CD45	Pan cytokeratin	S100	Vimentin
Negative	Positive	Negative	Negative



Tissue specific markers

Breast:

Gata3
Estrogen receptor
TRPS1
SOX10

Lung:

TTF
Napsin

Gastrointestinal:

CDX2
Cadherin 17
SATB2

Kidney:
PAX8

Prostate:

NKX3.1

Melanoma:

SOX10
MLA

Mesothelioma:

Calretinin
Podoplanin

Bladder:

GATA3
Uroplakin II

Germ cell:

OCT3/4
CD30
SALL4

Female genitals:

PAX8
WT1
ER

Pancreas:

SMAD4
Gata3

Neuroendocrine:

SYP
CGA
INSM1

Thyroid:

TTF
PAX8

Selected panels

Lung:
TTF
Napsin

Mesothelioma:
Calretinin
Podoplanin

Neuroendocrine:
SYP
CGA
INSM1

Female genitals:
PAX8
WT1
ER

Selected panels

All purpose cytokeratins:

CK5
CK7
CK20

Cytokeratins

CK5	Negative
CK7	Positive (dots)
CK20	Negative

Lung:

TTF
Napsin

Neuroendocrine:

SYP
CGA
INSM1

Female genitals:

PAX8
WT1
ER

Mesothelioma:

Calretinin
Podoplanin

Lung

TTF	Positive
Napsin	Negative

Neuroendocrine

SYP	Positive
CGA	Positive
INSM1	Positive

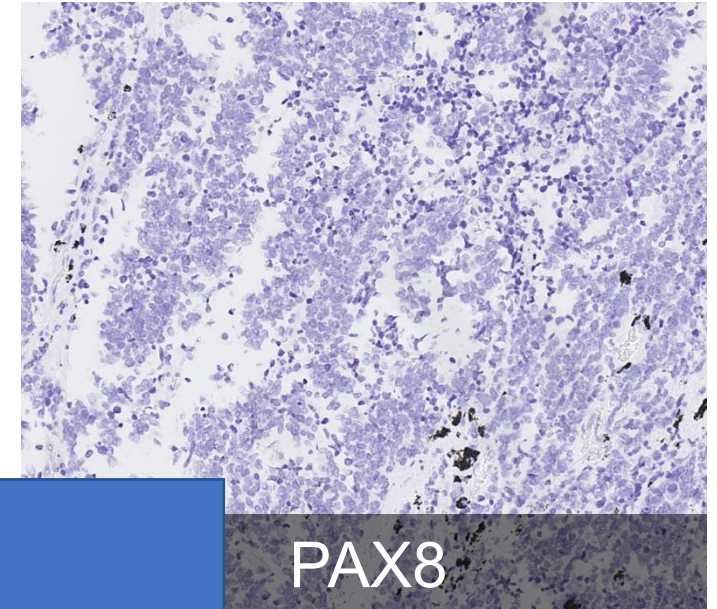
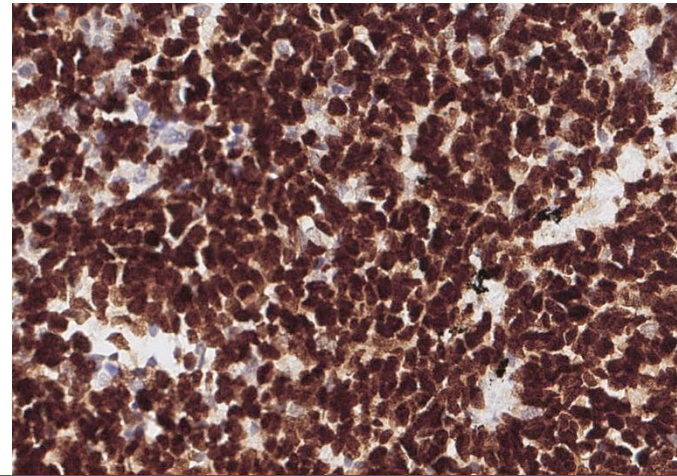
Female genitals

PAX8	Negative
WT1	Negative
ER	Negative

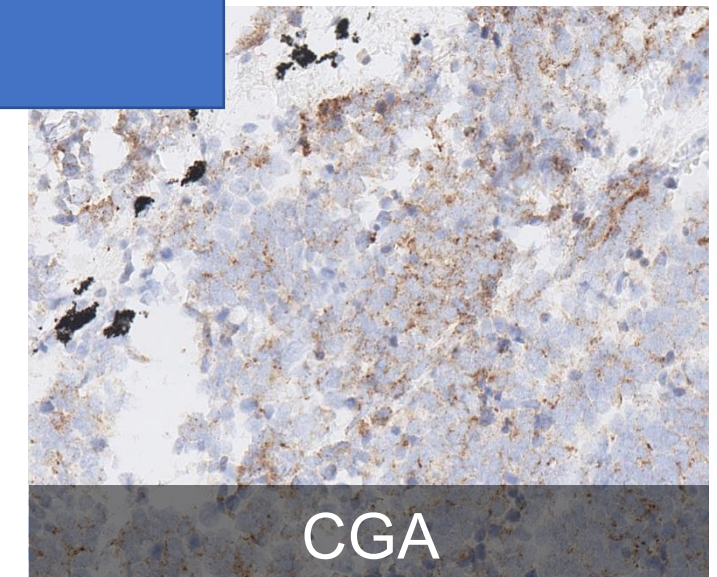
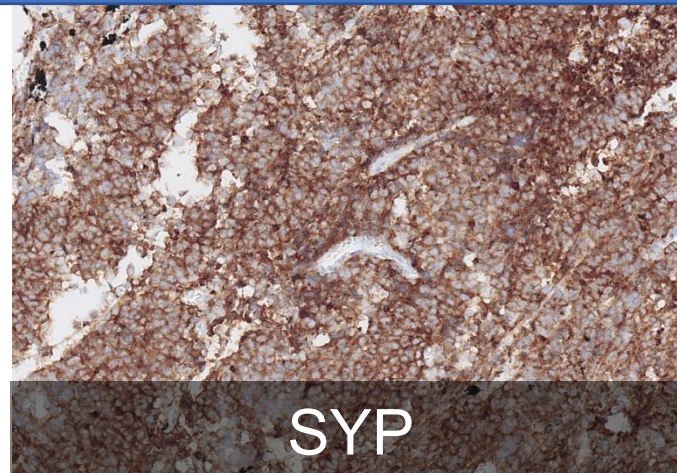
Mesothelioma

Calretinin	Negative
Podoplanin	Negative

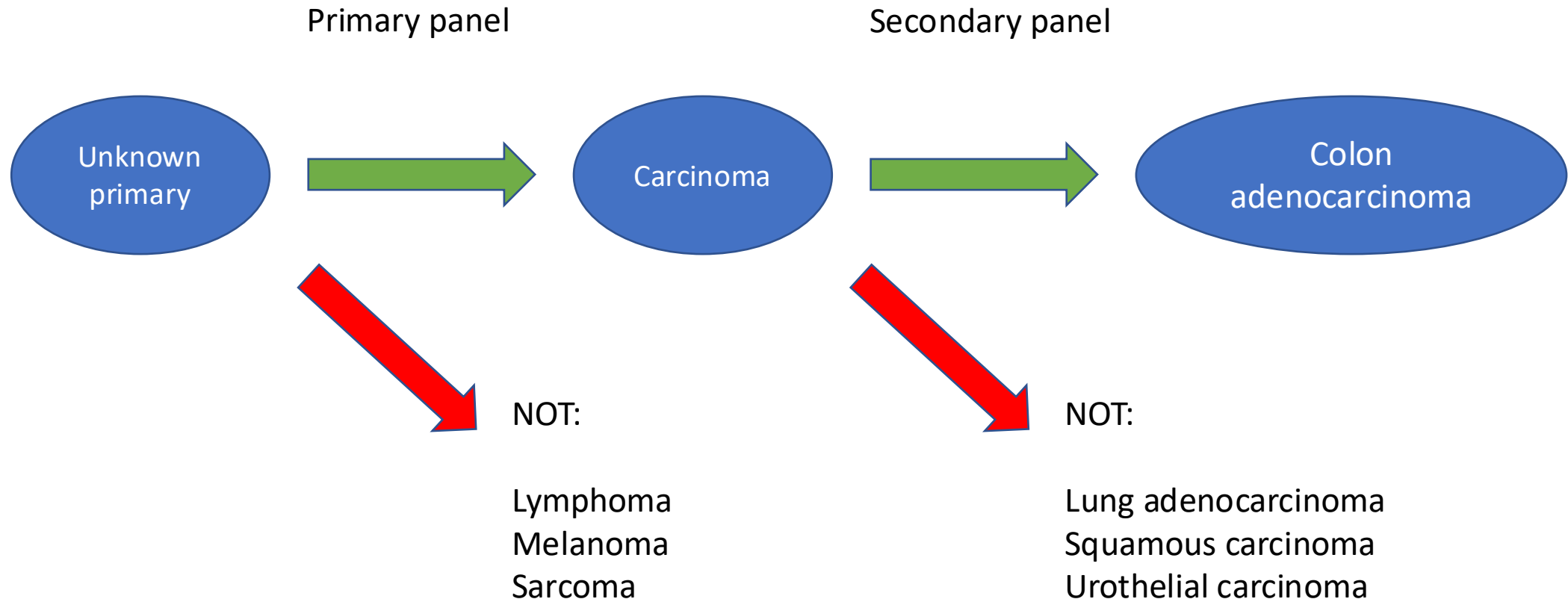
Case



Small cell lung carcinoma
(neuroendocrine)



IHC panels for unknown primary tumour



An Algorithmic Immunohistochemical Approach to Define Tumor Type and Assign Site of Origin

Andrew M. Bellizzi, MD

TABLE 1. Next-generation Immunohistochemical Markers Discussed in This Review

Marker	Useful in Diagnosis of	Next-generation IHC "Qualifications"
PAX8	Müllerian, renal, and thyroid carcinoma; thymic neoplasms (with polyclonal antibody); pancreatic origin of well-differentiated neuroendocrine tumor (with polyclonal antibody)	Lineage-restricted transcription factor
MDM2/CDK4	Well- and dedifferentiated liposarcoma	Protein correlate of molecular genetic event
GATA-3	Breast and urothelial carcinoma; also expressed by pheochromocytoma/paraganglioma, choriocarcinoma, mesonephric carcinoma, parathyroid tumors, and pituitary gonadotroph and TSH-expressing tumors; often expressed by mesothelioma, chromophobe renal cell carcinoma, and cutaneous epithelial neoplasms; variably expressed by yolk sac tumor	Lineage-restricted transcription factor
ERG	Vascular neoplasms; also expressed by subsets of prostate cancer, Ewing sarcoma, and acute leukemia; antibodies to N-terminus label epithelioid sarcoma	Lineage-restricted transcription factor
Islet 1	Pancreatic origin of well-differentiated neuroendocrine tumor	Protein correlate of molecular genetic event
PAX6	Pancreatic origin of well-differentiated neuroendocrine tumor	Lineage-restricted transcription factor
SALL4	Germ cell neoplasia; also expressed by hepatoid adenocarcinoma; may be frequently expressed by rhabdoid and Wilms tumor; aberrant expression in a significant minority (20%-30%) of serous, gastric, urothelial, and biliary carcinomas	Lineage-restricted transcription factor
SOX10	Melanocytic, nerve sheath, and myoepithelial tumors; also often (60%) expressed by triple-negative breast cancer	Lineage-restricted transcription factor
INI1/SMARCB1 (loss)	Epithelioid sarcoma; malignant rhabdoid tumors of soft tissue, kidney, and CNS, and medullary carcinoma of kidney; subset of epithelioid MPNST, myoepithelial carcinoma of soft tissue, and extraskeletal myxoid chondrosarcoma	Protein correlate of molecular genetic event
p40	Squamous, urothelial, and myoepithelial tumors; myoepithelial/basal cell marker in breast and prostate	Lineage-restricted transcription factor
ATRX (loss)	Diffuse astrocytoma and pancreatic origin of well-differentiated neuroendocrine tumor	Protein correlate of molecular genetic event
SATB2	Colorectal origin of adenocarcinoma, lower GI origin of well-differentiated neuroendocrine tumor, and possibly cutaneous origin of a poorly differentiated neuroendocrine carcinoma; osteoblastic lineage; <i>BCOR</i> -rearranged sarcoma	Lineage-restricted transcription factor
OTP	Bronchopulmonary origin of well-differentiated neuroendocrine tumor	Lineage-restricted transcription factor; identified through gene expression profiling
Rb protein (loss)	Poorly differentiated neuroendocrine carcinoma; spindle cell/pleomorphic lipoma, cellular angiofibroma, and mammary-type myofibroblastoma	Protein correlate of molecular genetic event
SMAD4 (loss)	Pancreatic origin of adenocarcinoma; also frequently lost in colorectal cancer	Protein correlate of molecular genetic event
BAP1 (loss)	Mesothelioma (especially epithelioid) and intrahepatic cholangiocarcinoma; loss in ocular melanoma and renal cell carcinoma prognostically adverse	Protein correlate of molecular genetic event
SF1	Adrenal cortical and sex cord-stromal neoplasms	Lineage-restricted transcription factor
NKX3.1	Prostate cancer	Lineage-restricted transcription factor
INSM1	Neuroendocrine neoplasms	Lineage-restricted transcription factor

CNS indicates central nervous system; GI, gastrointestinal; IHC, immunohistochemistry; MPNST, malignant peripheral nerve sheath tumor; OTP, orthopedia homeobox; SF1, steroidogenic factor 1.

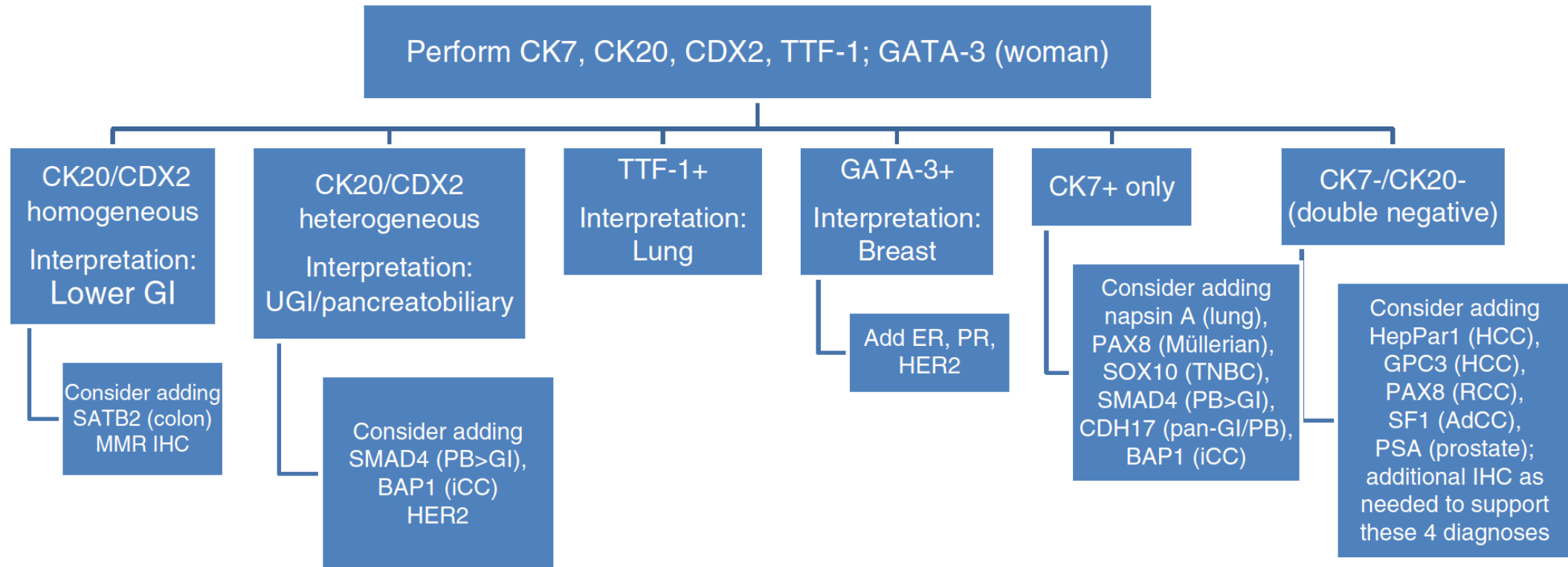


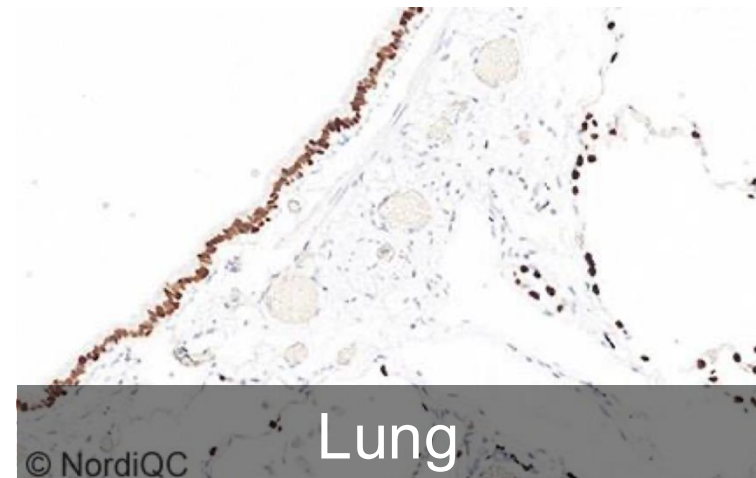
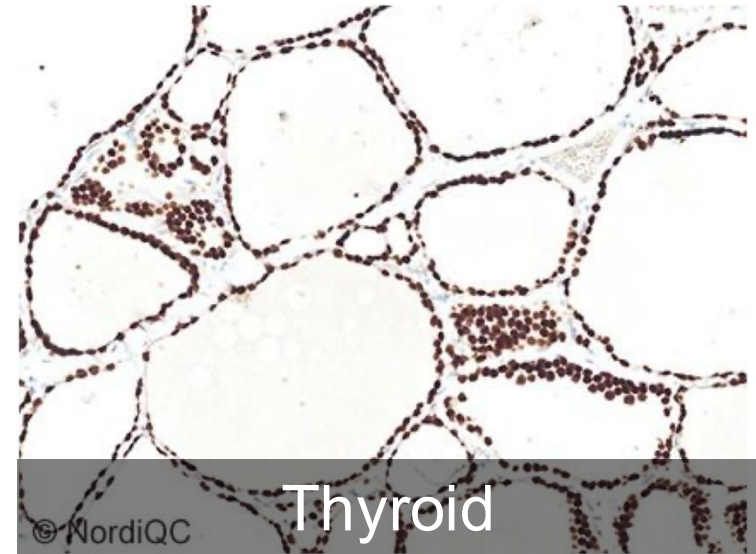
FIGURE 2. Immunohistochemical algorithm for “garden variety” adenocarcinoma in the liver. AdCC indicates adrenal cortical carcinoma; ER, estrogen receptor; GI, gastrointestinal; GPC3, glypican-3; HCC, hepatocellular carcinoma; iCC, intrahepatic cholangiocarcinoma; IHC, immunohistochemistry; PB, pancreatobiliary; PR, progesterone receptor; RCC, renal cell carcinoma; TNBC, triple-negative breast cancer; UGI, upper gastrointestinal. Please see this image in color online.

Thyroid transcription factor 1 (TTF)

- NKX2.1
- Nuclear transcription factor
- Expressed in lung, thyroid and brain

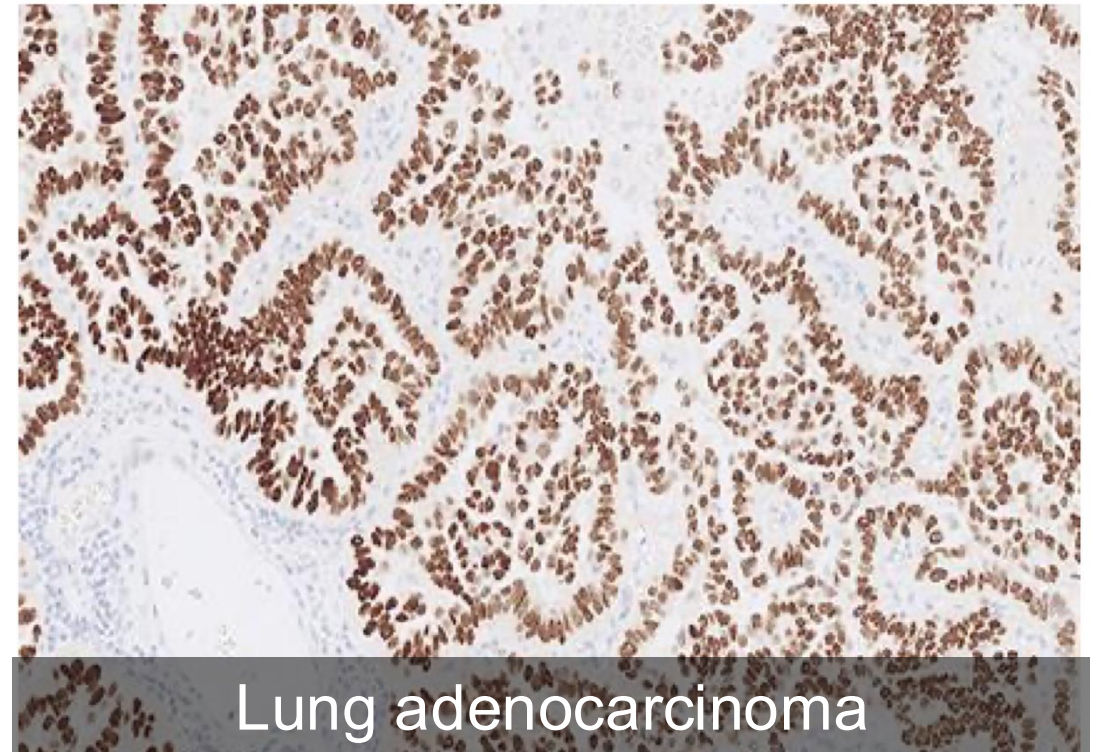
Lung:
TTF
Napsin

Thyroid:
TTF
PAX8



TTF

- Lung carcinomas
 - Adenocarcinomas: +
 - Small- and large cell: +
 - (Squamous): -/+
- Thyroid
 - Papillary, follicular, medullary: +
 - (Anaplastic): -/+
- Neuroendocrine tumors /
neuroendocrine differentiation:
weak, partially or focal



Lung:
TTF
Napsin

Thyroid:
TTF
PAX8

PAX8

- Nuclear transcription factor
- Involved in development and regulation of thyroid, kidney and Müllerian system
- Expression in normal cells
 - Thyroid glands
 - Kidney
 - Fallopian tubes
 - Uterus

Kidney:
PAX8

Female genitals:
PAX8
WT1
ER

Thyroid:
TTF
PAX8



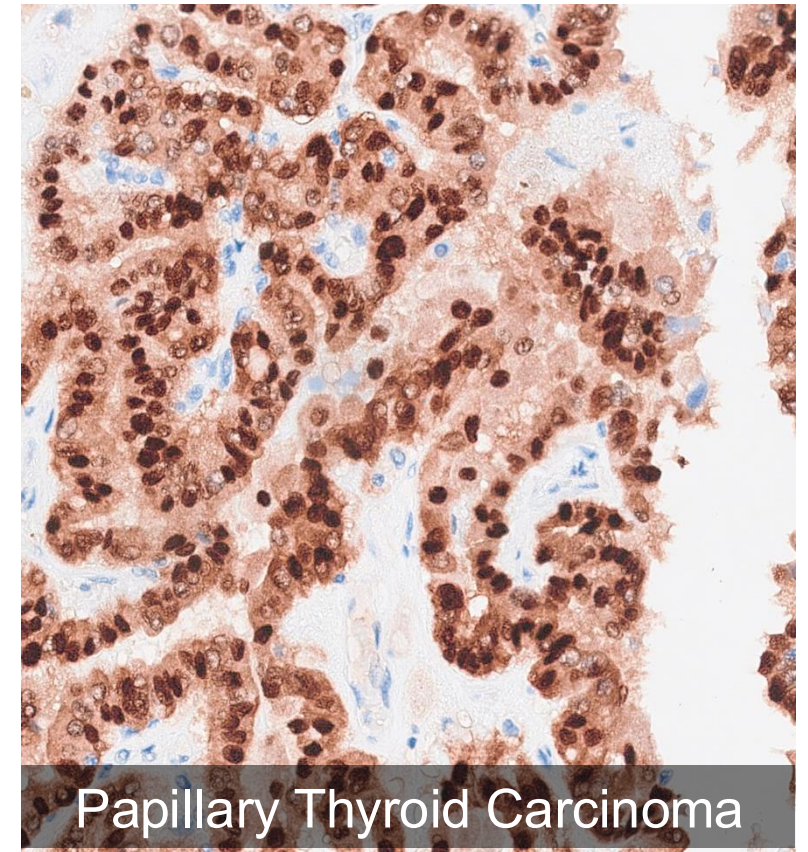
PAX8

- Nuclear transcription factor
- Involved in development and regulation of thyroid, kidney and Müllerian system
- Expression in neoplastic cells
 - Thyroid carcinoma
 - Renal carcinoma
 - Ovarian carcinoma
 - Endometrial carcinoma

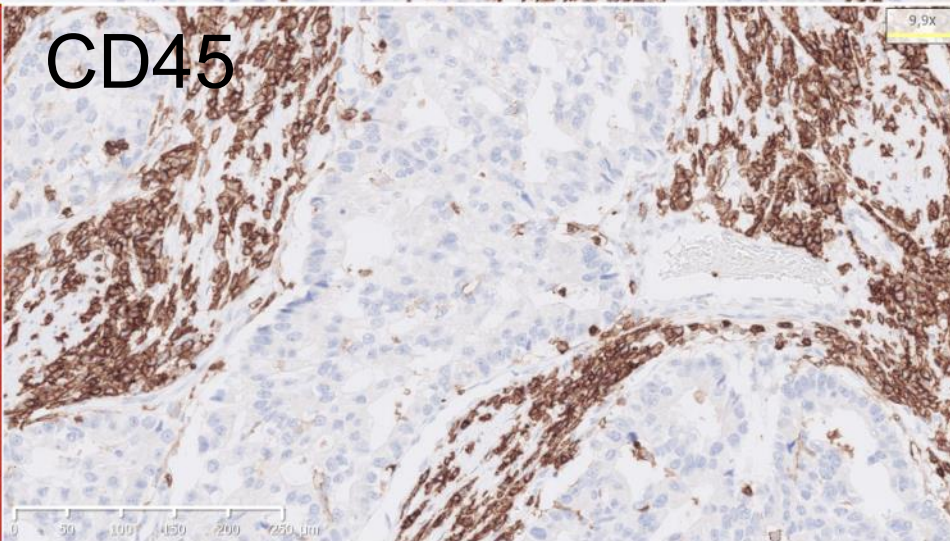
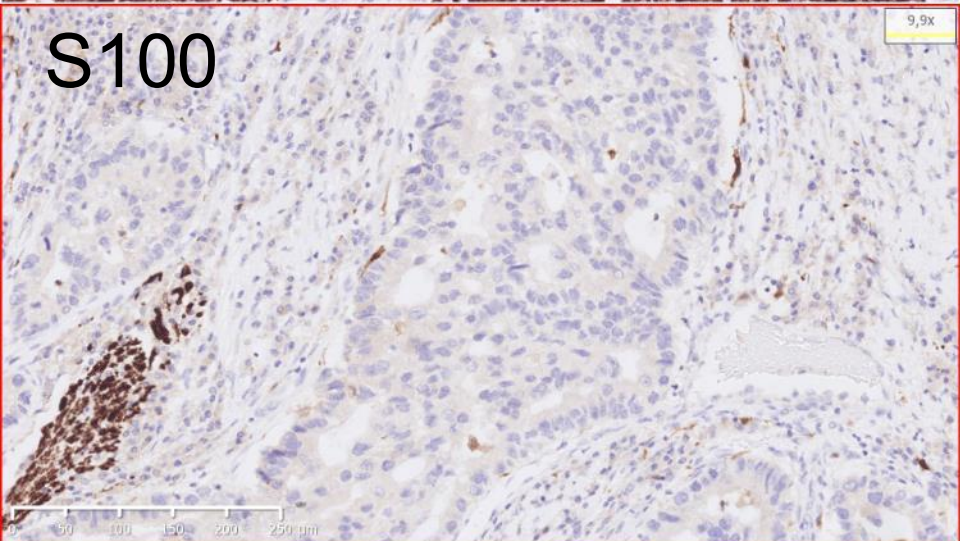
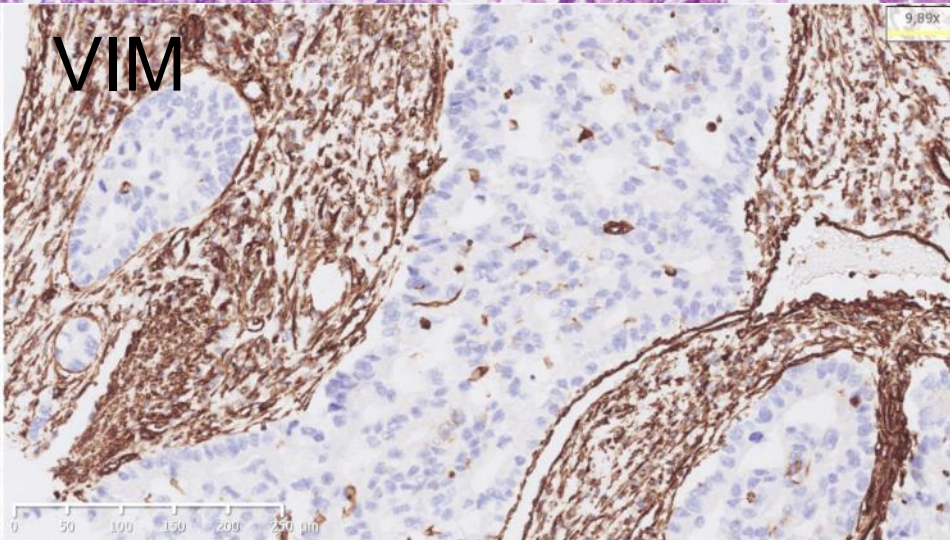
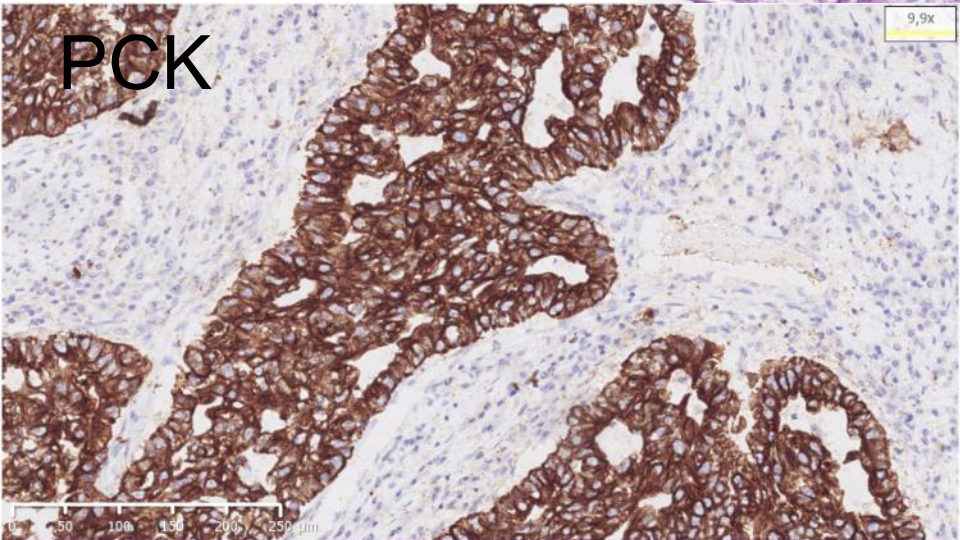
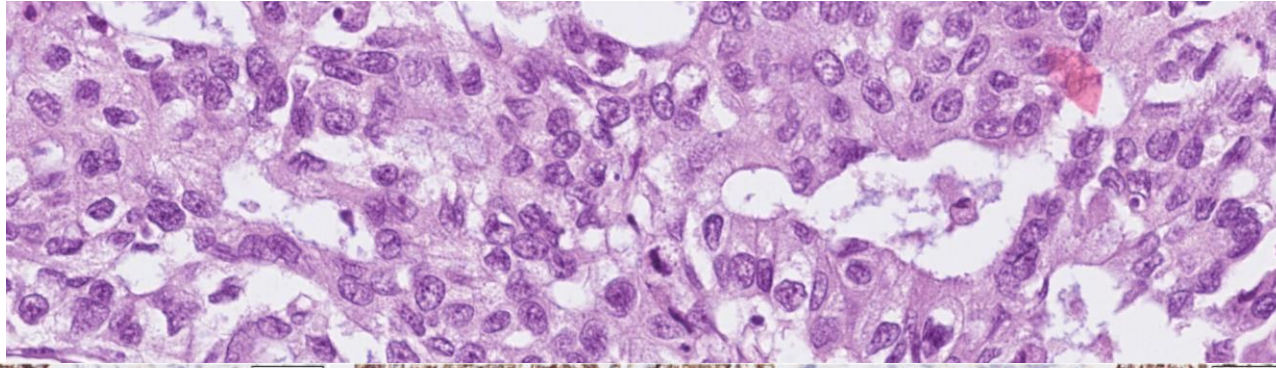
Kidney:
PAX8

Female genitals:
PAX8
WT1
ER

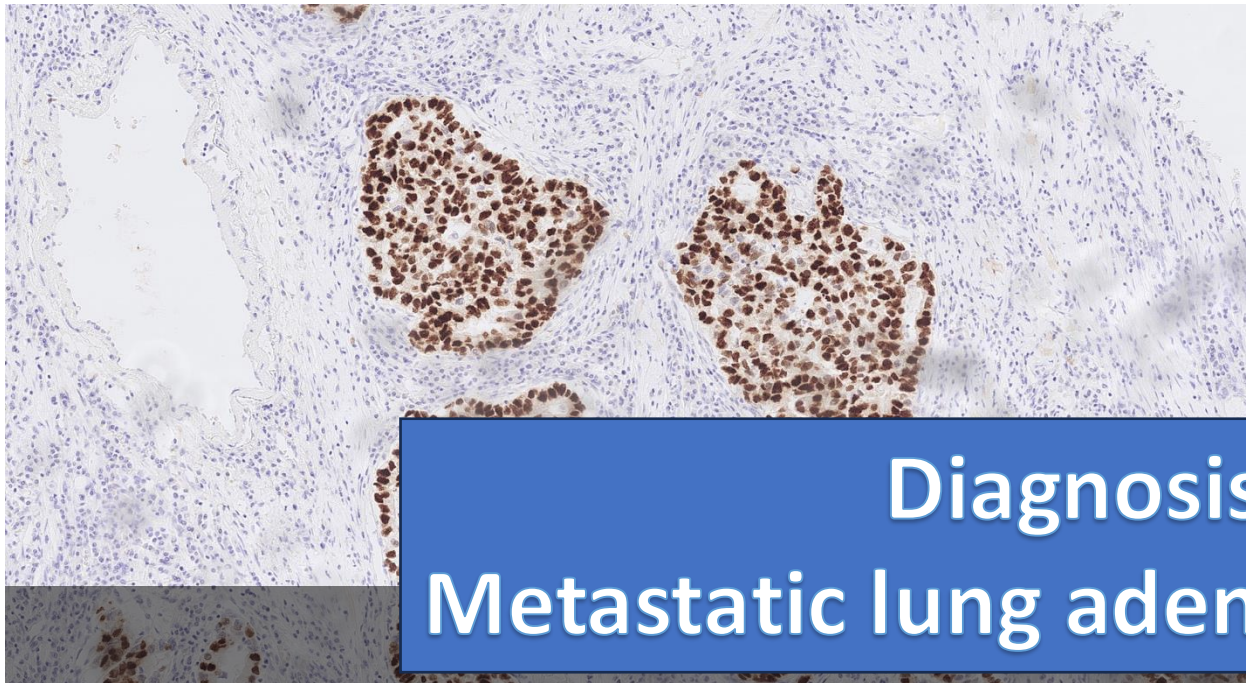
Thyroid:
TTF
PAX8



Papillary Thyroid Carcinoma



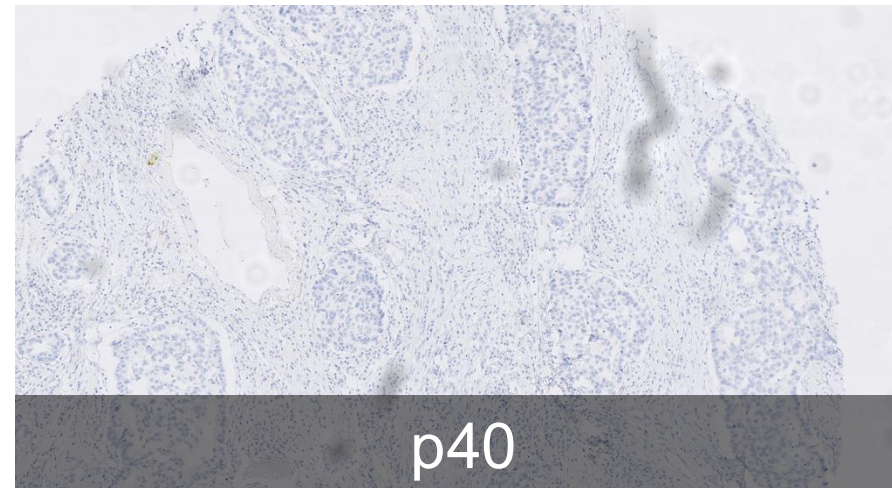
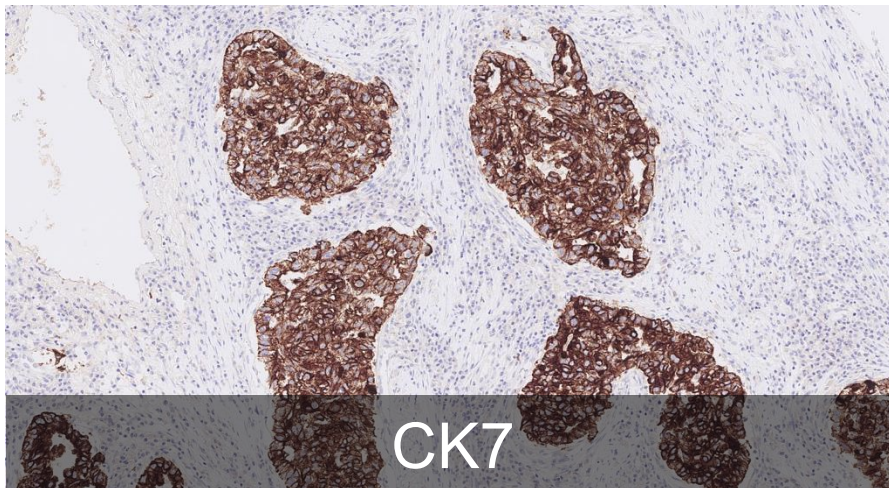
- 68 y.o. man
- Smoker
- Bloody expectorate
- Multiple lung tumours
- Biopsy from lymph node



Diagnosis

Metastatic lung adenocarcinoma

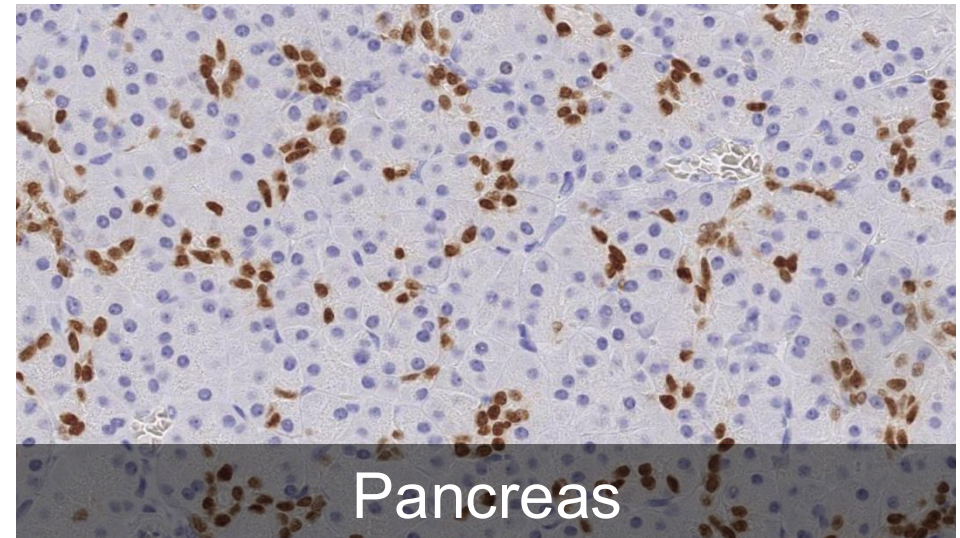
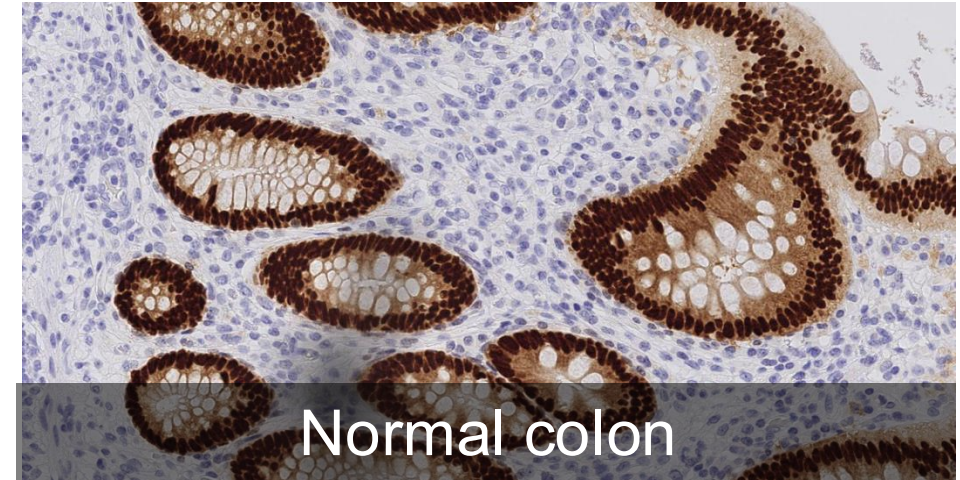
- 68 y.o. man
- Smoker
- Bloody expectorate
- Multiple lung tumours
- Biopsy from lymph node



CDX2

- Caudal-type homeobox 2
- Nuclear transcription factor that regulates intestinal differentiation
- Expression in normal cells
 - Intestinal epithelium
 - Heterogeneous in pancreas and bile ducts

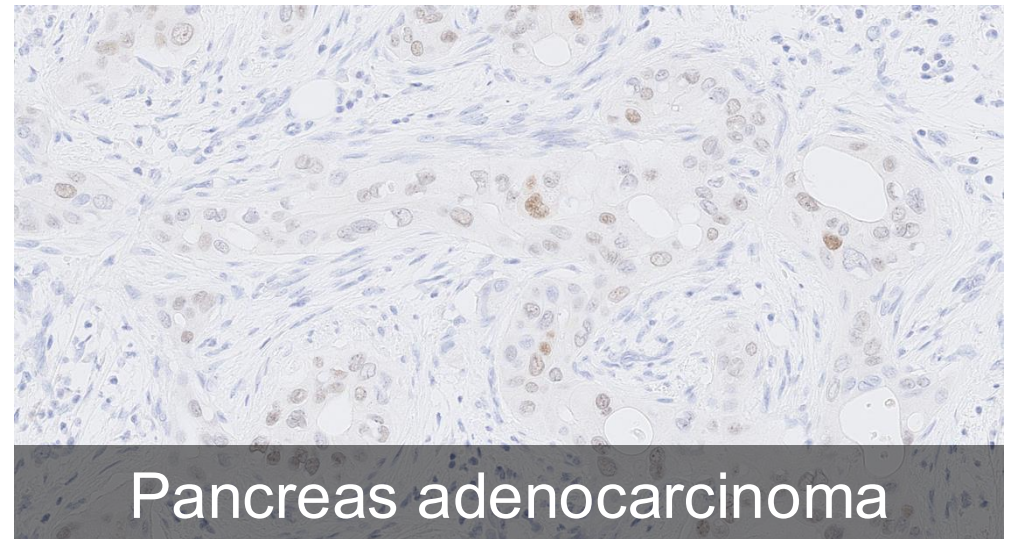
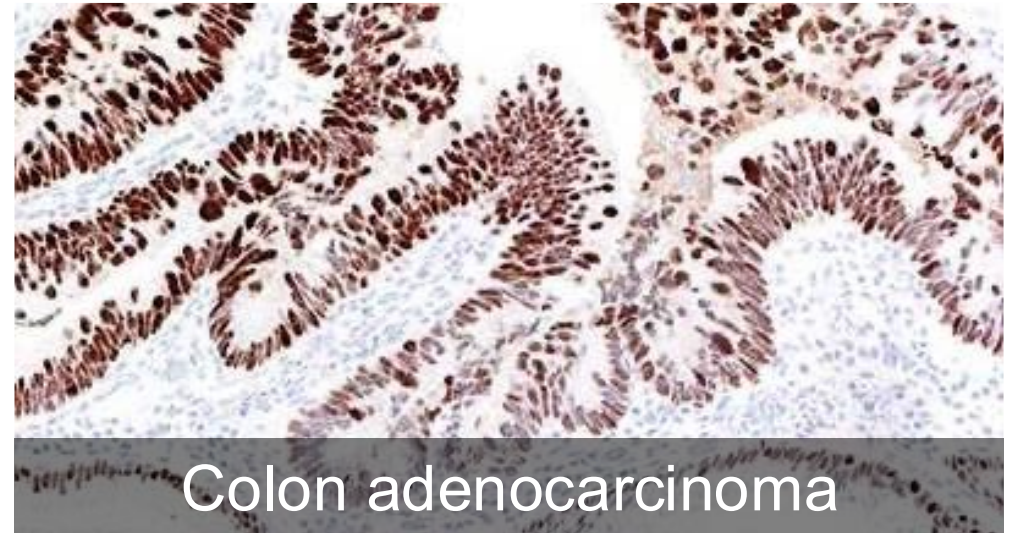
Gastrointestinal:
CDX2
Cadherin 17
SATB2



CDX2

- Caudal-type homeobox 2
- Nuclear transcription factor that regulates intestinal differentiation
- Expression in neoplasias
 - Colorectal adenocarcinomas
 - Pancreas
 - Intestinal differentiation

Gastrointestinal:
CDX2
Cadherin 17
SATB2



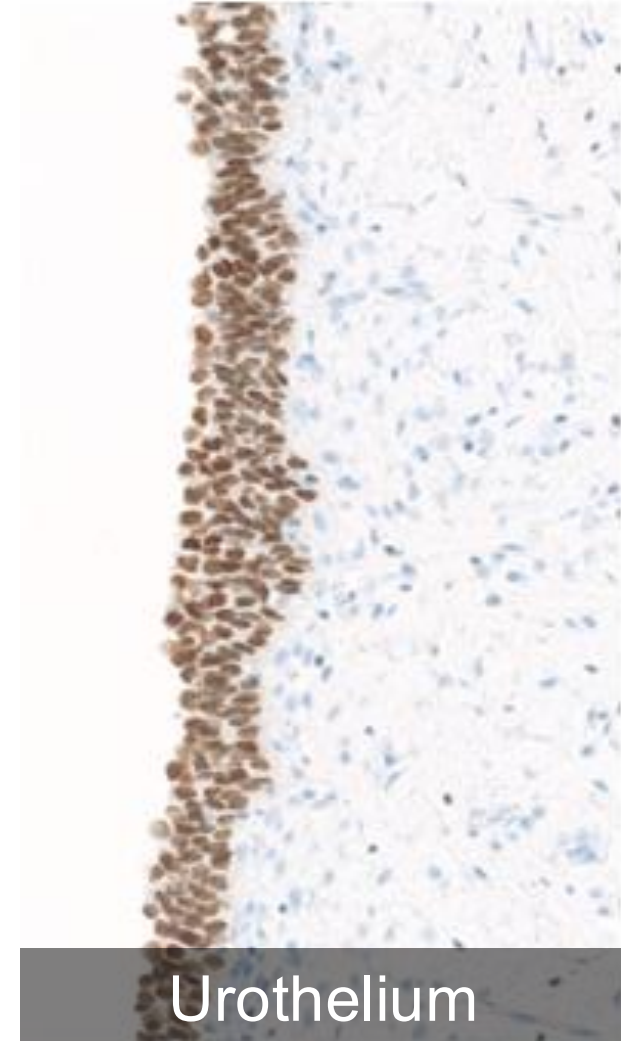
GATA3

- Nuclear transcription factor
- Involved in embryonic development and regulator of epithelial and immune cells
- Expression in normal cells:
 - Breast
 - Urothelium
 - Some T-cells
 - Skin

Breast:
GATA3
Estrogen receptor
TRPS1
SOX10

Bladder:
GATA3
Uroplakin II

Pancreas:
SMAD4
Gata3



Urothelium

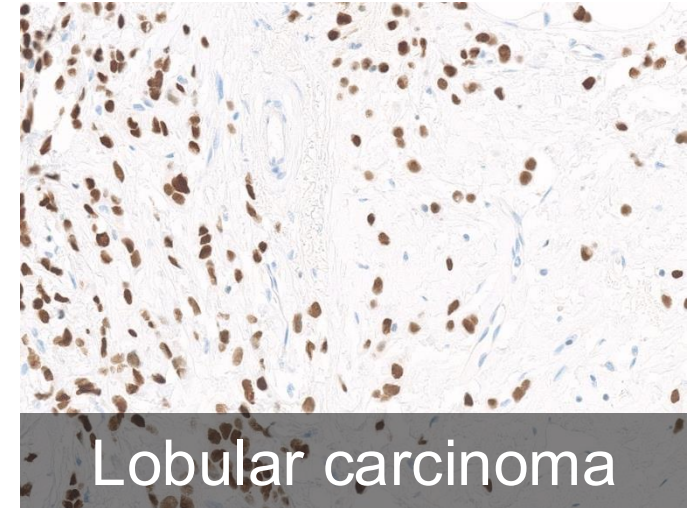
GATA3

- Nuclear transcription factor
- Expression in neoplastic cells
 - Breast carcinoma
 - Urothelial carcinomas
 - Skin cancers
 - Yolk sac tumours
 - Pancreatic (heterogenous)
 - Mesotheliomas (heterogenous)
 - Some lung adenocarcinomas

Breast:
GATA3
Estrogen receptor
TRPS1
SOX10

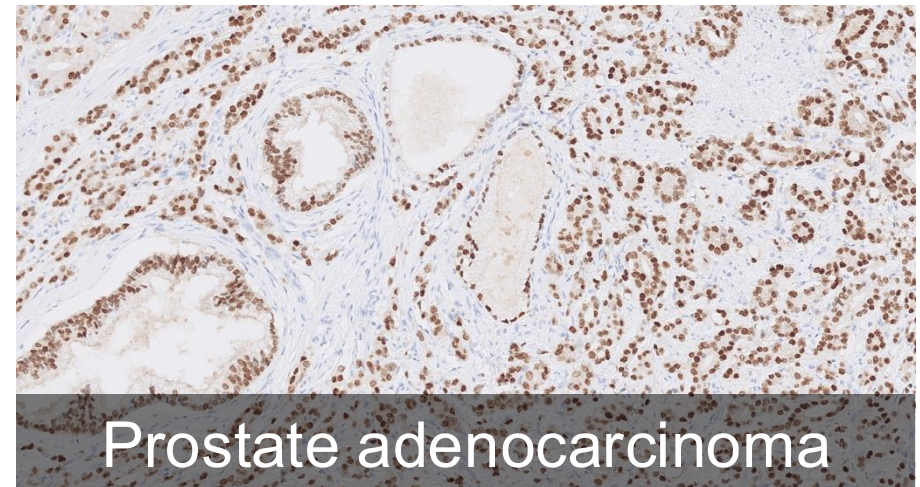
Bladder:
GATA3
Uroplakin II

Pancreas:
SMAD4
Gata3



NKX3.1

- Homeobox protein Nkx-3.1
- Androgen-regulated, prostate specific homeobox gene
- Transcription factor and tumor suppressor gene
- Expressed in normal prostate epithelium and some spermatogonia
- More sensitive than PSA in metastatic prostate adenocarcinomas



NKX3.1

TABLE 4

Sensitivity of NKX3.1, PSA and PSAP for Metastatic Prostate Carcinoma

	NKX3.1	PSA	PSAP
Lymph Node	59/59 (100%)	58/59 (98.3%)	59/59 (100%)
Distant	9/10 (90%)	7/10 (70%)	9/10 (90%)
Overall	68/69 (98.6%)	65/69 (94.2%)	68/69 (98.6%)

Only cases in which the same cores were able to be evaluated for each of the 3 markers w

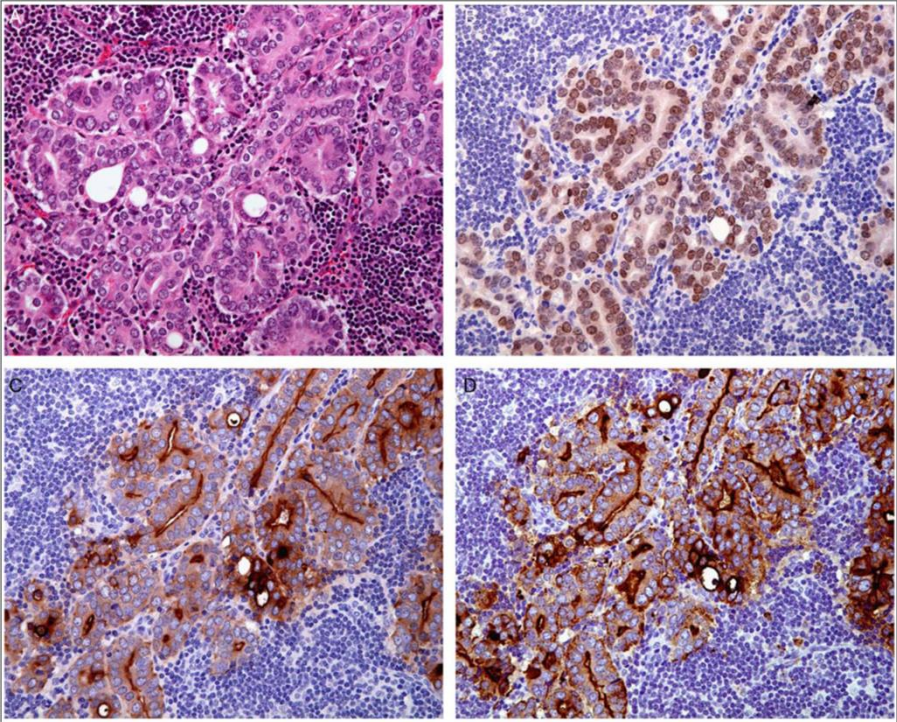
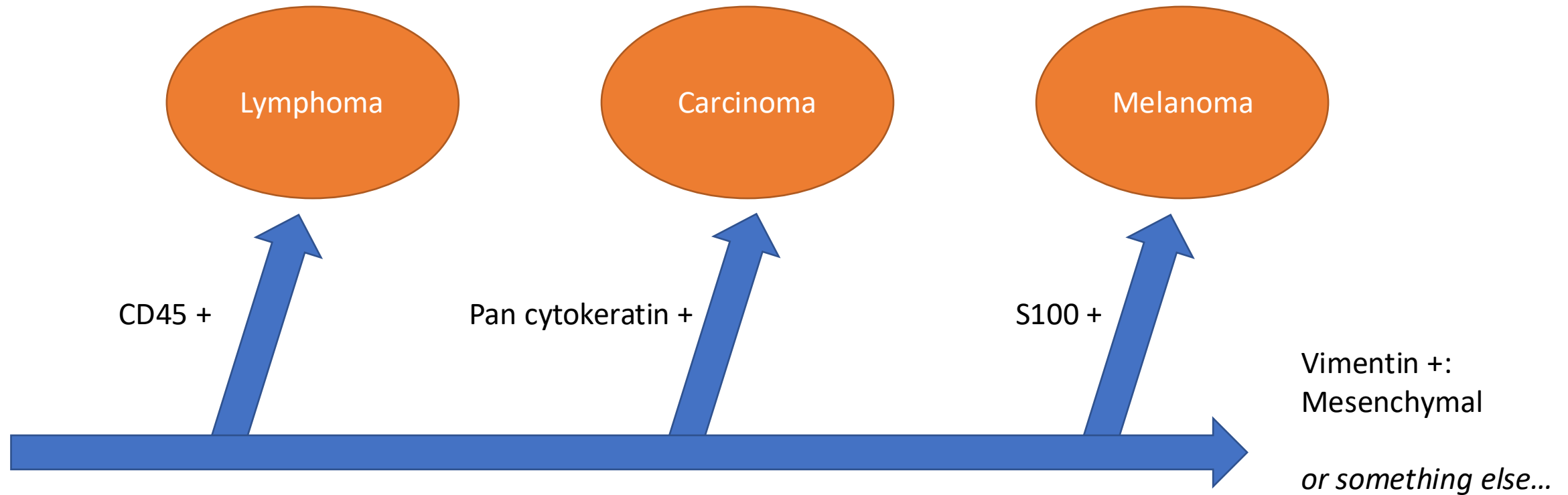


FIGURE 3
A prostate carcinoma metastasis to a lymph node stained with H&E (A), NKX3.1 (B), PSA (C) and PSAP (D).

NKX3.1 as a Marker of Prostatic Origin in Metastatic Tumors
[Am J Surg Pathol. 2010 Aug; 34\(8\): 1097–1105.](#) Author manuscript.

Primary panel in action

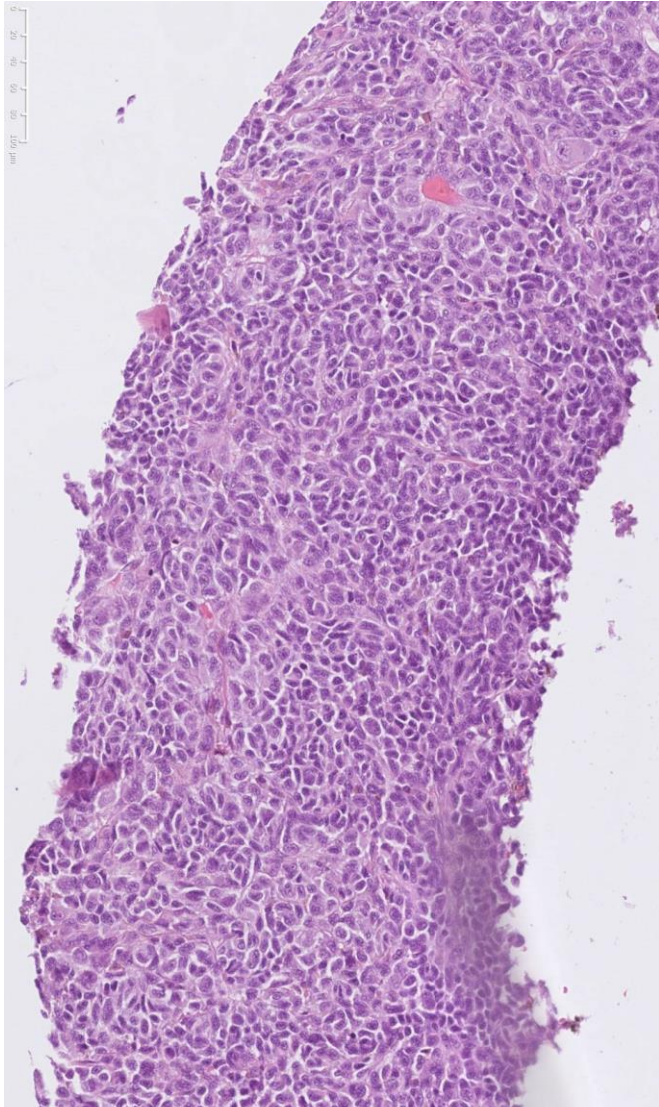


Other melanocytic markers

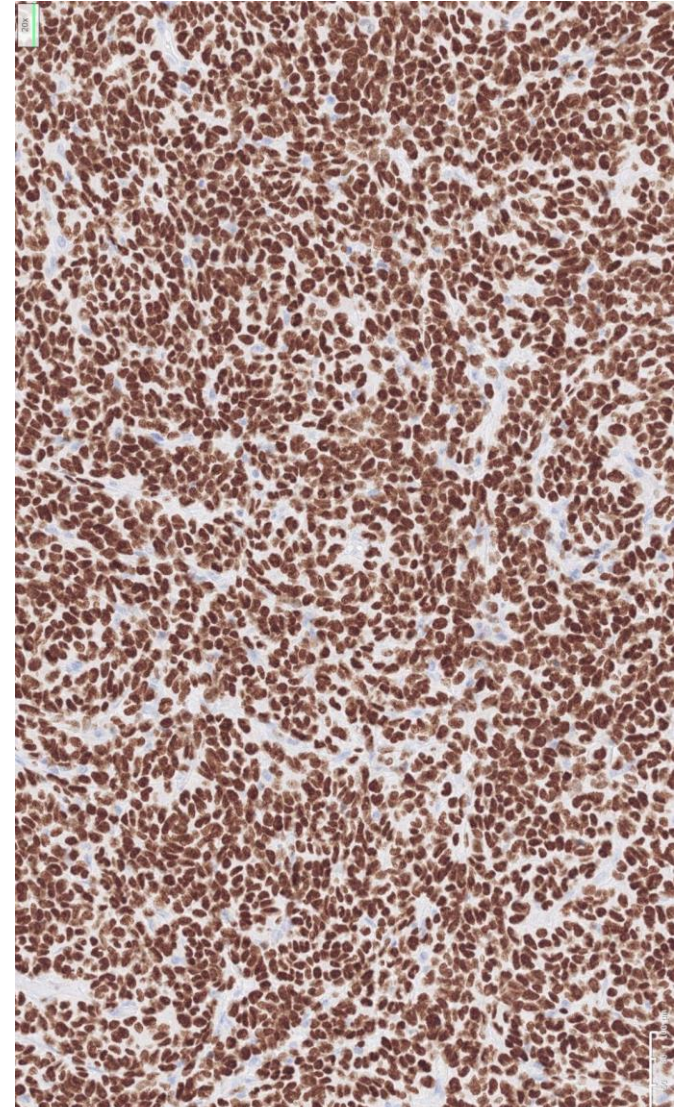
Unknown primary tumour

SOX10

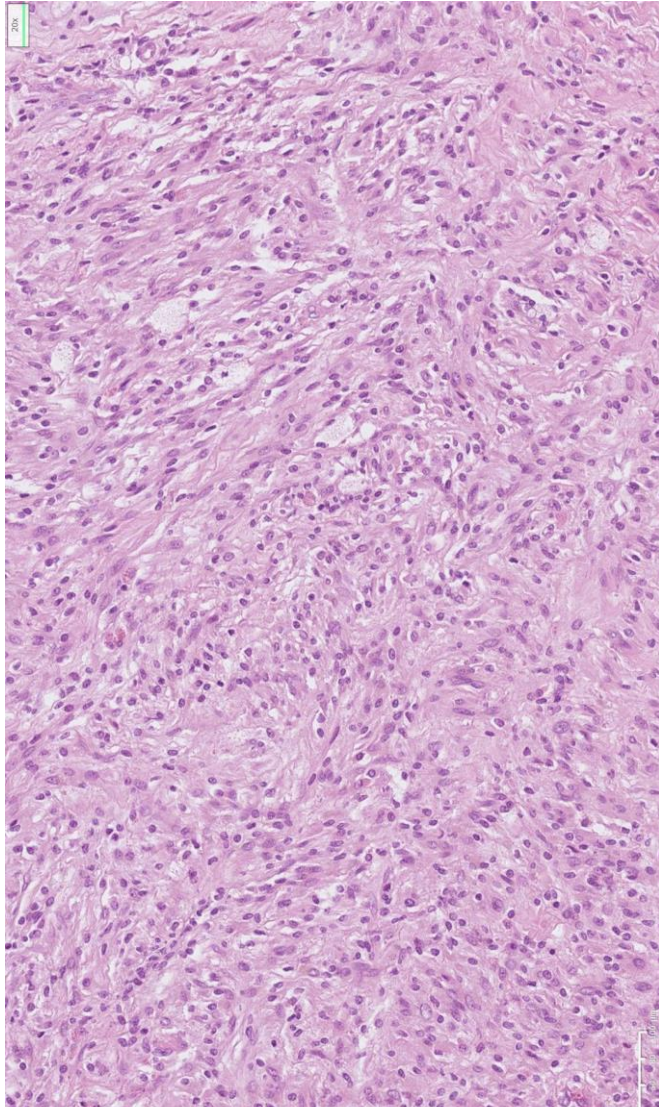
- Nuclear transcription factor
- Expression in normal cells
 - Melanocytes
 - Schwann cells
 - Myoepithelial cells
- Expression in neoplasias
 - Melanomas
 - Triple negative breast carcinoma
 - Acinic cell and adenoid cystic carcinoma



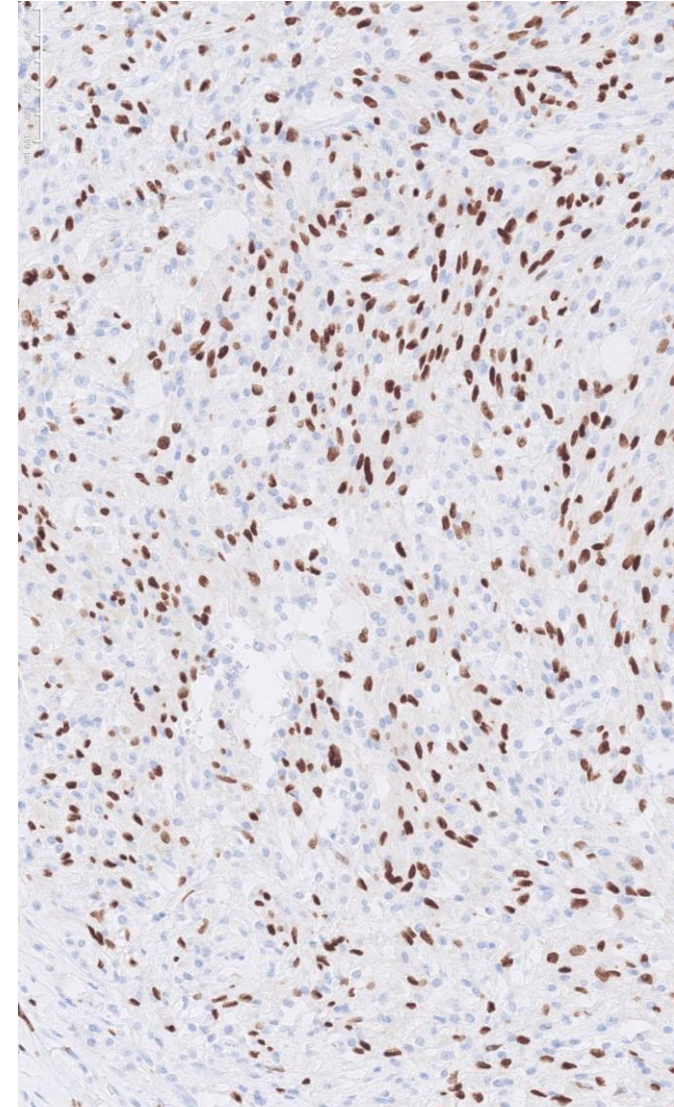
Malignant melanoma



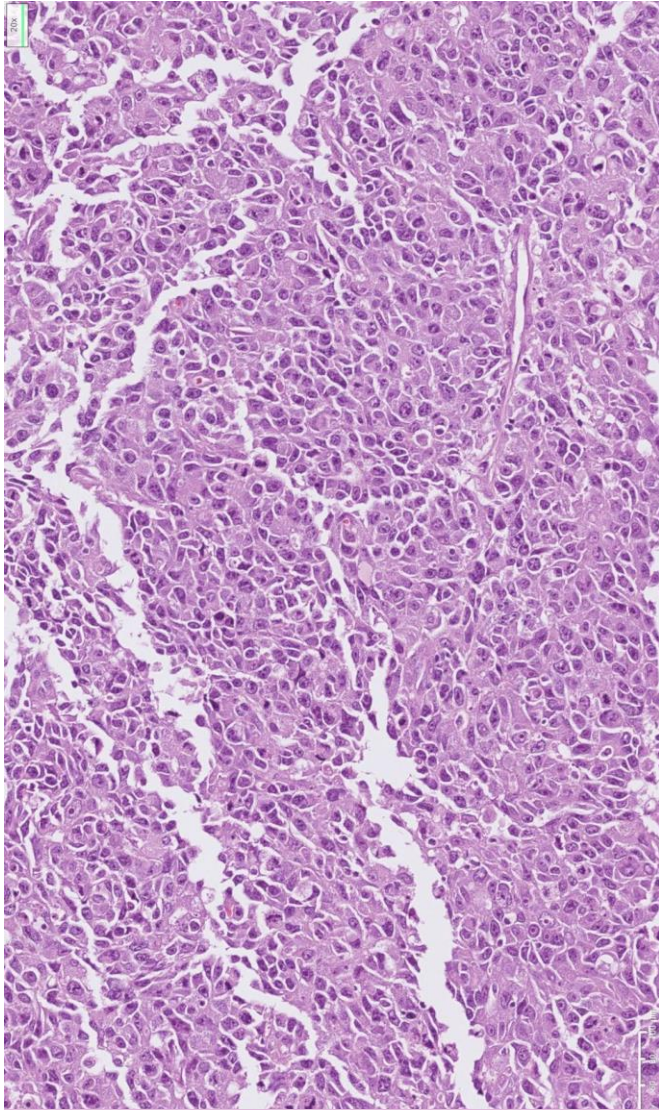
SOX10



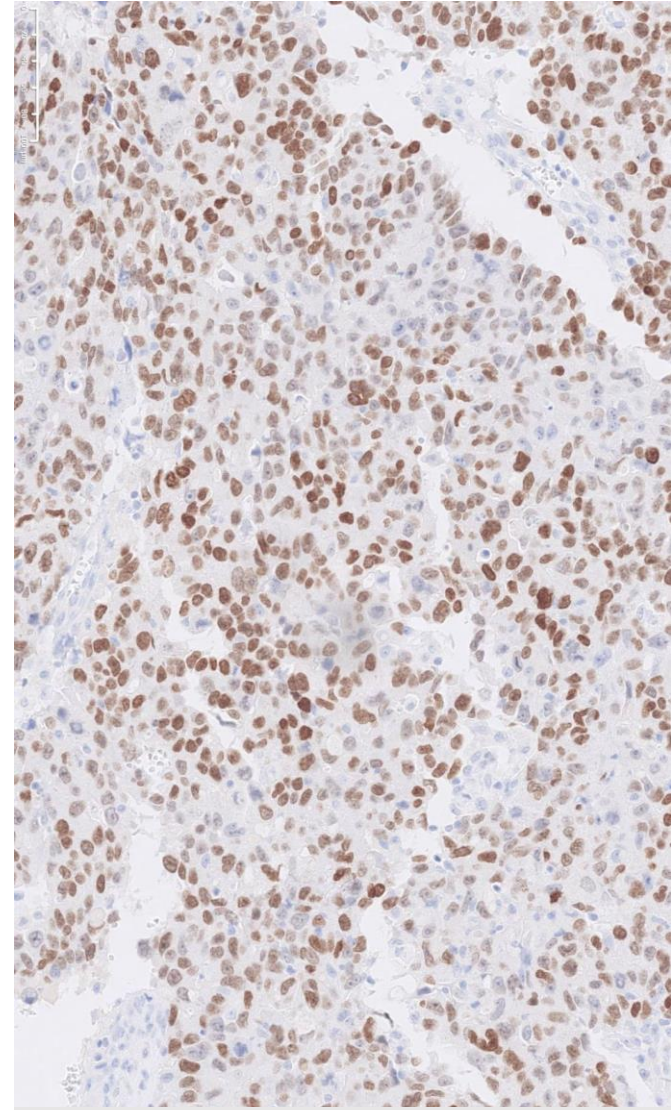
Schwannoma



SOX10



Triple negative breast carcinoma



SOX10

S100 versus SOX10

Table 4.7 Comparison of the performance of S-100 versus SOX10 in tumor diagnosis

S-100–, SOX10+	S-100+, SOX10+
Some MPNSTs, melanomas, and carcinomas with myoepithelial differentiation (sensitivity issue)	- Melanoma - Nerve sheath tumors - Neoplasms with myoepithelial differentiation
S-100–, SOX10–	S-100+, SOX10–
- Most carcinomas, sarcomas, lymphomas - Mesothelioma - Germ cell tumor - Pheochromocytoma/paraganglioma (though both markers expressed by sustentacular cells)	- Tumors of adipocytic/chondroid lineage - Chordoma - Ossifying fibromyxoid tumor - Biphenotypic sinonasal sarcoma - Lipofibromatosis-like neural tumor - Infantile fibrosarcoma-like tumor - Rare cases of Ewing, RMS, SS - S-100+ carcinomas without myoepithelial differentiation (S-100A1 and/or S-100A6+) - S-100+ histiocytic/dendritic cell tumors (Langerhans cell histiocytosis, Rosai-Dorfman, interdigitating dendritic cell tumor (100%); histiocytic sarcoma, Erdheim-Chester, blastic plasmacytoid dendritic cell tumor (30%); follicular dendritic cell sarcoma, juvenile xanthogranuloma (occ.))

Polyclonal S-100 (S-100B >> S-100A1 >> S-100A6)

SOX10 vs. MLA / S100

SOX10

- Nuclear staining
- More sensitive og specific

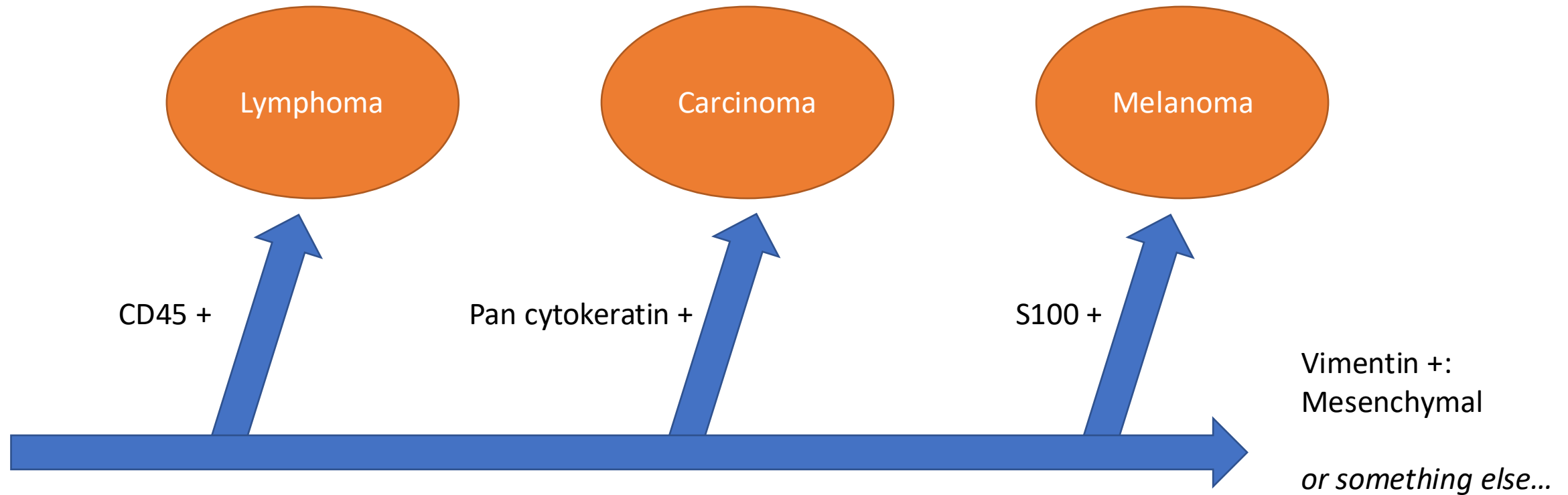
MLA (MART1)

- Cytoplasmic
- Sex cord tumours (A103 only)
- Adrenal (A103 only)

S100

- Cytoplasmic

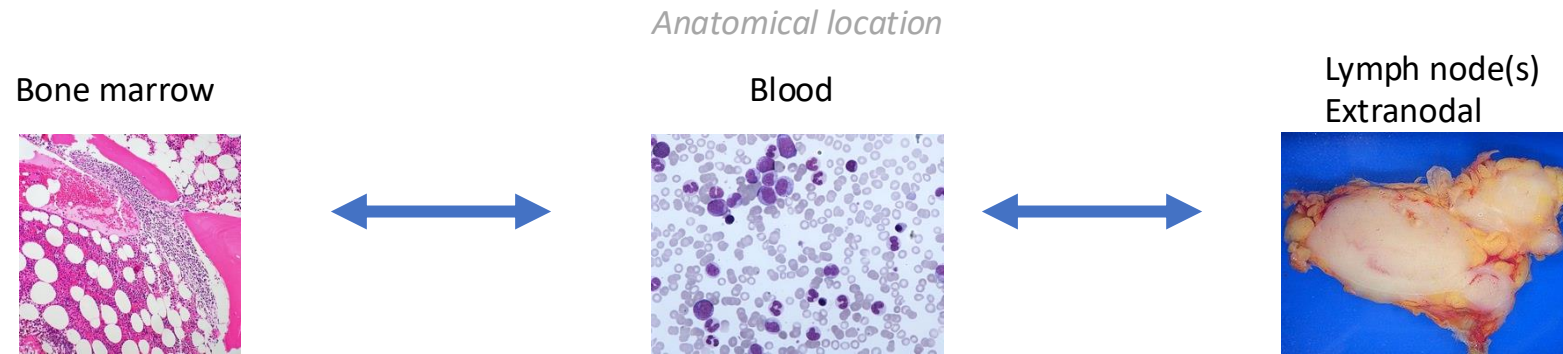
Primary panel in action



Lymfomas and leukemia

Unknown primary tumour

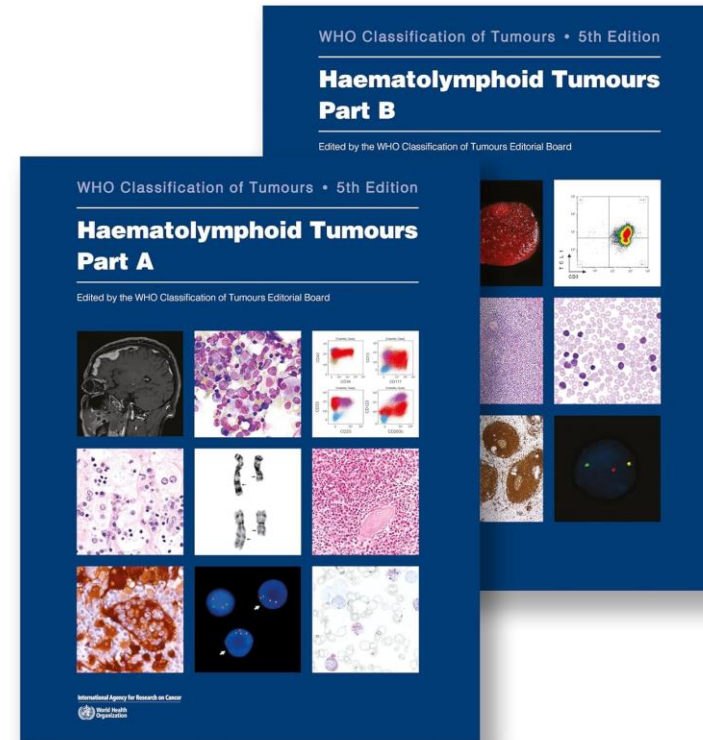
Hematopoietic and lymphoid neoplasias



Adapted from S. Hamilton

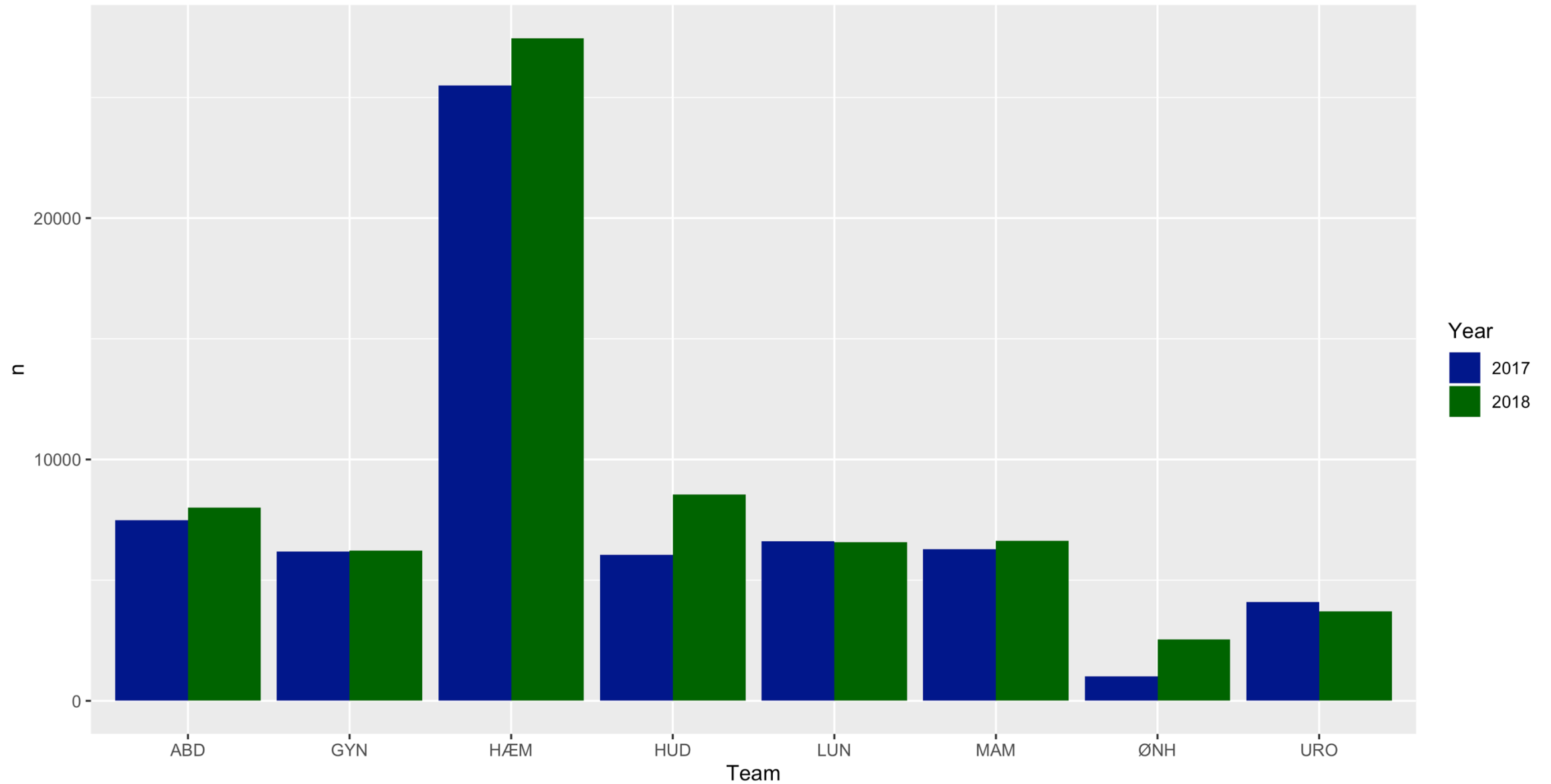
WHO Classification – update 2024

- “WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues - 5th edition”
- More than 100 lymphoma entities!
- Contains
 - Clinical features
 - Morphology
 - **Immunophenotypes**
 - Molecular genetics
 - Mutation profile

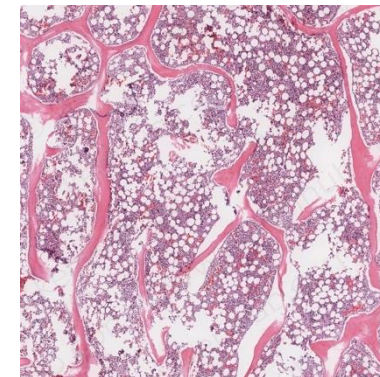
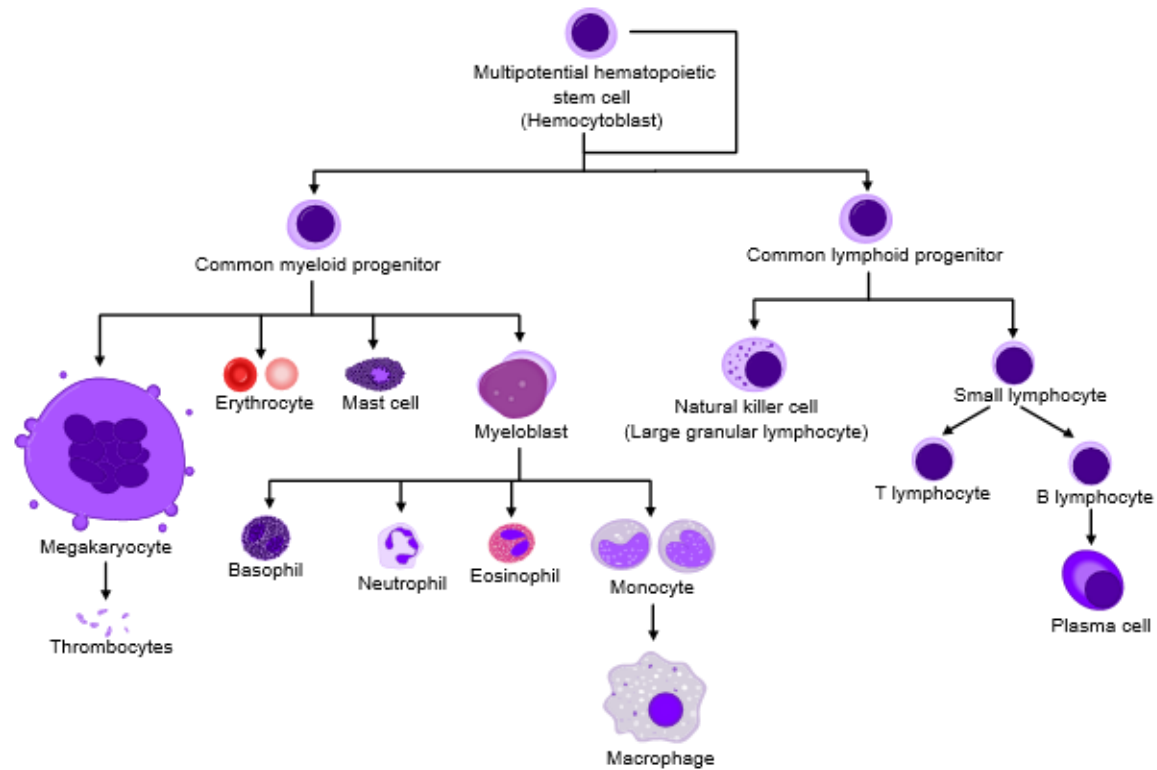


WHO5 vs ICC

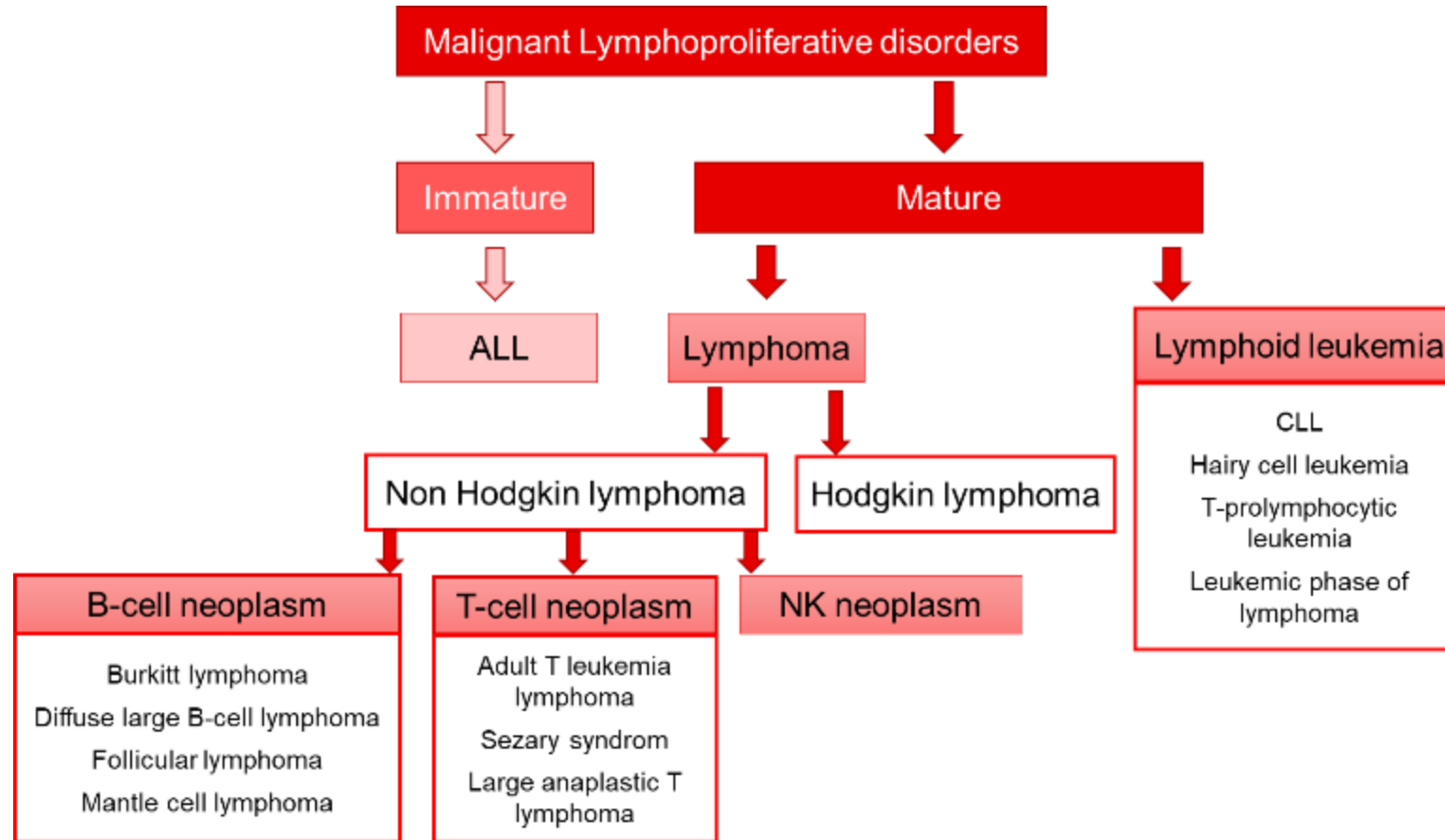
IHC stains - Department of pathology - Aalborg University Hospital



Hematopoiesis

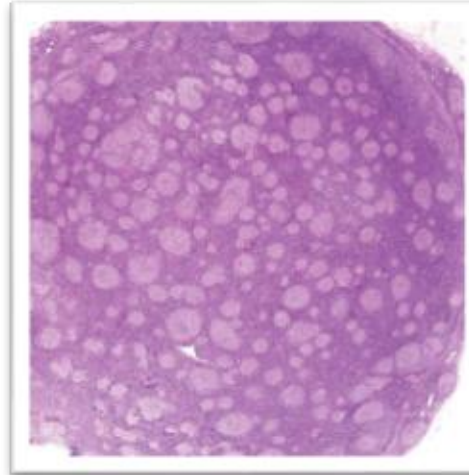


Lymphoproliferative disorder

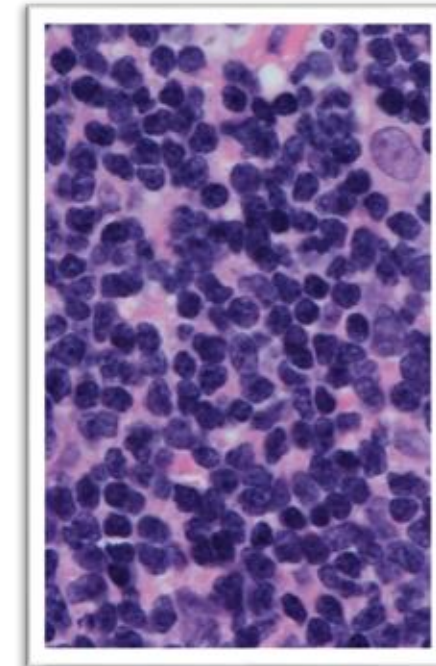


How are lymphomas diagnosed

- Morphology
 - Cell size
 - Cell types (uniform or different)
 - Mitosis
- Architecture
 - Normal architecture?
 - Growth through capsule?
 - Sclerosis?
- Immunophenotype
 - IHC
 - Flow cytometry
- Material
 - Biopsy (excision of lymph node preferred)
 - No fine needle aspiration

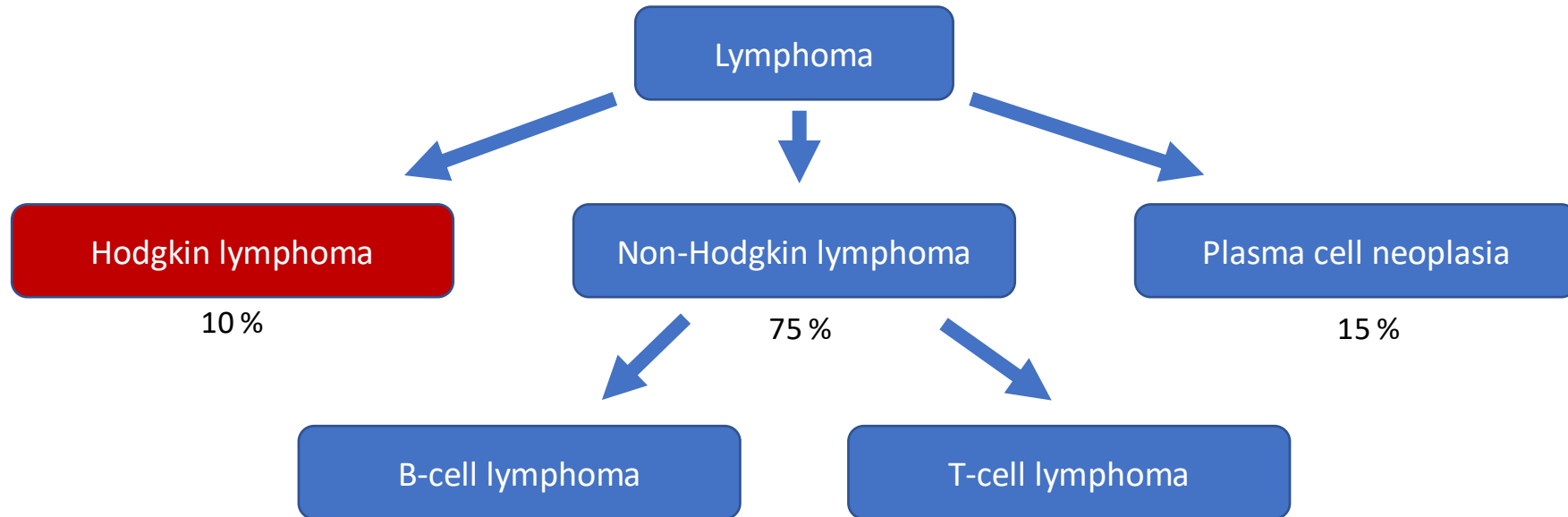


Abnormal architecture in follicular lymphoma



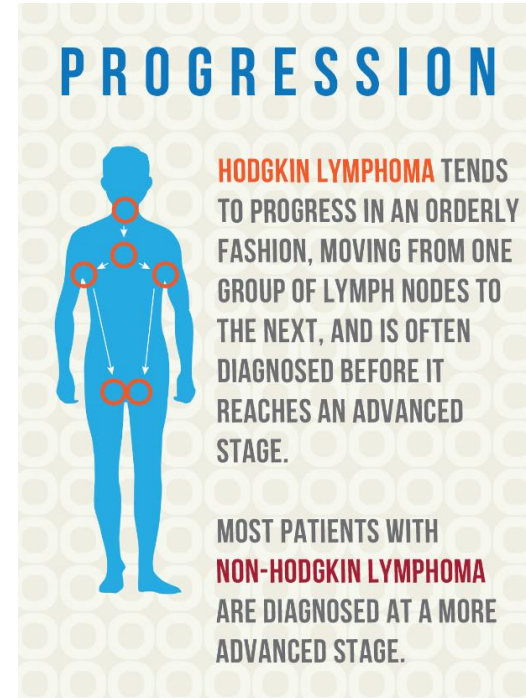
Irregular nuclei in mantle cell lymphoma

Classification of lymphomas

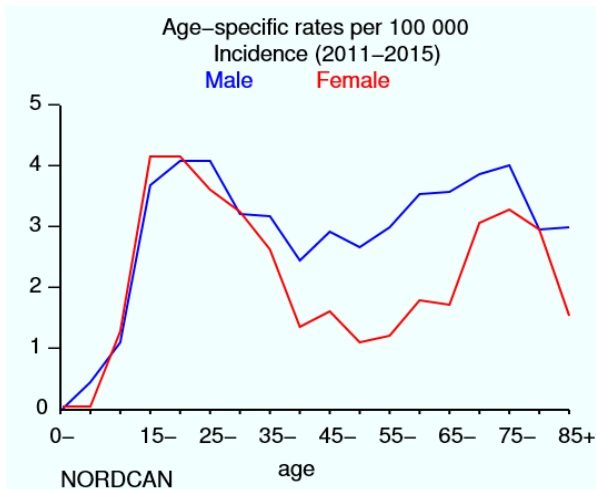


Hodgkin versus non-Hodgkin lymphoma

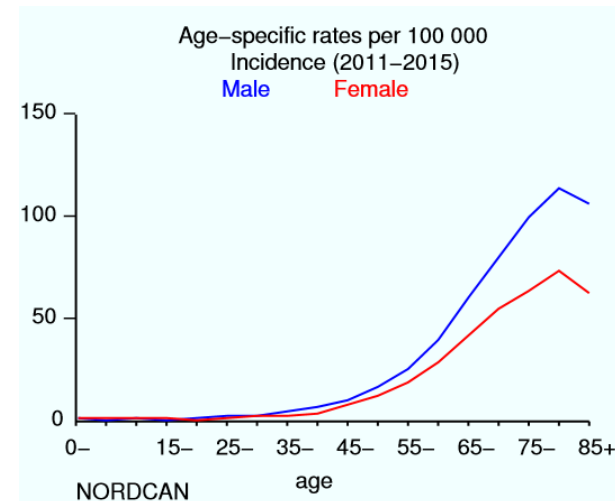
- Both types of lymphoma are developed from lymphocytes
- Age of onset is different
- HL develop on neck or chest, while non-HL can arise throughout the body
- Non-HL is diagnosed at a more advanced stage than HL
- HL have better survival (5-year: 90%) than non-HL (overall)



Hodgkin lymphoma

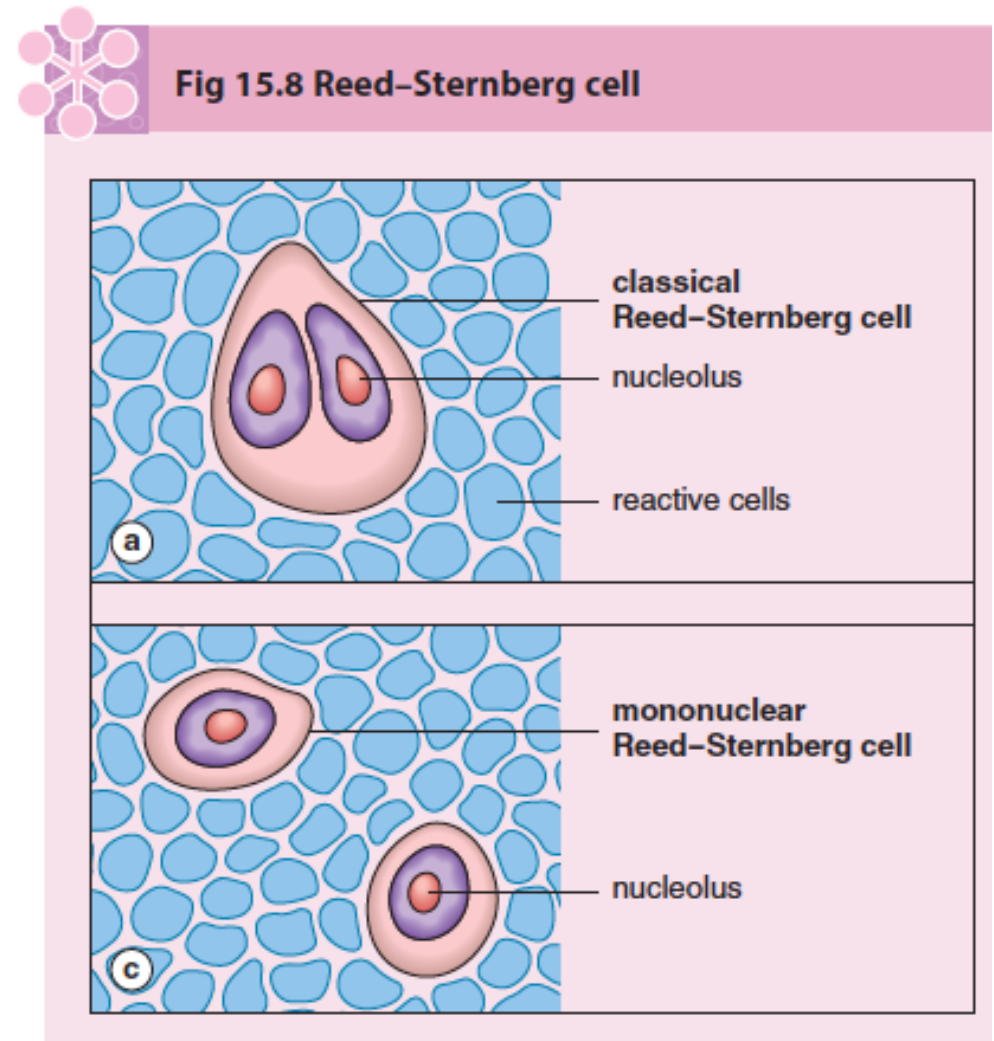


Non-Hodgkin lymphoma

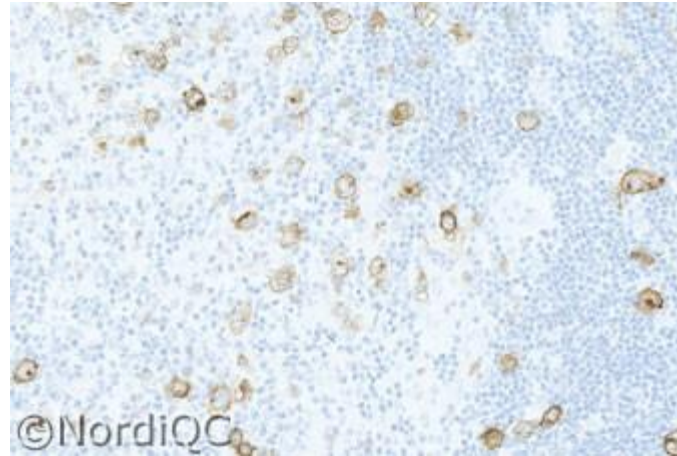
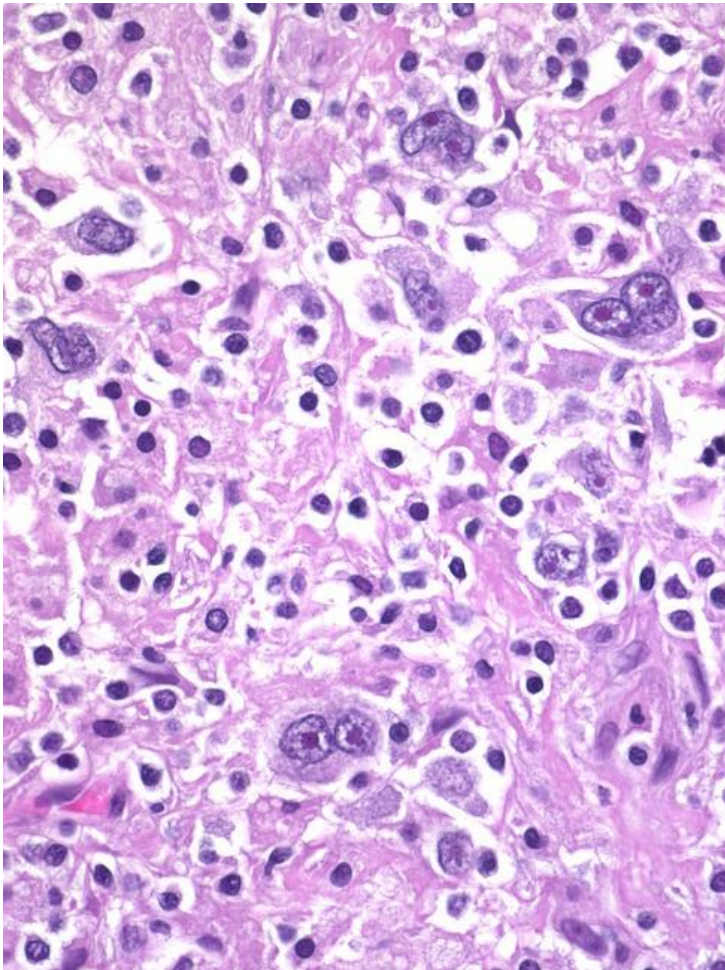


Hodgkin's lymphoma

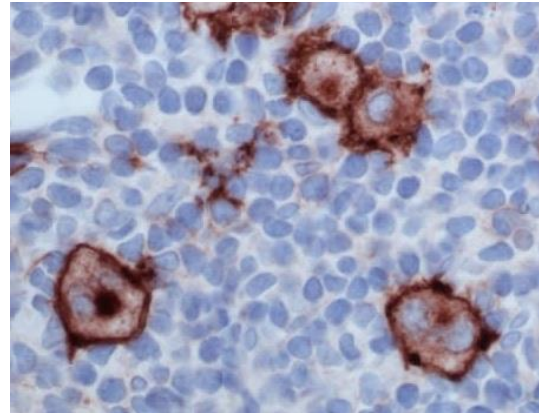
- Hodgkin and Reed-Sternberg cells
- Reactive (non-neoplastic) background of:
 - Lymphocytes
 - Macrophages
 - Eosinophils
- Association with EBV
- Most commonly in cervical, mediastinal and axillary lymph nodes



Hodgkin's lymphoma



CD30 / CD15 positive



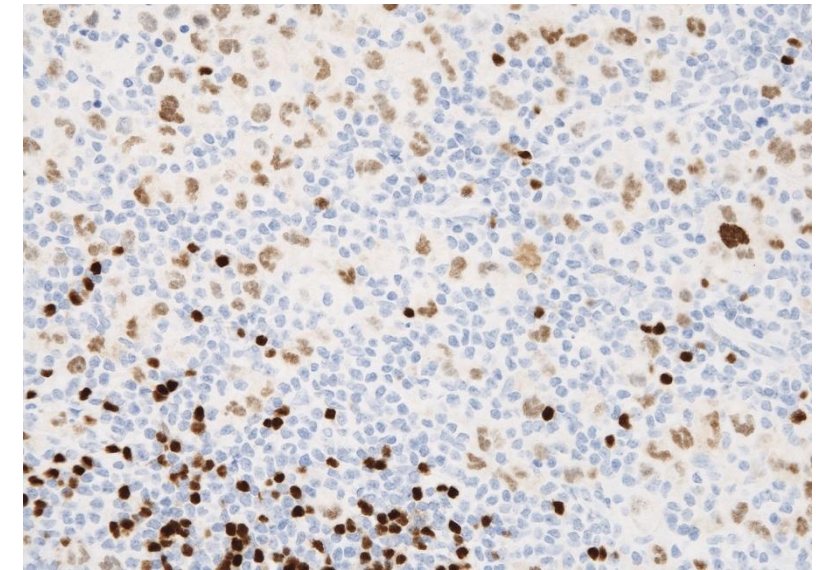
Hodgkin lymphoma

CD30

CD15

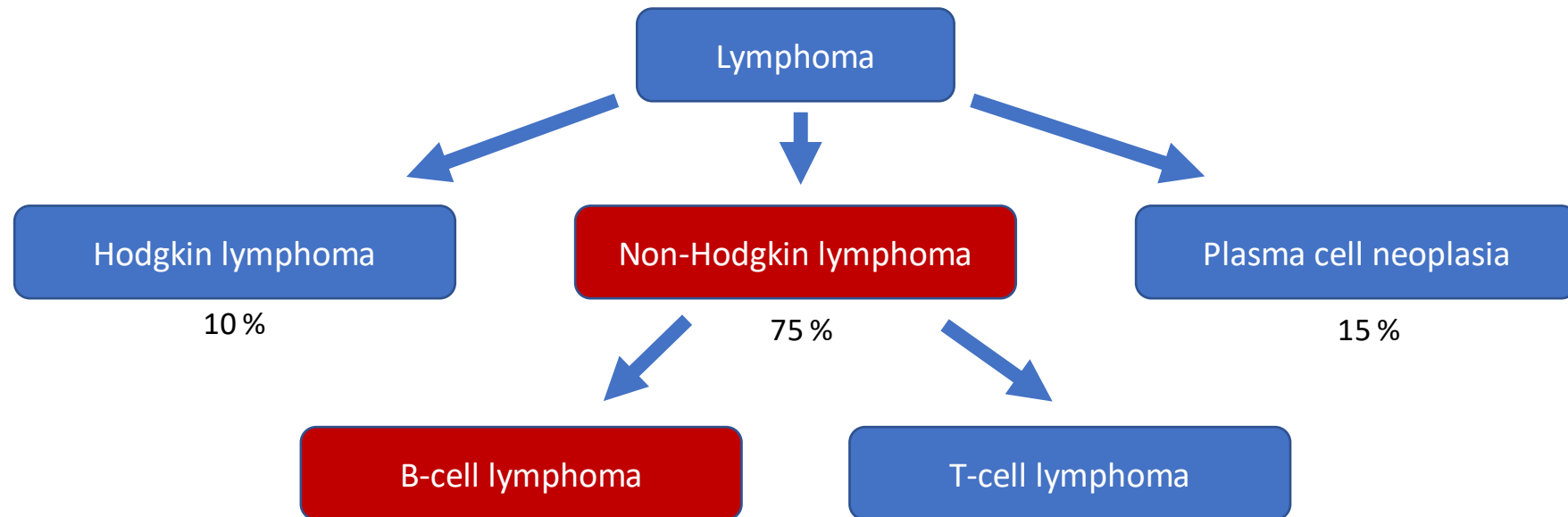
PAX5 (weak)

CD20 Negative

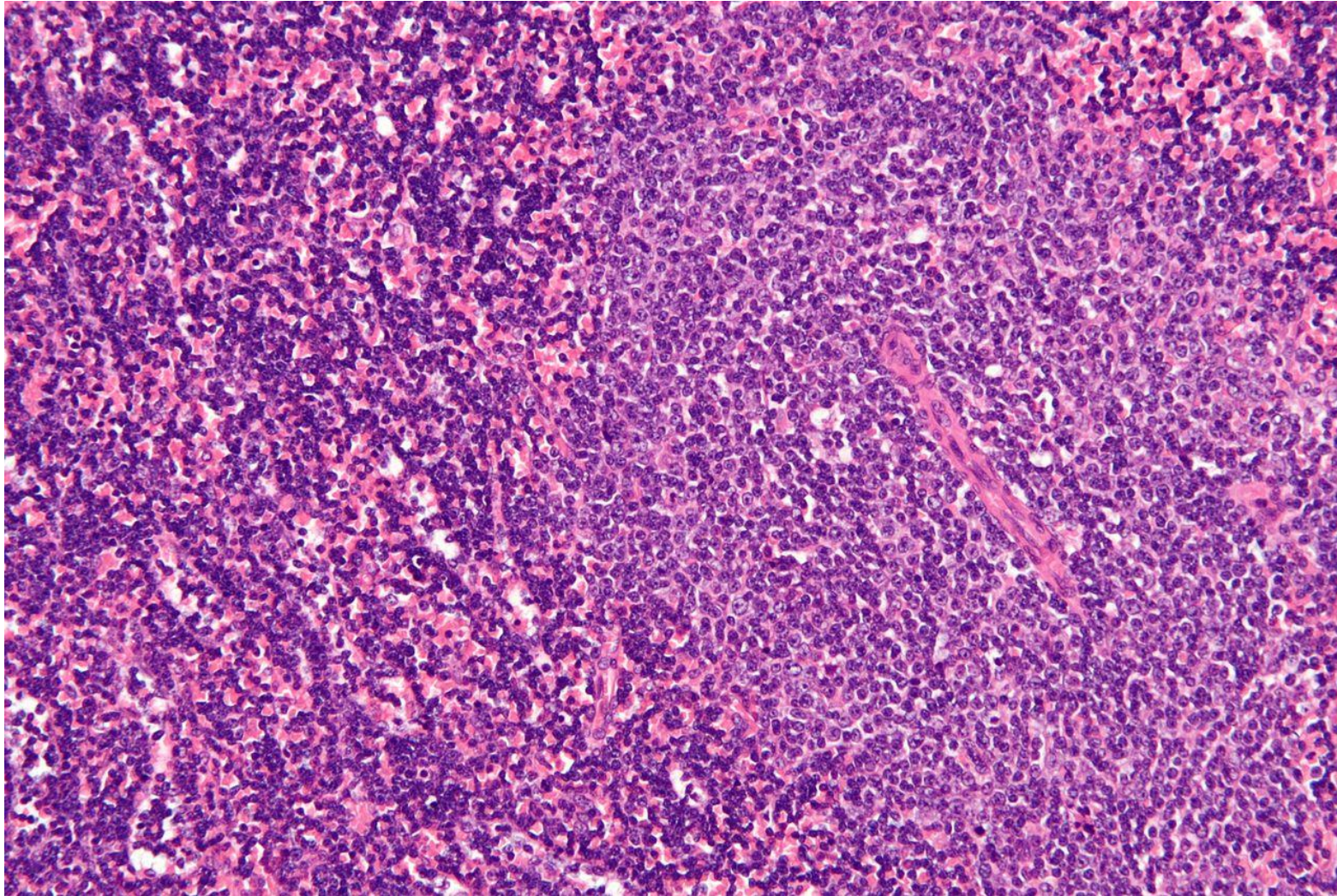


PAX5 weak

Classification of lymphomas



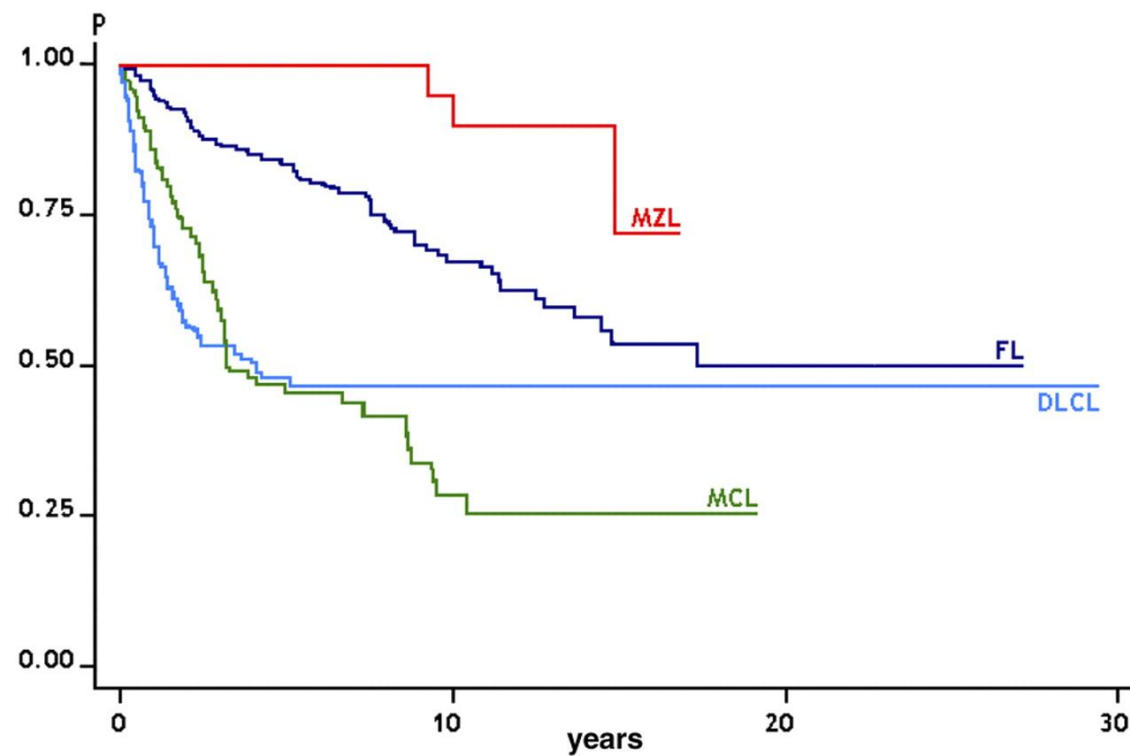
Small B-cell lymphomas



Survival B-cell lymphomas

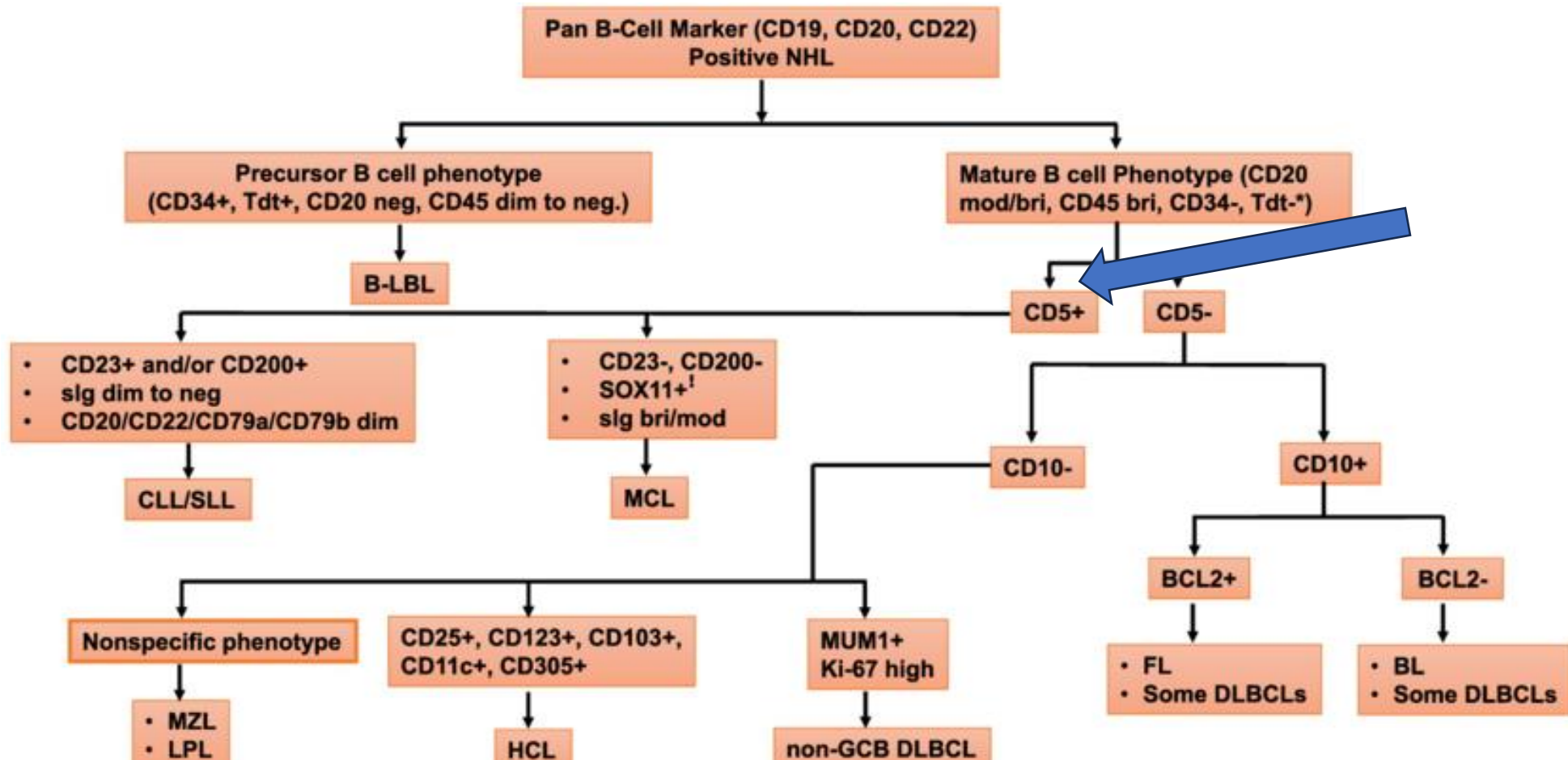
Michele Ghilmini and Emanuele Zucca

Blood 2009 114:1469-1476; doi: <https://doi.org/10.1182/blood-2009-02-179739>



Cause-specific survival of the main B-cell lymphoma subtypes in the series of the Oncology Institute of Southern Switzerland, 1980-2006.

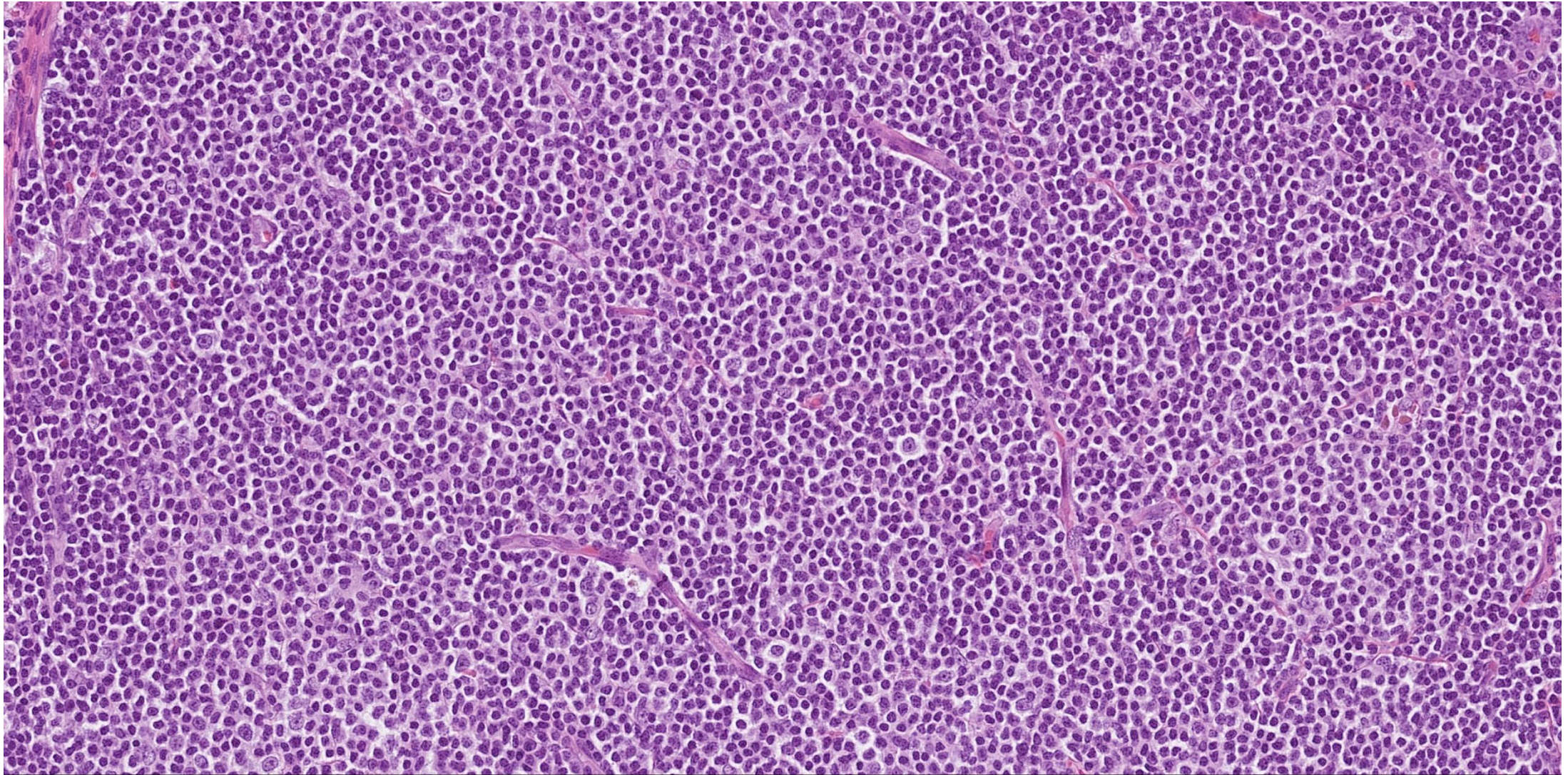
B-cell neoplasia



Chronic lymphocytic leukemia (B-CLL) / Small-cell lymphocytic lymphoma (B-SLL)

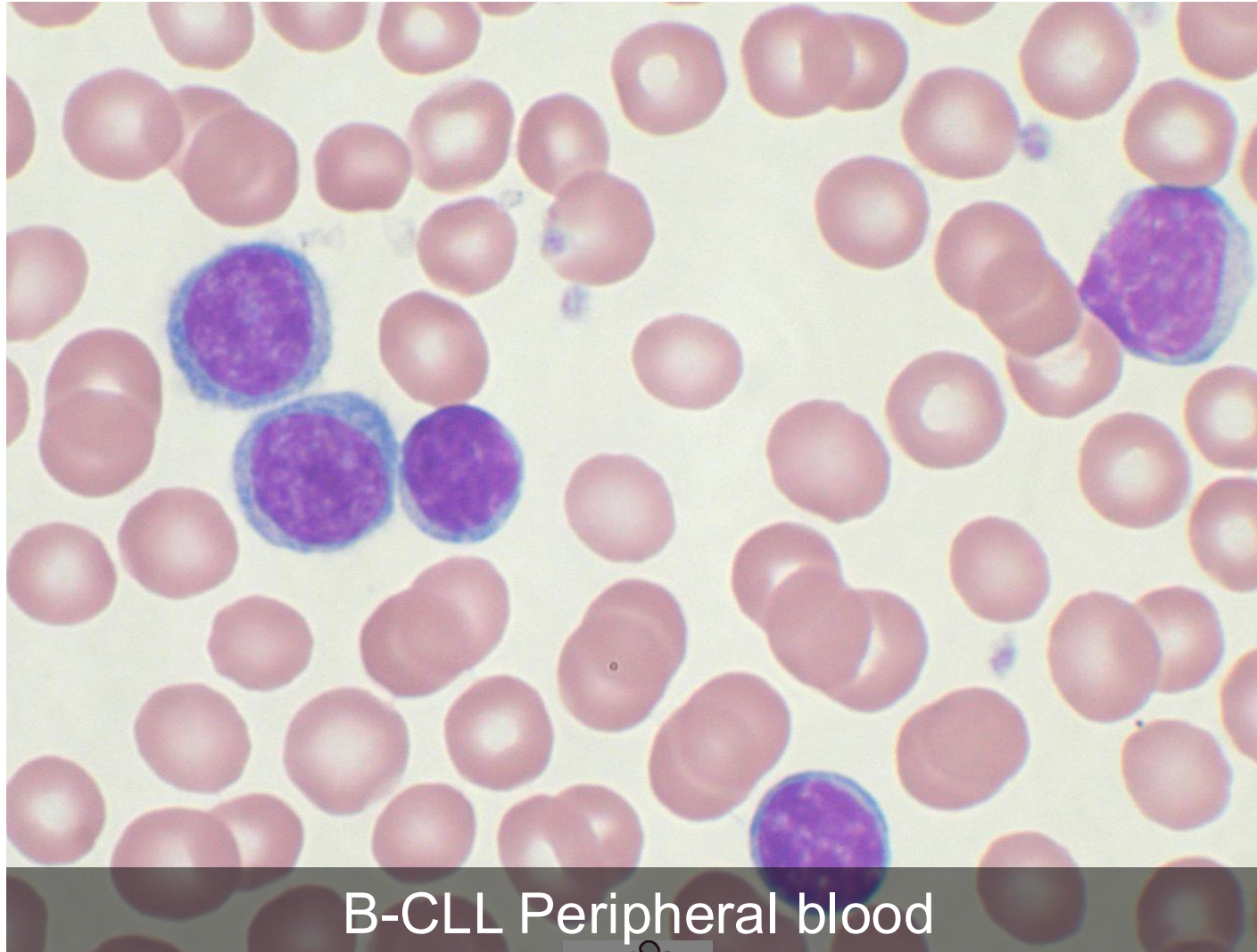
B-CLL / SLL
CD20, PAX5
CD5
CD23

- B-cell derived neoplasia
- Presentation: If present in blood (alone $>5 \times 10^9$ in 3 months), BM and spleen = B-CLL. If $<5 \times 10^9$, but lymph node involvement = B-SLL
- Morphology: small lymphocytes, small amount of cytoplasm, dense chromatin
- Symptoms: very few
- Good prognosis, but no cure.
- May transform to DLBCL (Richter transformation).



Small lymphocytic lymphoma (SLL) in lymph node

B-CLL

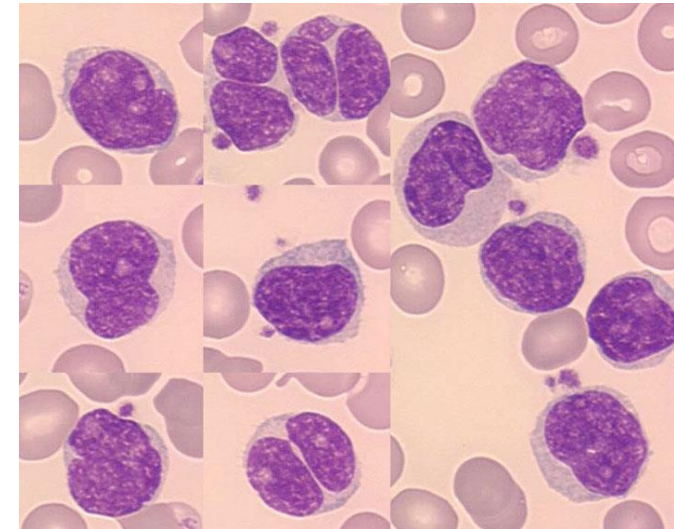


B-CLL Peripheral blood

Mantle cell lymphoma

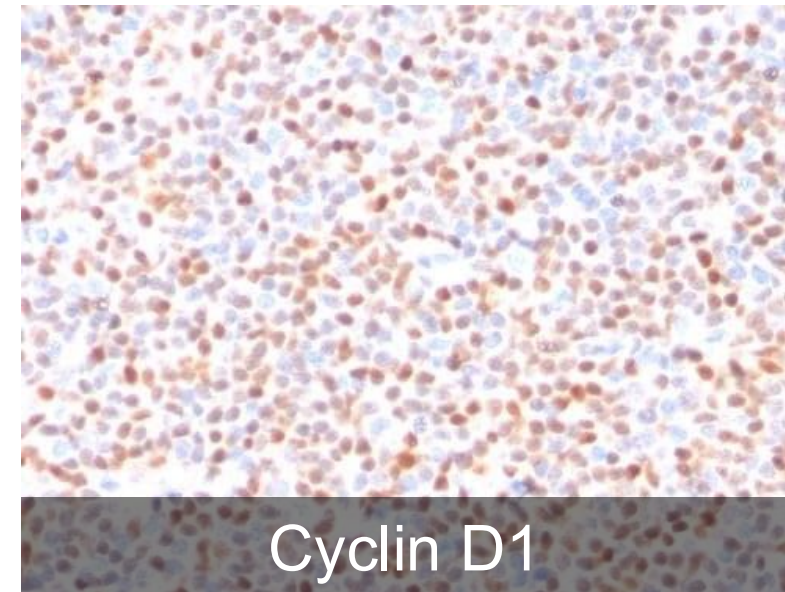
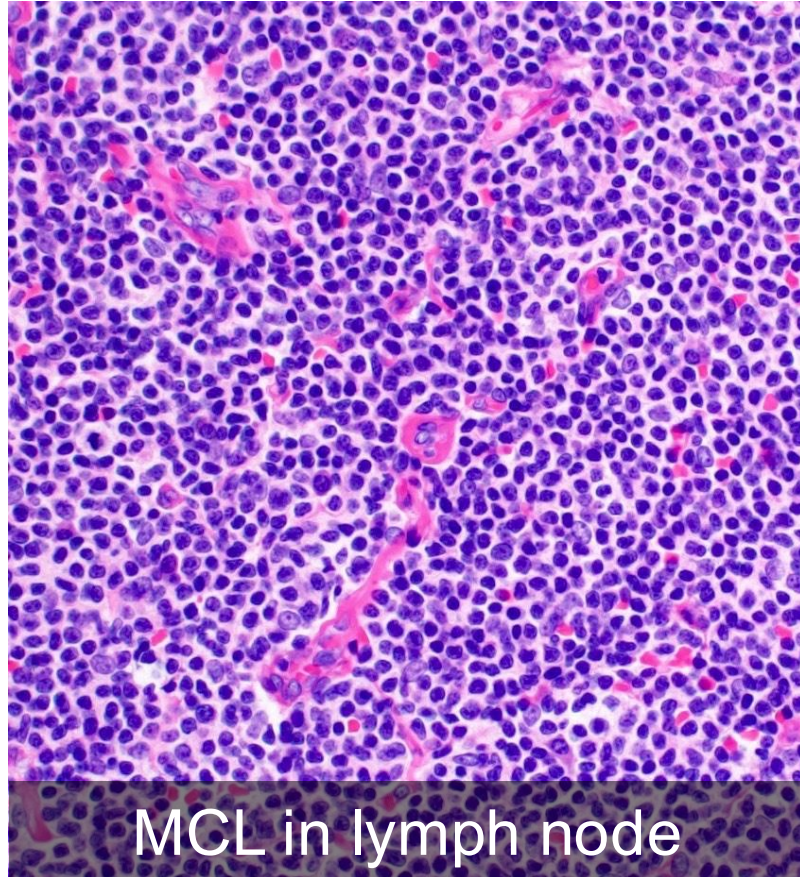
MCL
CD20, PAX5
CD5
Cyclin D1
SOX11

- B-cell neoplasia, monomorphic cells with irregular nuclei.
- Cytogenetics: translocation t(11,14) (q13;q32) induces Cyclin D1 expression that, in turn, stimulates cell proliferation
- Prognosis: 5 year: 50-70%

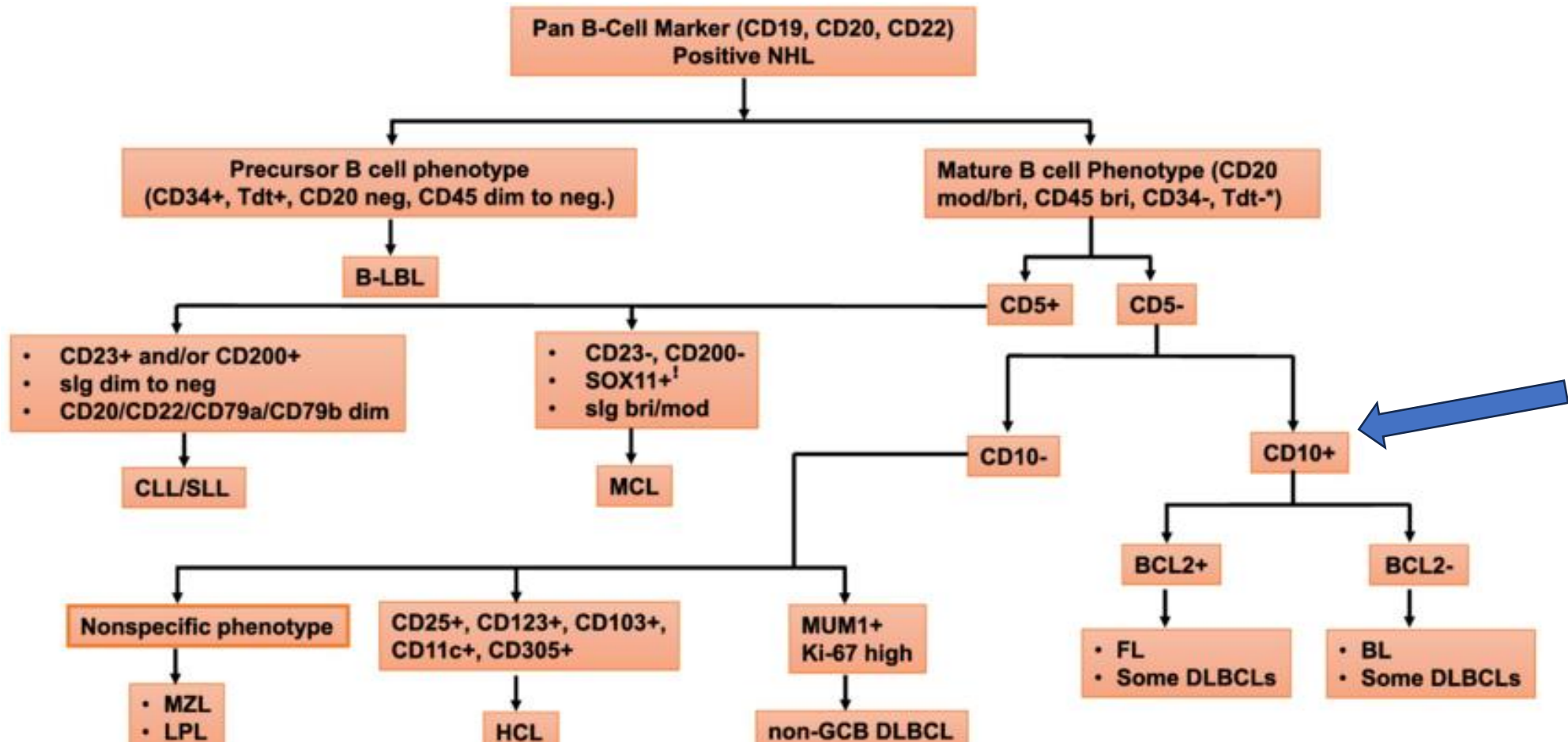


Mantle cell lymphoma

MCL
CD20, PAX5
CD5
Cyclin D1
SOX11



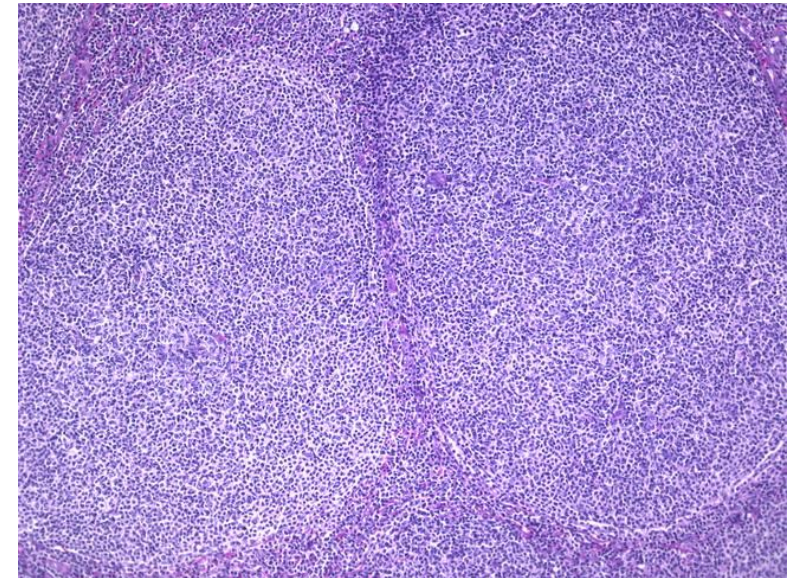
B-cell neoplasia



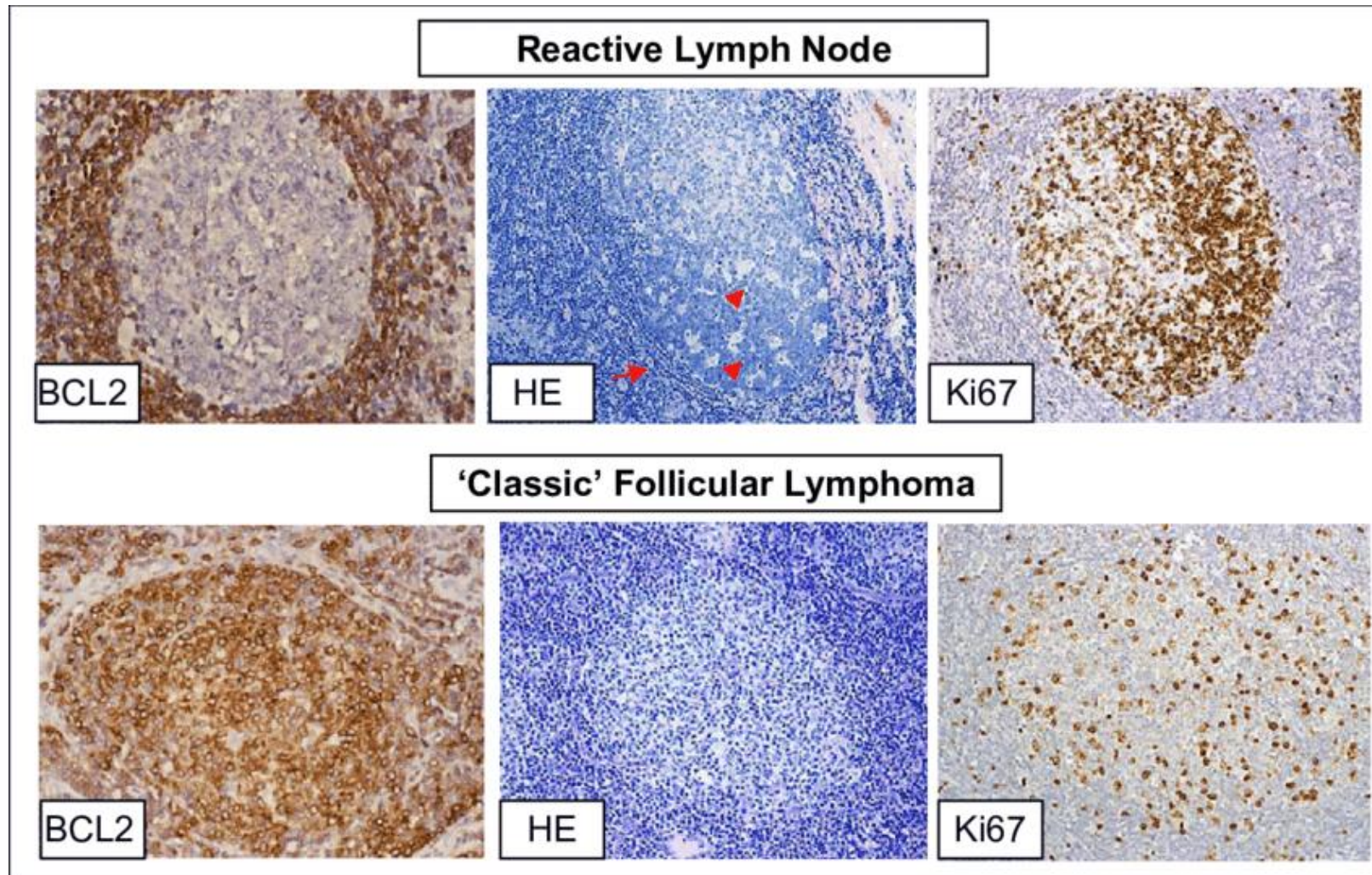
Follicular lymphoma

- B-cell lymphoma
- Morphology: similar to germ center cells (both centrocytes and centroblasts). Growth pattern with abnormal follicles
- Cytogenetics: 90% with translocation t(14;18), (q32;q21), which creates overexpression of Bcl-2.
- Most common lymphoma
- Good prognosis

FL
CD20
PAX5
CD10
BCL2
BCL6



Follicular lymphoma



Burkitt's lymphoma

MCL
CD20
PAX5
CD10
CMYC
Ki67: high

- B-cell neoplasia, often as leukemia or extranodal.
 - Endemic: African population, associated to EBV
 - Sporadic: global, young adults and children
 - Immunodeficiency associated: HIV
- Morphology: Moderate large cells with monotonous round nuclei, many mitosis, but many apoptotic bodies in macrophages = 'starry sky'
- Cytogenetics: 90% t(8;14)(q24;q32) MYC-translocation
- Prognosis: 70-90% depending on stage
- Medical emergency!

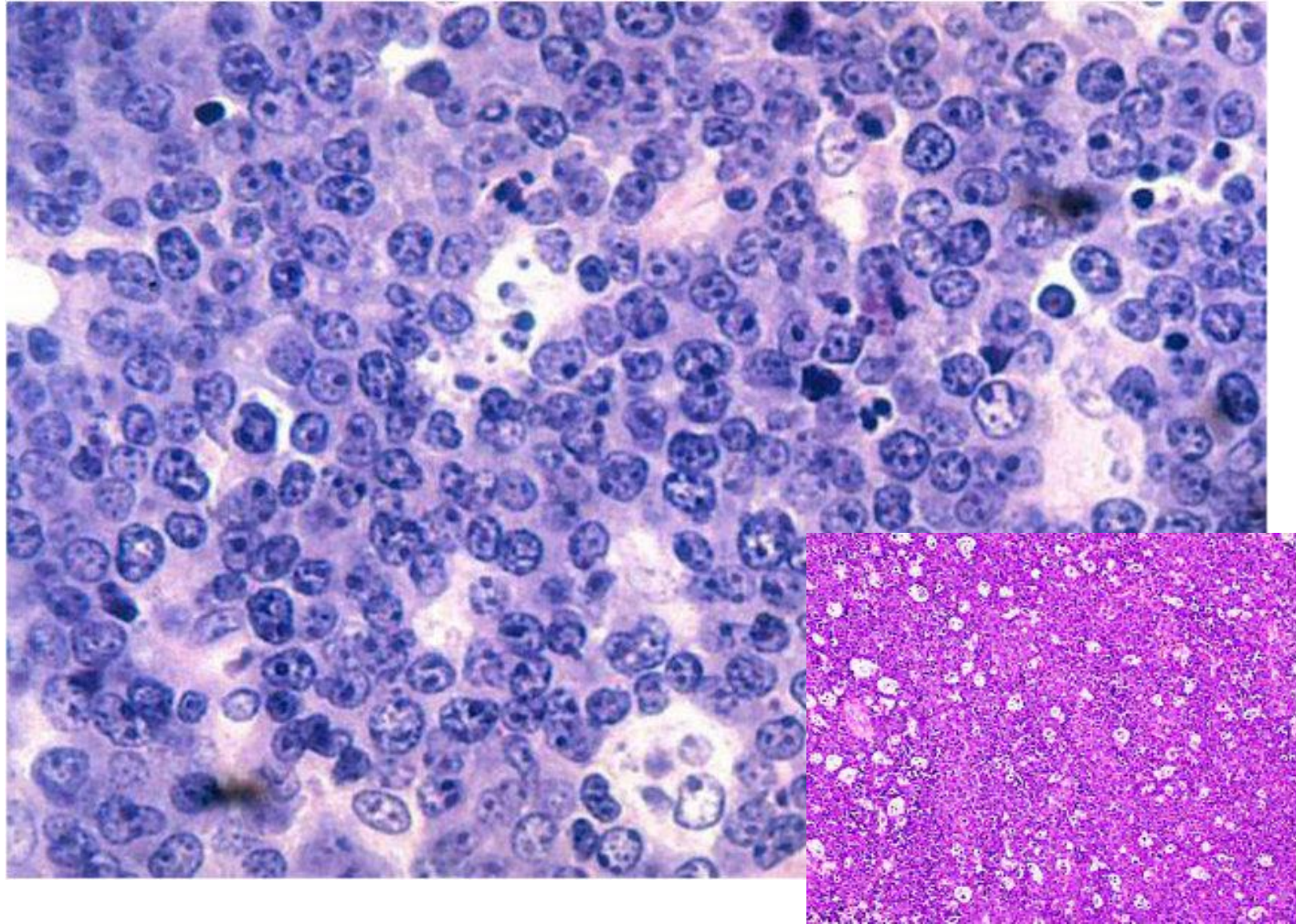
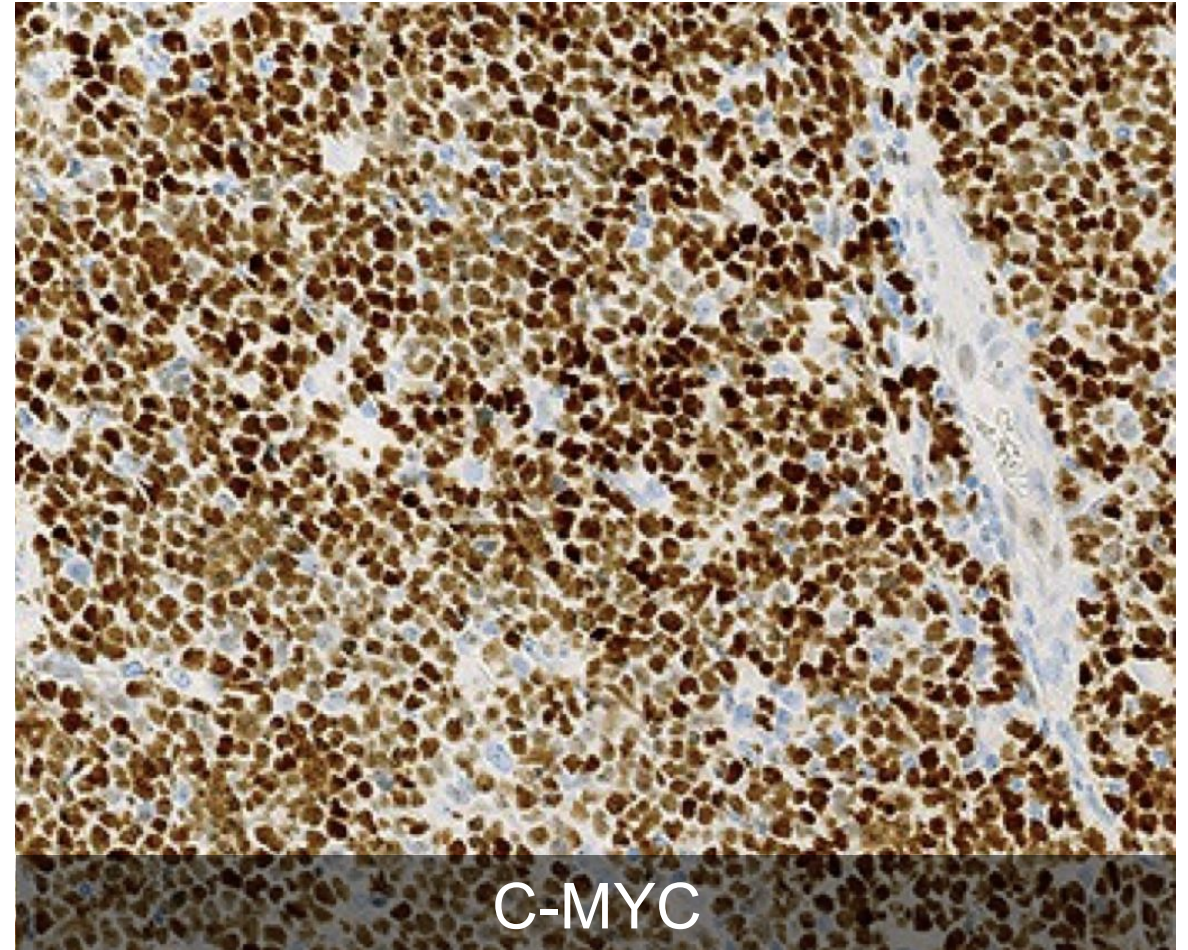
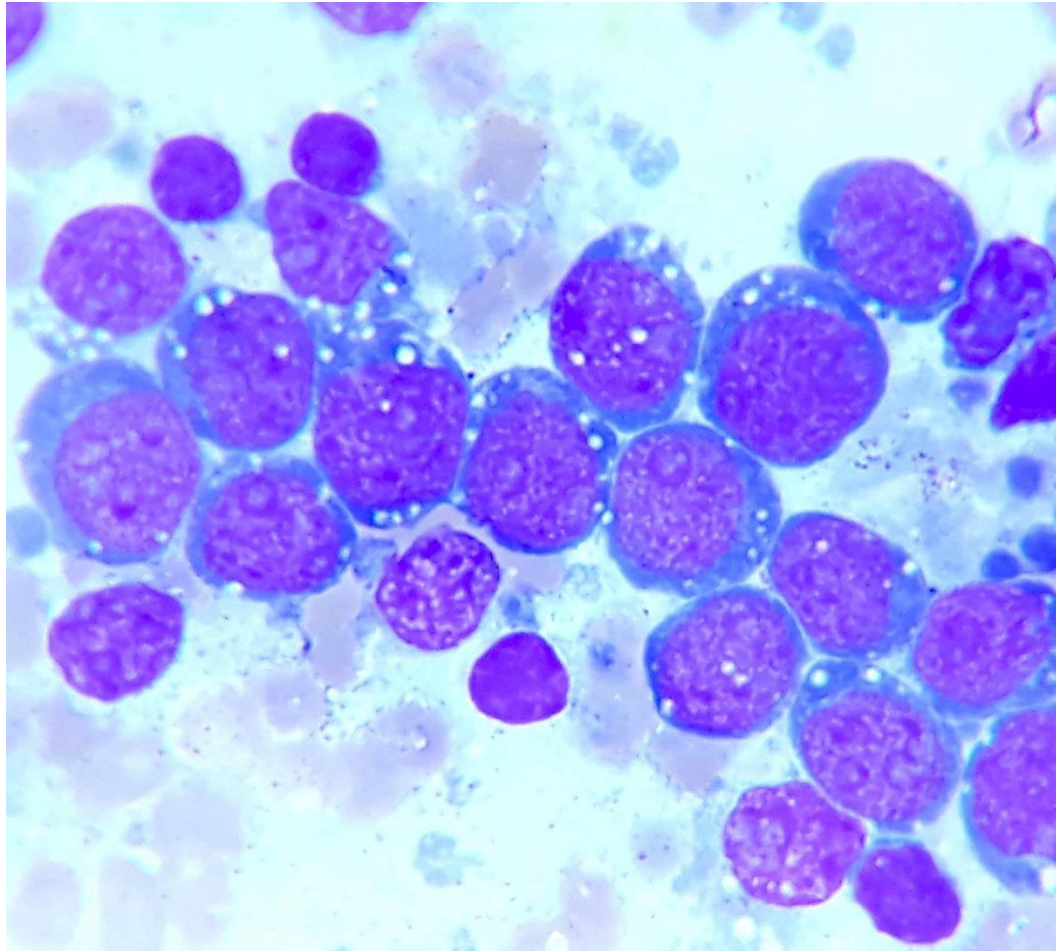
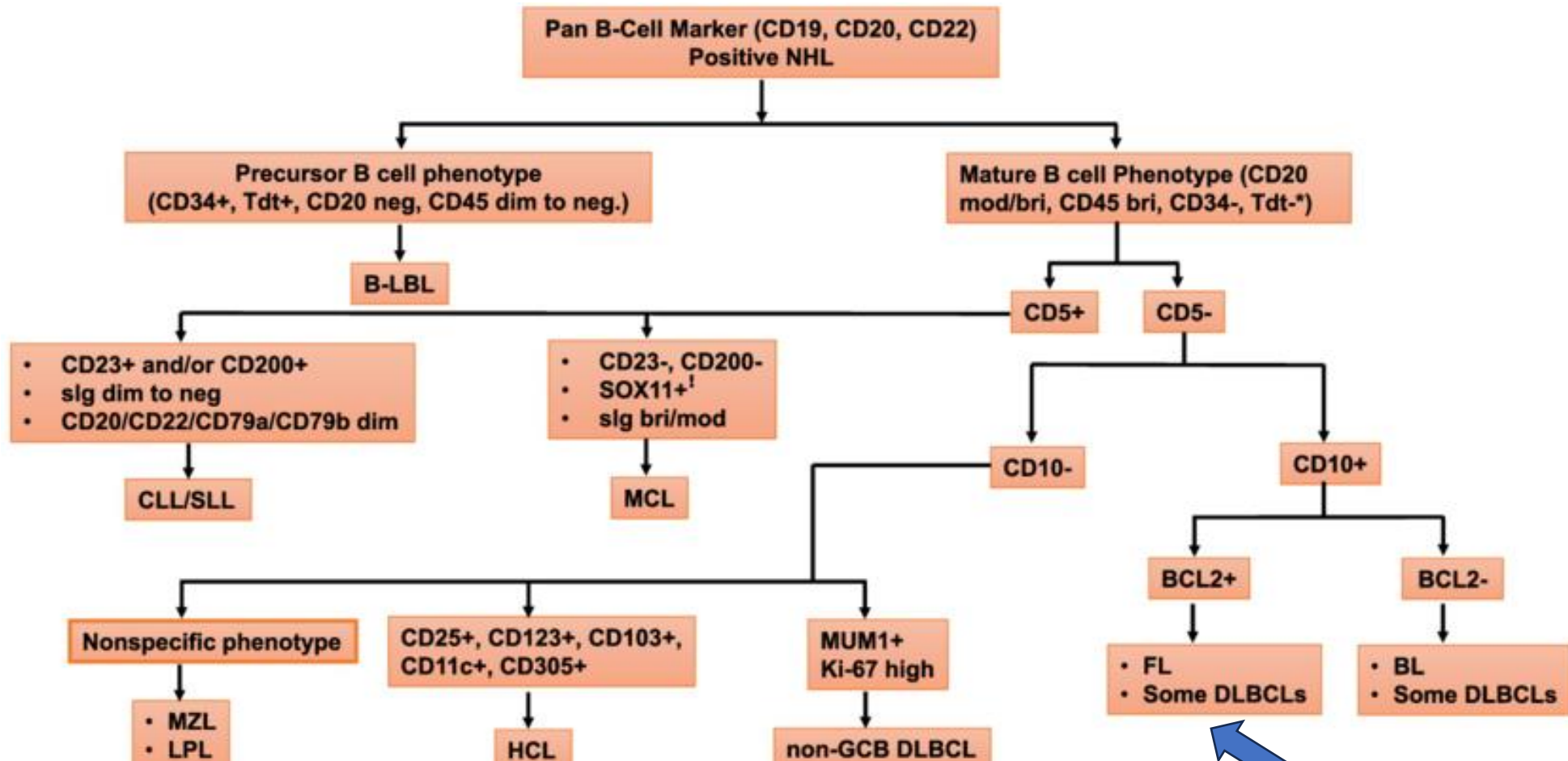


Fig. 15.15 Burkitt's lymphoma. In Burkitt's lymphoma a characteristic feature is the presence of large macrophagic cells (**M**) scattered through the tumor, likened to a 'starry sky'.

Burkitt lymphoma

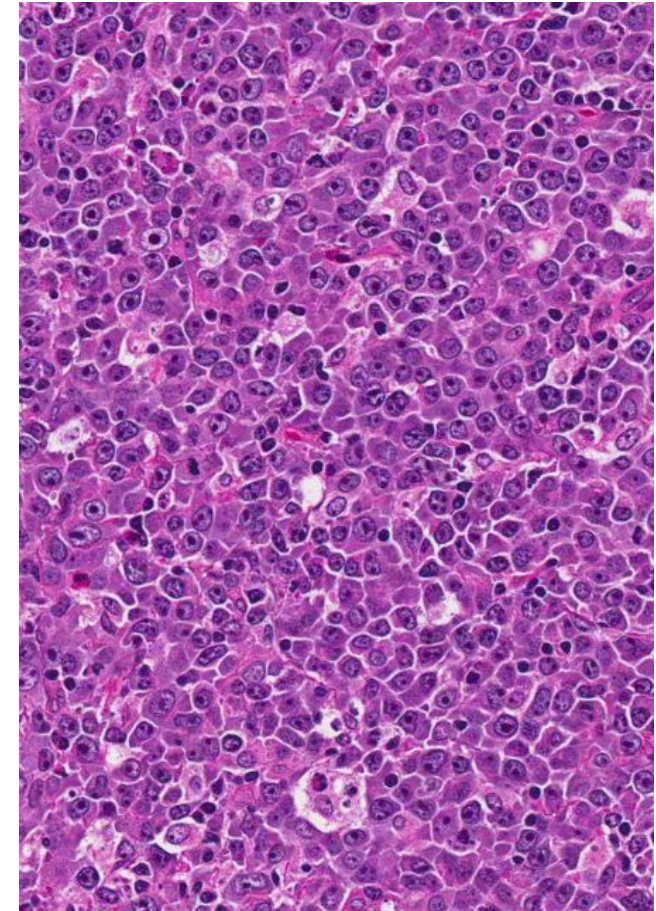


B-cell neoplasia



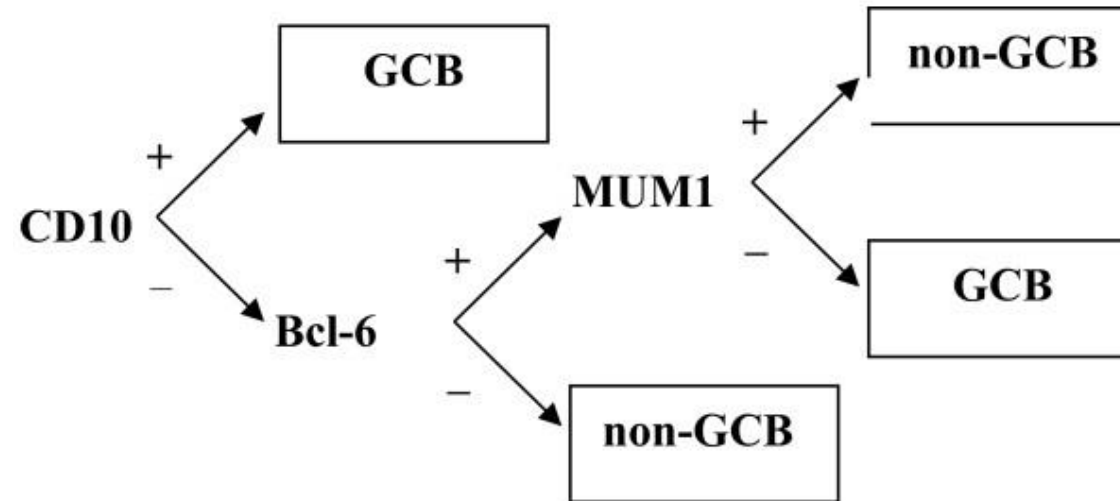
Diffuse Large B-cell Lymphoma

- Malignant B-cell neoplasia. May be primary or transformation of other B-cell lymphomas
- Morphology: Large lymphoid cells with diffuse growth.
- Both nodal and extra nodal.
- Second most common lymphoma
- Prognosis: Dependent of stage



Diffuse Large B-cell Lymphoma

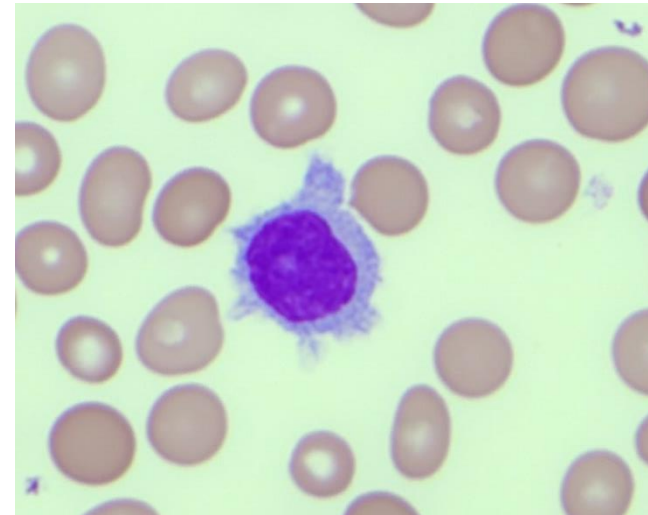
Germinal center like (GCB) or non-GCB



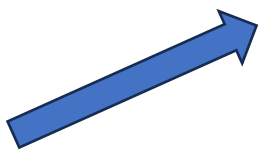
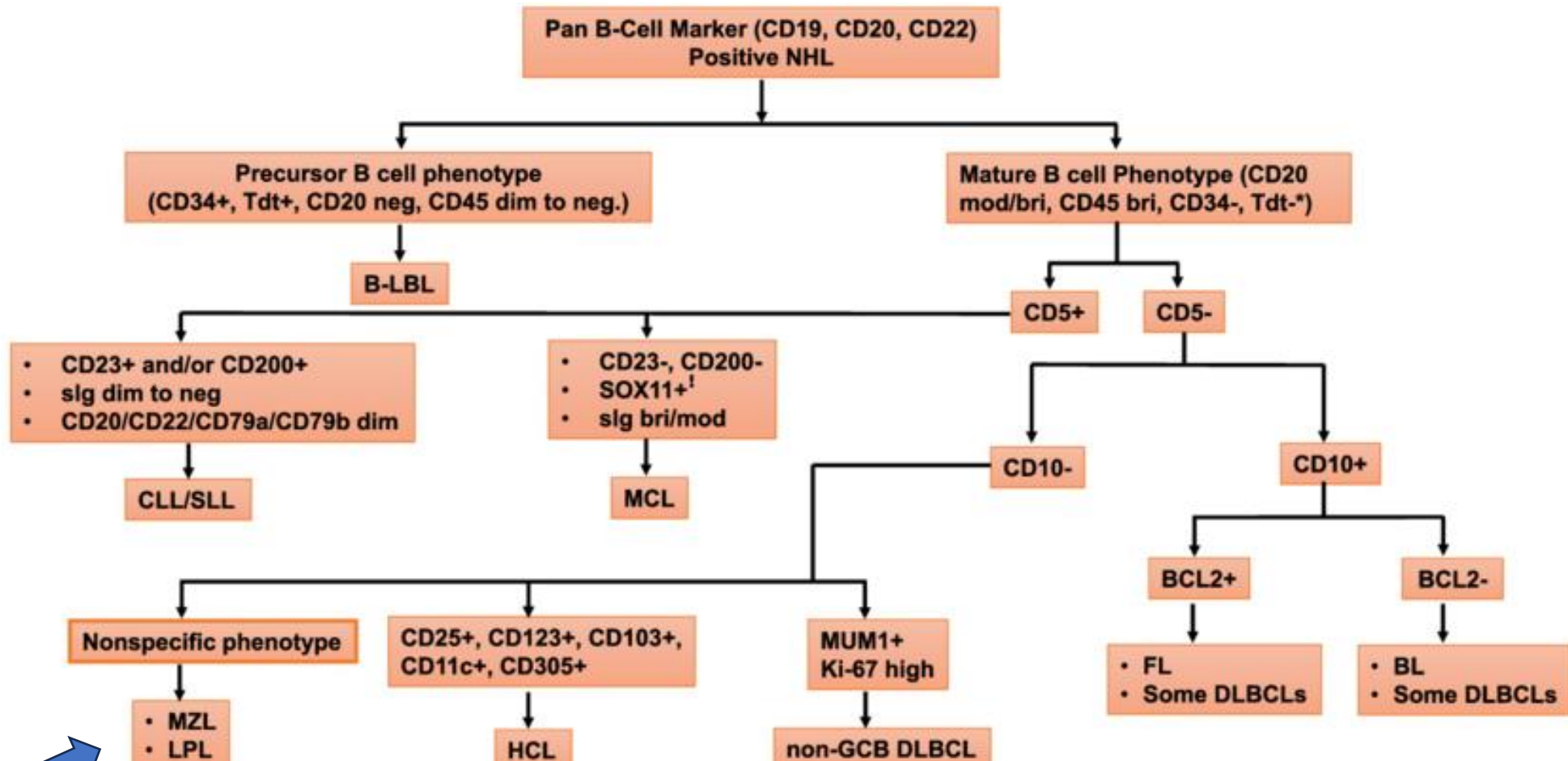
Hairy cell leukemia (HCL)

- Pancytopenia and splenomegaly
- Peripheral blood: small–medium lymphoid cells with pale cytoplasm and projections (“hairy cells”).
- BRAF V600E mutation present in nearly all classic HCL cases
- Good prognosis (indolent)

HCL
CD20, PAX5
CD103, CD123
CD11c
BRAF V600E

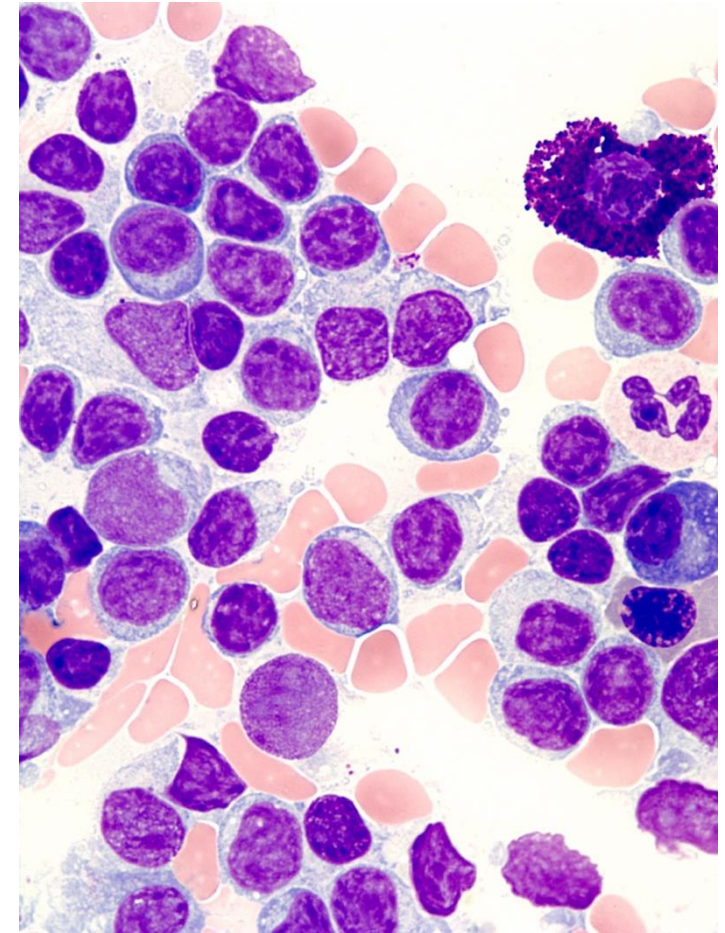


B-cell neoplasia



Lymphoplasmacytic lymphoma (LPL)

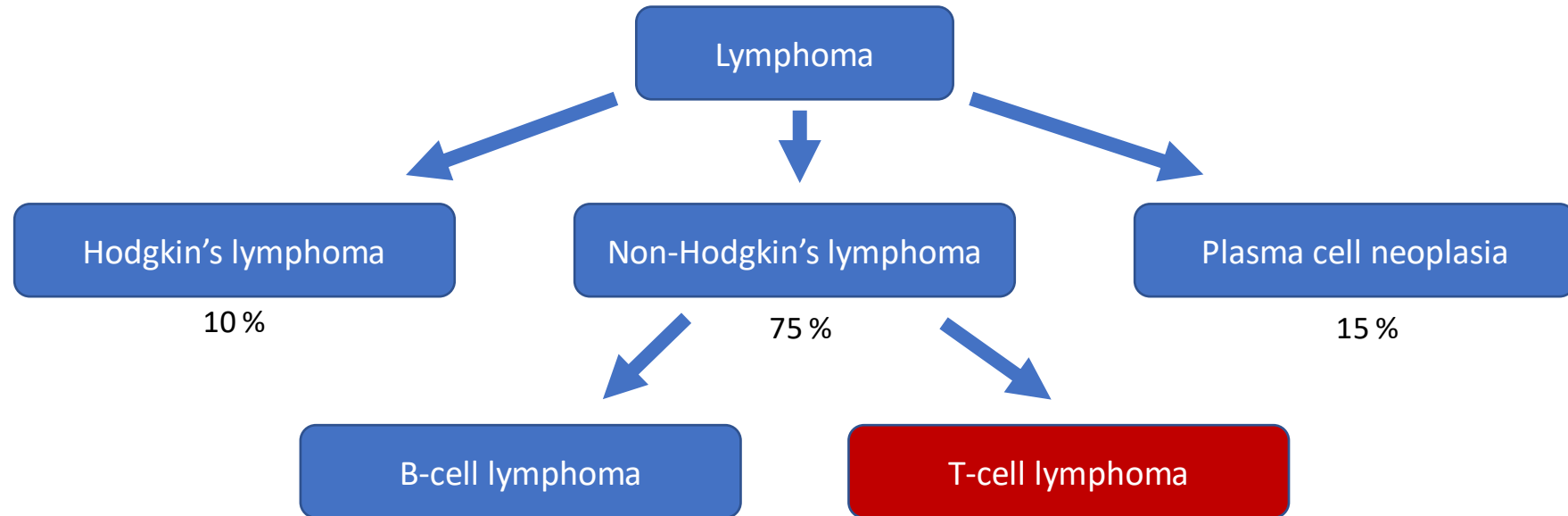
- Small B-lymphocytes, lymphoplasmacytoid and plasma cells not fulfilling criteria for other B-cell neoplasia
- MYD88 L265P mutation in ~90% of cases
- Prognosis: Long-term survival but more malignant transformation may be seen.
- No aberrant IHC profile



Marginal zone lymphoma

- Similar appearance as marginalzone lymphocytes. Three types
 - Splenic marginal zone lymphoma (rare)
 - Nodal marginal zone B-cell lymphoma (rare)
 - Extranodal marginal zone lymphoma (MALT – Mucosa Associated Lymphoid Tissue lymphoma): frequently gastric: associated with chronic inflammation (h. pylori).
- Prognosis: elimination of H. pylori lead to 95% remission of gastric MALT-lymphoma

Classification of lymphomas

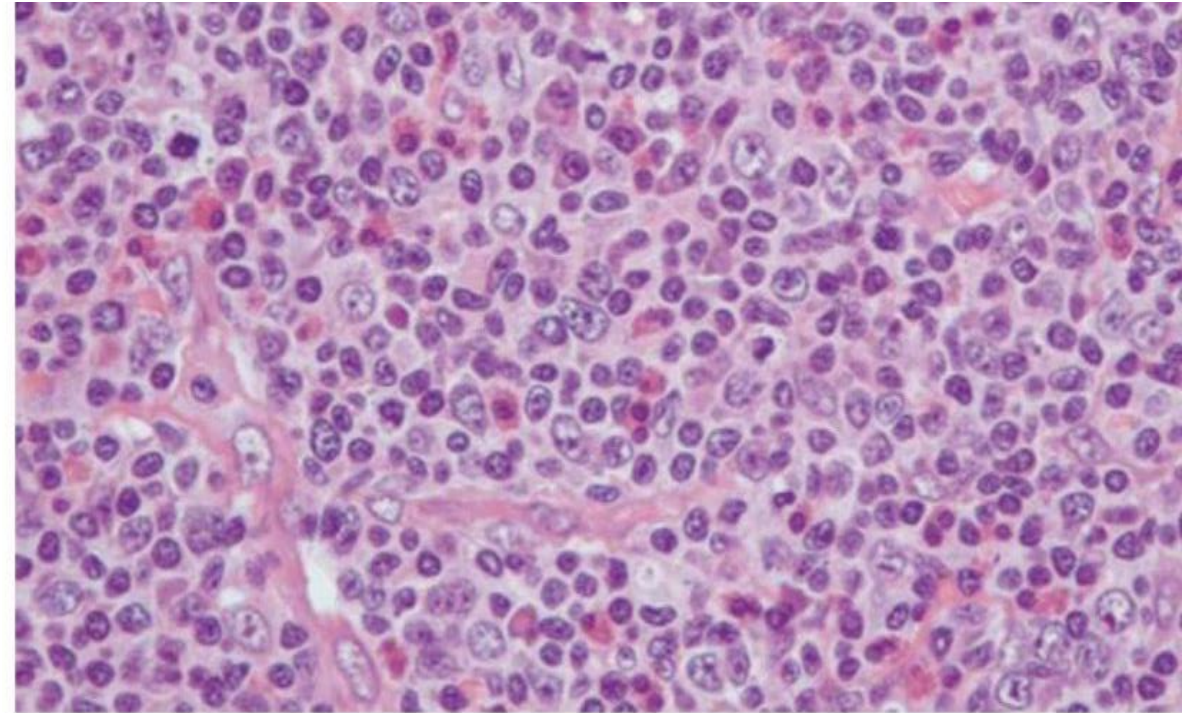


T-cell lymphomas

- Heterogeneous group of non-Hodgkin lymphomas derived from mature (post-thymic) T-cells and NK-cells
- Less common than B-cell lymphomas
- Often aggressive with poorer prognosis than B-cell lymphomas
- Pan-T-cell markers: **CD2, CD3, CD5, CD7** (loss is diagnostic clue)
- Other markers: CD4, CD8, ALK, EBV

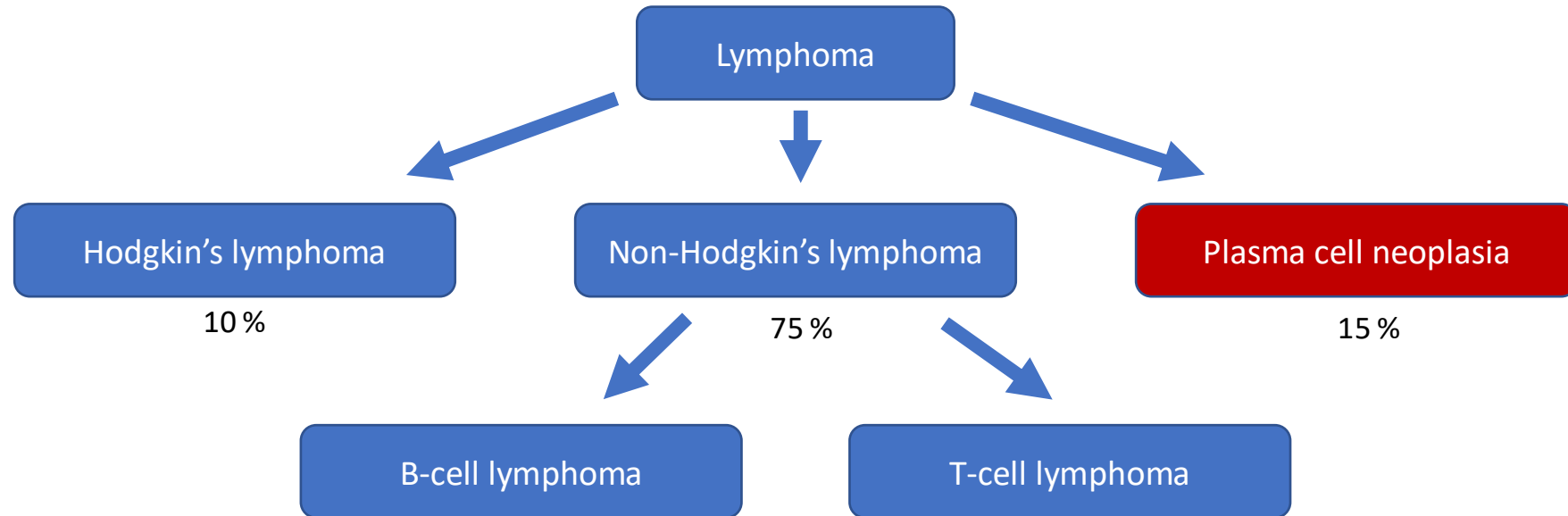
Nodal T-follicular helper cell lymphoma, angioimmunoblastic type

- Most common nodal T-cell lymphoma
- Polymorphic infiltrate lymphocytes, plasma cells, eosinophils, histiocytes
- Proliferation of venules
- EBV-associated



nTFHL-AIT
CD3
CD10
CXCL13, ICOS
BCL6, GATA3, PD1

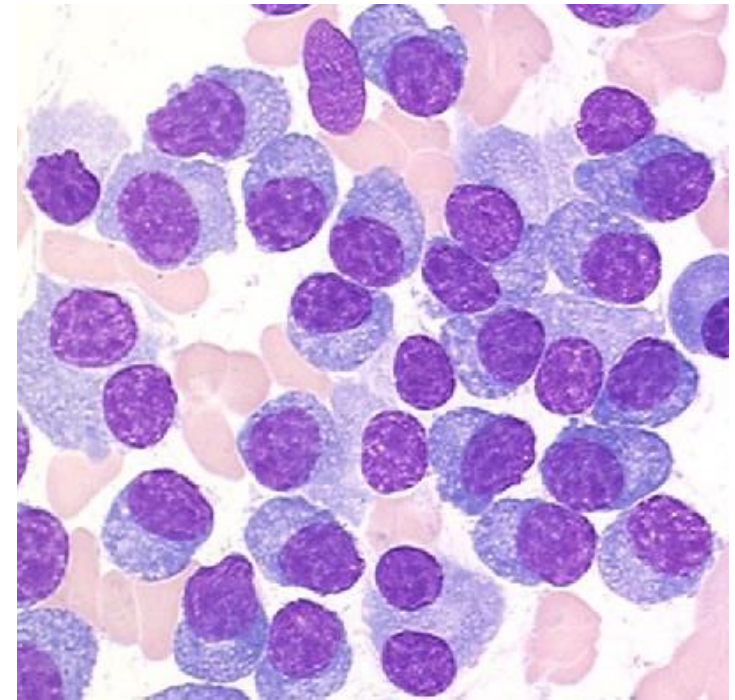
Classification of lymphomas



Multiple myeloma

- Malignant plasma cell neoplasm
- Clonal proliferation of plasma cells producing a monoclonal immunoglobulin
- Median survival: 5-7 years
- Typically, all either kappa or lambda positive

MM
CD138, MUM1
"CD56, Cyclin D1, CD117"
Kappa/lamba
Heavy Chain Ig
CD45 negative



Thank you for your attention!

NordiQC team

Heidi Lykke Kristoffersen

Tanya Julio

Louise Rønnow Holler

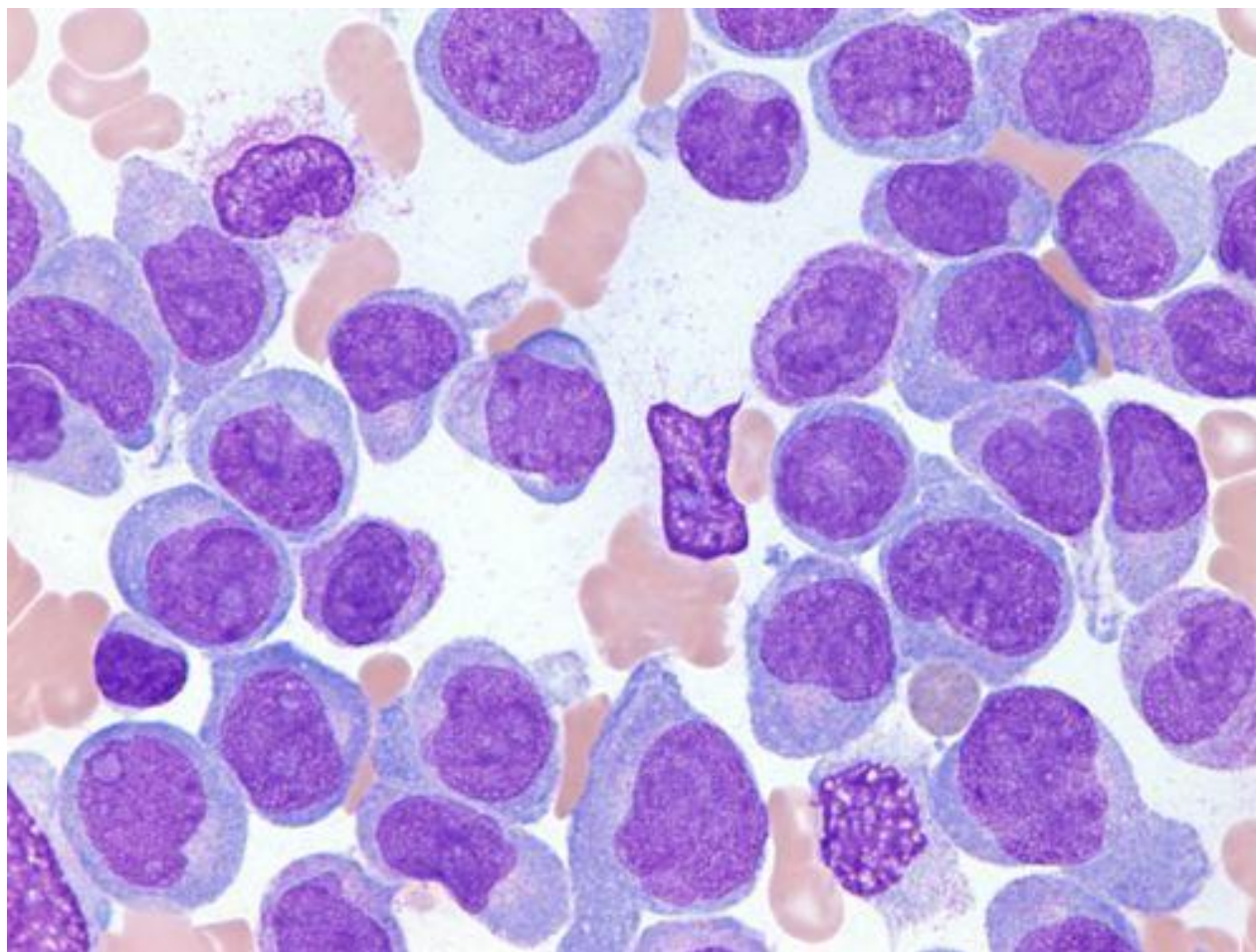
Lilli Guldager Malaca

Kristi Bøgh Andersson

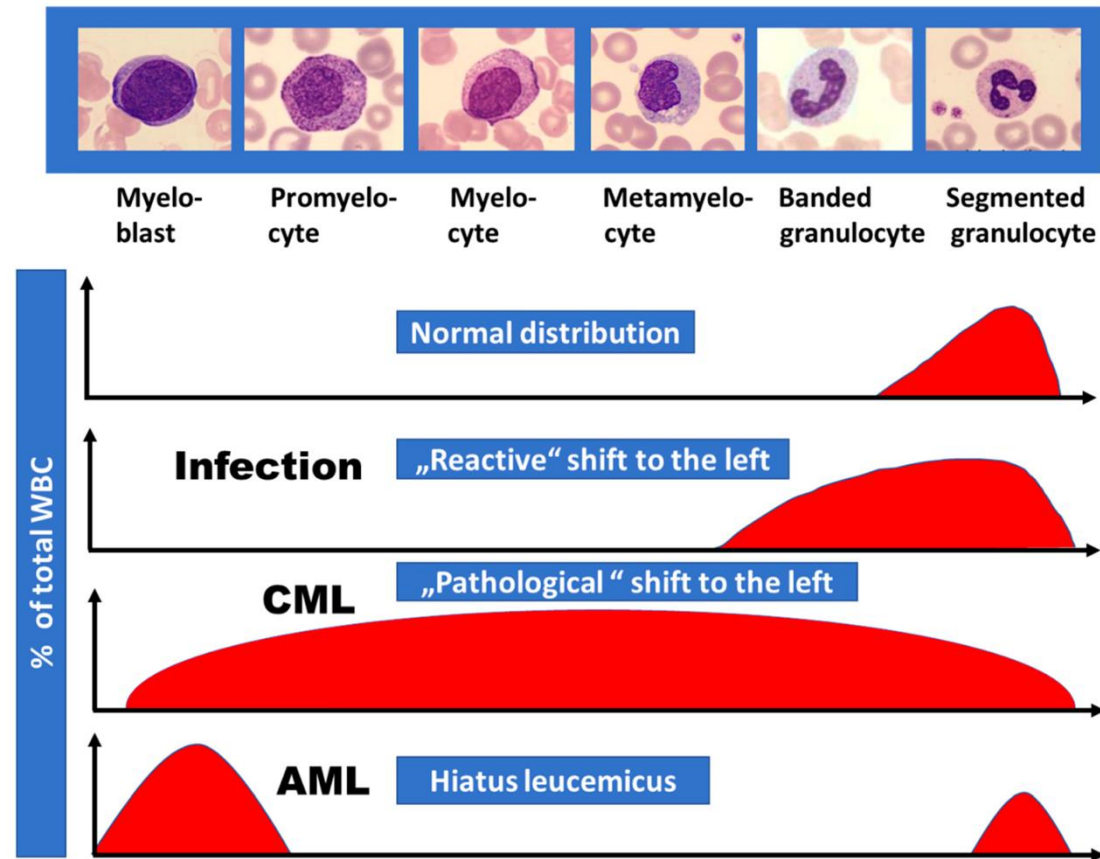
Søren Nielsen



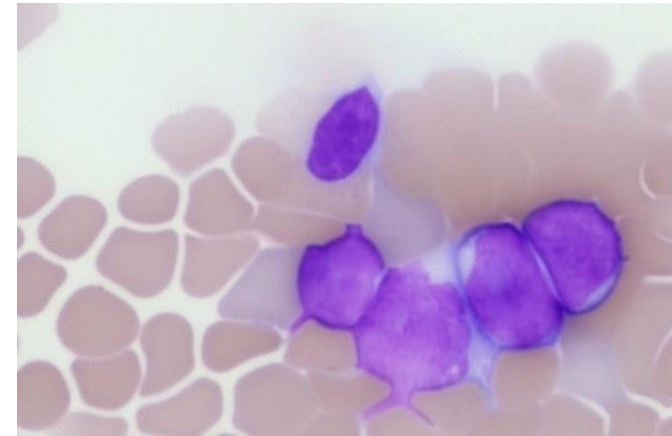
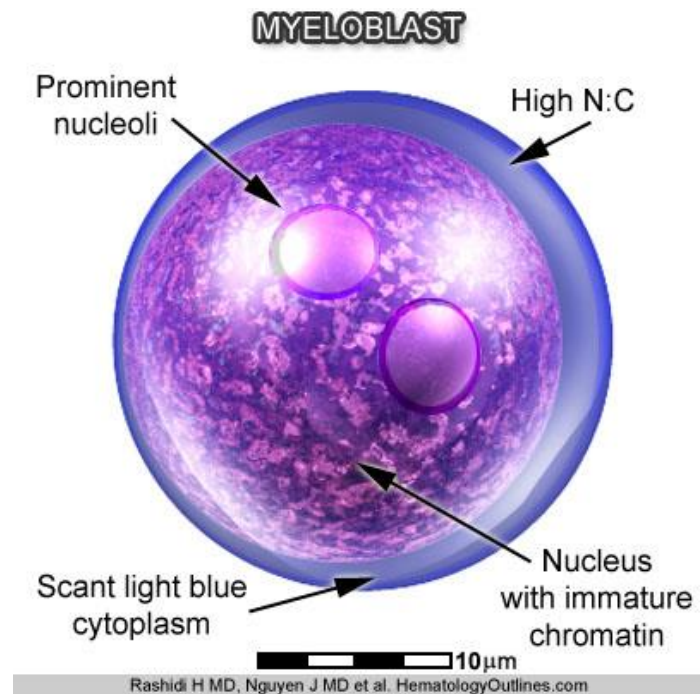
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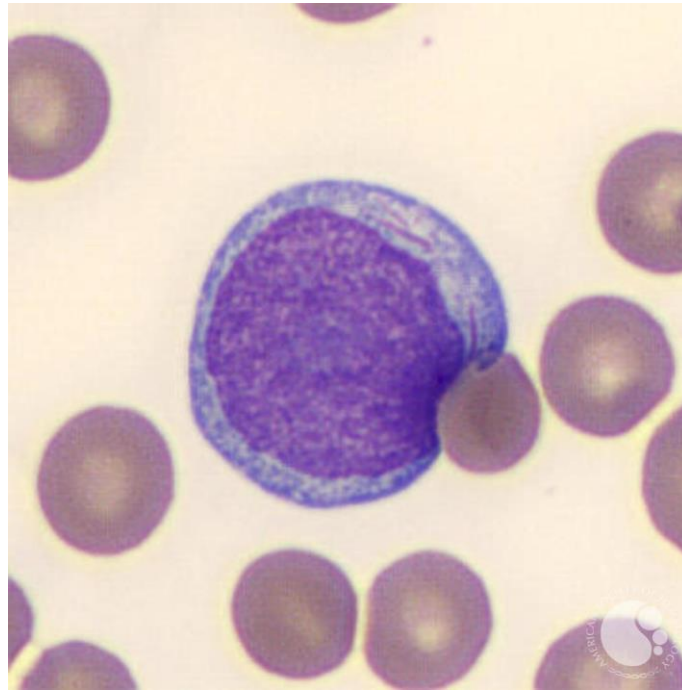
Leucocytosis / immature cells



Blasts



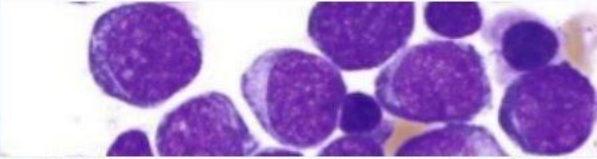
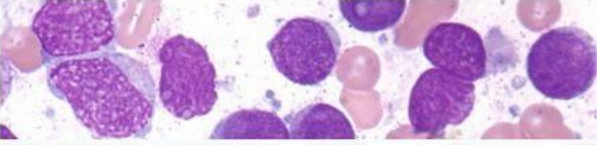
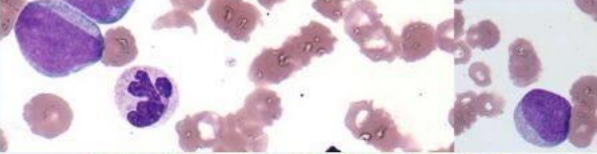
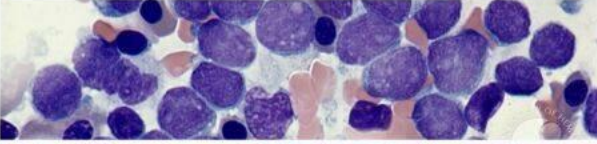
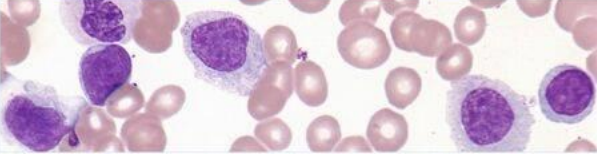
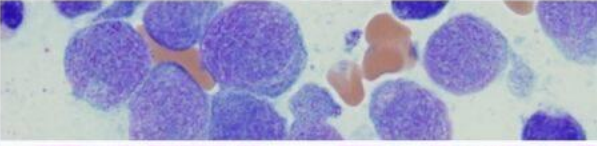
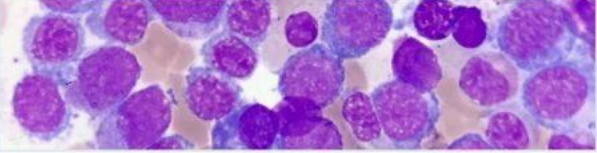
Auer rods



AML - Causes

- "Preleukemic" disorders may evolve into AML
 - Myelodysplastic syndrome
 - Myeloproliferative diseases
- Chemical exposure
 - Chemotherapy
 - Benzene and organic solvents
- Radiation

FAB CLASSIFICATION SYSTEM OF ACUTE MYELOID LEUKAEMIA

M0	AML with minimal differentiation	
M1	AML without maturation	
M2	AML with maturation	
M3	Acute promyelocytic leukaemia	
M4	Acute myelomonocytic leukaemia	
M5	Acute monoblastic and monocytic leukaemia	
M6	Pure erythroid leukaemia	
M7	Acute megakaryoblastic leukemia	