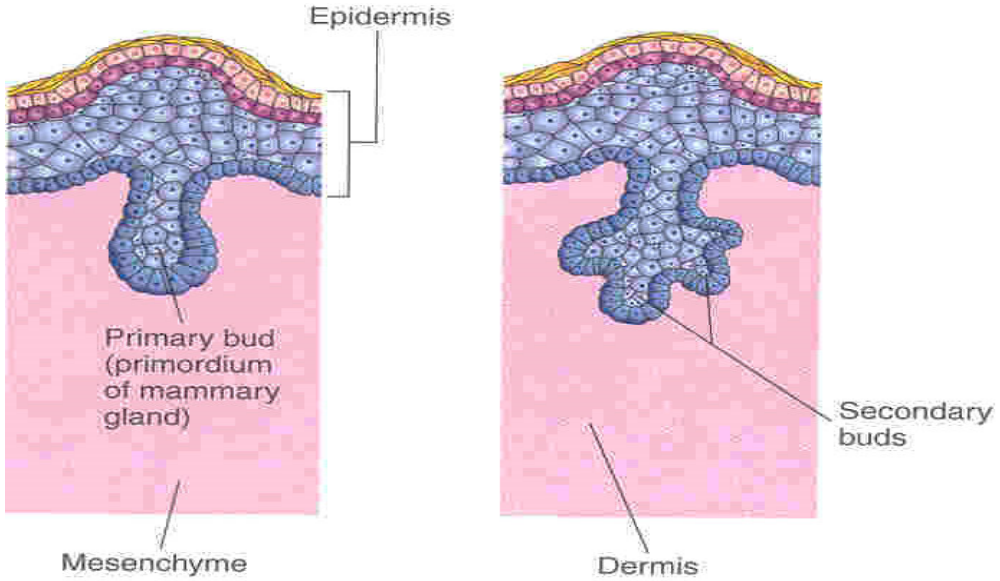
Learning objectives

- Structure, histology and development of the mammary gland
- Breast cancer: subtypes and main drivers, histology and staging
- Hematopoietic tissues and histology
- Forms of cell death and its histological recognition

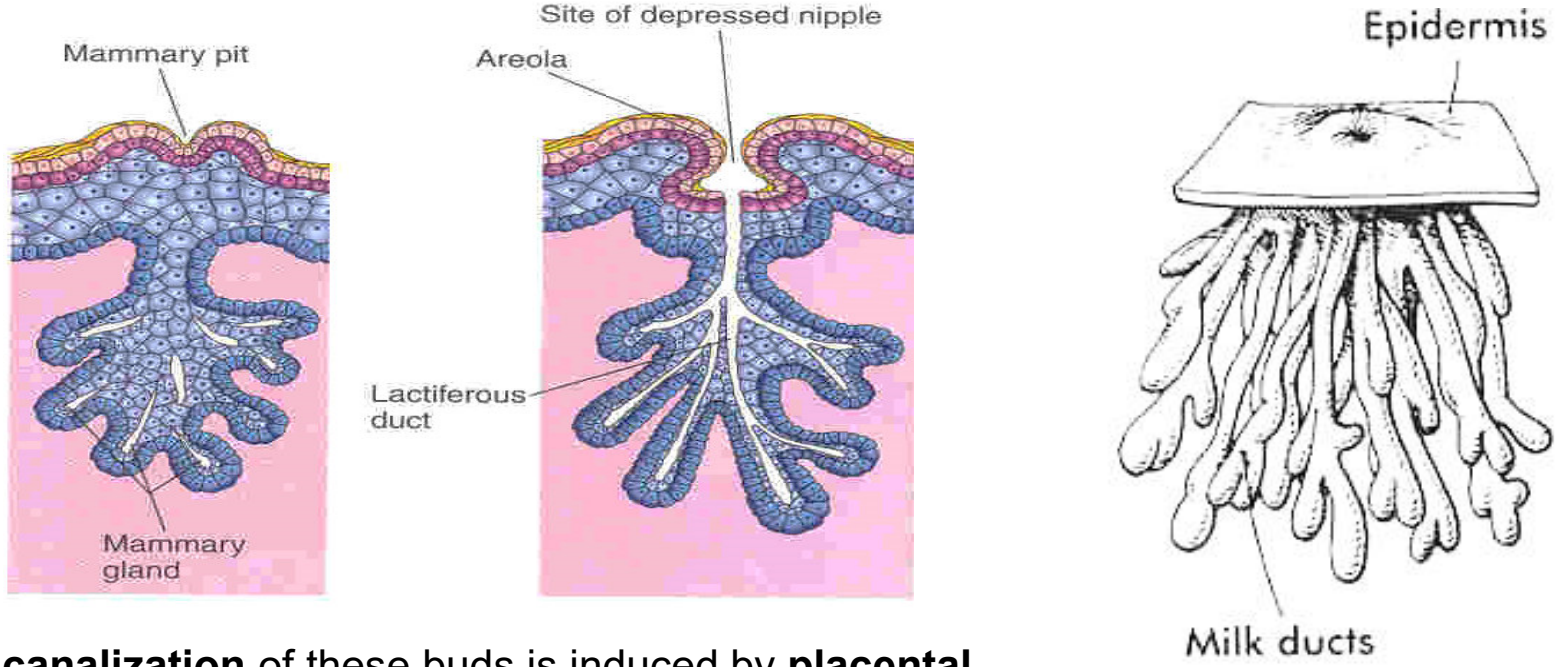
Development of the mammary gland

- mammary glands develop during the 6th week as a solid down-growth of the epidermis that infiltrate the underlying mesenchyme
- formation occurs along the mammary ridges - two thickened strips of ectoderm that run from the axillary to the inguinal regions
- depending on the species, one or more of the segments of these ridges persist on each side
- in human embryos, mammary ridges occur during the 4th week, but except for the part in the pectoral area rapidly disappear

- each **primary mammary bud** soon gives rise to several **secondary buds** that develop into **lactiferous ducts** and their branches



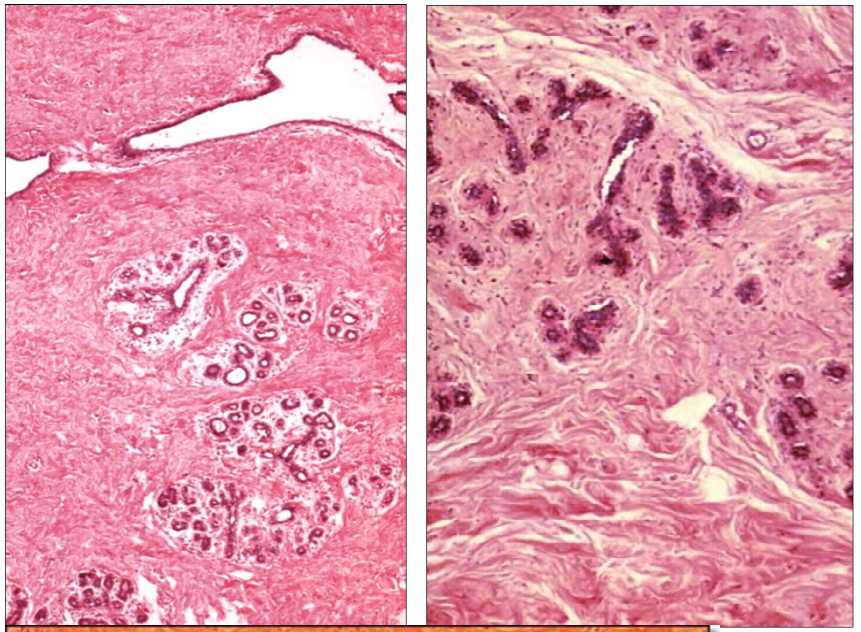
Development of the mammary gland

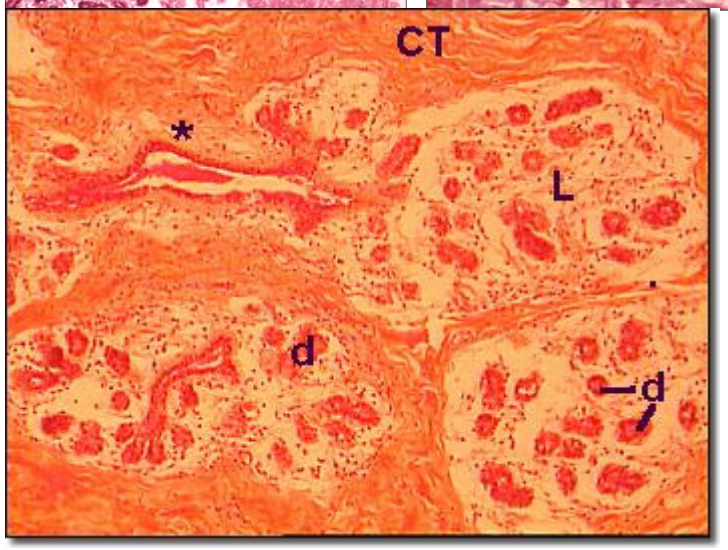


- **canalization** of these buds is induced by **placental sex hormones**. By full term, 15 to 20 lactiferous ducts are formed
- Except for these main ducts the gland will not further develop until puberty when growth of the duct system occurs because of the raised levels of circulating estrogens, prolactin, corticoids and growth hormone
- the fibrous connective tissue and fat develop from the surrounding mesenchyme
- during late fetal period the epidermis at the site of origin of the mammary gland becomes depressed forming a shallow **mammary pit**. In newborn infants the nipple is depressed. After birth the **nipples** rise from the mammary pits due to proliferation of the surrounding connective tissue of the **areola**
- the smooth muscle fibers of the nipple and areola differentiate from surrounding mesenchymal cells. Full development occurs at about 20 years

Mammary glands

- are modified apocrine sweat glands producing **milk**
- each mammary gland consists of 15-25 lobes which are separated from each other by dense connective tissue
- lobes are drained into excretory **lactiferous ducts** that open in the nipple (15-25 openings)
- **histological structure of mammary glands varies according to sex, age, and physiologic status**



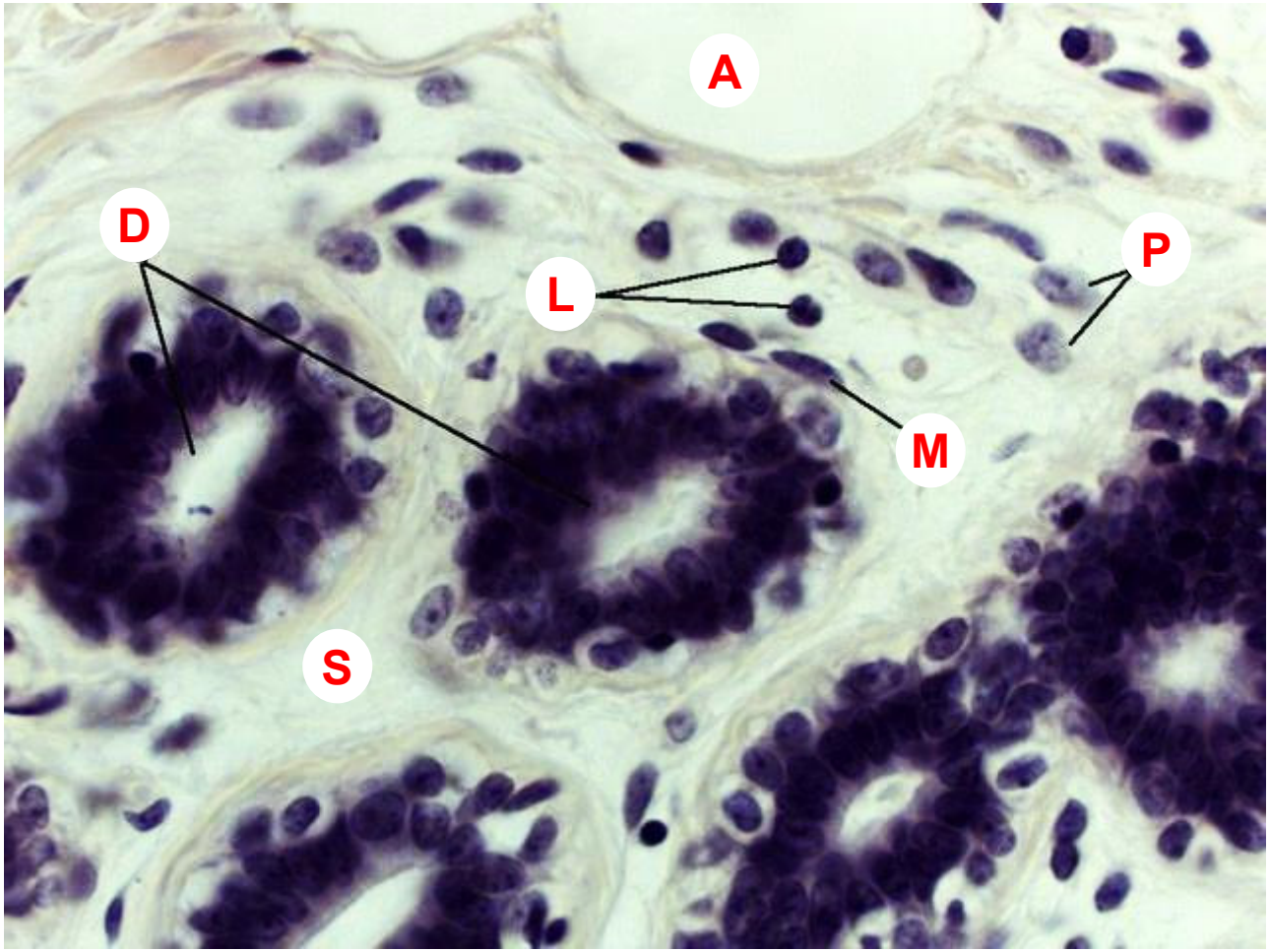


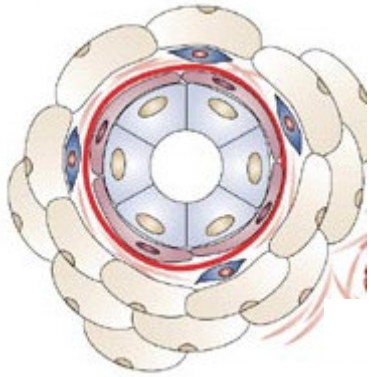
Nonlactating mammary glands

- glandular tissue is reduced to only the duct system, e.g. **lactiferous ducts, terminal interlobular ducts (*)** and **intralobular ducts (d)**

- an area of one interlobular duct is a **lobule (L)**
- the lobules are separated by a denser, less cellular interlobular connective tissue (CT)
- spaces within lobules are filled with loose intralobular stroma tissue abundant in cells

Mammary gland duct: histology

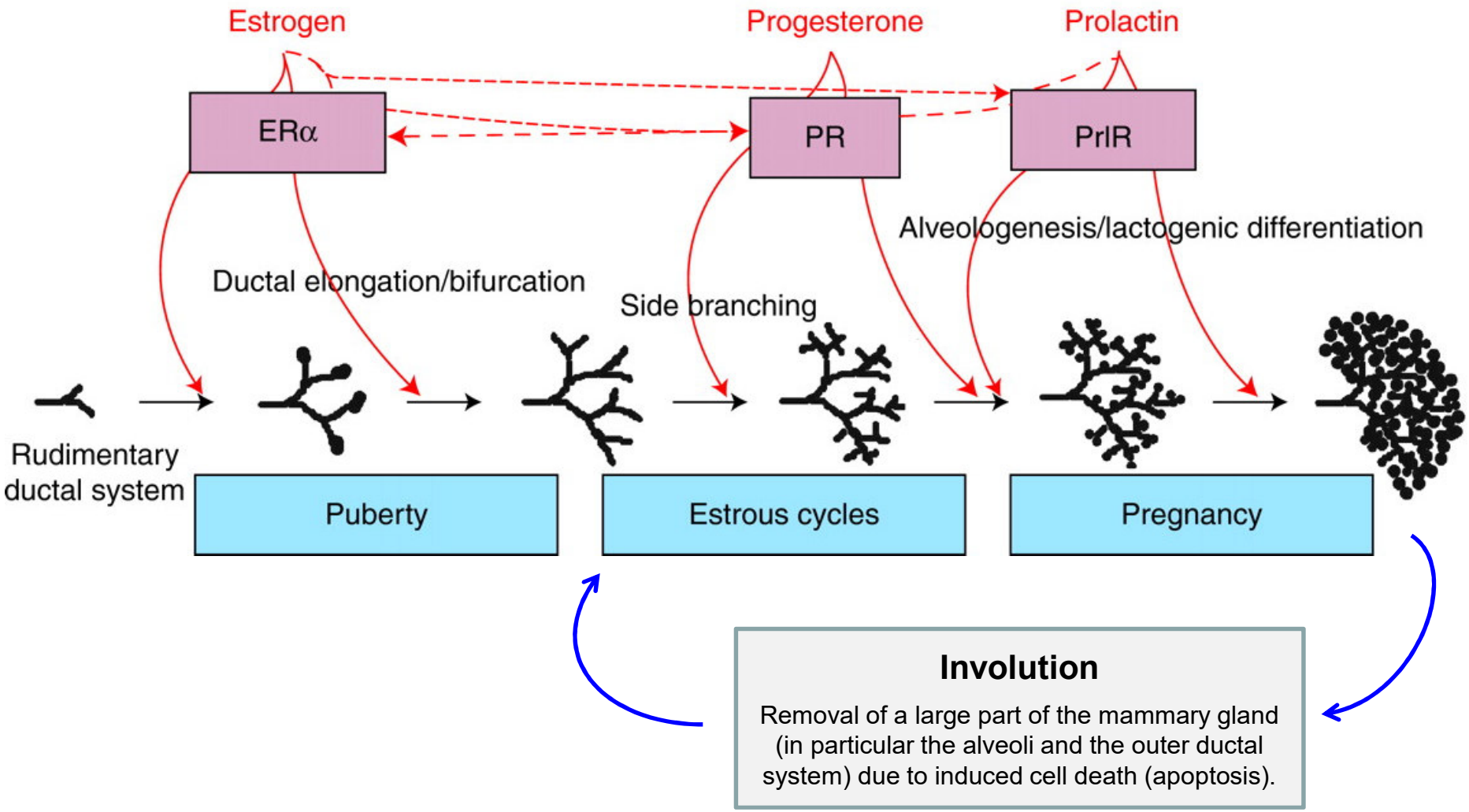


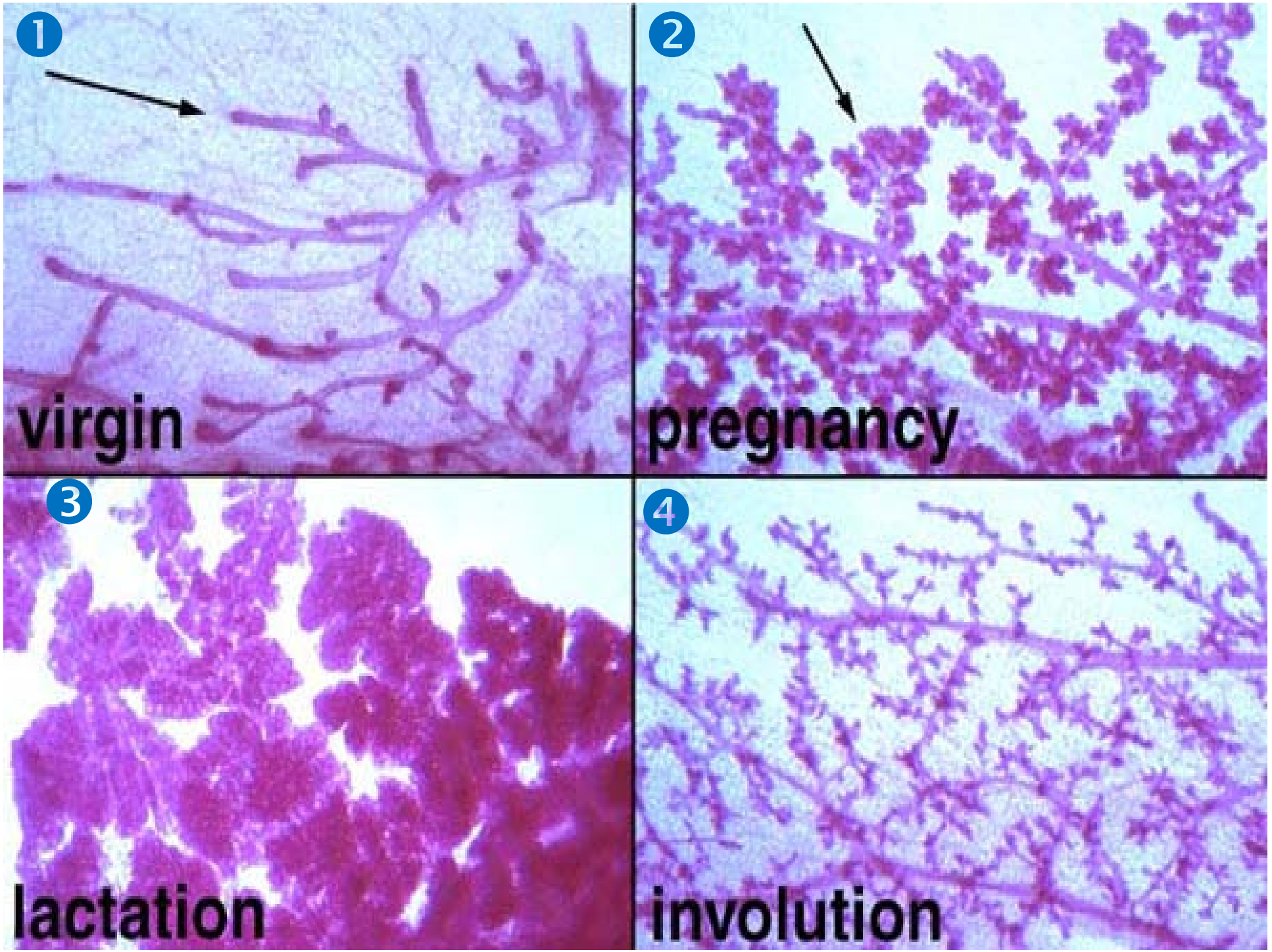


D - Ducts
L - Lymphocytes
A - Adipocytes
M - Myoepithelium
P - Plasma cell
S - Connective tissue septa

Ducts of the mammary gland comprise an outer tube of myoepithelial cells juxtaposing an inner tube of luminal epithelial cells surrounding a central lumen.

Hormonal control of mammary gland growth

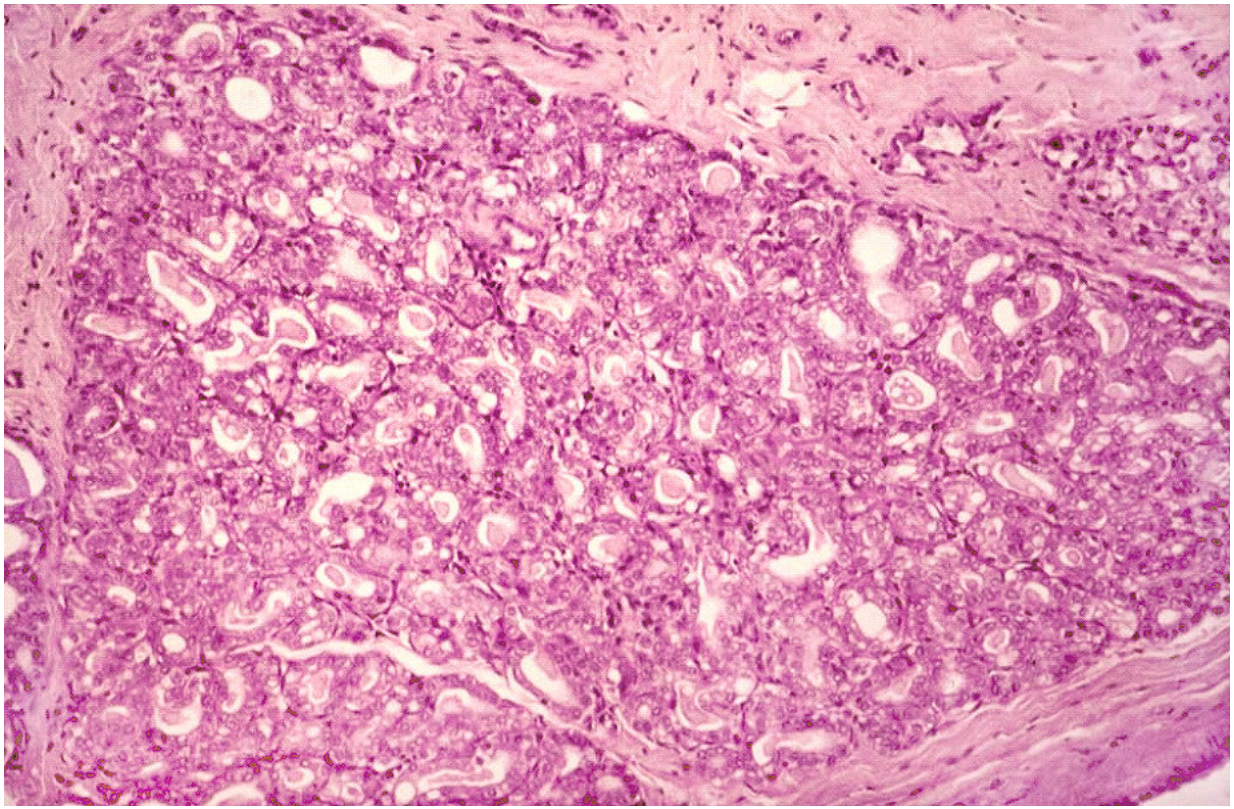



1
virgin
2
pregnancy
3
lactation
4
involution

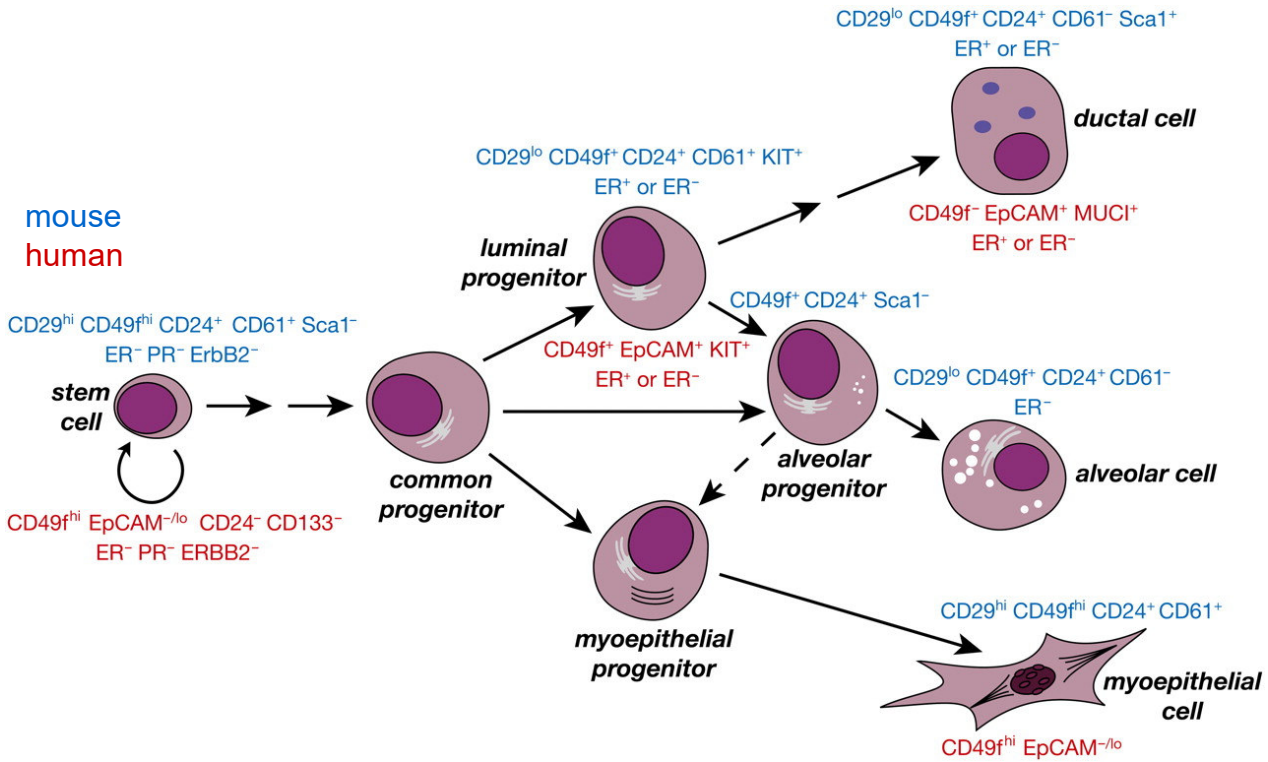
Lactating mammary glands

- upon pregnancy, the mammary glands complete their development; the intralobular ducts undergo rapid development forming buds that become alveoli
- the glandular parenchyma is divided into the lobules by thin connective tissue septa
- lobules contain spherical to elongated **acini** (alveoli) which can differ in size
- the first secretion appearing after birth is called the **colostrum**, it contains less fat and more protein than regular milk and is rich in antibodies (secIgA)

- **the wall of acini** consist of
  - the basement membrane
  - cuboidal or columnar secretory cells and
  - contractile myoepithelial cells located between the basement membrane and the bases of secretory cells
- **ducts**
  - a) **intralobular ducts** lined by a simple cuboidal to columnar epithelium
  - b) **lactiferous ducts** lined by two layers of columnar cells which, in the lactiferous sinus, changes into stratified squamous epithelium



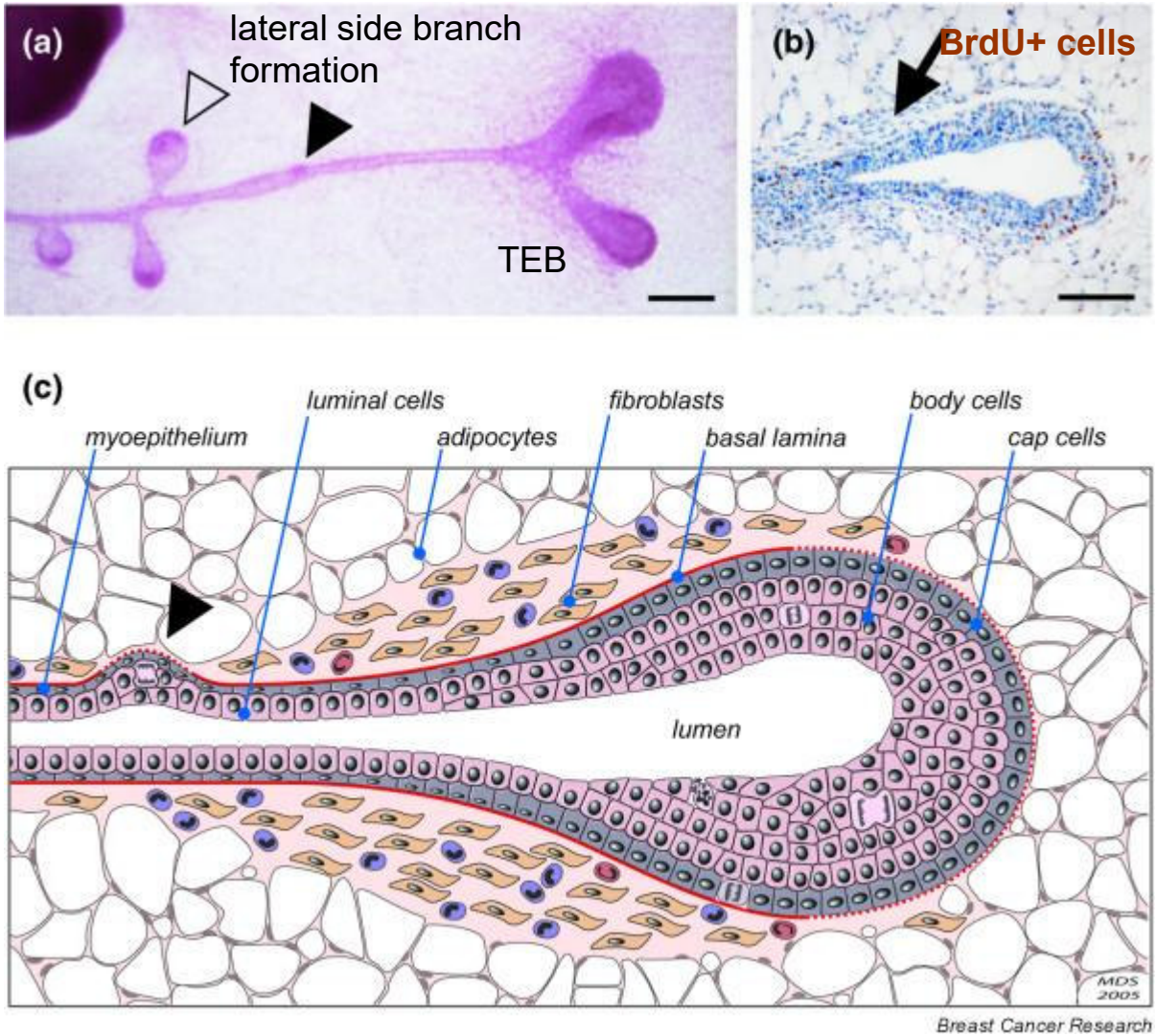
Cell lineages in the mammary gland



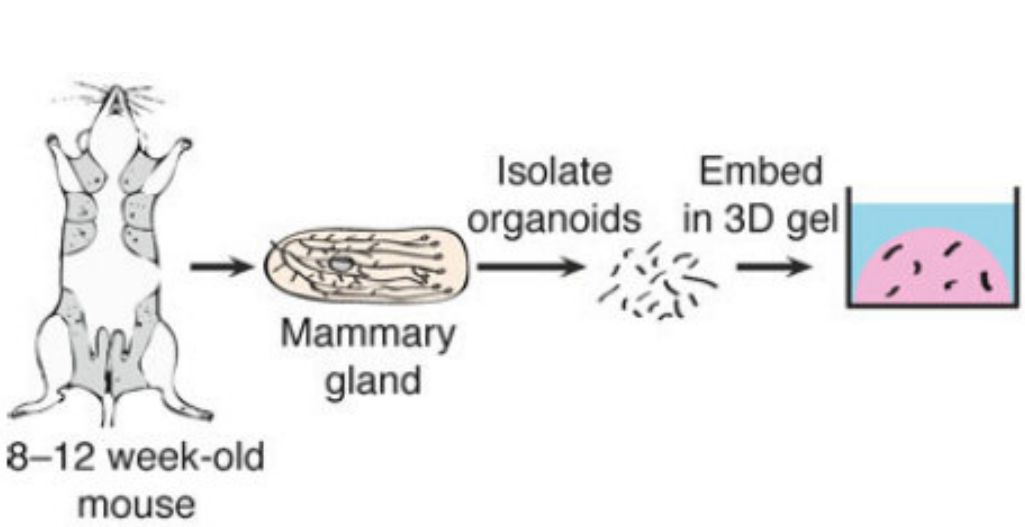
- Basal cells expressing α_6 and β_1 intergrin, CD24 and K14/K5 were shown to be highly enriched for mammary repopulation activity following transplantation into cleared mammary fat pads
- Lineage-tracing experiments using a large panel of different inducible CRE^{ER} lines expressing in the basal (K5, K14 and Lgr5) and luminal (K8 and K18) cells demonstrated that during embryonic development, multipotent $K14^+$ progenitors give rise to all the different epithelial lineages of the mammary tissue
- epithelial expansion after birth occurring during puberty and the multiple cycles of pregnancy, lactation and involution is sustained by unipotent basal and luminal stem cells rather than by multipotent stem cells
- however, transplantation of adult basal cells (which are unipotent *in vivo* according to lineage-tracing experiments) into the mammary fat pad induced the regeneration of functional mammary gland containing basal and luminal lineages, demonstrating that the transplantation experiments can expand the normally restricted differentiation potential of basal stem cells

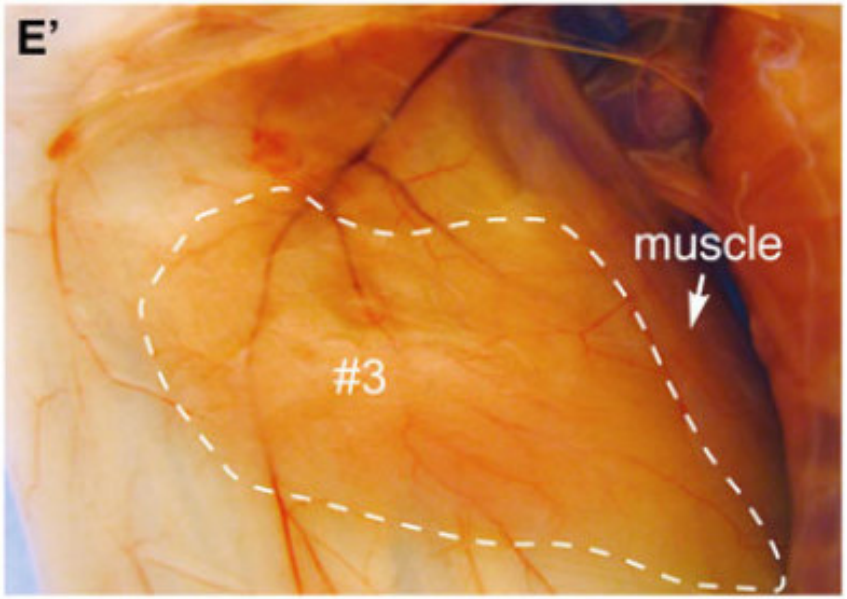
Mammary gland development: terminal end buds (TEBs)

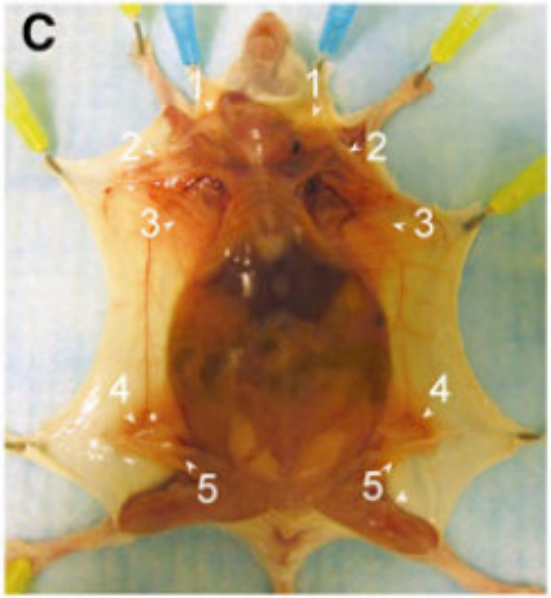
- during development, the motile TEB invades into the stromal fat pad at a rapid pace (0.5 mm/day), establishing the complex ductal architecture of the virgin mammary gland
- growth is driven by the proliferation of a single layer of cells, termed cap cells, at the tip of the TEB, and by the underlying preluminal epithelium
- as the TEB grows, cap cells translocate laterally to differentiate into myoepithelial cells and a fraction of cap cells detach and move into the subjacent preluminal cell population, where they are thought to give rise to luminal epithelial cells
- though there is no evidence that normal ductal cells ever cross the basal lamina, thinning of the basement membrane (dotted lines) does seem to occur at the tips of invading TEBs as a result of their partial enzymatic degradation and/or incomplete de novo synthesis

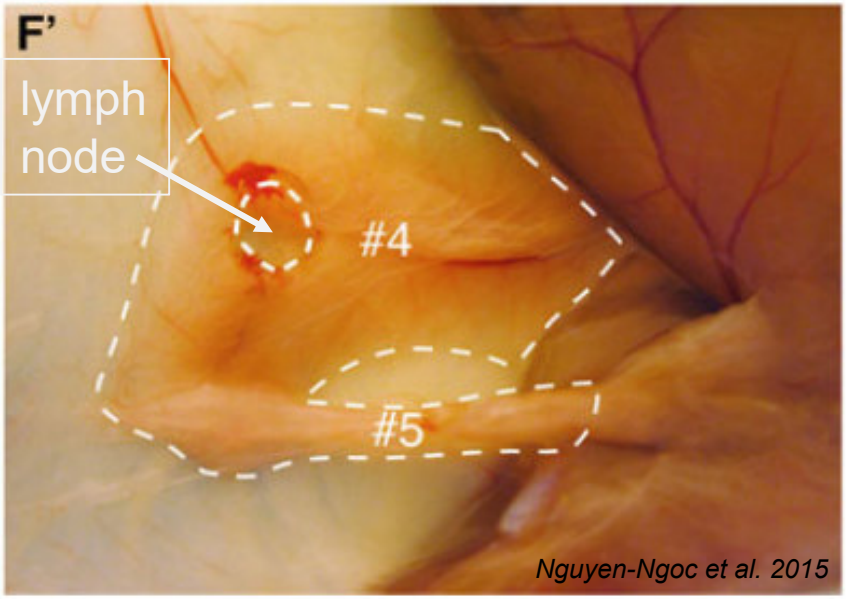


Isolation and culture of mammary glands

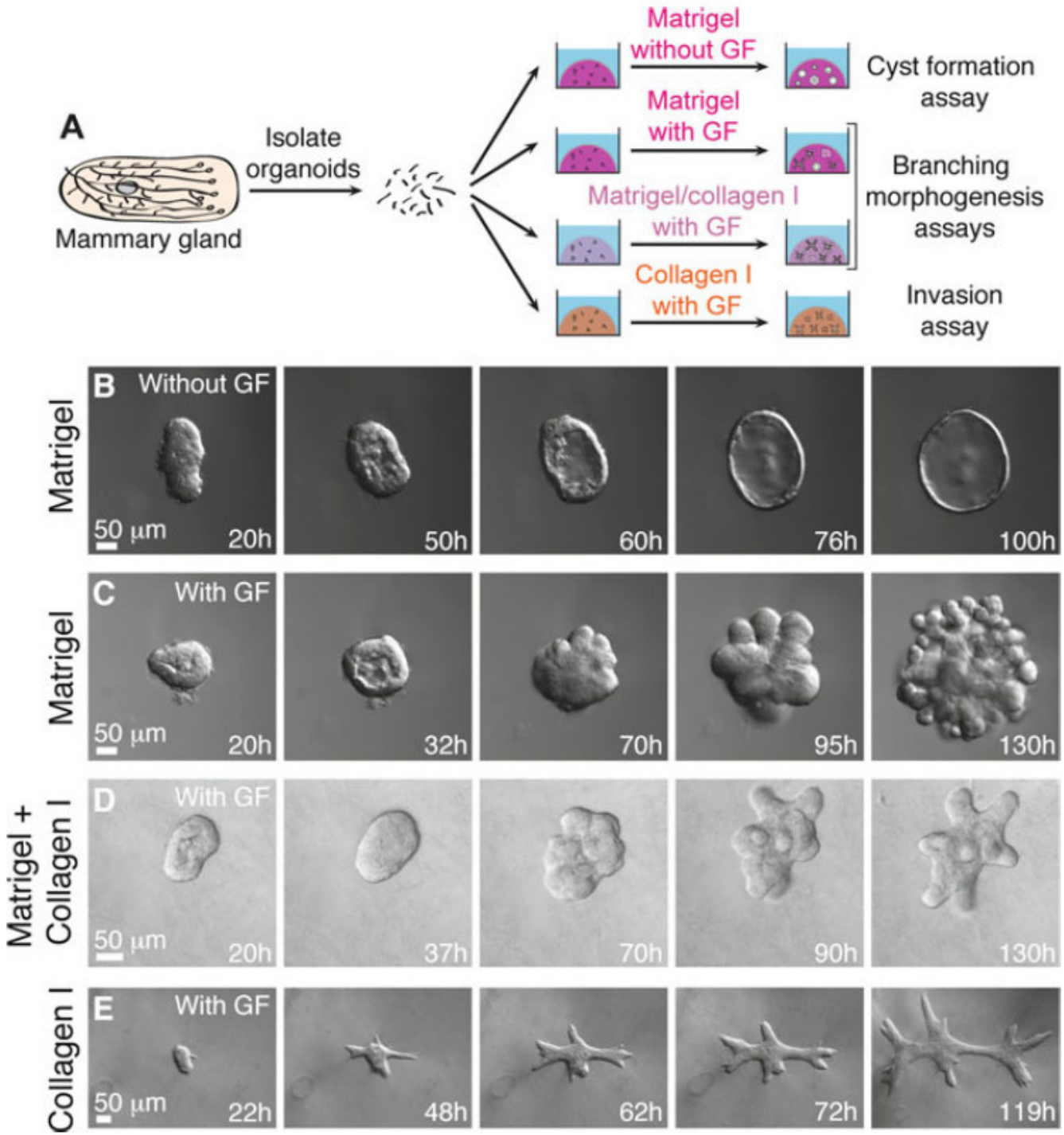








Nguyen-Ngoc et al. 2015



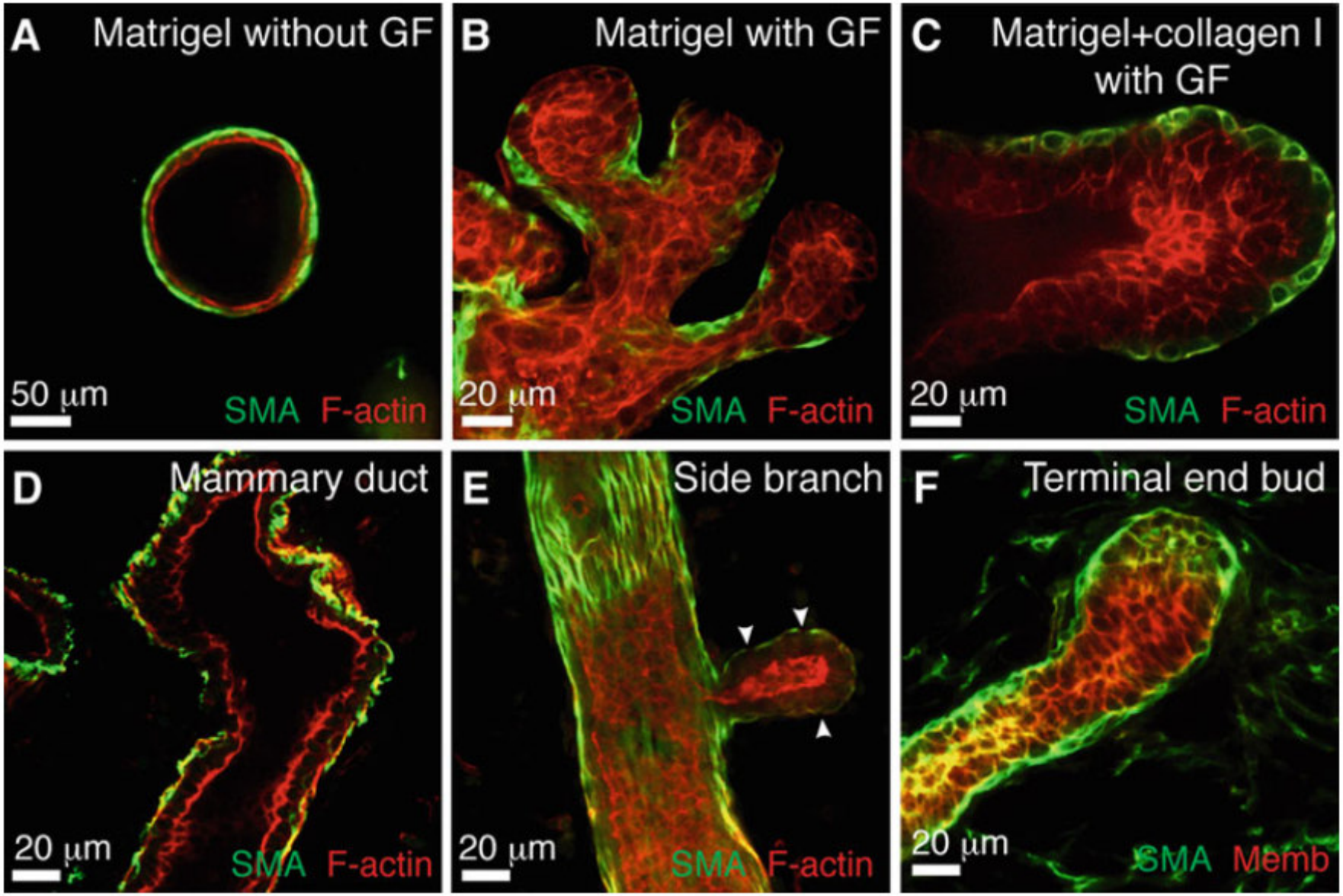
- matrigel is a basement membrane-rich ECM preparation secreted by Engelbreth–Holm–Swarm (EHS) mouse sarcoma cells. It contains structural proteins such as laminin, entactin, collagen and HSPGs
- without supplemental growth factors simple, bi-layered cysts are formed
- culture with growth factor (FGF2) induces a stereotyped program of branching morphogenesis
- collagen1-rich microenvironments induce a conserved program of invasion and dissemination
- defined mixtures of Matrigel and collagen1 can reproduce a more physiological organization of the elongating TEBs

Nguyen-Ngoc et al. 2015

Comparable structures in 3D cultures

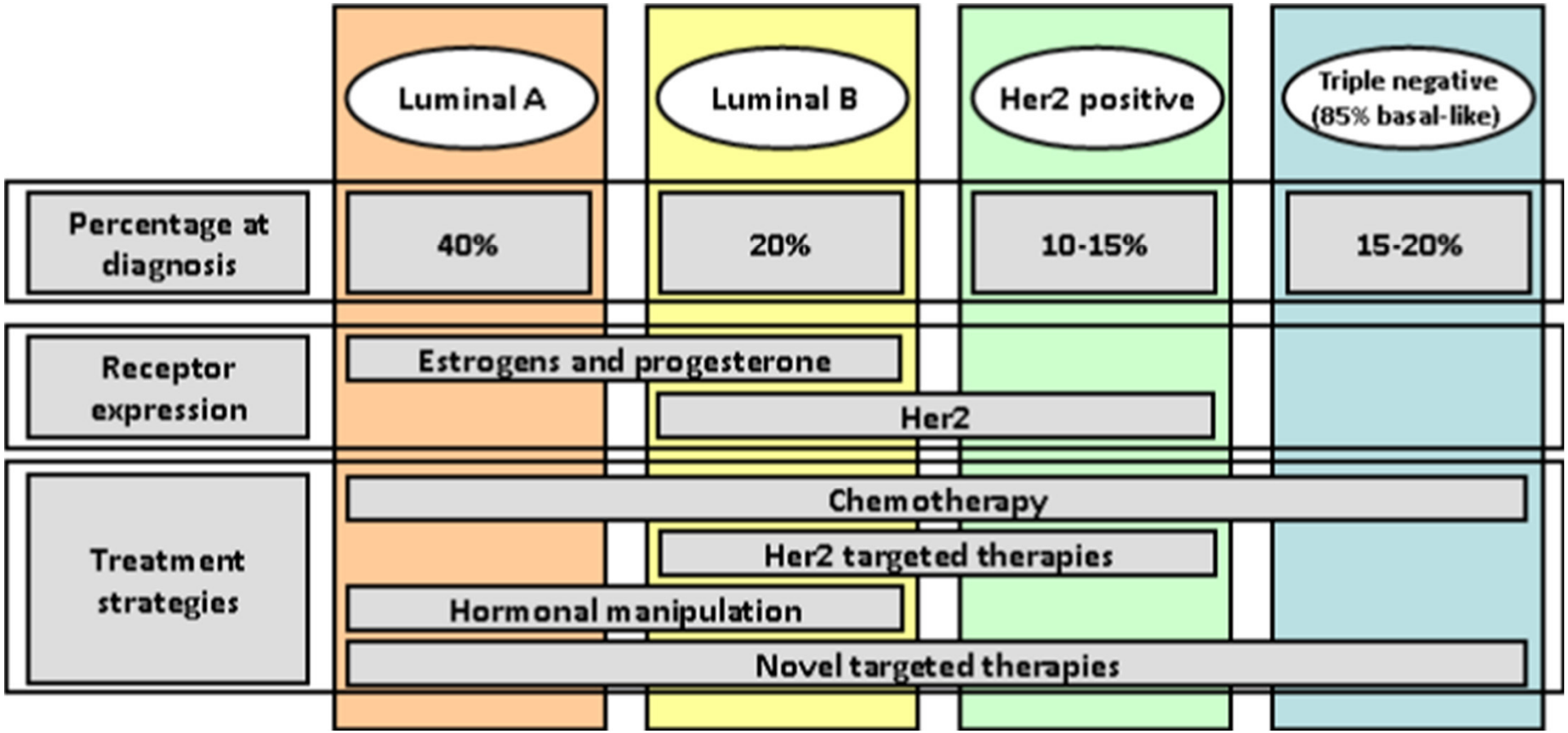
3D culture

gland

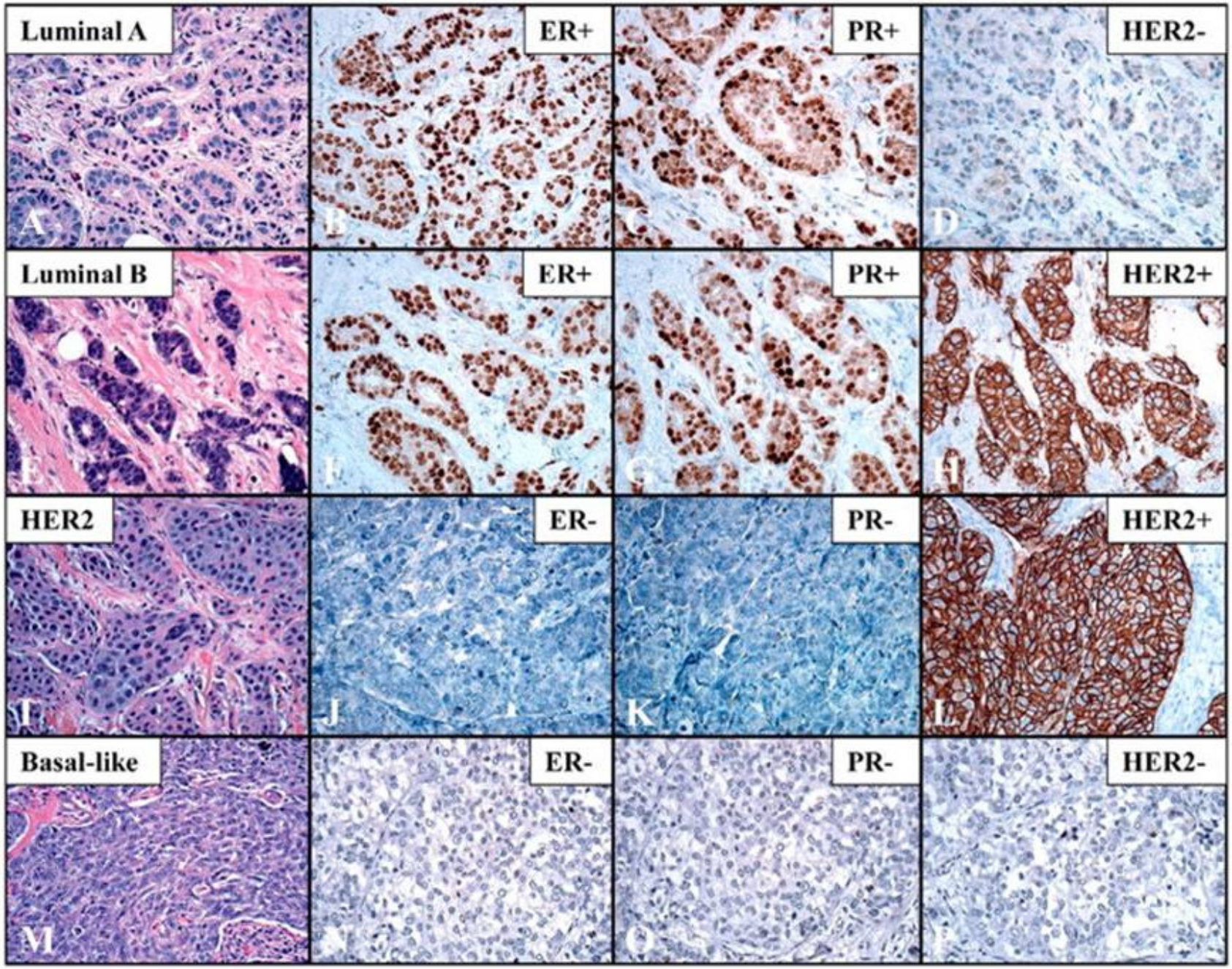


Nguyen-Ngoc et al. 2015

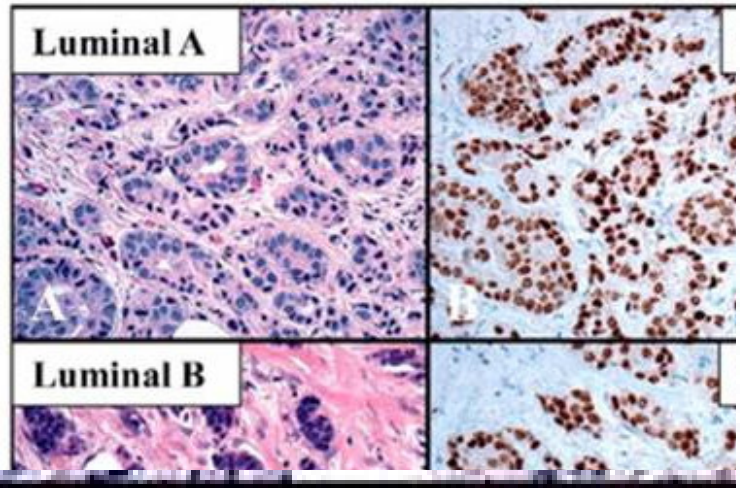
Major subtypes of breast cancer

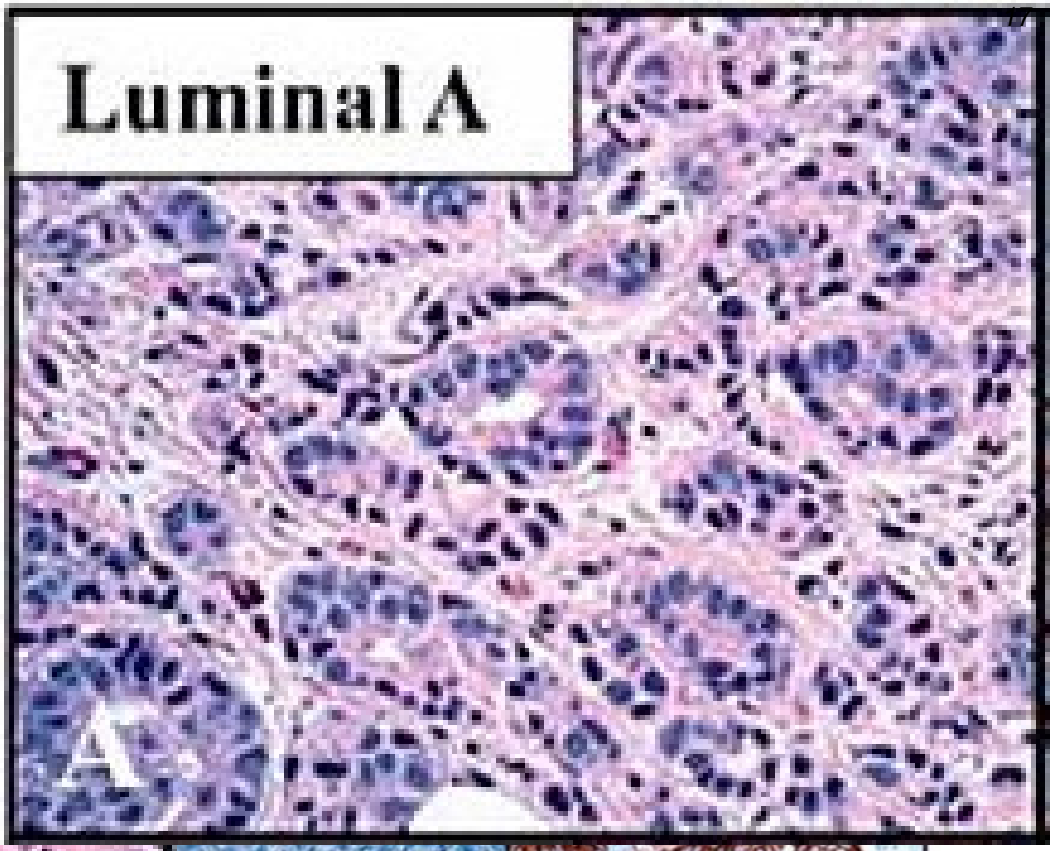


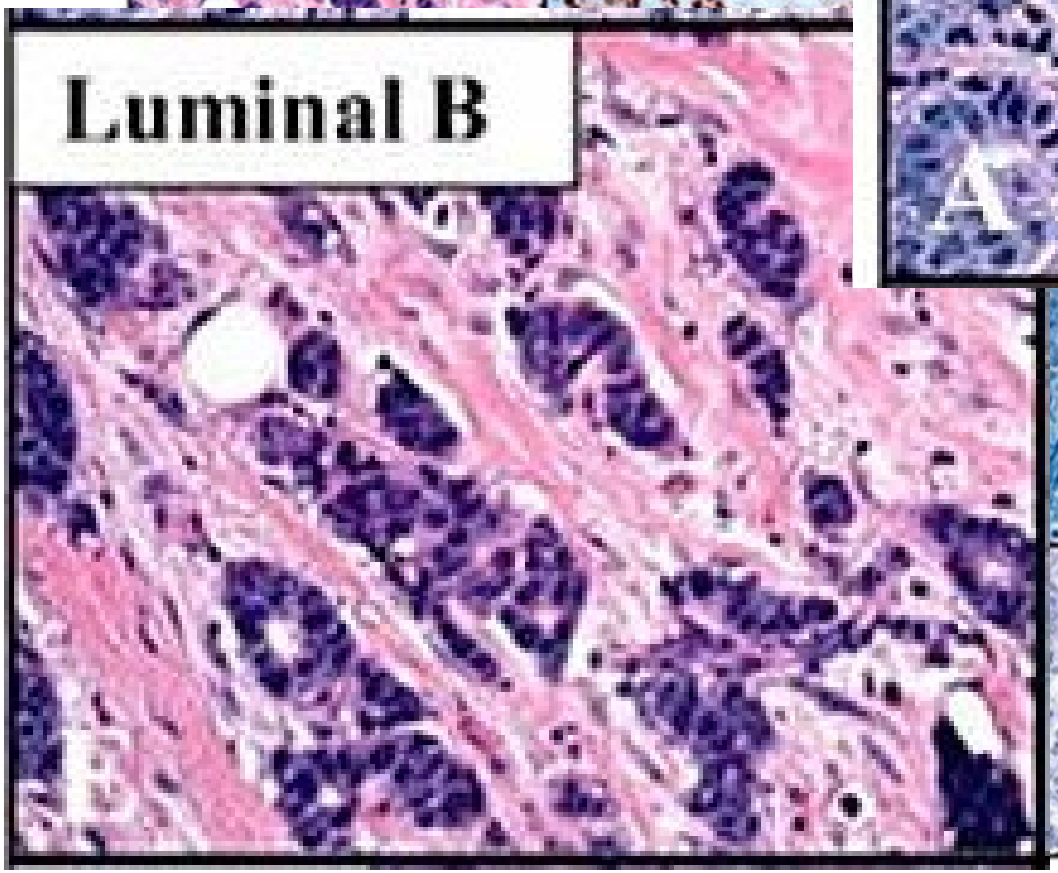
Major subtypes of breast cancer

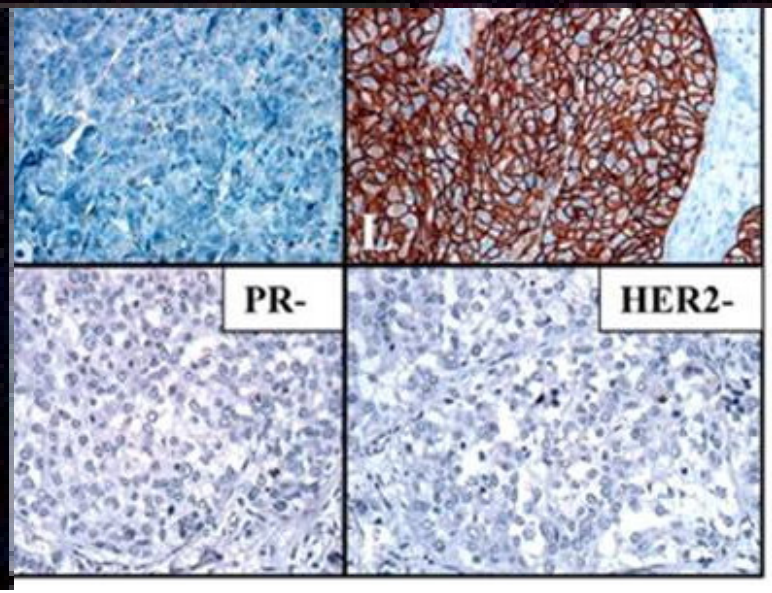


Major subtyp

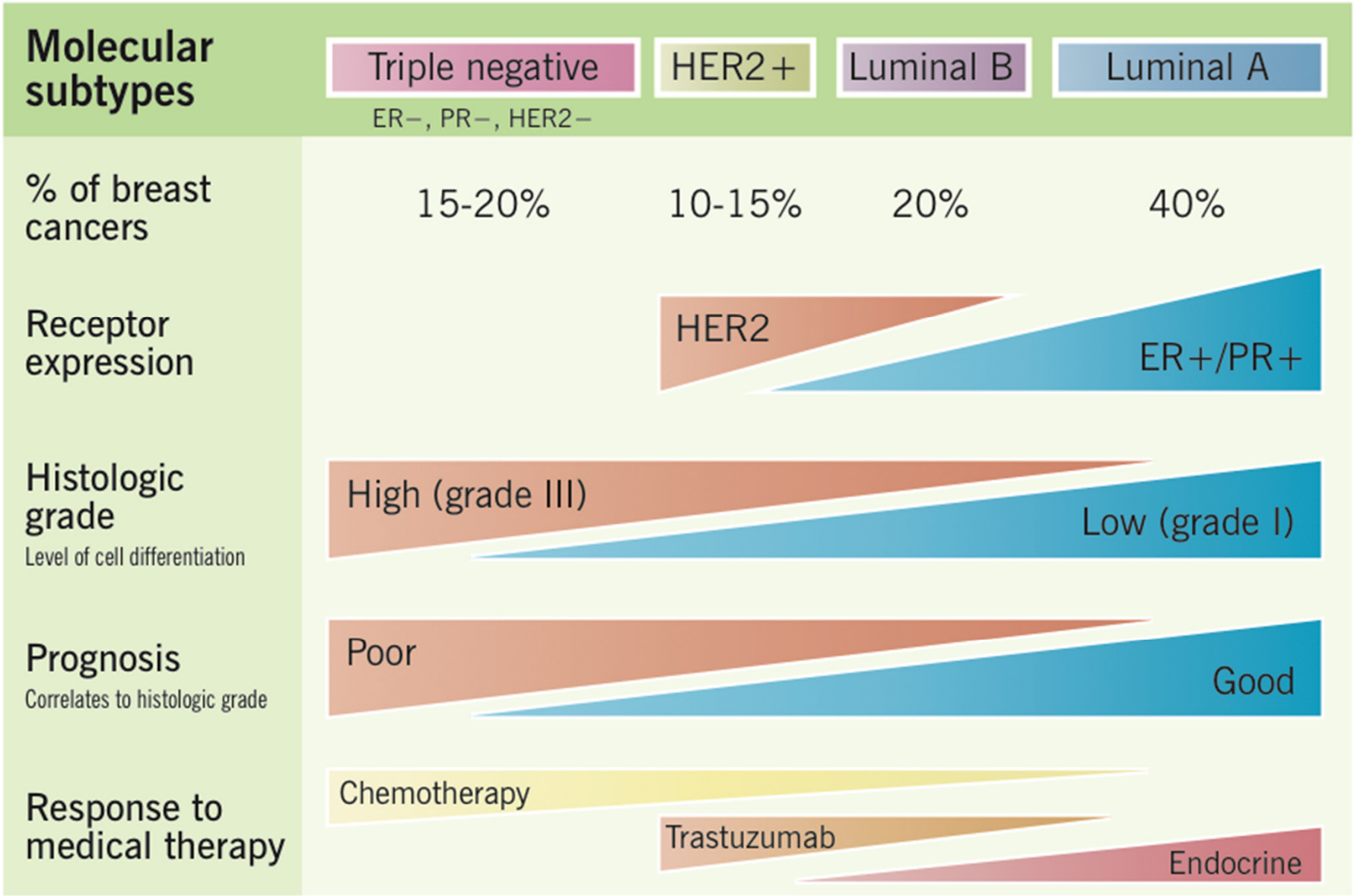




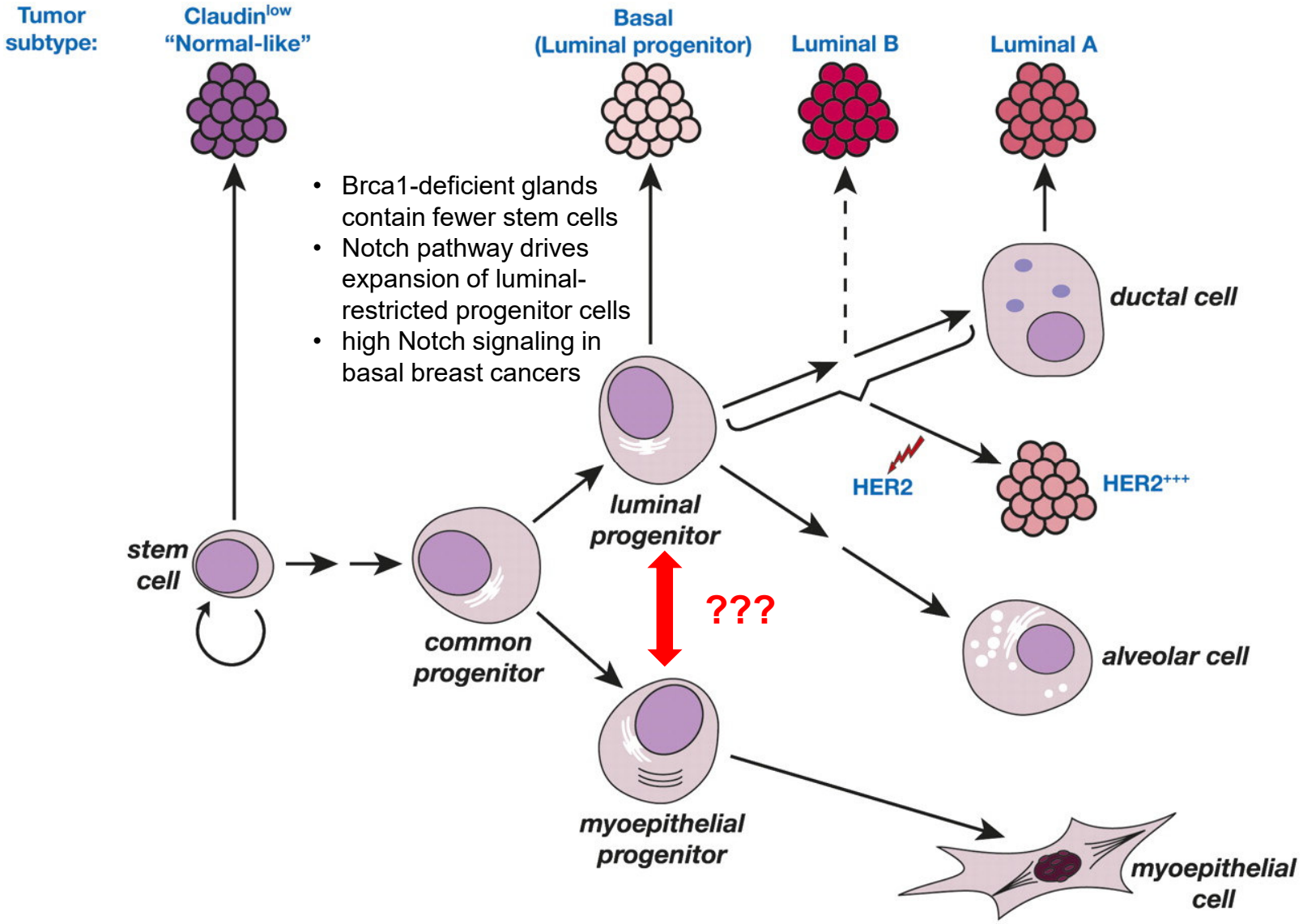




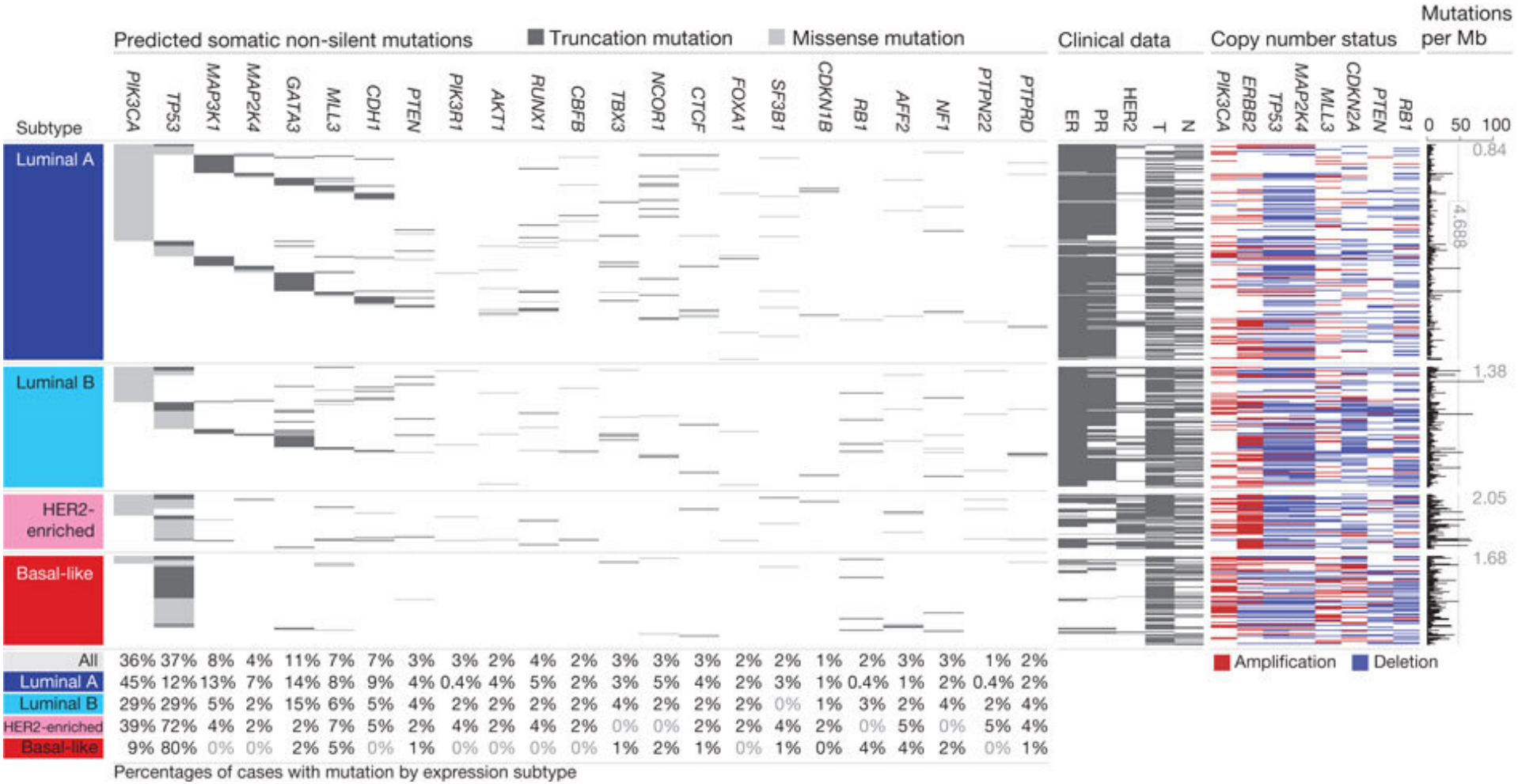
Major subtypes of breast cancer



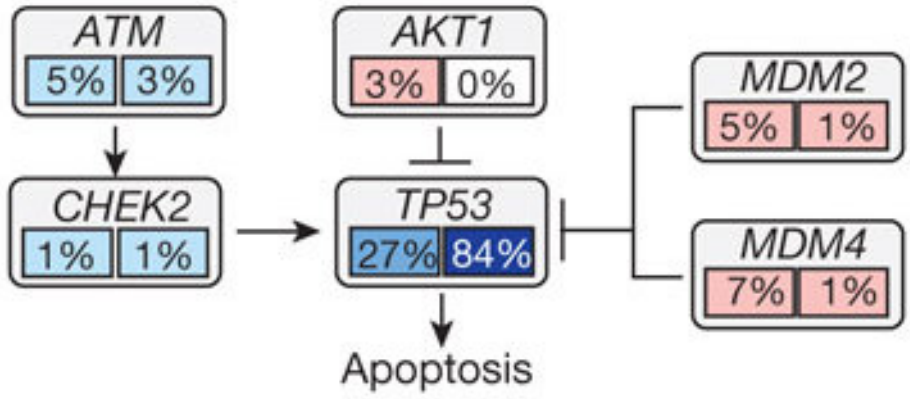
Potential relation between normal and cancer cells

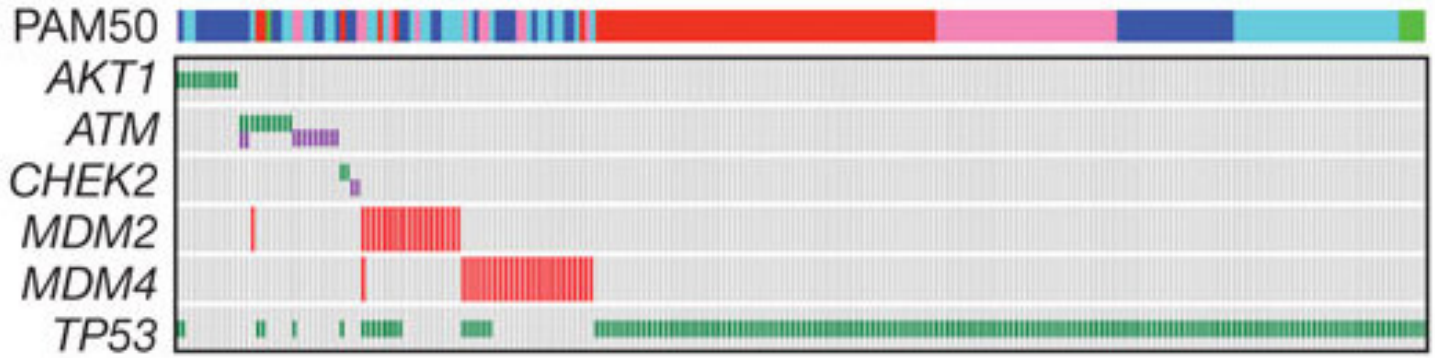


Significantly mutated genes and correlations with genomic and clinical features

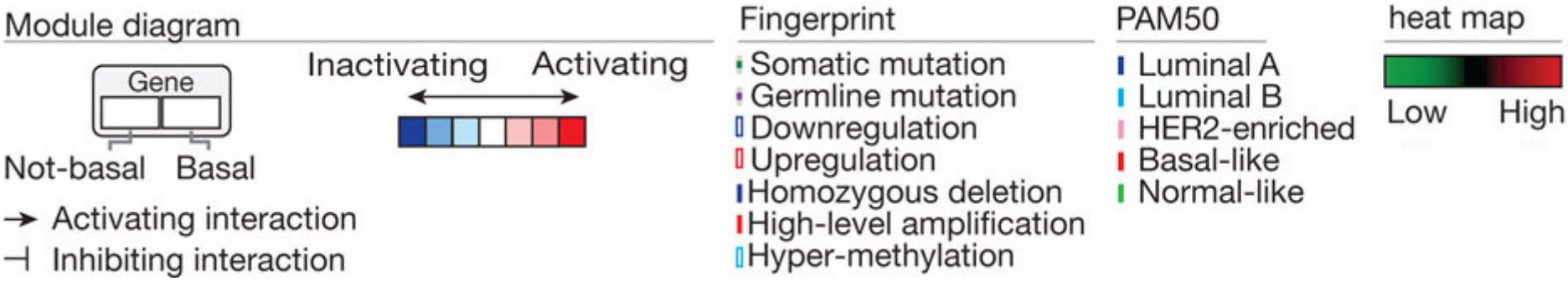


Mutual exclusivity modules: P53

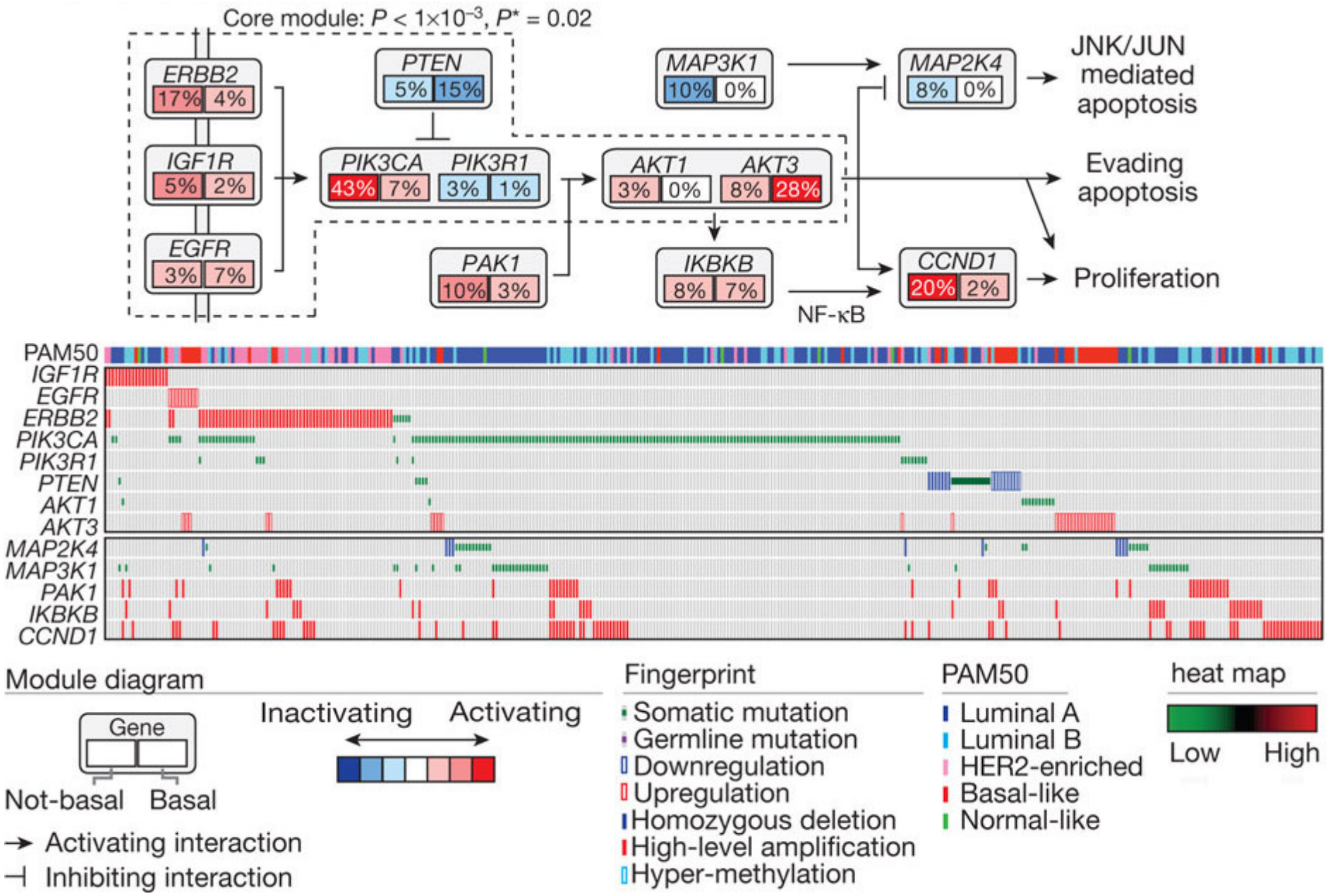




PAM50 gene expression signature outputs a risk of recurrence (ROR) score, risk category, and intrinsic subtype (Luminal A/B, HER2-enriched, Basal-like)

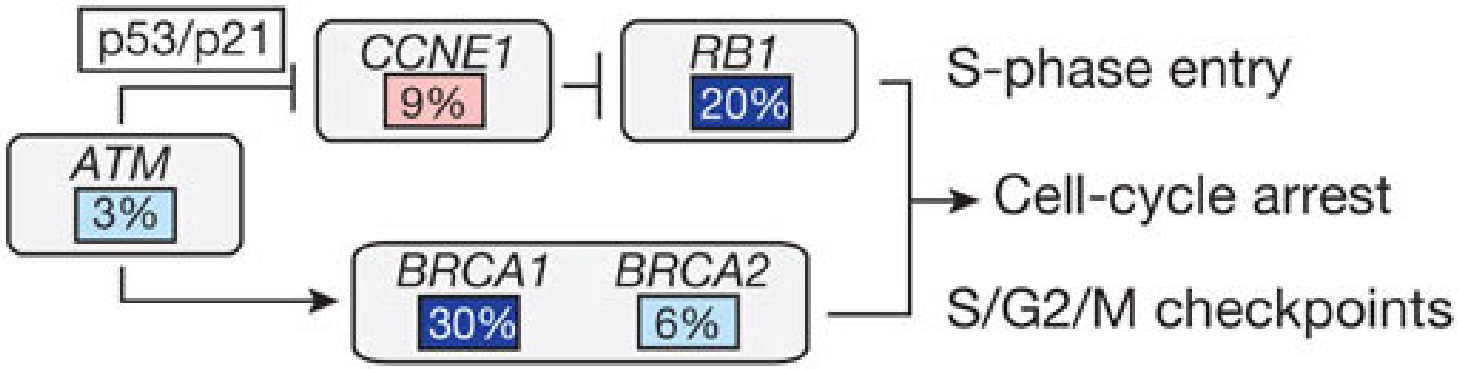


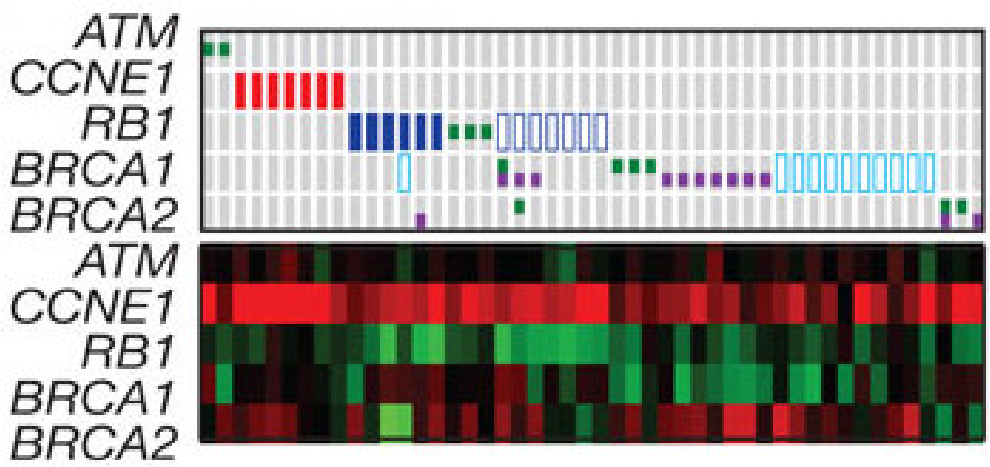
Mutual exclusivity modules: PI3K/AKT



Mutual exclusivity modules: cell cycle check points

(Basal tumors only)





Inactivating Activating

tion
ion

Fingerprint
- Somatic mutation
- Germline mutation
- Downregulation
- Upregulation
- Homozygous deletion
- High-level amplification
- Hyper-methylation

PAM50
- Luminal A
- Luminal B
- HER2-enriched
- Basal-like
- Normal-like

heat map

Low High

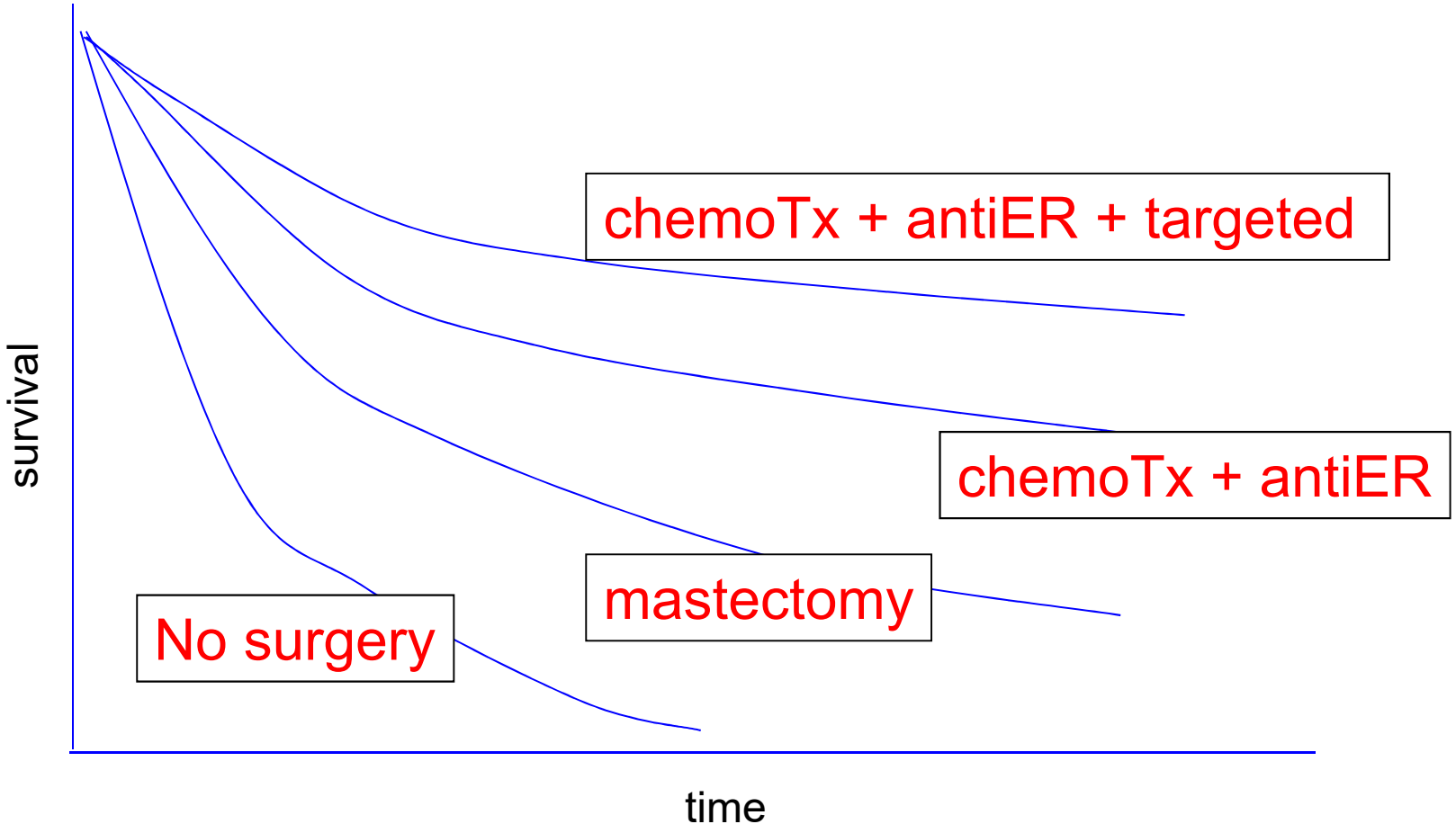
Breast cancer subtypes summary

Subtype	Luminal A	Luminal B	Basal-like	HER2+
$ER^+/HER2^-$	87 %	82 %	10 %	20 %
$HER2^+$	7 %	15 %	2 %	68 %
TNBCs	2 %	1 %	80 %	9 %
TP53 pathway	TP53 mut (12%); gain of MDM2 (14%)	TP53 mut (32%); gain of MDM2 (31%)	TP53 mut (84%); gain of MDM2 (14%)	TP53 mut (75%); gain of MDM2 (30%)
PIK3CA PTEN pathway	PIK3CA mut (49%); PTEN mut/loss (13%); INPP4B loss (9%)	PIK3CA mut (32%) PTEN mut/loss (24%) INPP4B loss (16%)	PIK3CA mut (7%); PTEN mut/loss (35%); INPP4B loss (30%)	PIK3CA mut (42%); PTEN mut/loss (19%); INPP4B loss (30%)
RB1 pathway	Cyclin D1 amp (29%); CDK4 gain (14%); low expression of CDKN2C; high expression of RB1	Cyclin D1 amp (58%); CDK4 gain (25%)	RB1 mut/loss (20%); cyclin E1 amp (9%); high expression of CDKN2A; low expression of RB1	Cyclin D1 amp (38%); CDK4 gain (24%)
mRNA	High ER cluster; low proliferation	Lower ER cluster; high proliferation	Basal signature; high proliferation	HER2 amplicon signature; high proliferation
Copy number	Most diploid; many with quiet genomes; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (24%)	Most aneuploid; many with focal amp; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (51%); 8p11.23 amp (28%)	Most aneuploid; high genomic instability; 1q, 10p gain; 8p, 5q loss; MYC focal gain (40%)	Most aneuploid; high genomic instability; 1q, 8q gain; 8p loss; 17q12 focal ERRB2 amp (71%)
DNA mutations	PIK3CA (49%); TP53 (12%); GATA3 (14%); MAP3K1 (14%)	TP53 (32%); PIK3CA (32%); MAP3K1 (5%)	TP53 (84%); PIK3CA (7%)	TP53 (75%); PIK3CA (42%); PIK3R1 (8%)
DNA methylation	–	Hypermethylated phenotype for subset	Hypomethylated	–
Protein expression	High oestrogen signalling; high MYB; RPPA reactive subtypes	Less oestrogen signalling; high FOXM1 and MYC; RPPA reactive subtypes	High expression of DNA repair proteins, PTEN and INPP4B loss signature	High expression of EGFR and HER2

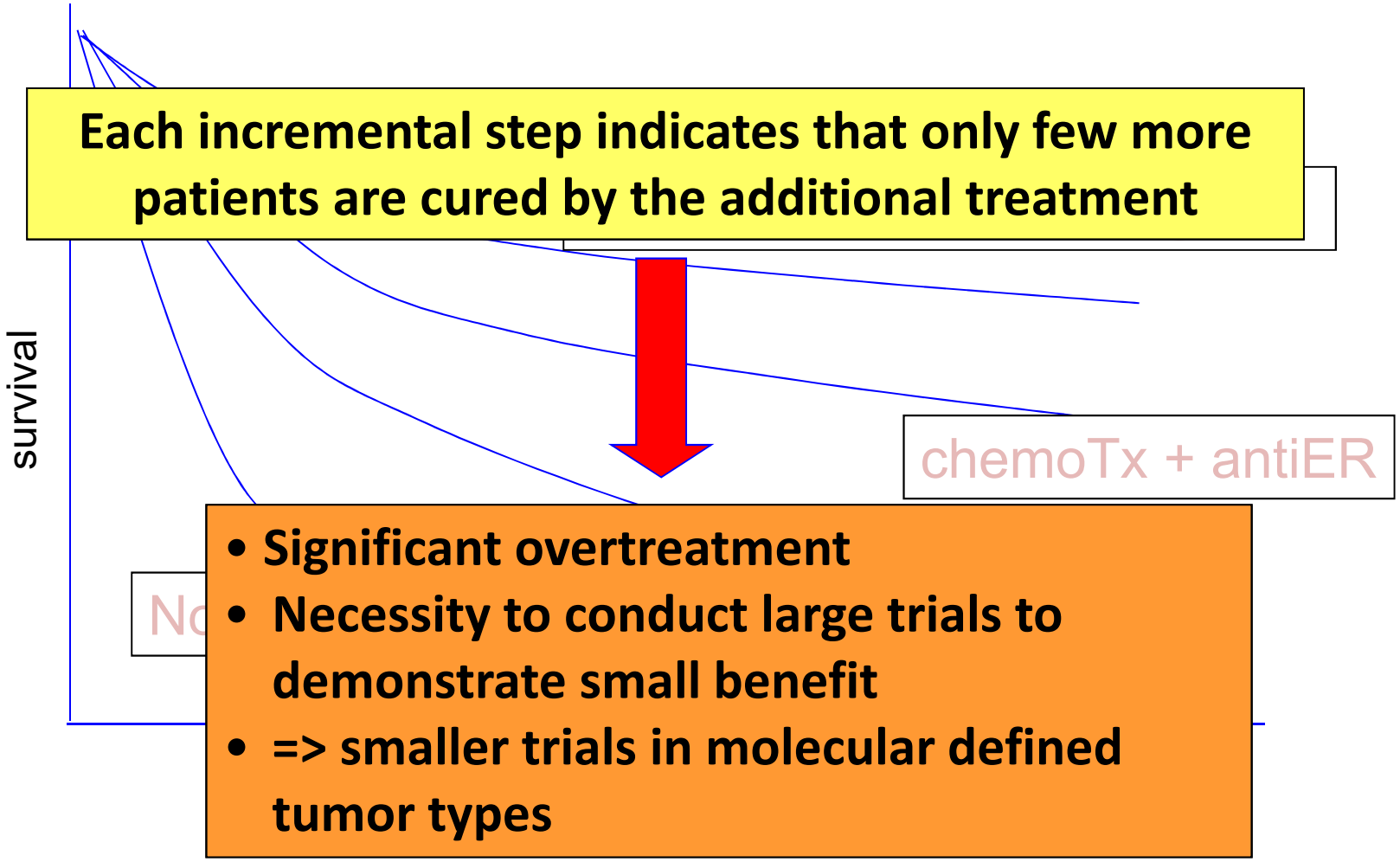
Treatment: luminal breast cancer (A and B subtypes)

- respond to **estrogen (hormonal) manipulation**
- therapies include tamoxifen, aromatase inhibitors (anastrozole, exemestane and letrozole) and fulvestrant
  - tamoxifen is an anti-estrogen binding to and inhibiting the estrogen receptor
  - aromatase inhibitors prevent the conversion of androgens to estrogens in post-menopausal women thus reducing the amount of estrogen available
  - fulvestrant binds the estrogen receptor preventing dimerization and nuclear localization
- **LUM A** shows an excellent ten year survival rate of approximately 90%; however, tumor **dormancy and late recurrences** beyond 10 years are characteristic of LUM A
- endocrine treatment is typically given adjuvant for 5 years, but may be extended for even 10 years
- **LUM B** is inherently more aggressive and requires more aggressive therapy: **combination of endocrine and chemo- or targeted therapies** against Her2
- upon **relapse, 50% of patients fail to respond to hormonal manipulation**; this may be due to increased hormonal receptor expression, alterations in co-regulator proteins or activation of alternative cell signaling pathways

Incremental benefit of therapies



Incremental benefit of therapies



TAILORx: personalized therapy for breast cancer

(using Oncotype DX 21 gene recurrence score (RS) assay)

large trial (>11'000 patients) started in 2006
first results reported in 2015

Sixteen cancer genes and five reference genes from three studies

Proliferation group
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

Estrogen group
ER
PR
Bcl-2
SCUBE2

Invasion group
Stromelysin 3
Cathepsin L2

HER2 group
GRB7
HER2

GSTM1

BAG1

CD68

Reference group
Beta-actin
GAPDH
RPLPO
GUS
TFRC

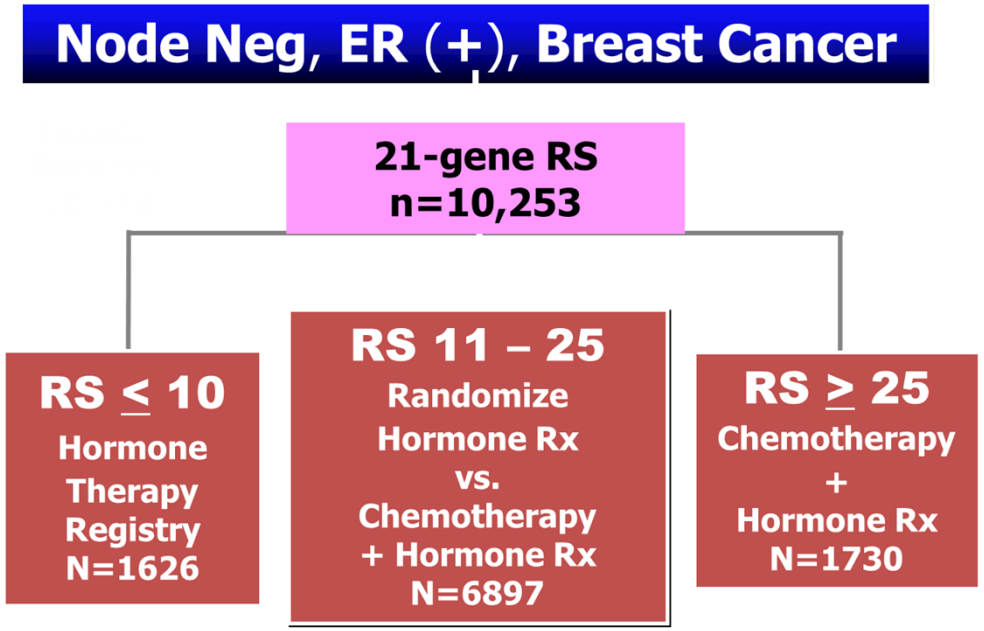
Calculation of gene recurrence score

RS = + 0.47 × HER2 group score
– 0.34 × estrogen group score
+ 1.04 × proliferation group score
+ 0.10 × invasion group score
+ 0.50 × CD68
– 0.08 × GSTM1
– 0.07 × BAG1

Interpretation of recurrence score

CATEGORY	RS (0–100)
Low risk	RS < 18
Intermediate risk	RS ≥ 18 and < 31
High risk	RS ≥ 31

Scheme: TAILORx



Of 10,253 eligible women enrolled, 1626 women (15.9%) who had a recurrence score of 0 to 10 were assigned to receive endocrine therapy alone without chemotherapy.
At 5 years, in this patient population, the rate of invasive disease–free survival was 93.8%, the rate of freedom from recurrence of breast cancer at a distant site was 99.3%, the rate of freedom from recurrence of breast cancer at any site was 98.7%, and the rate of overall survival was 98.0%.
=> a favorable gene-expression profile predicts very low rates of recurrence at 5 years with endocrine therapy alone

9-year outcome: TAILORx

- For midrange recurrence score of 11 to 25, **endocrine was not inferior to chemo-endocrine therapy**, which provides evidence that adjuvant **chemotherapy was not beneficial** in these patients
- Exploratory analyses indicated that **chemotherapy was associated with benefit** for women 50 years of age or younger who had a recurrence score of 16 to 25, which may be at least partly explained by an antiestrogenic effect associated with premature menopause induced by chemotherapy.

Table 2. Estimated Survival Rates According to Recurrence Score and Assigned Treatment in the Intention-to-Treat Population.*

End Point and Treatment Group	Rate at 5 Yr	Rate at 9 Yr
	percent	
Invasive disease–free survival†		
Score of ≤10, endocrine therapy	94.0±0.6	84.0±1.3
Score of 11–25, endocrine therapy	92.8±0.5	83.3±0.9
Score of 11–25, chemoendocrine therapy	93.1±0.5	84.3±0.8
Score of ≥26, chemoendocrine therapy	87.6±1.0	75.7±2.2
Freedom from recurrence of breast cancer at a distant site		
Score of ≤10, endocrine therapy	99.3±0.2	96.8±0.7
Score of 11–25, endocrine therapy	98.0±0.3	94.5±0.5
Score of 11–25, chemoendocrine therapy	98.2±0.2	95.0±0.5
Score of ≥26, chemoendocrine therapy	93.0±0.8	86.8±1.7
Freedom from recurrence of breast cancer at a distant or local–regional site		
Score of ≤10, endocrine therapy	98.8±0.3	95.0±0.8
Score of 11–25, endocrine therapy	96.9±0.3	92.2±0.6
Score of 11–25, chemoendocrine therapy	97.0±0.3	92.9±0.6
Score of ≥26, chemoendocrine therapy	91.0±0.8	84.8±1.7
Overall survival		
Score of ≤10, endocrine therapy	98.0±0.4	93.7±0.8
Score of 11–25, endocrine therapy	98.0±0.2	93.9±0.5
Score of 11–25, chemoendocrine therapy	98.1±0.2	93.8±0.5
Score of ≥26, chemoendocrine therapy	95.9±0.6	89.3±1.4

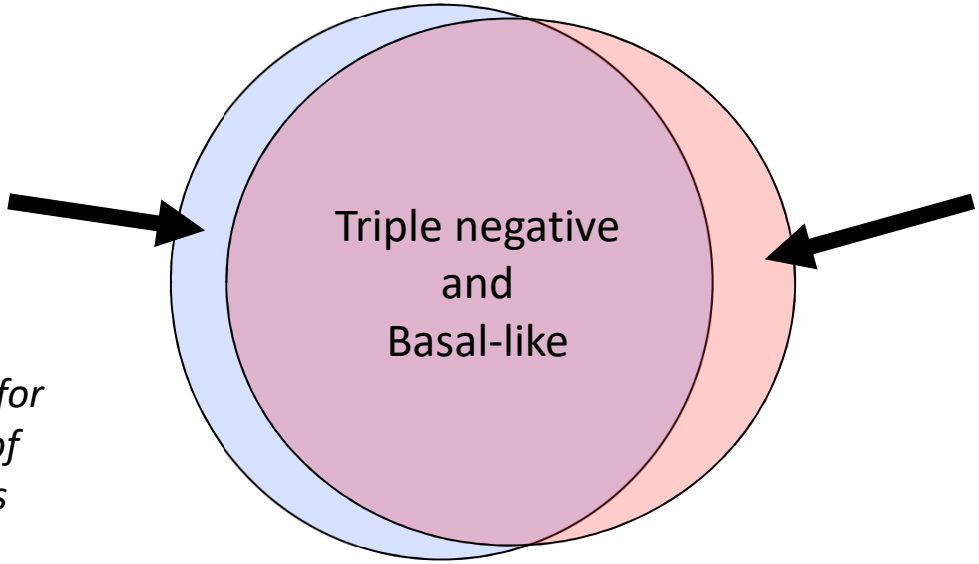
* Plus–minus values are Kaplan–Meier estimates ±SE.
† Invasive disease–free survival was defined as freedom from invasive disease recurrence, second primary cancer, or death.

Worse outcome for Triple-Negative Breast Cancer

- **Tumors that do not express the ER, PR, and HER2/neu markers are more likely to be basal-subtype tumors**
  - Inherently aggressive biologic behavior
  - Triple-negativity used as a surrogate for basal subtype

- **Tumors that do not express the ER, PR, and HER2/neu markers have fewer systemic therapy options**
  - Endocrine/hormonal therapy will be ineffective
  - Herceptin therapy will be ineffective
  - Chemotherapy and other targeted therapies ***ARE*** effective, however often not curative

Triple negative but not basal
10-30%
Can also include "claudin-low", a subtype notable for high expression of stem cell markers



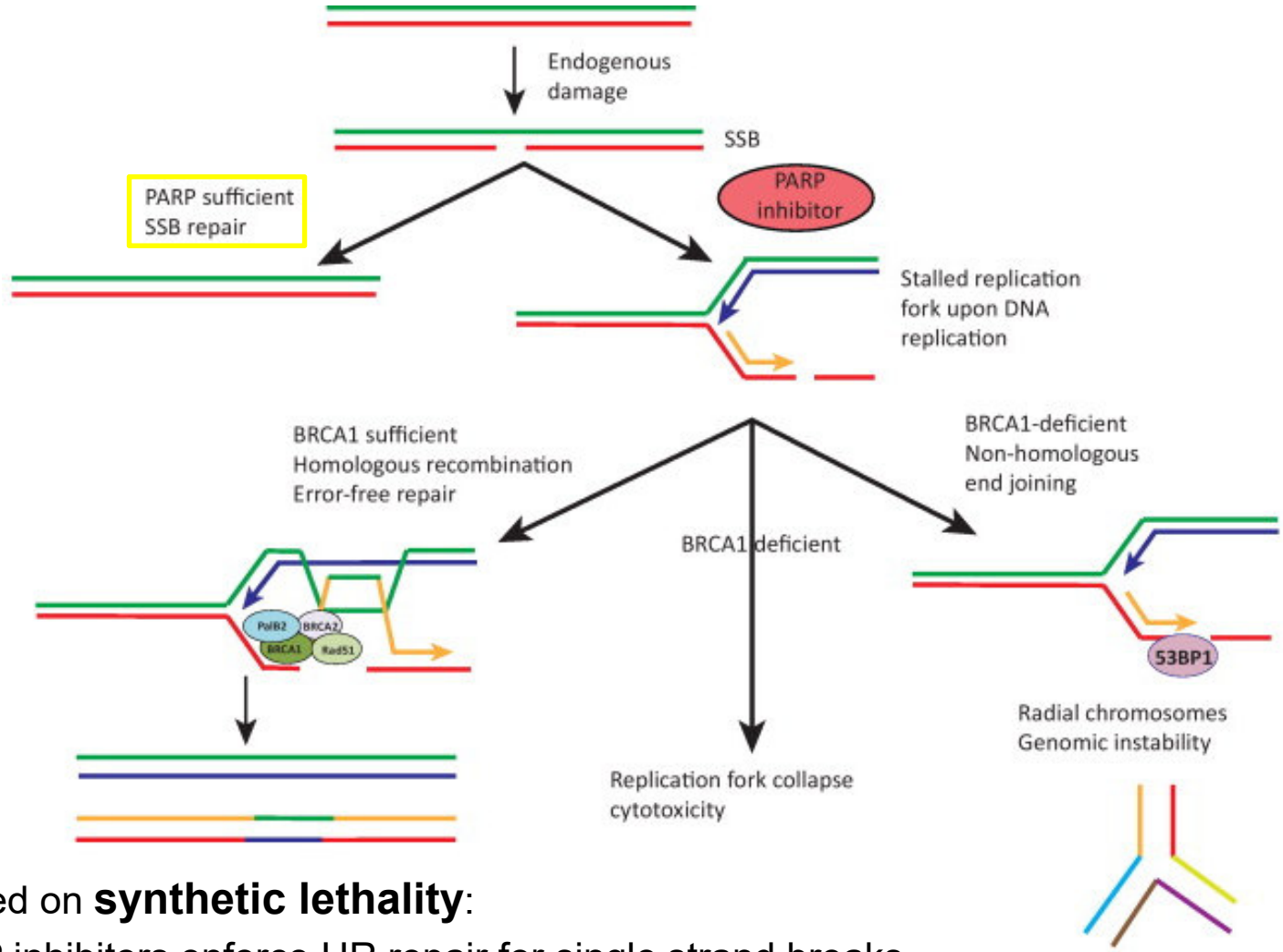
Basal but not triple negative

15-40% are ER+, PR+, or HER2+

TNBC Shares Clinical and Pathologic Features with BRCA-1-Related Breast Cancers

Characteristics	Hereditary *BRCA1*	Triple Negative/Basal-Like[1,2,3]
ER/PR/HER2 status	Negative	Negative
TP53 status	Mutant	Mutant
BRCA1 status	Mutational inactivation*	Diminished expression*
Gene-expression pattern	Basal-like	Basal-like
Tumor histology	Poorly differentiated (high grade)	Poorly differentiated (high grade)
Sensitivity to DNA-damaging agents	Highly sensitive	Highly sensitive

PARP inhibitor treatment for Brca-mutant cancers



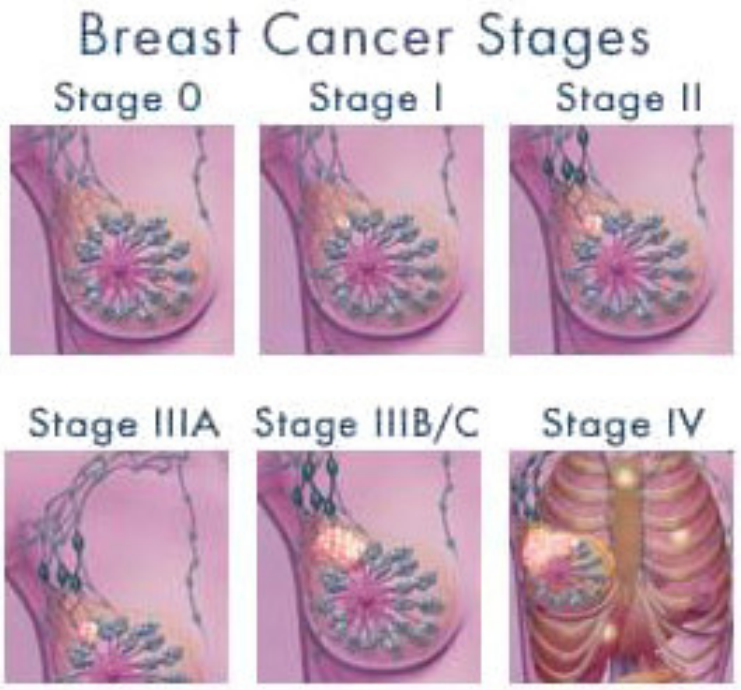
=> based on **synthetic lethality**:

- PARP inhibitors enforce HR repair for single strand breaks
- in the absence of HR due to Brca1 mutations cells will be sensitized to DNA damaging therapy (platinum drugs, irradiation)
- benefit: 3.5 months better PFS in phase2 trial, not confirmed in phase 3 (however, patients were not stratified based on Brca status or PARP status)

Grading and staging of breast cancer

- histological grade provides important prognostic and management information
- rules defined by Elston and Ellis
- assess acinar formation, nuclear size/pleomorphism and mitotic activity (each element is given a score of 1 – 3)
- the sum of scores is equivalent to the tumor grade:
  - Total score 3 - 5 = Grade 1
  - Total score 6 or 7 = Grade 2
  - Total score 8 or 9 = Grade 3

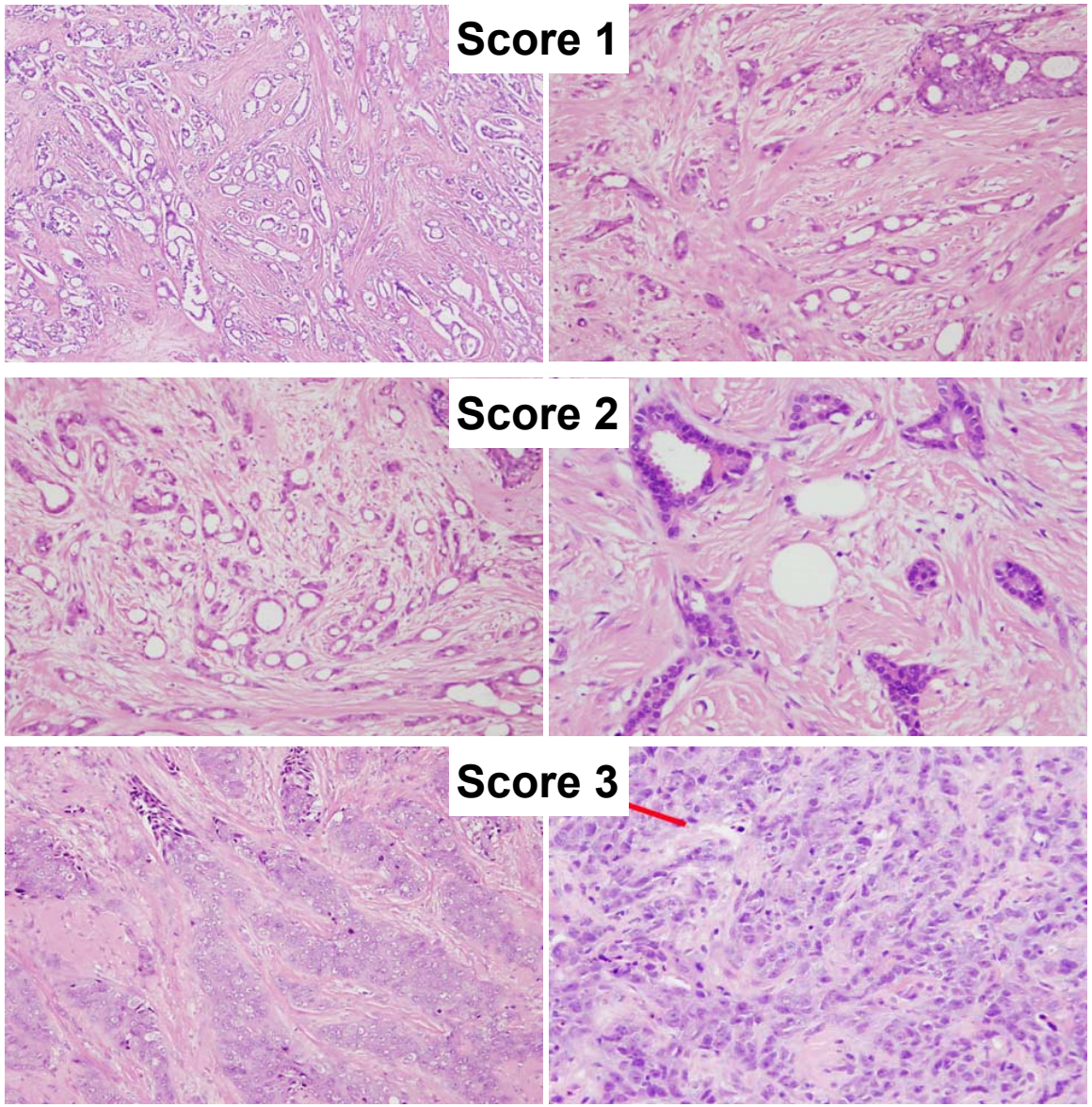
- **<u>Manchester staging system:</u>**
  - **Stage 1**: confined to the breast.
  - **Stage 2**: confined to the breast but palpable, mobile lymph nodes are present in the axilla
  - **Stage 3**: local invasion, extends beyond the mammary parenchyma:
    (a) skin invasion or fixation over an area large in relation to the size of the breast or skin ulceration;
    (b) tumor fixation to the underlying muscle or fascia; axillary nodes, if present, are mobile
  - **Stage 4**: extends beyond the breast area as shown by fixation or matting of the axillary nodes, complete fixation of the tumor to chest wall, deposits in supra-clavicular nodes or in the opposite breast, or distant metastases.



Grading of breast cancer

tuble/acini formation:

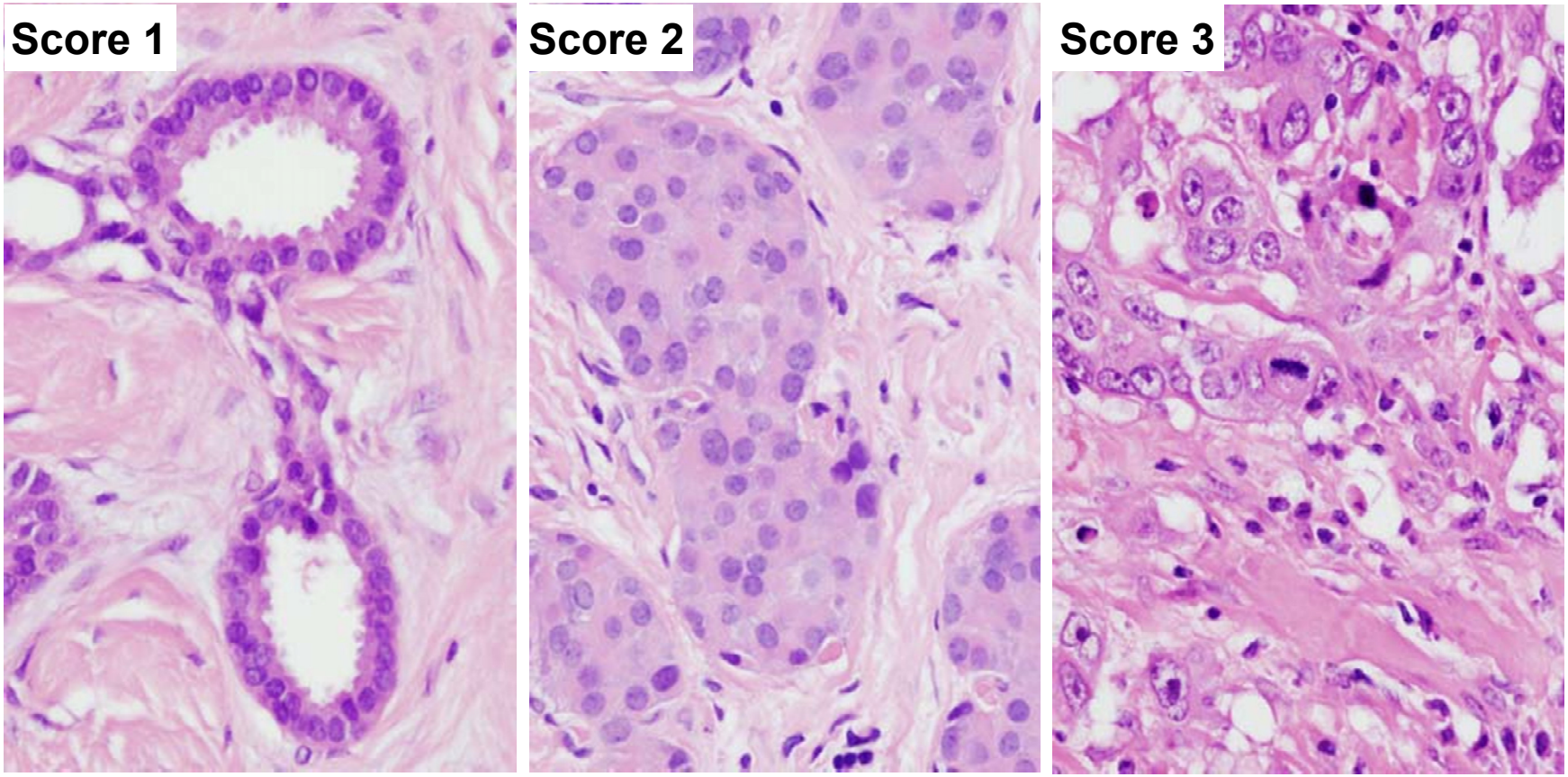
> 75% - Score 1
10 - 75% - Score 2
<10% - Score 3



Grading of breast cancer

Nuclear atypia / pleomorphism:

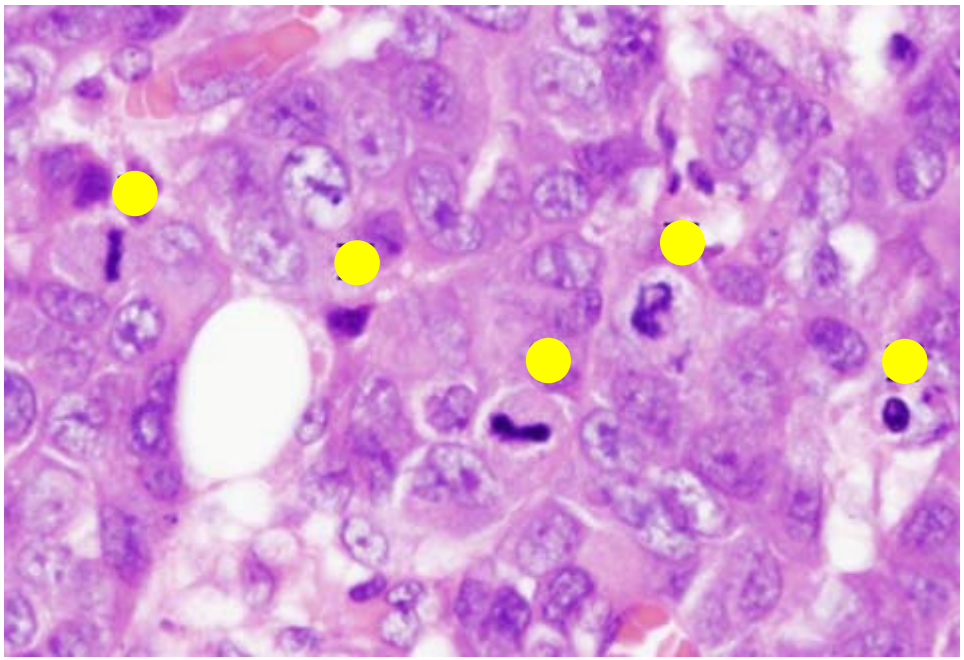
- Score1 Small nuclei very similar in size to benign ductal/acinar epithelial cells
  Minimal pleomorphism and even chromatin pattern
  Nucleoli very inconspicuous
- Score 2 Larger nuclei with mild to moderate pleomorphism
  Nucleoli visible but small and inconspicuous
- Score 3 Vesicular nuclei often with prominent nucleoli
  Marked variation in size and shape

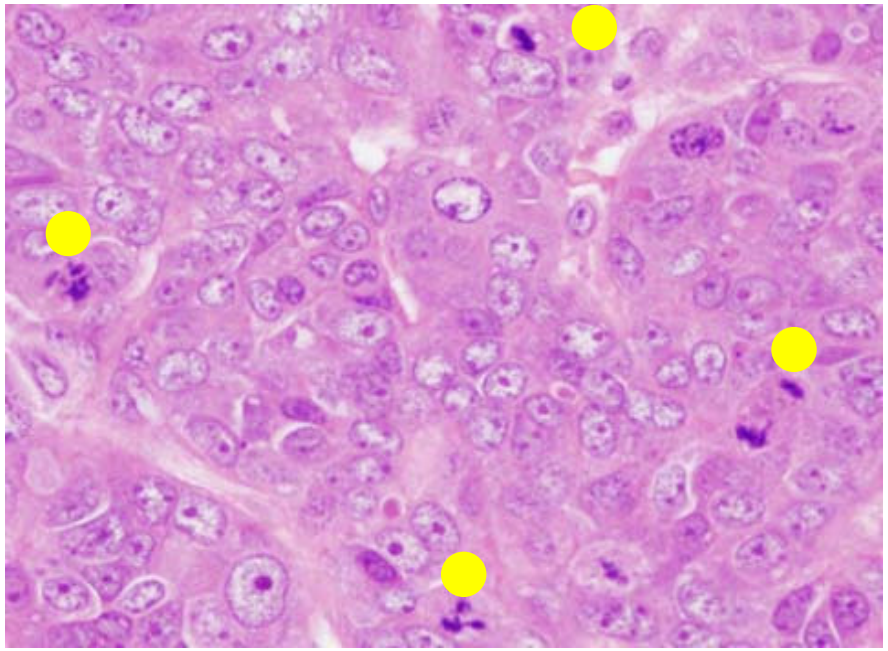


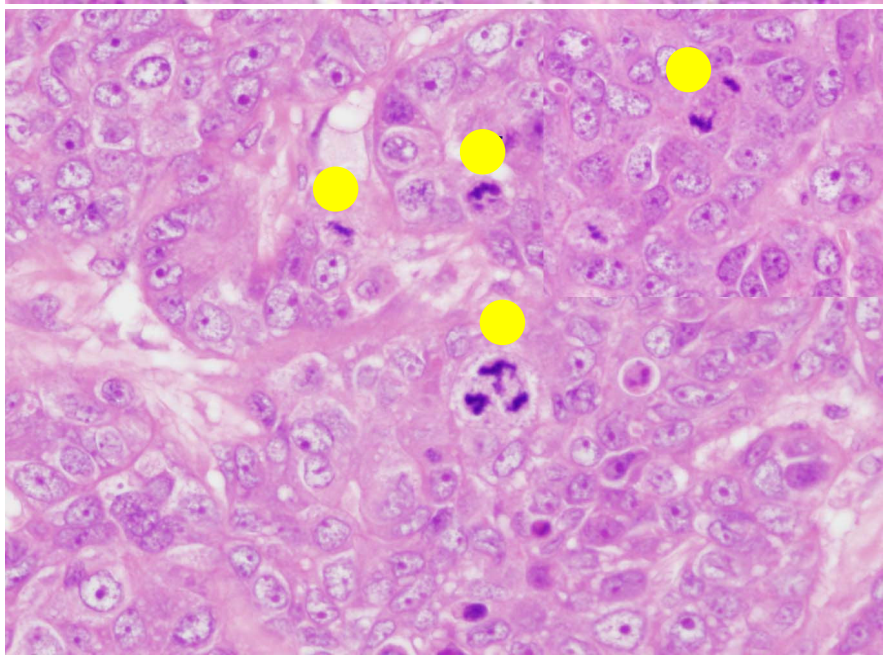
Grading of breast cancer

mitosis:

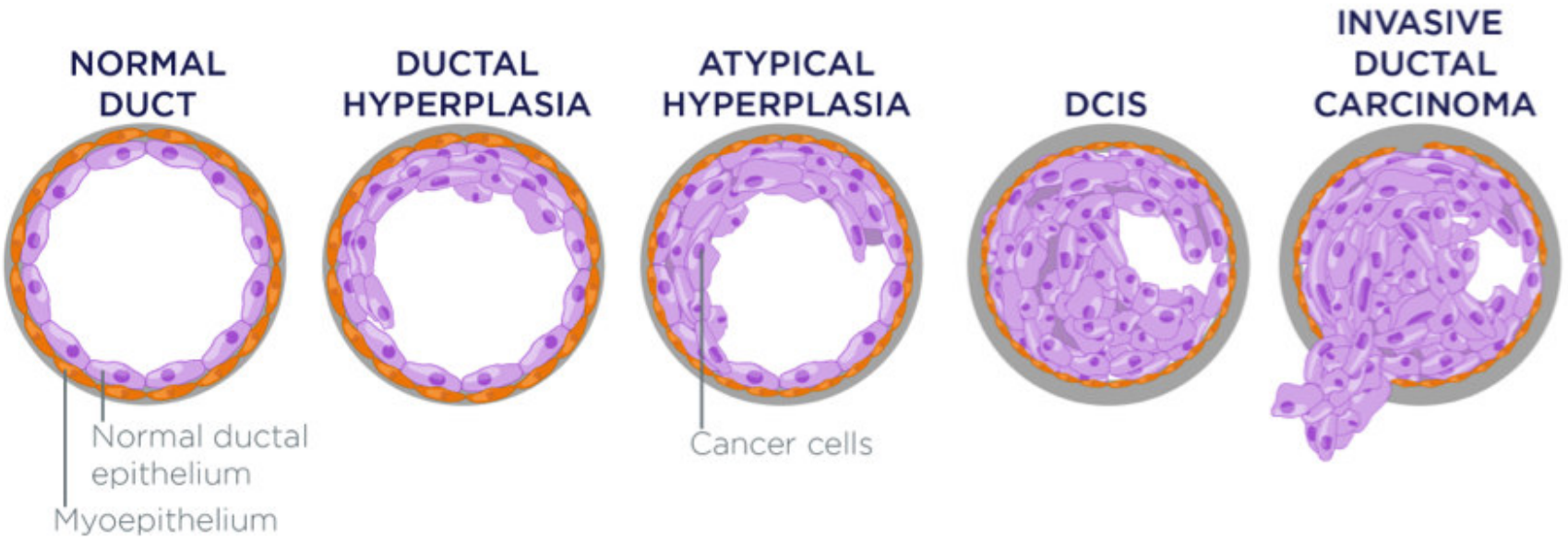
Score 1: <= 7 per 10 fields
Score 2: 8-14 per 10 fields
Score 3: > 15 per 10 fields







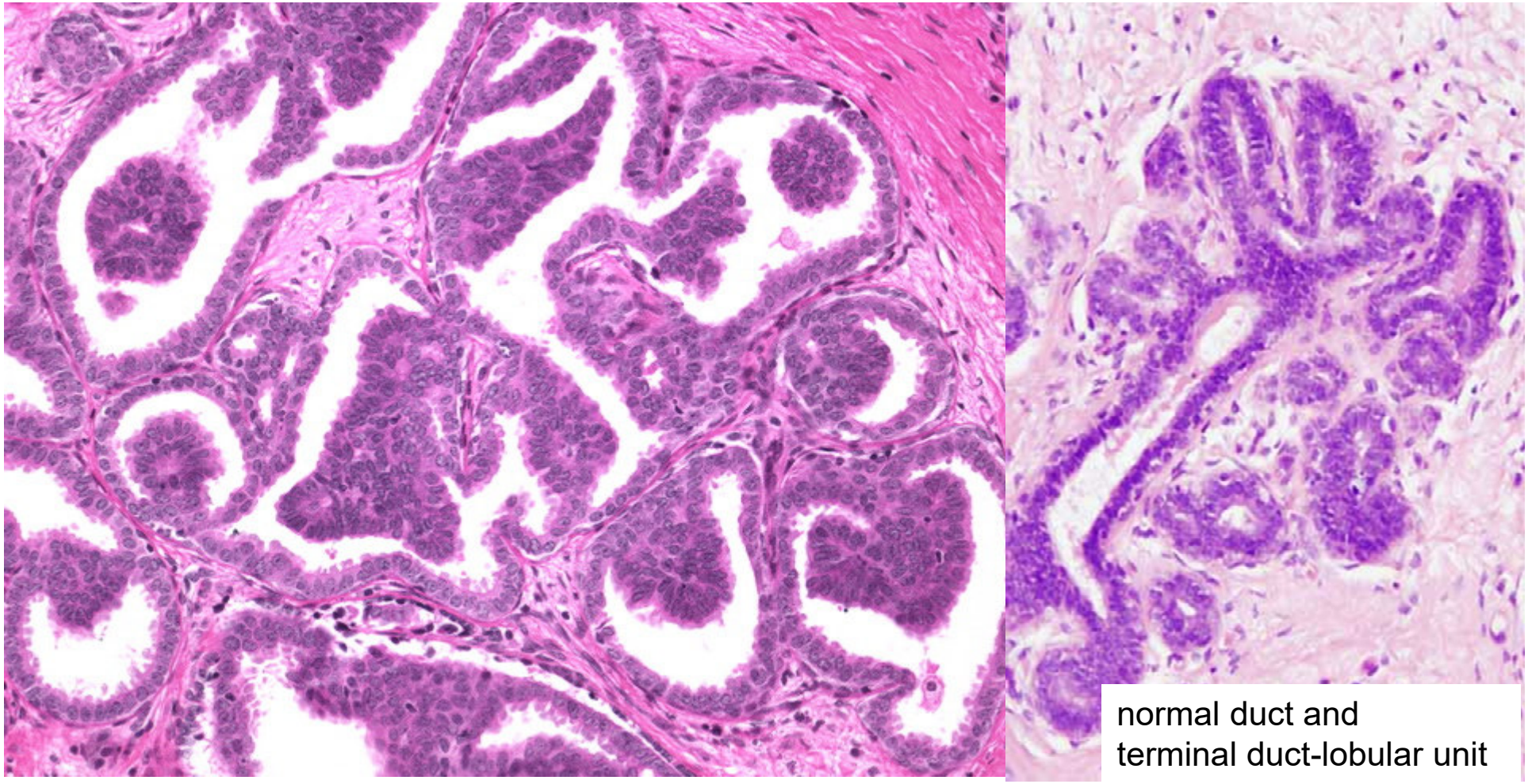
Tumor growth patterns during cancer progression



ADH (Atypical Ductal Hyperplasia) is formed from a uniform population of small or medium-sized, round, cuboidal or polygonal hyperchromatic cells, which are regularly arranged. The nuclei are evenly distributed and may form a rosette-like pattern. Only single, small nucleoli are present. Mitoses, particularly abnormal forms, are infrequently seen.

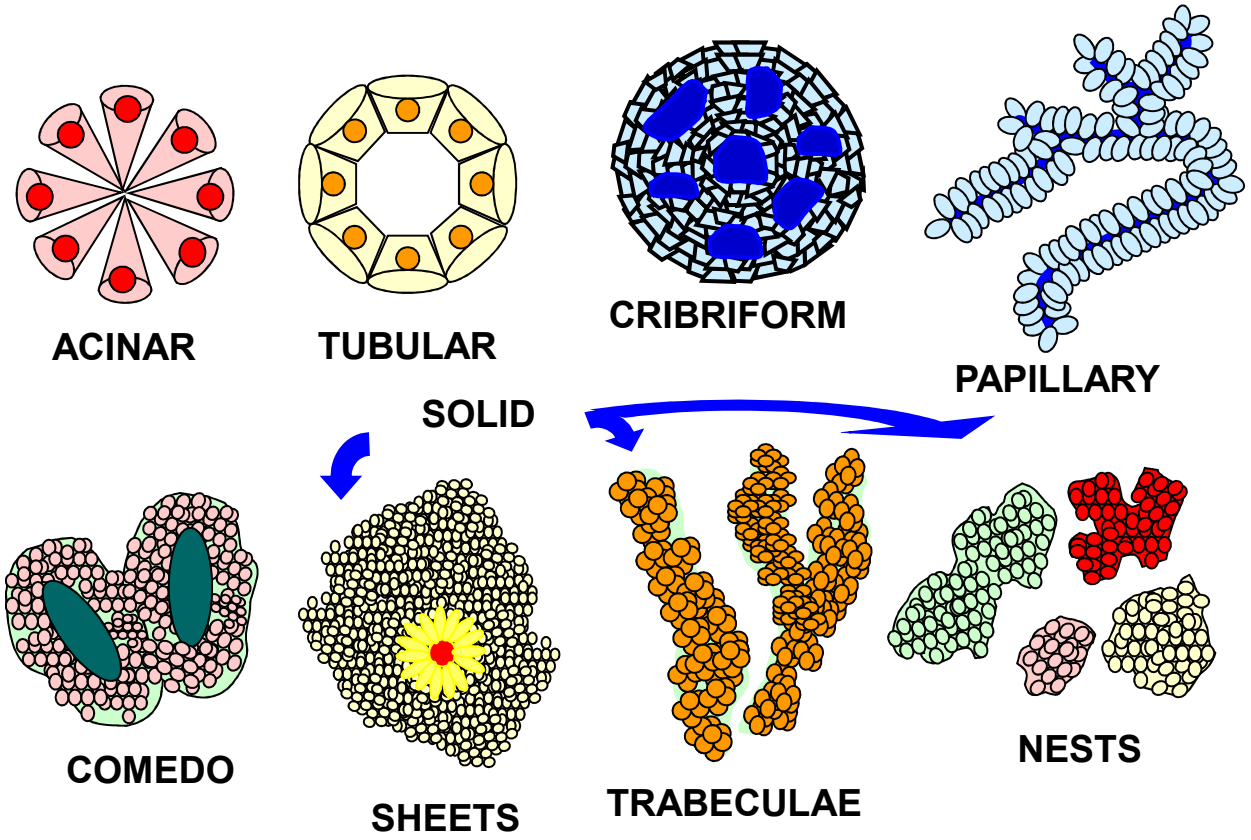
DCIS (Ductal Carcinoma In Situ) is defined as the proliferation of malignant epithelial cells within the breast parenchymal structures with no evidence of invasion across the basement membrane.

Ductal Hyperplasia



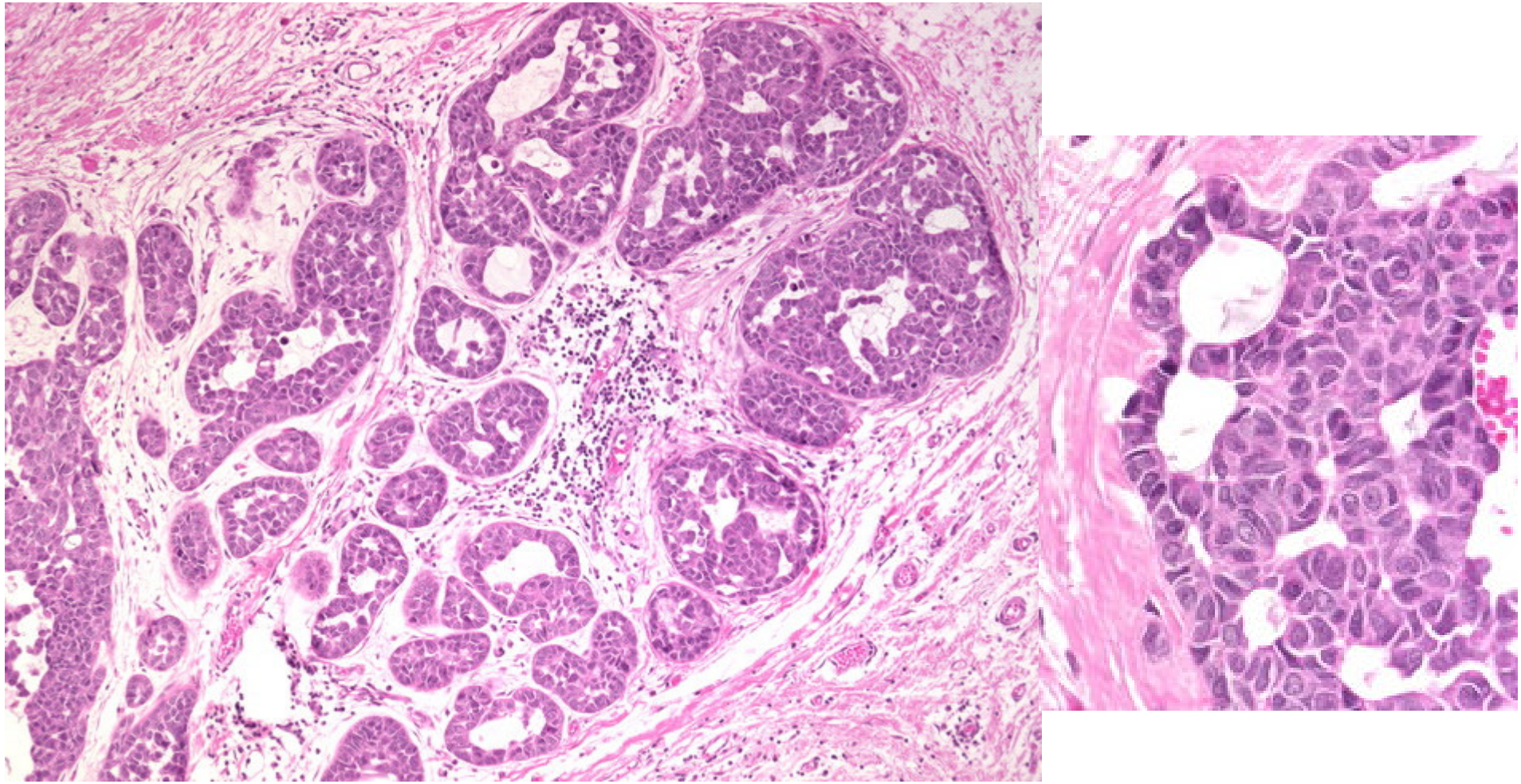
- intraluminal proliferation may create a solid ball of epithelium attached to one side of the duct and surrounded by a cleft-like space
- cells maintain normal morphology

Tumor growth patterns



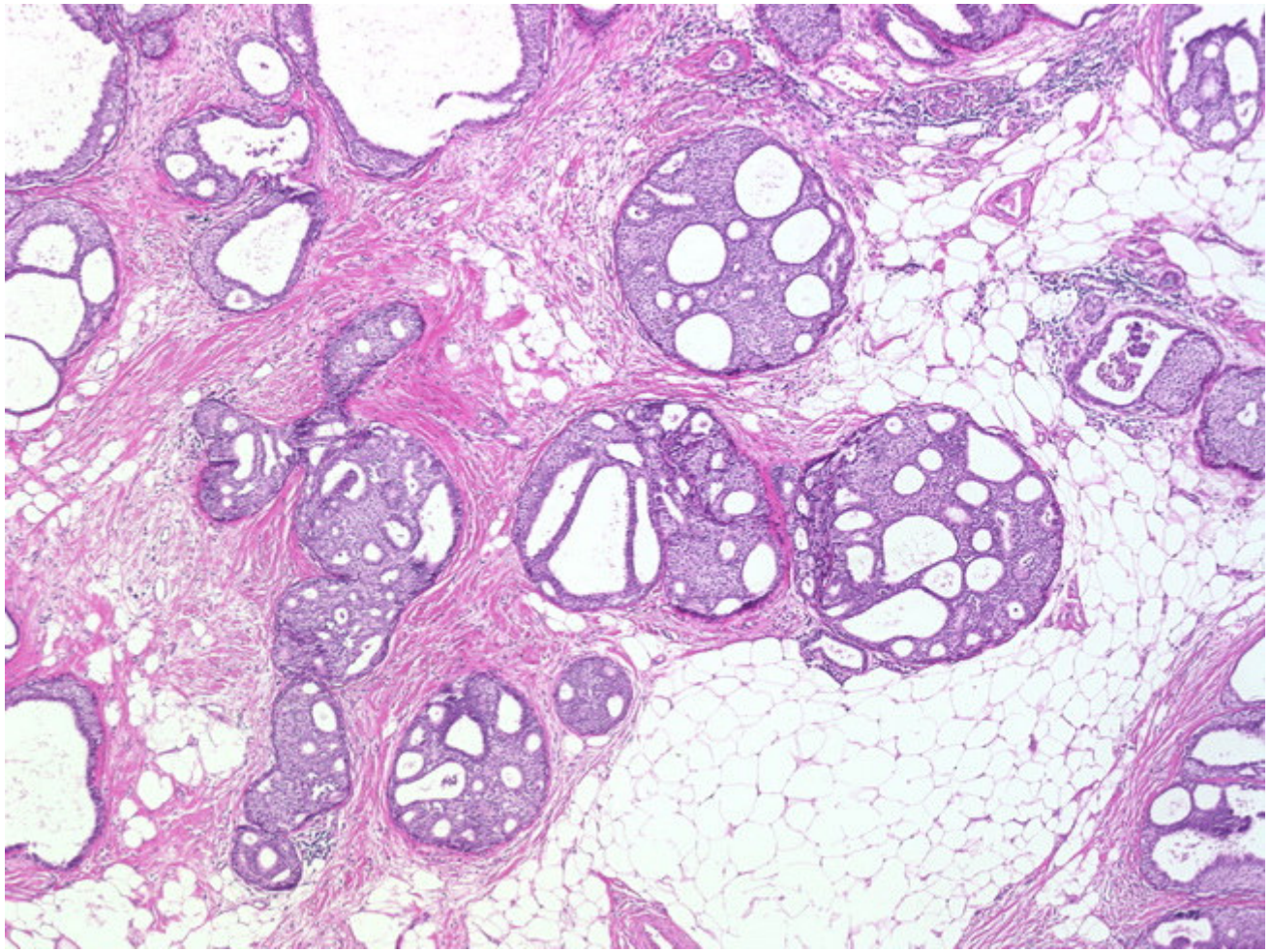
- variations in tumor growth patterns range from the well-differentiated acinar pattern to tubular, papillary, comedo (with central necrosis) and solid, forming sheets, trabeculae or nests
- a single tumor can display one or even all of these features. Acinar/alveolar structures can be absent and then it becomes difficult to classify the tumor. In such instances it may be useful to have an additional category, such as carcinoma NOS, meaning not otherwise specified

Ductal Carcinoma-In-Situ



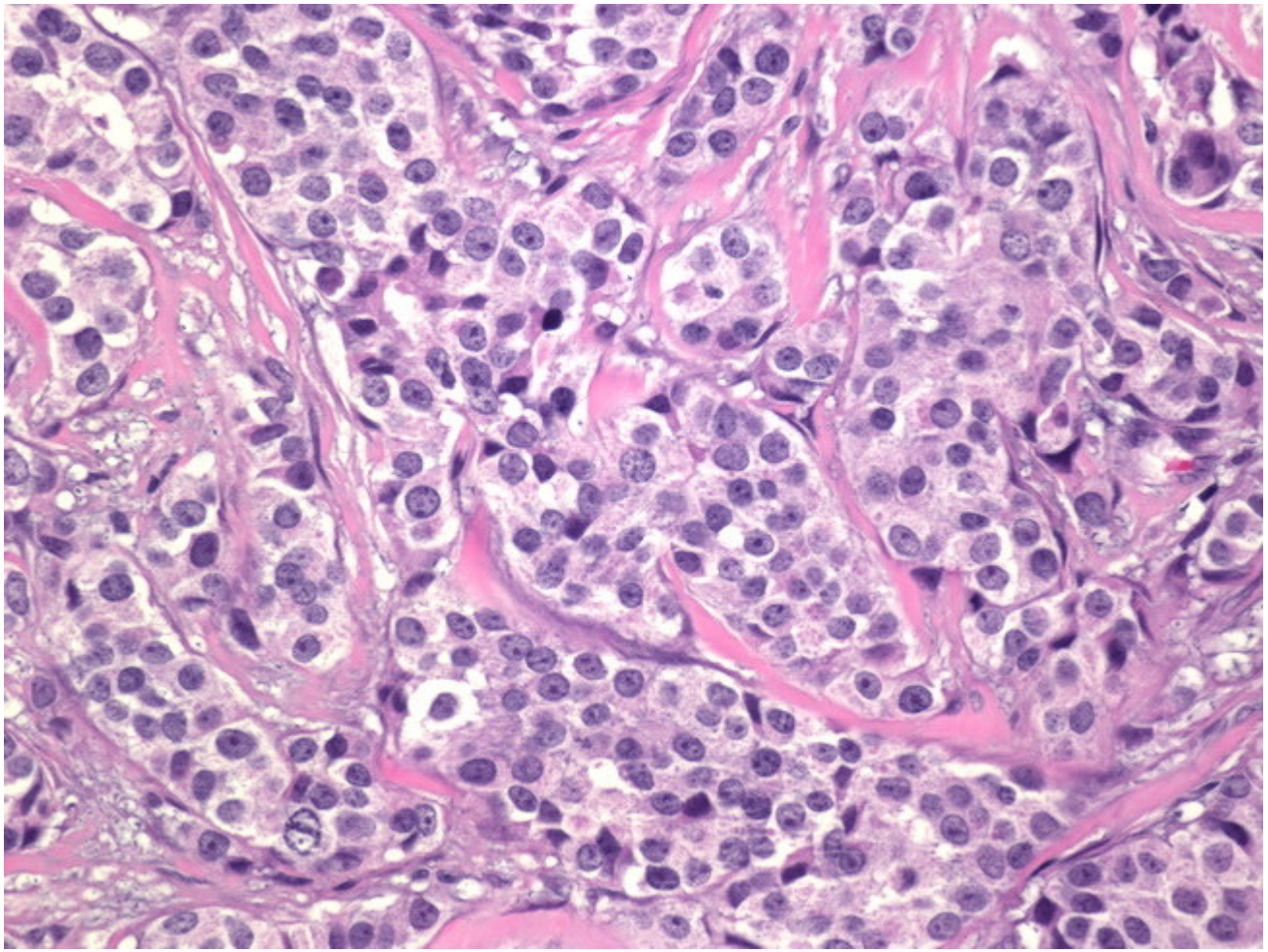
- non-invasive cancerous lesion, about 20–30% will progress to breast cancer without treatment
- carcinoma cells are present within structures clearly identifiable as a (greatly expanded) lobules
- tumor cells have high nuclear grade but maintain cell-to-cell adhesion

Ductal Carcinoma-In-Situ



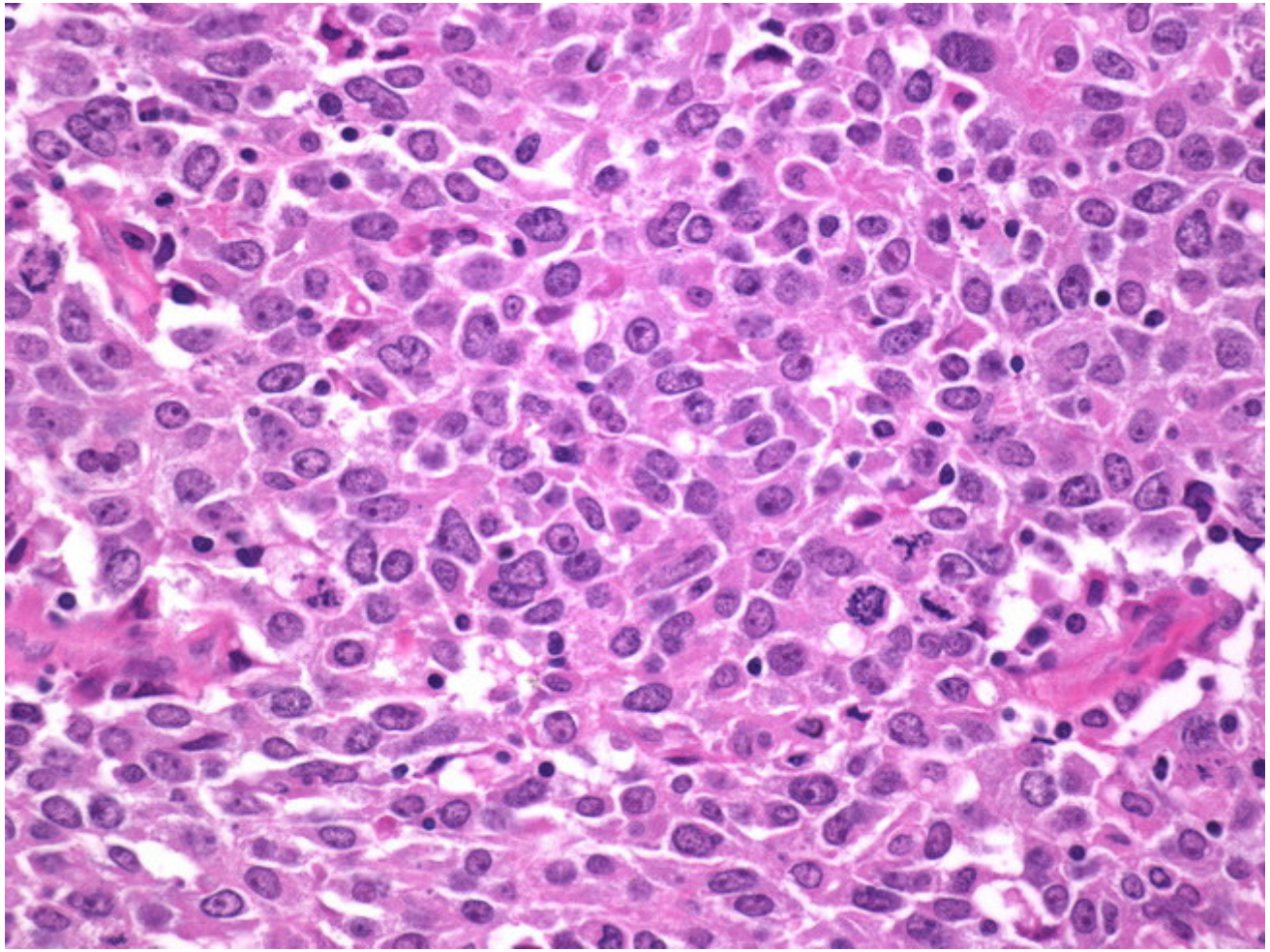
in **cribriform** DCIS, **round fenestrations** are found within the glands; the more regular these spaces are in size, shape and distribution, the more likely the lesion to be malignant

Infiltrating Ductal Carcinoma



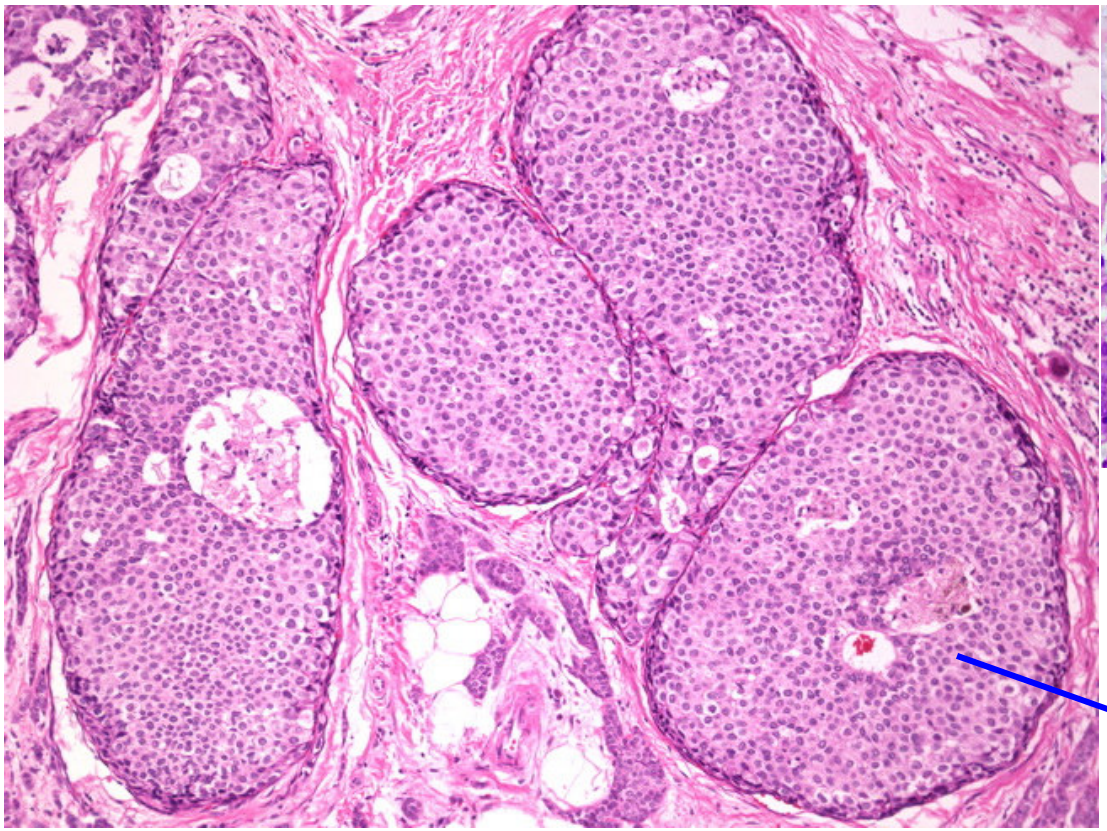
Grade II (using Nottingham modification of Bloom-Richardson system - the grade is obtained by adding up the scores for tubule formation, nuclear pleomorphism, and mitotic count)
tumor cells are arranged in cords, nests, and as individual cells.

Infiltrating Ductal Carcinoma

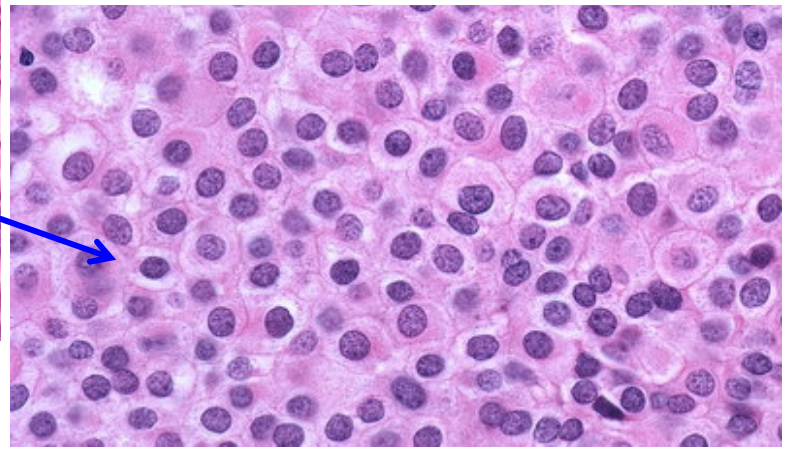


Grade III
tumor cells are highly pleomorphic and show frequent mitotic figures
no evidence of tubule formation

Lobular Carcinoma-In-Situ

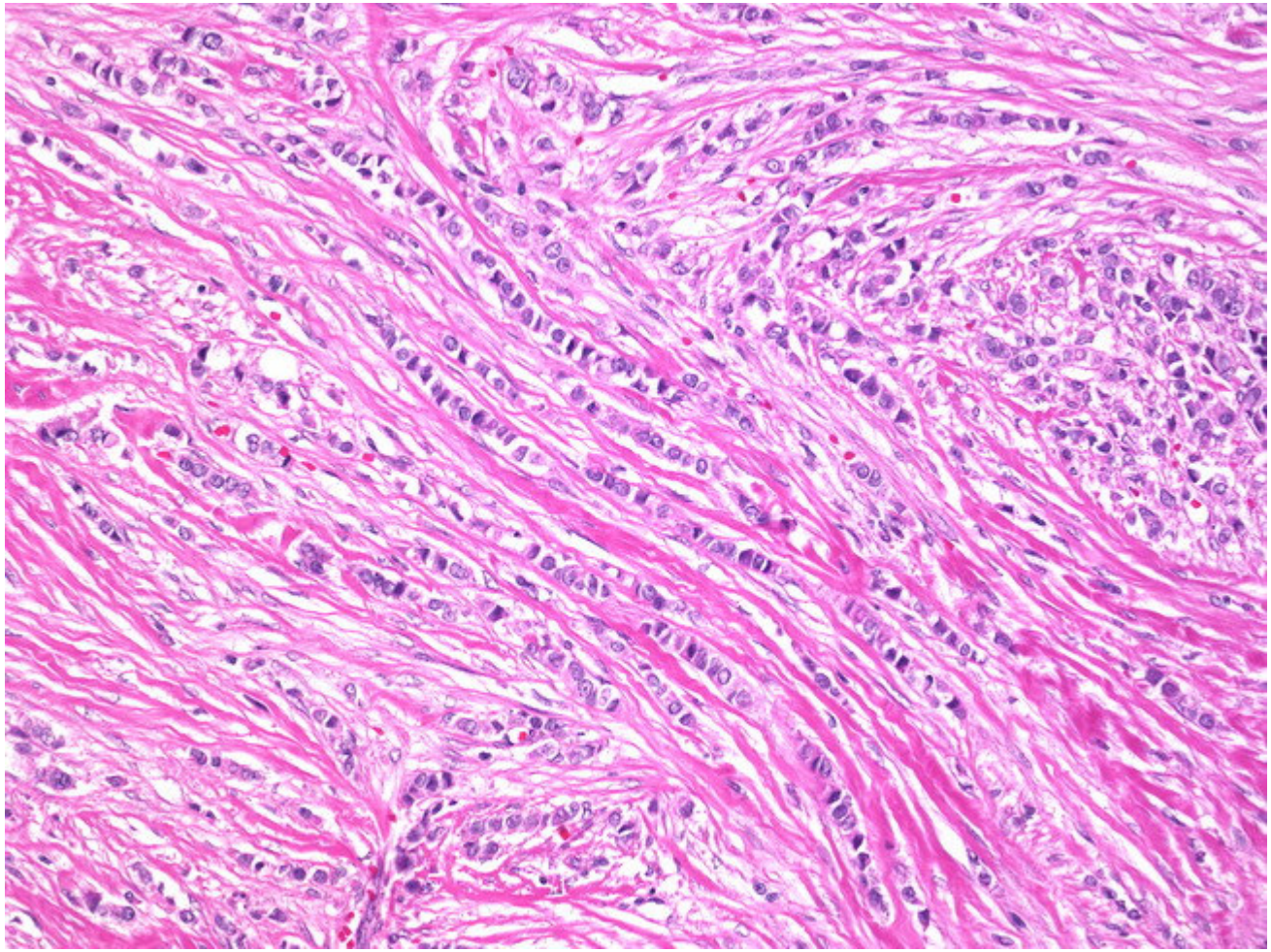


normal duct and
terminal duct-lobular unit



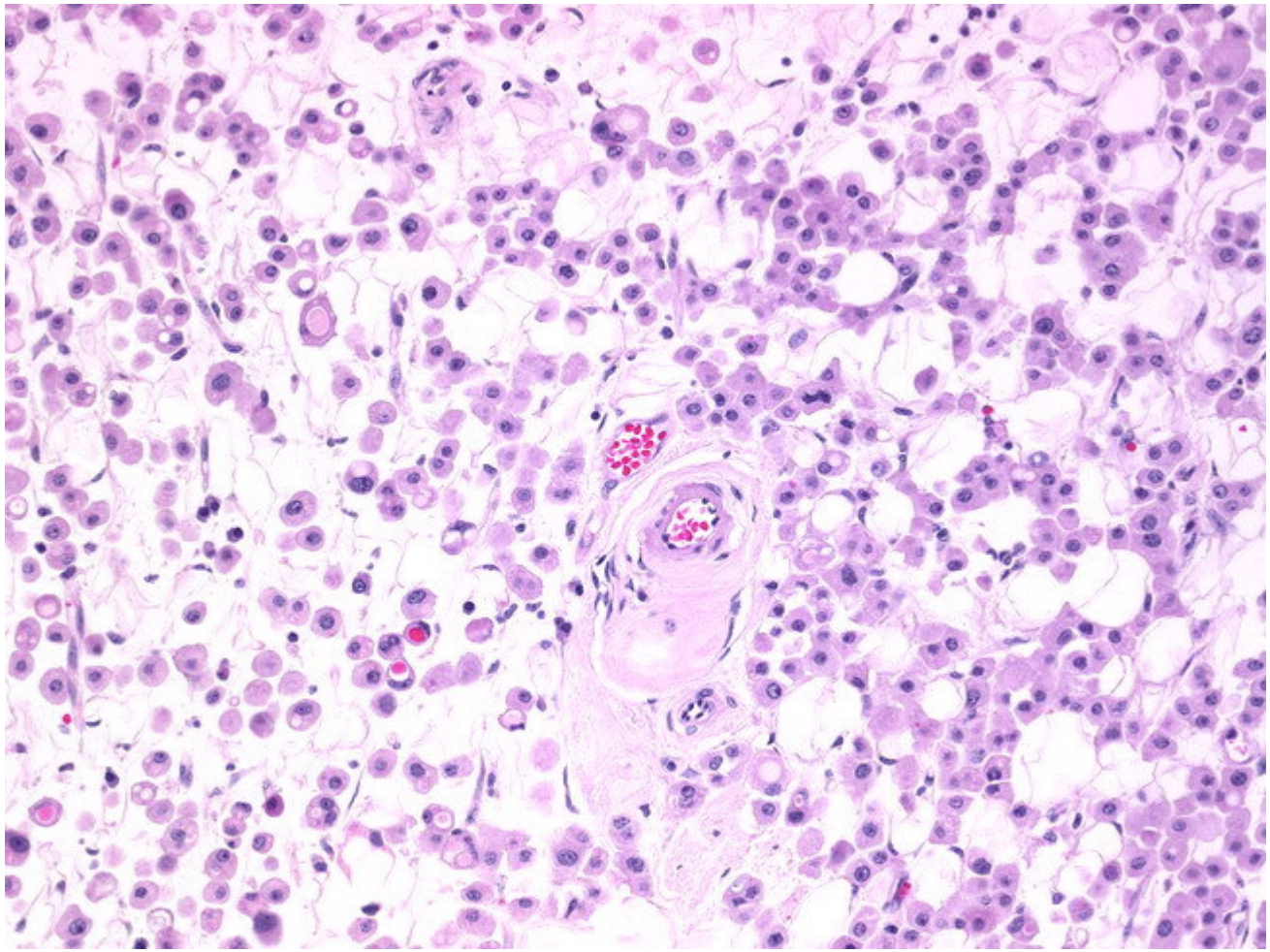
lobules are distended with uniform round or oval, medium sized cells
typically loss of E-cadherin mediates change in cell shape
nuclei are uniform and normochromatic
may progress to invasive carcinoma (~3x increased life-time risk)

Infiltrating Lobular Carcinoma



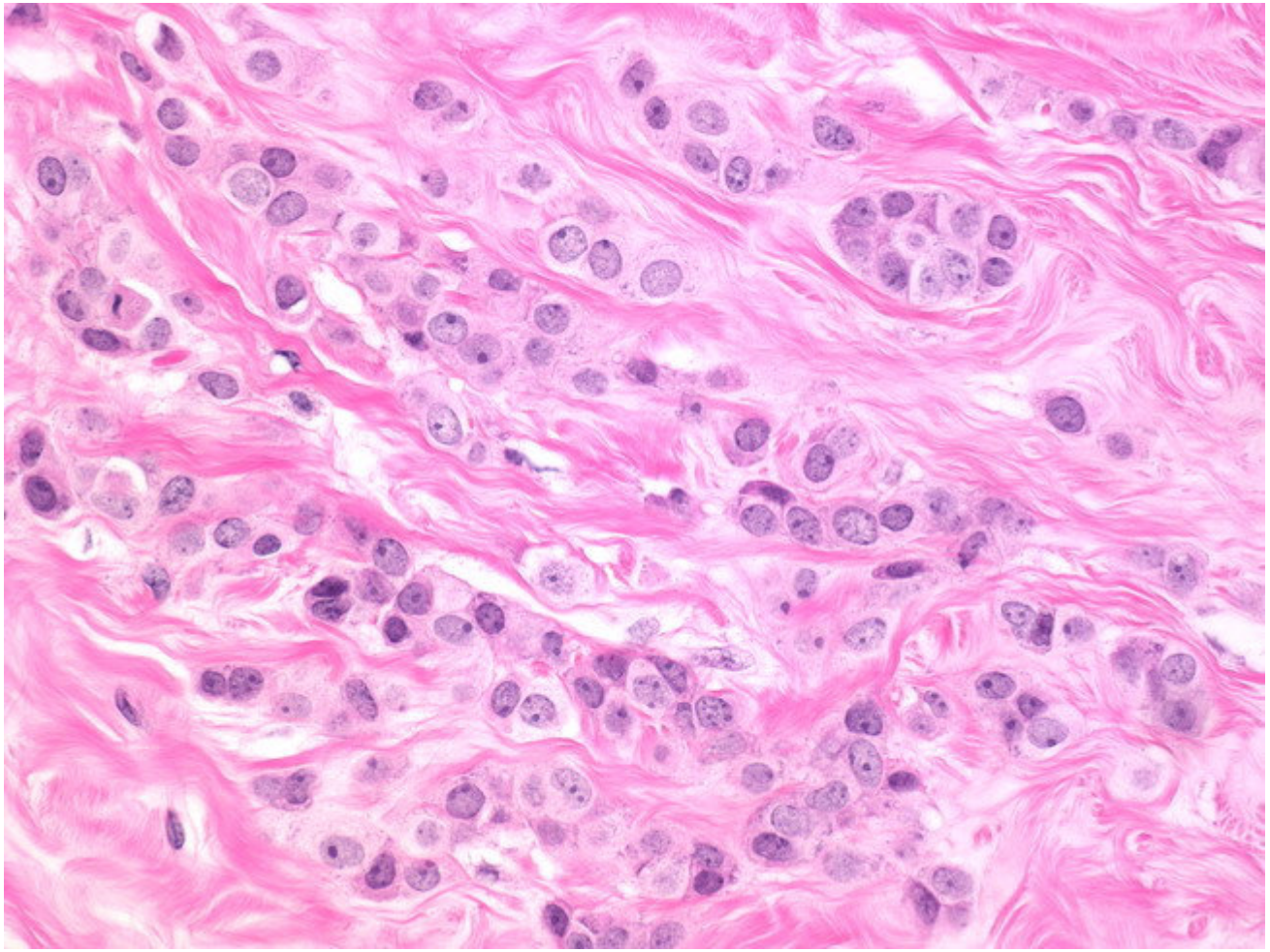
absence of solid, alveolar, papillary, or gland-forming units
tumor cells are arranged in slender linear strands (so-called Indian filing)

Infiltrating Lobular Carcinoma



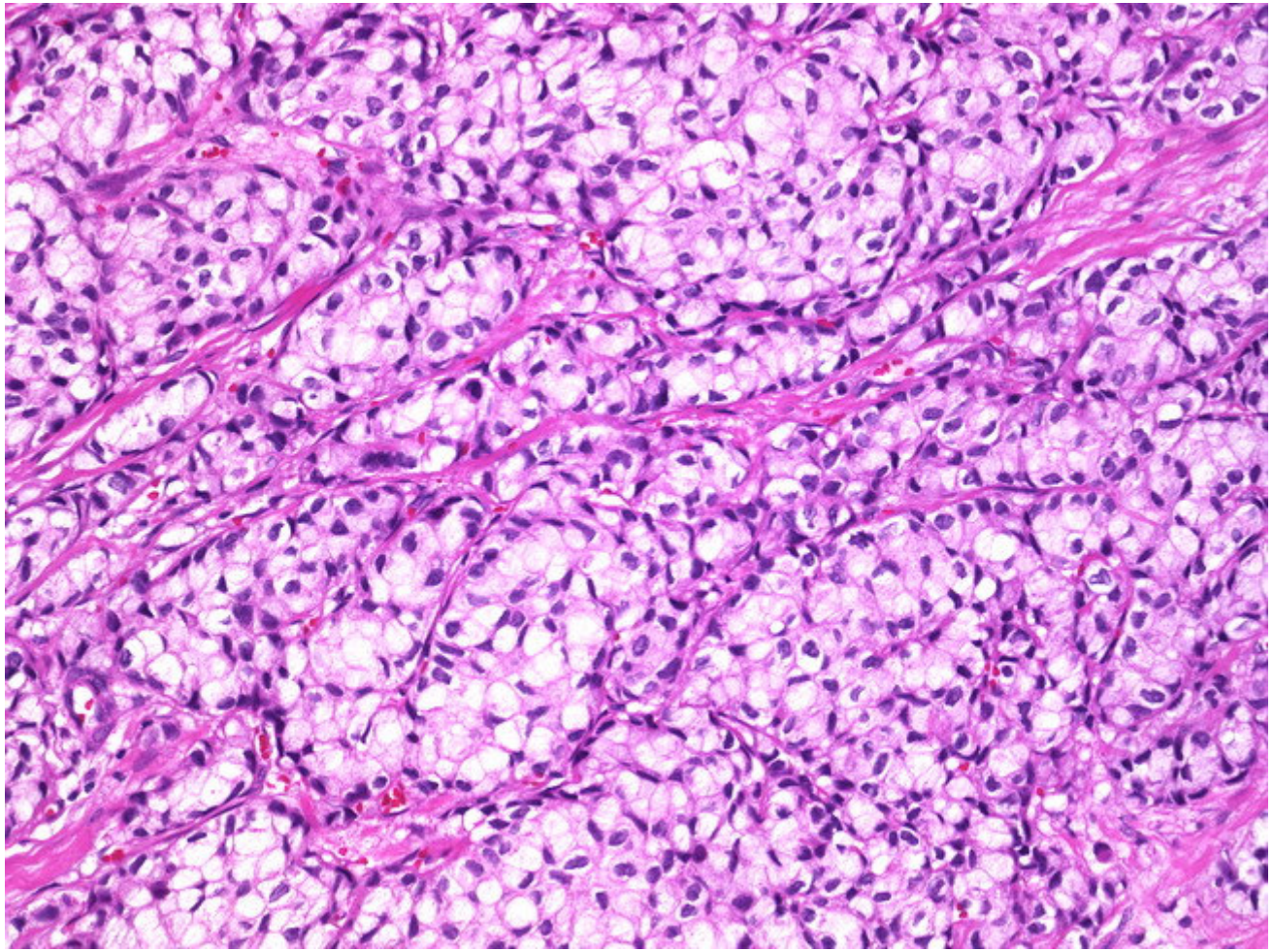
invasive carcinoma showing apocrine differentiation
tumor cells infiltrate singly or in small clusters and have abundant granular, foamy cytoplasm and enlarged hyperchromatic nuclei

Infiltrating Lobular Carcinoma



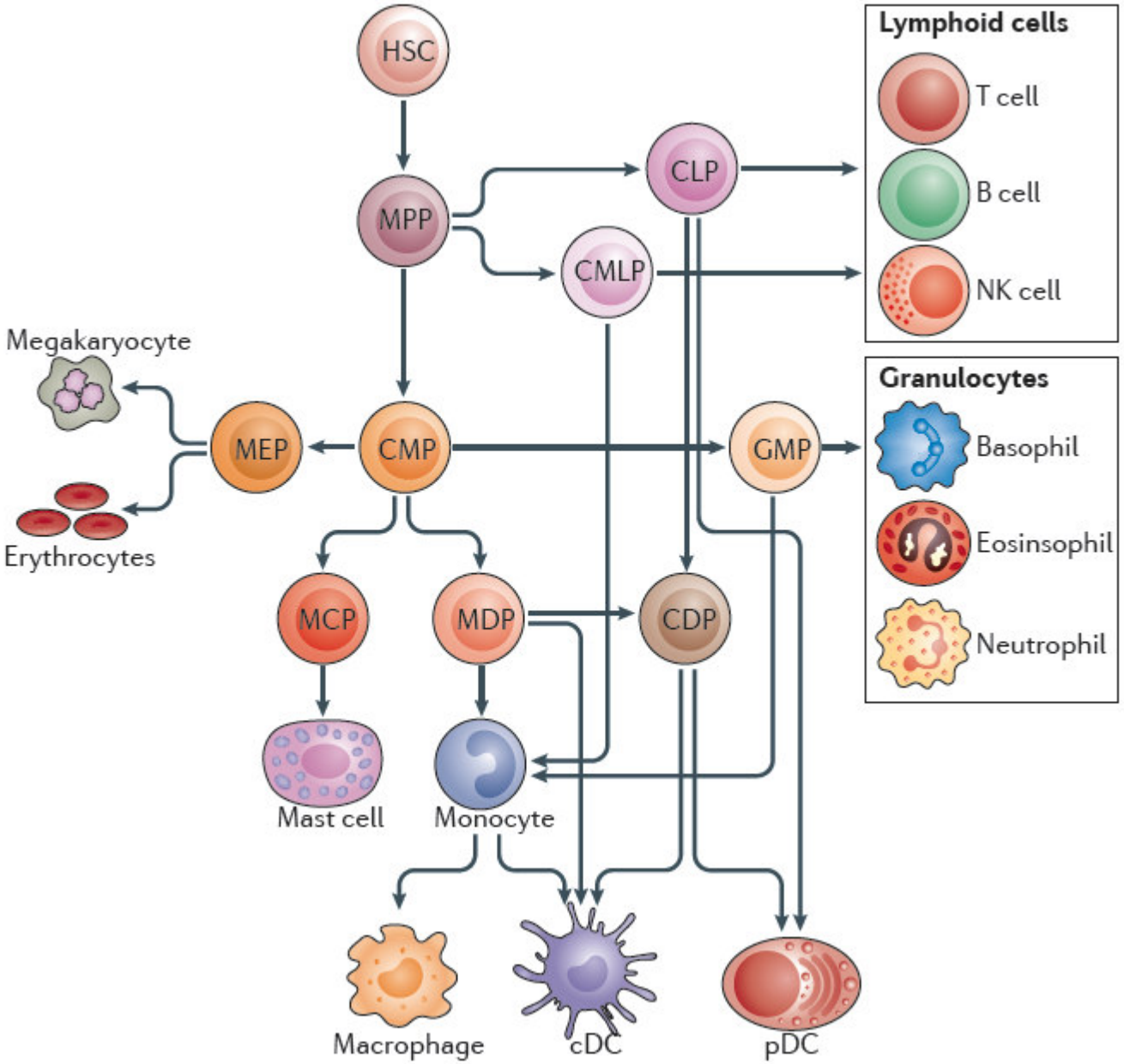
relatively uniform vesicular nuclei with small punctate nucleoli
abundant dense fibrous stroma

Infiltrating Lobular Carcinoma



signet ring morphology in this infiltrating lobular carcinoma

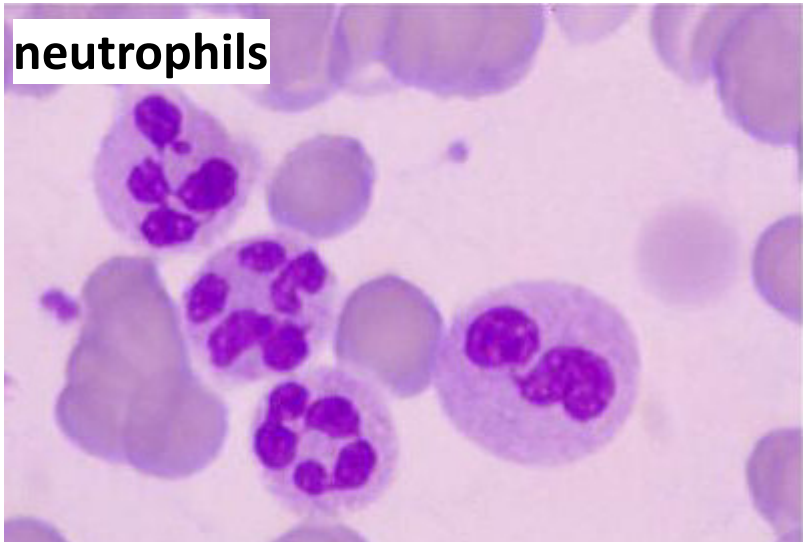
Adult hematopoiesis



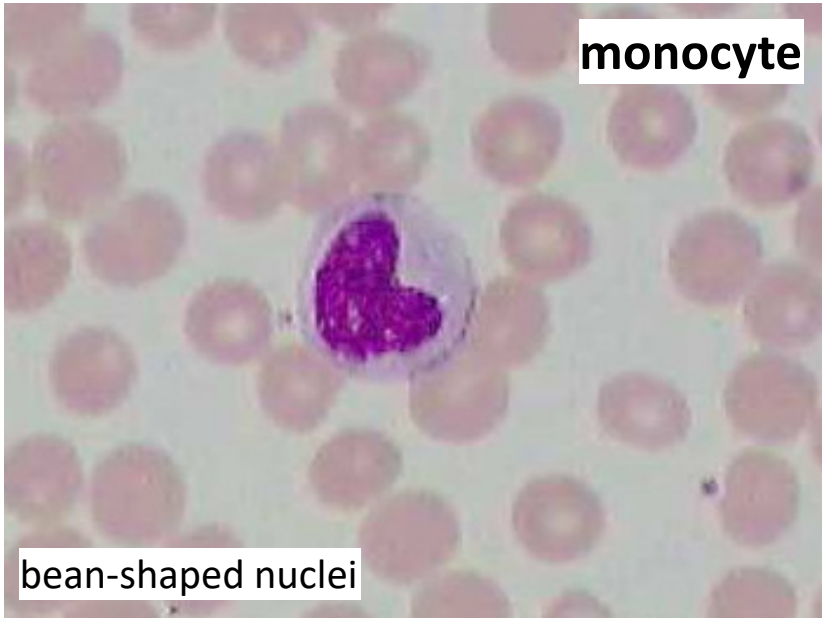
cDC	conventional DC
CDP	common DC progenitor
CLP	common lymphoid progenitor
CMLP	common myelolymphoid progenitor
CMP	common myeloid progenitor
DC	dendritic cell
GMP	granulocyte and macrophage progenitor
MCP	mast cell progenitor
MDP	macrophage and DC progenitor
MEP	megakaryocyte and erythroid progenitor
NK	natural killer
pDC	plasmacytoid DC

Gabrilovich et al. Nat. Rev. Imm. 2012

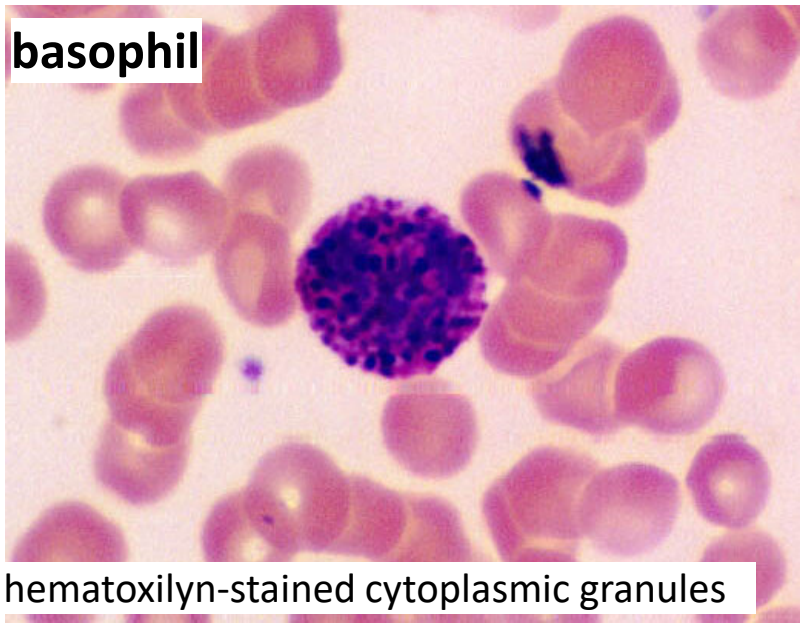
Innate immune cells



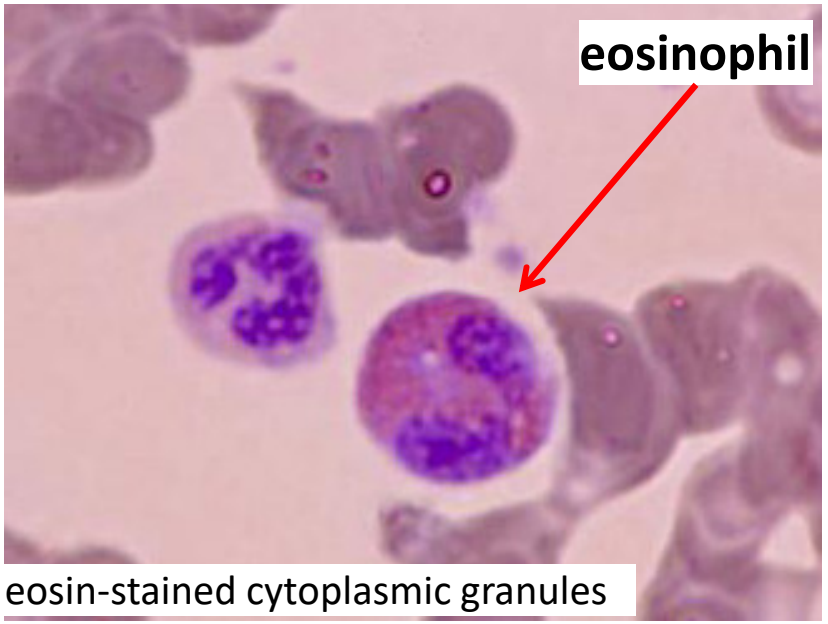
polymorphic nuclei to facilitate tissue invasion



bean-shaped nuclei



hematoxilyn-stained cytoplasmic granules

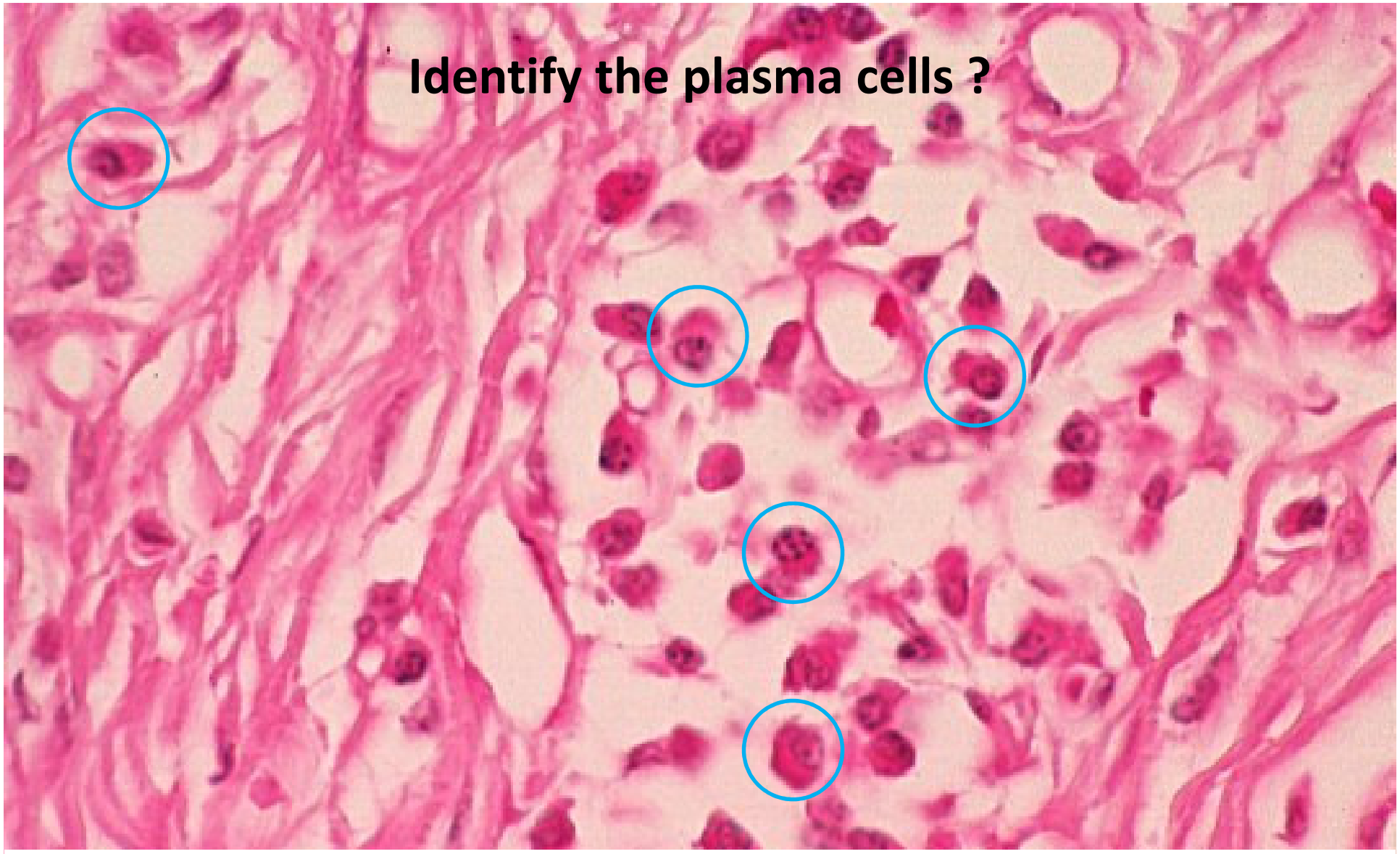


eosin-stained cytoplasmic granules

Identify the plasma cells ?

Plasma cells make antibodies and are identified by:

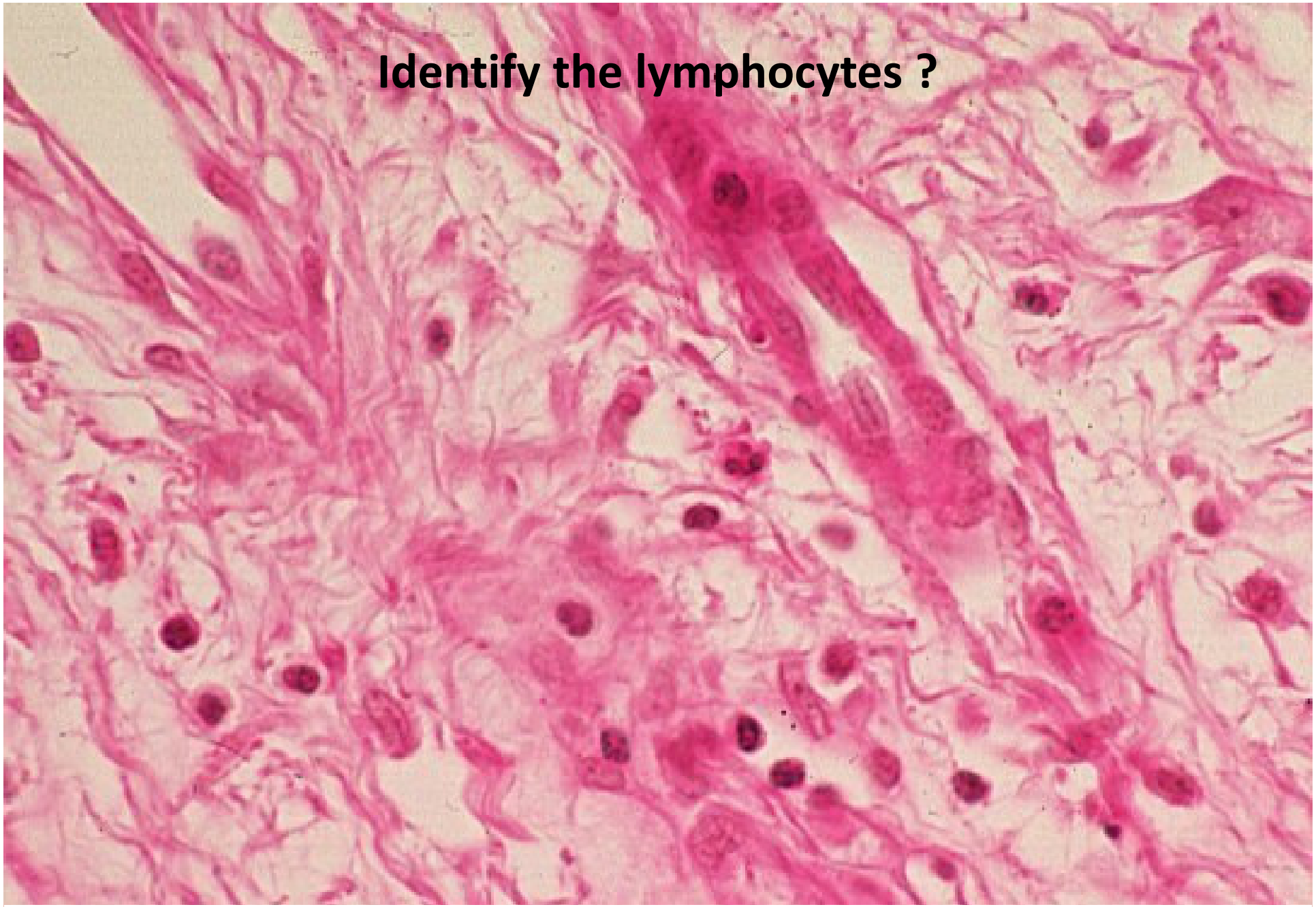
- abundant, deeply-staining (usually purple) cytoplasm
- a round, eccentrically-located nucleus
- clumps of chromatin arranged around the edge of the nucleus, like the numbers on a clock or a soccer ball
- a pale spot next to the nucleus; this is the Golgi apparatus



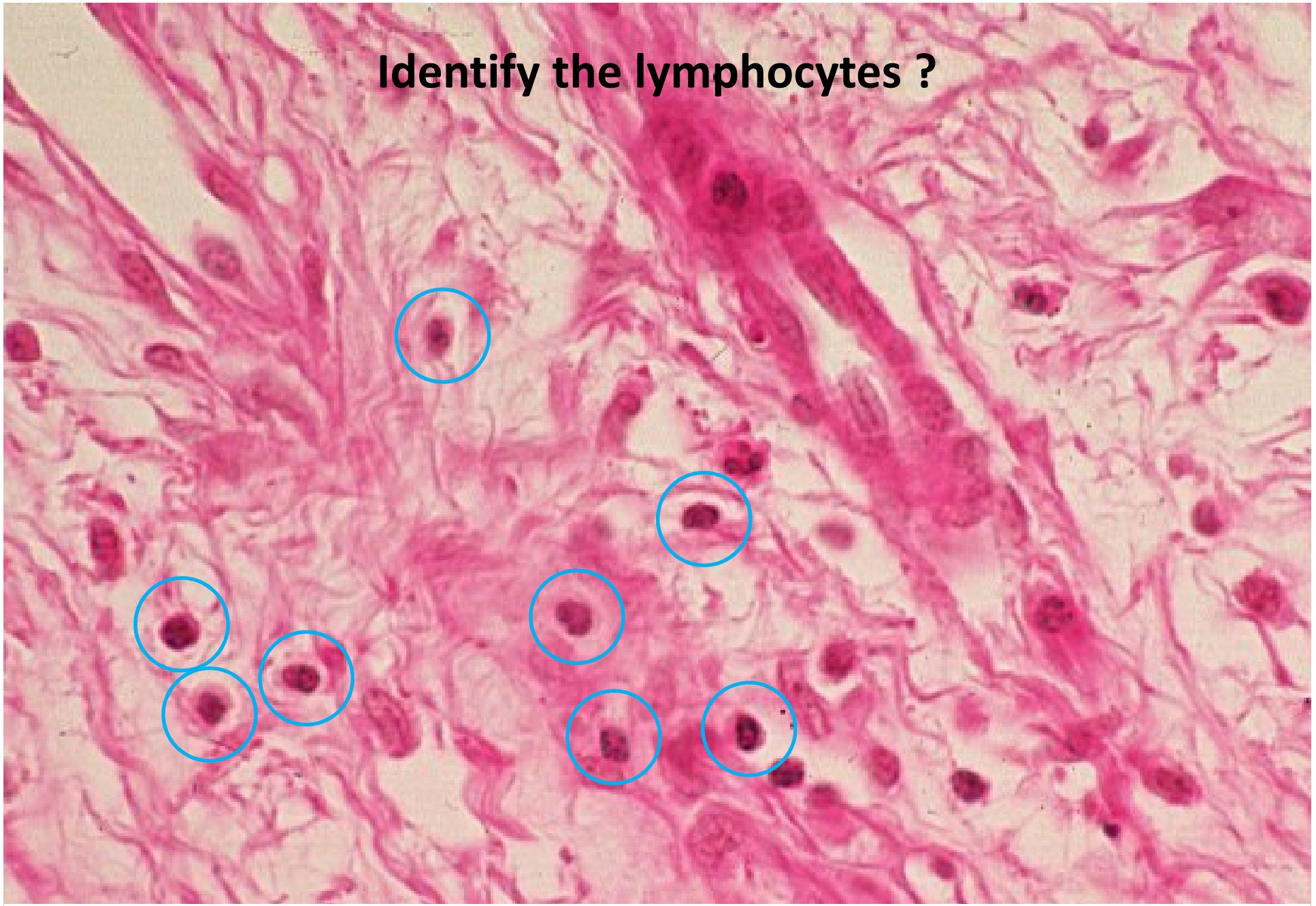
Plasma cells make antibodies and are identified by:

- abundant, deeply-staining (usually purple) cytoplasm
- a round, eccentrically-located nucleus
- clumps of chromatin arranged around the edge of the nucleus, like the numbers on a clock or a soccer ball
- a pale spot next to the nucleus; this is the Golgi apparatus

Identify the lymphocytes ?

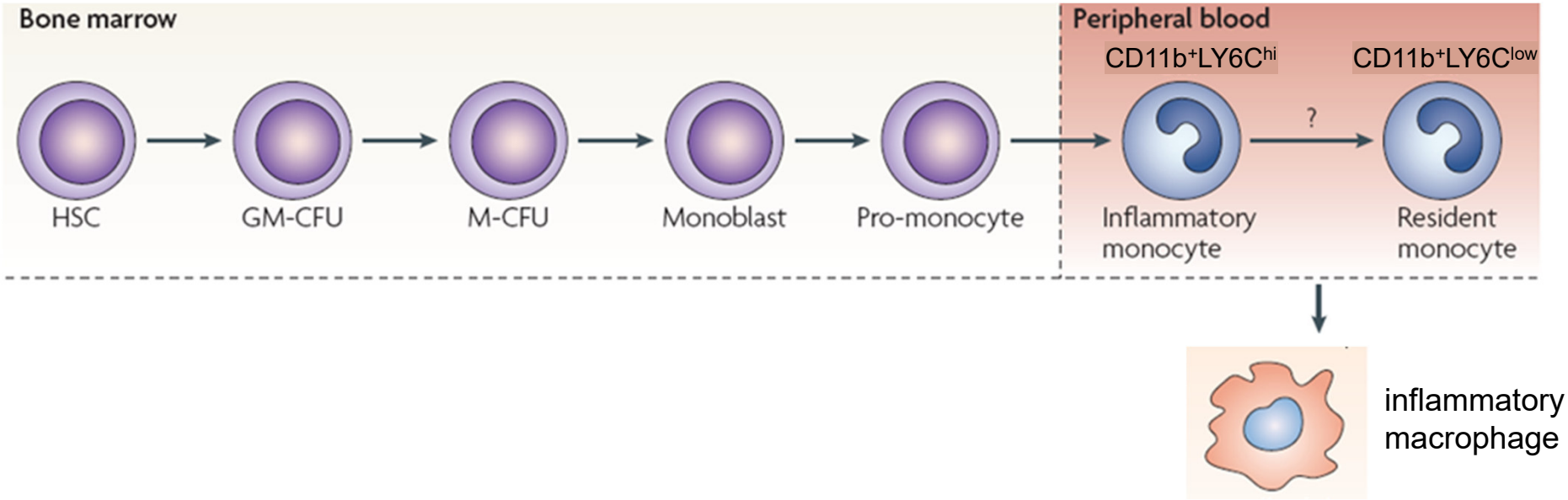


lymphocytes have scant cytoplasm and nuclei with a preponderance of heterochromatin being always clumpy and separated by thin strips of euchromatin



lymphocytes have <u>scant cytoplasm</u> and nuclei with a preponderance of heterochromatin being always clumpy and separated by thin strips of euchromatin

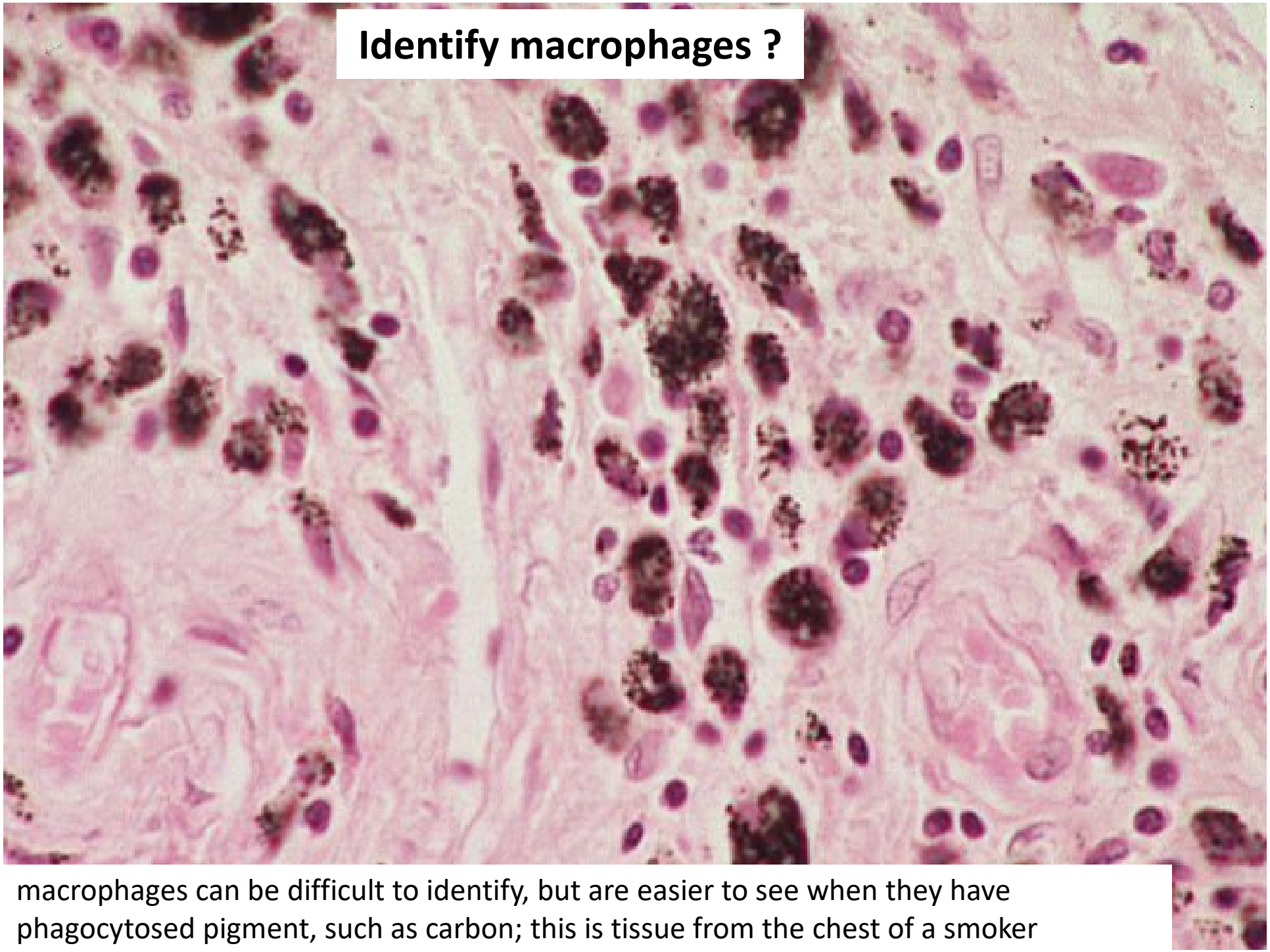
Origin of monocytes and inflammatory macrophages



monocytes are constantly generated in the bone marrow and enter the bloodstream as two distinct populations:

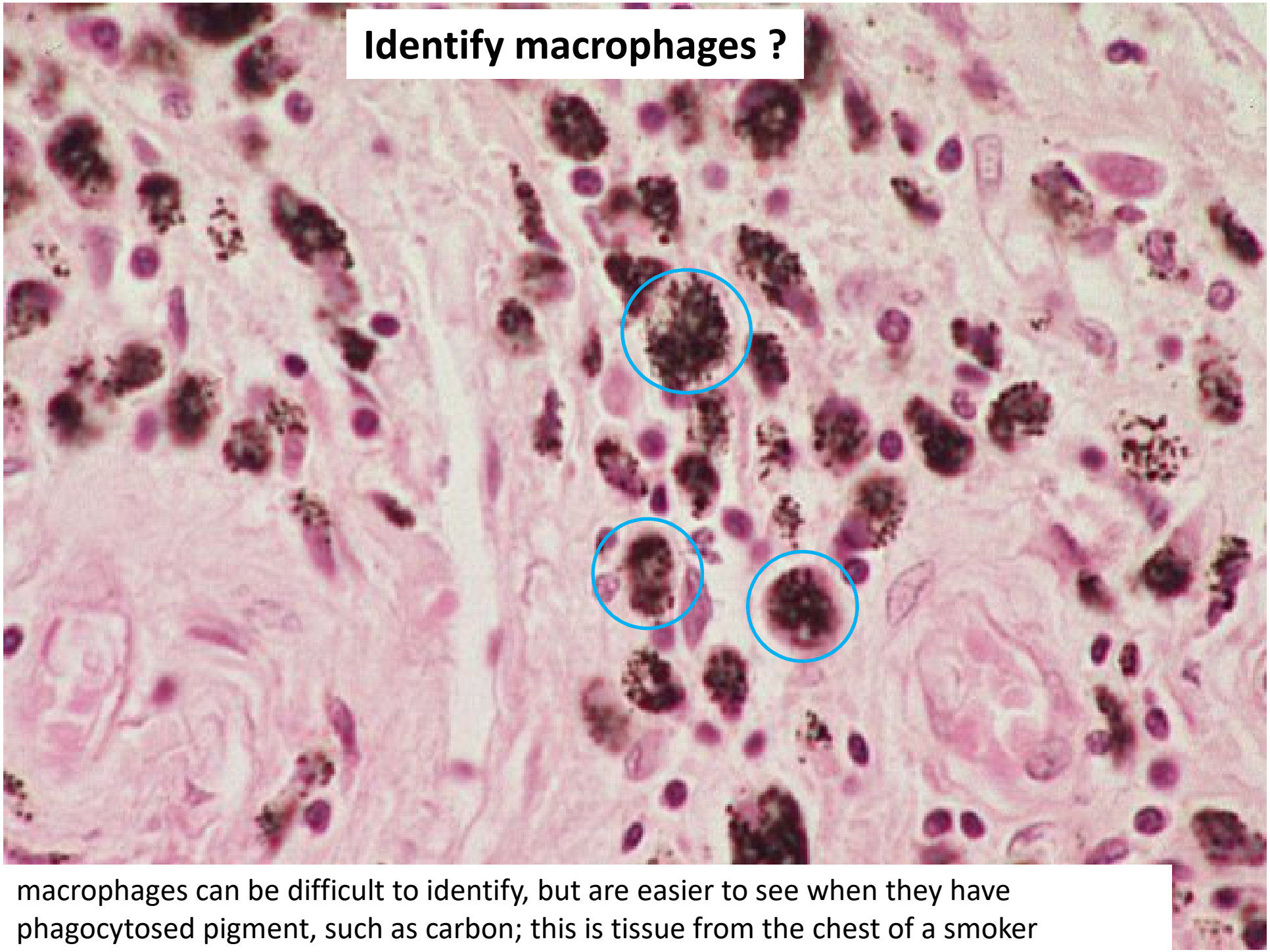
- **inflammatory monocytes** ($CD11b^{+}LY6C^{hi}CCR2^{+}$) which rapidly exit the blood
- **blood-resident monocytes** ($CD11b^{+}LY6C^{-low}CX3CR1^{+}$) which circulate and patrol the luminal side of the endothelium of small blood vessels

monocytes migrate to different tissues, where they differentiate into inflammatory macrophages (with a bias to M1-type for inflammatory monocytes and M2-type for resident monocytes) characterized by often large size with an extended cytoplasm.

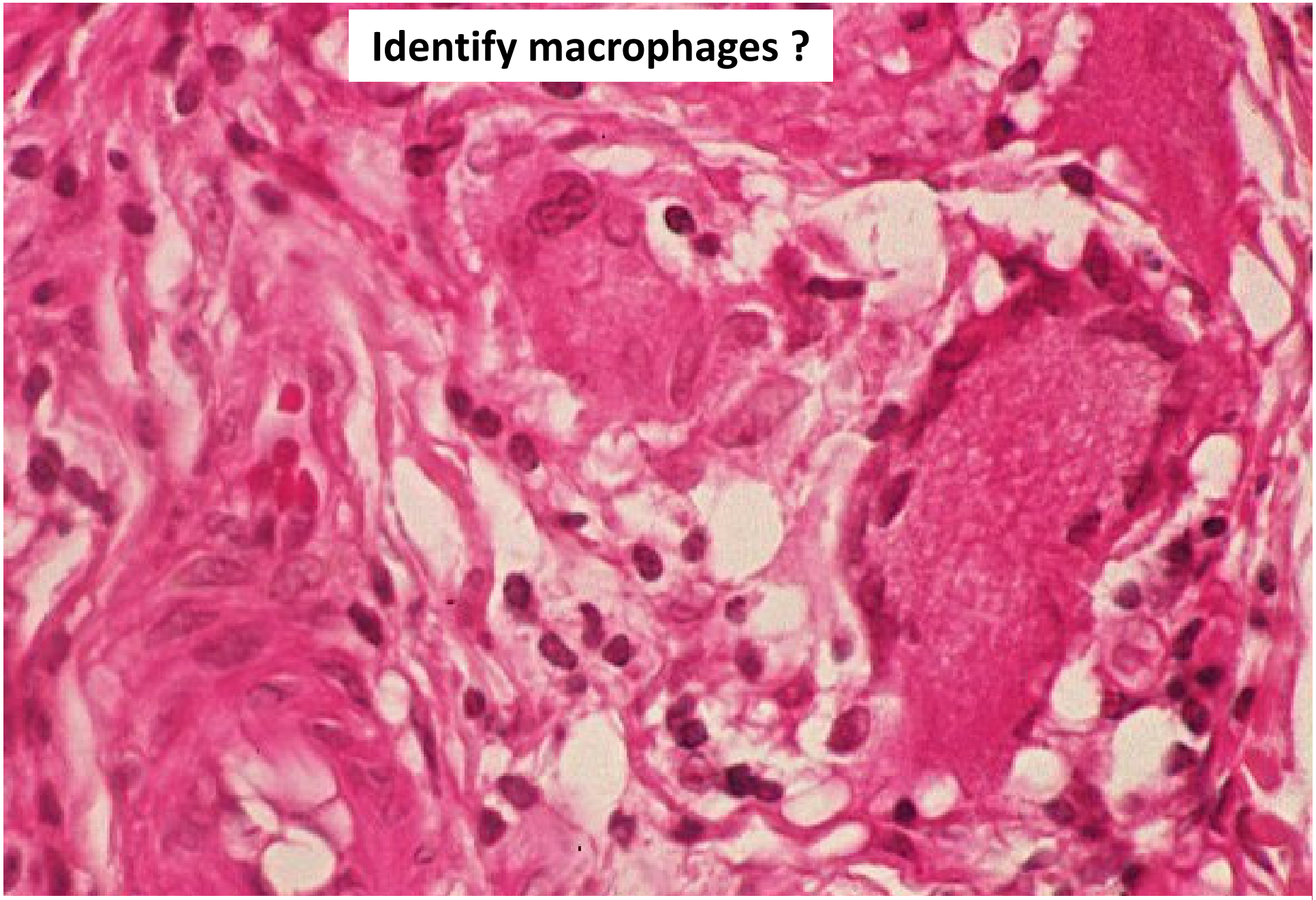


macrophages can be difficult to identify, but are easier to see when they have phagocytosed pigment, such as carbon; this is tissue from the chest of a smoker

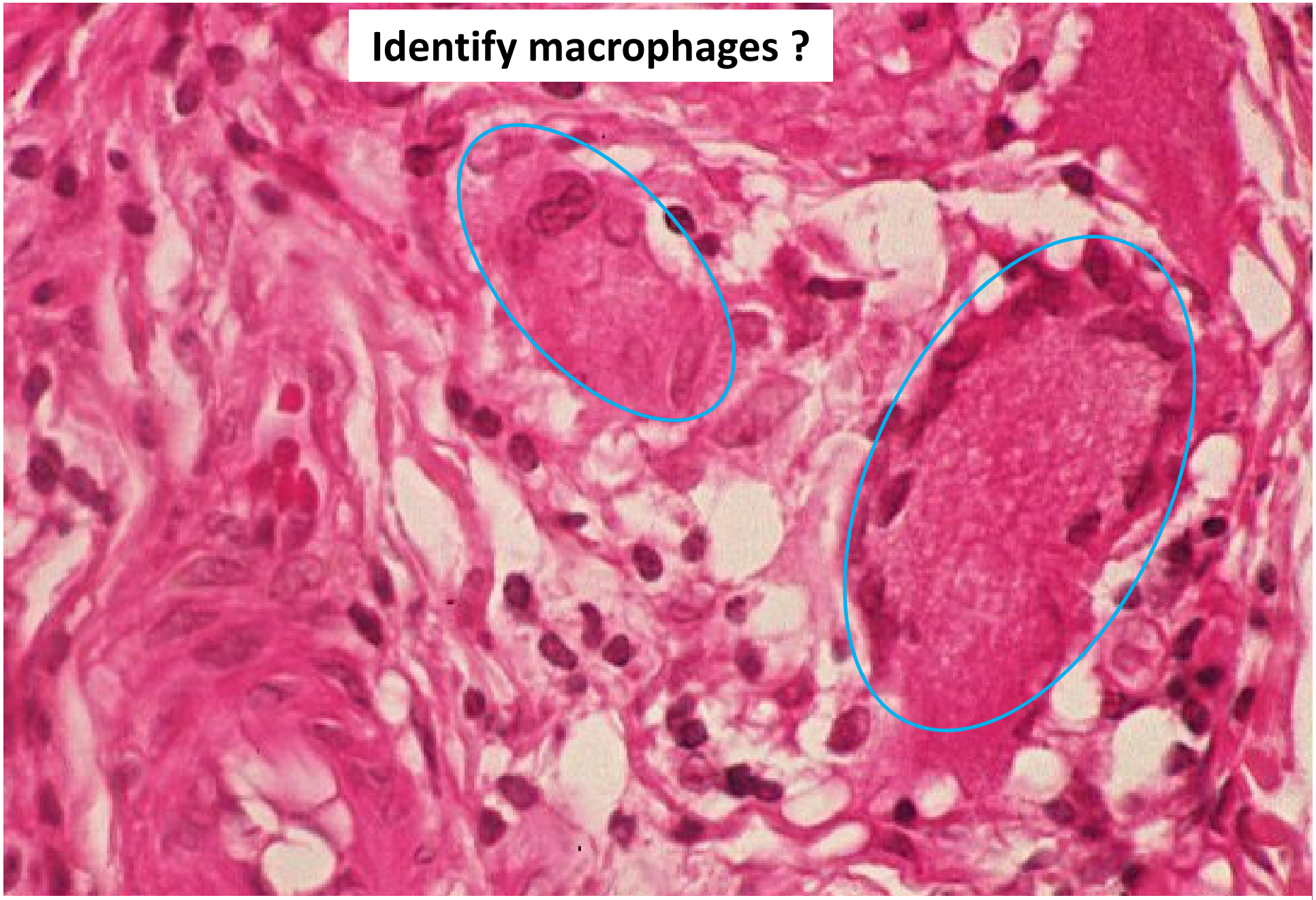
Identify macrophages ?



macrophages can be difficult to identify, but are easier to see when they have phagocytosed pigment, such as carbon; this is tissue from the chest of a smoker



if macrophages get activated they can fuse to form **giant cells**, with nuclei which seem to overlap within a mass of cytoplasm; often the nuclei are arranged around the edge of the cytoplasm



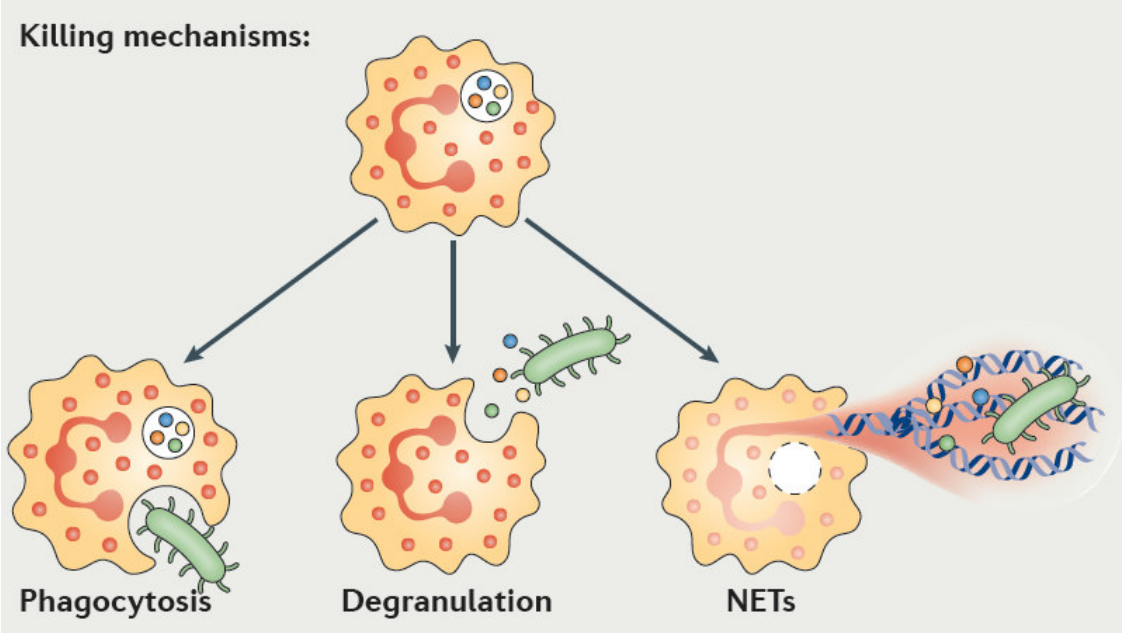
if macrophages get activated they can fuse to form giant cells, with nuclei which seem to overlap within a mass of cytoplasm; often the nuclei are arranged around the edge of the cytoplasm

Neutrophils

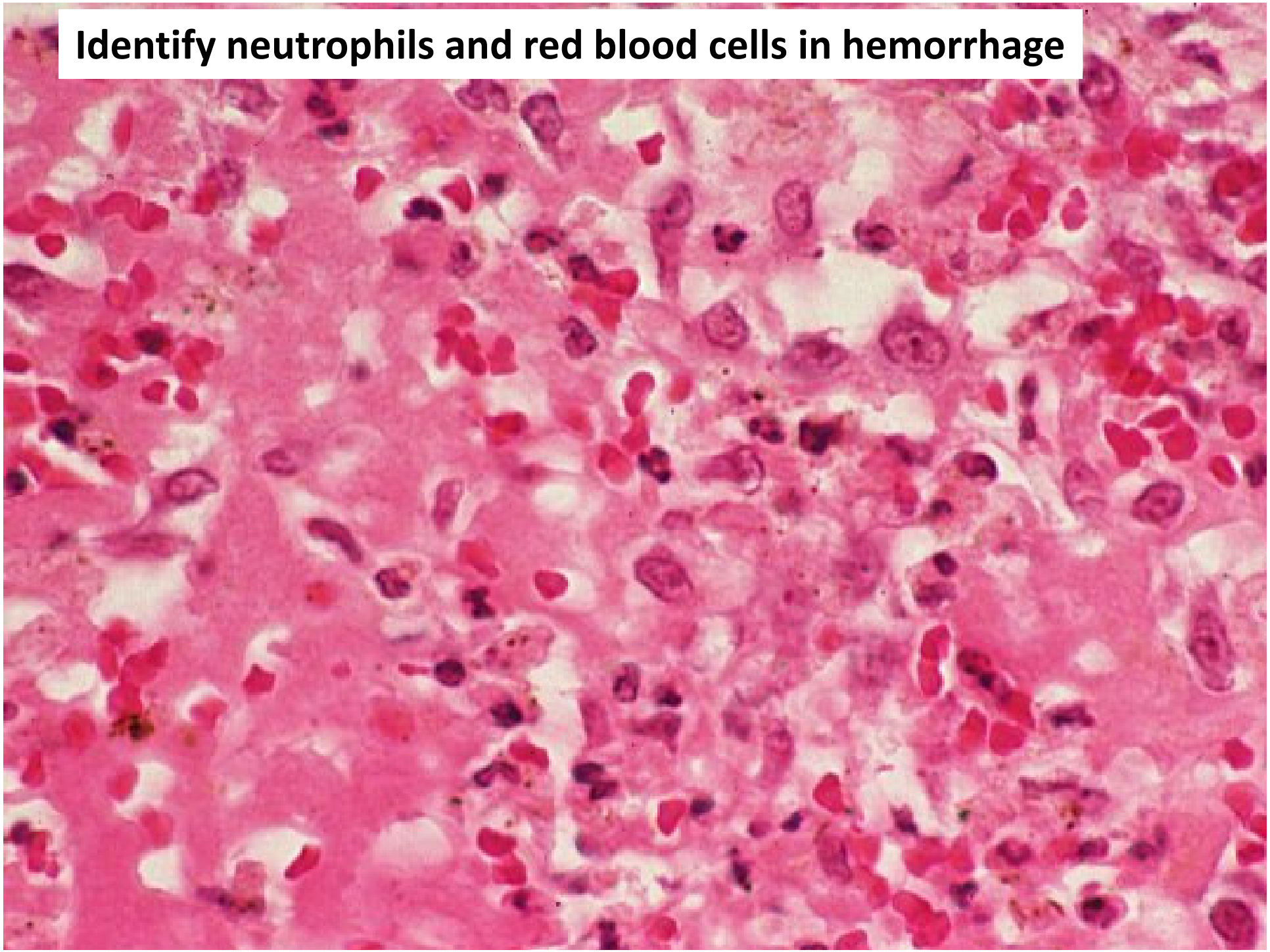
Origin and maturation:

- neutrophils are continuously generated in the bone marrow from myeloid precursors
- daily production can reach up to 2×10^{11} cells
- granulocyte colony stimulating factor (G-CSF) is the major cytokine driving production
- short lifespan (12h) which is increased up to several days upon activation in tissues
- characteristic **polymorphic, multi-lobed nucleus**
- recruitment via CXCR2 chemokines (CXCL1, 2, 5, 8)
- activated via pattern recognition receptor (PRR) and TLRs
  e.g. FPR1 (receptor for N-formylmethionine found in bact. and mitochondrial proteins) => response to tissue damage or necrosis

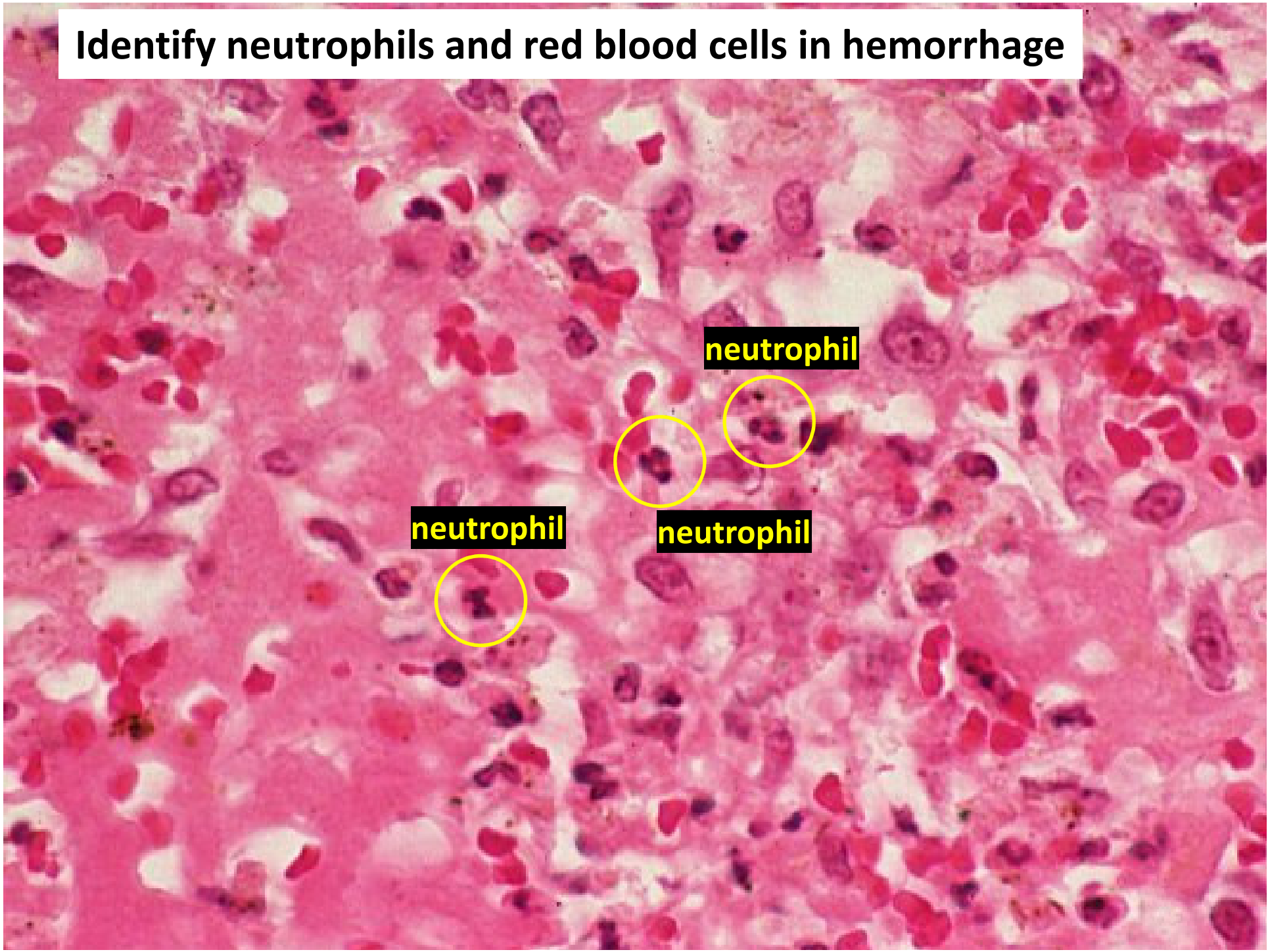
effector mechanisms:

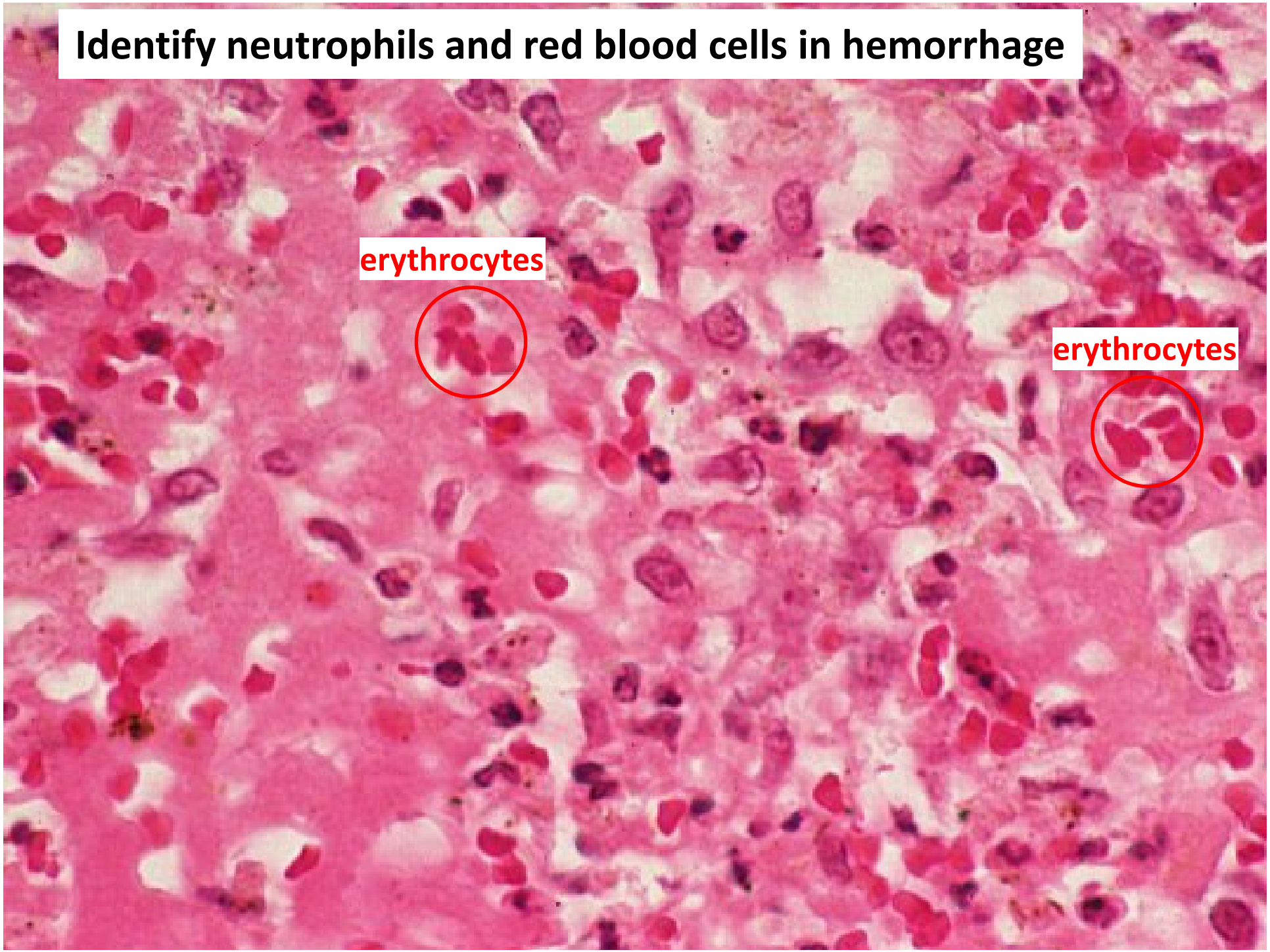


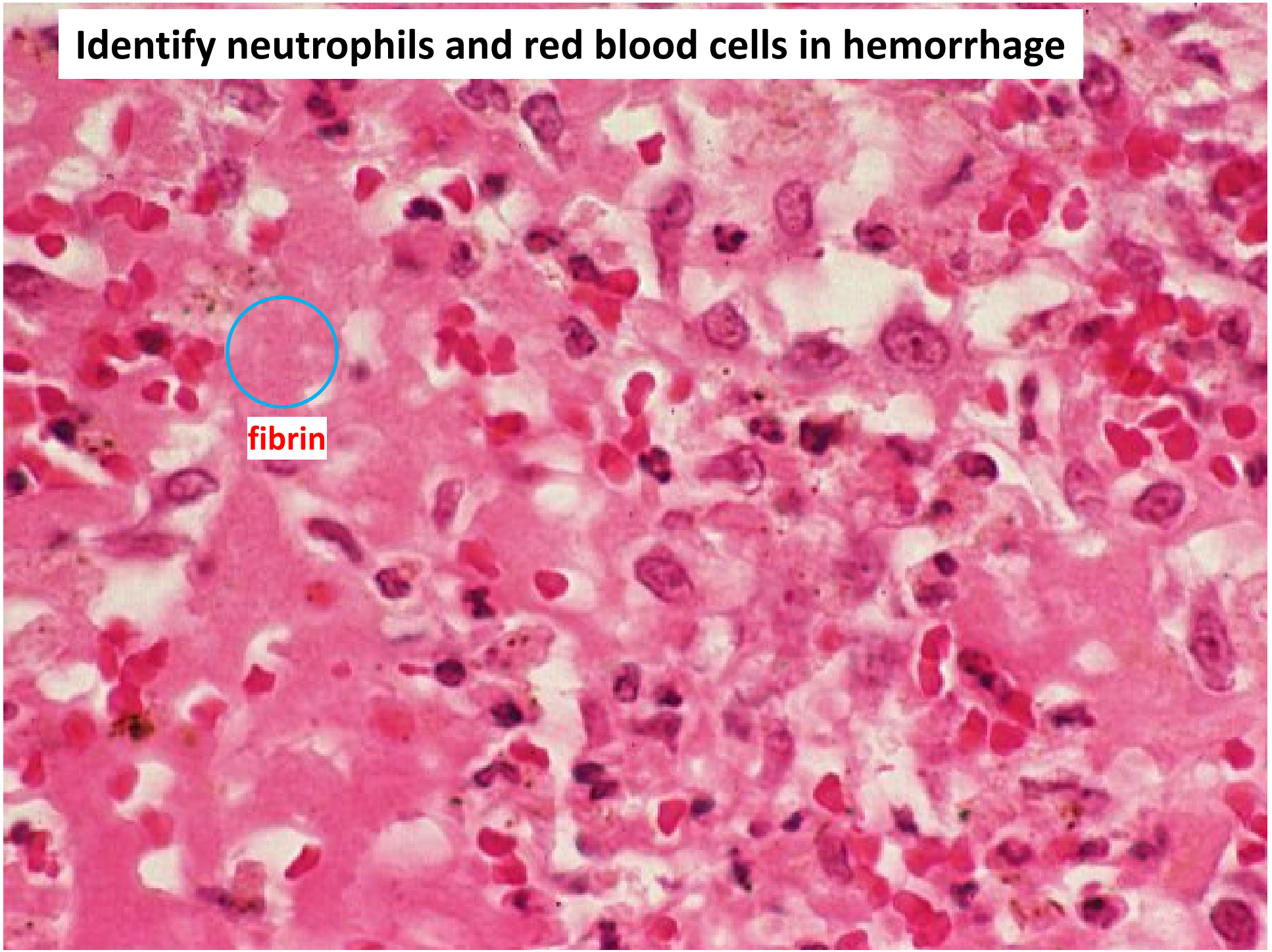
Identify neutrophils and red blood cells in hemorrhage



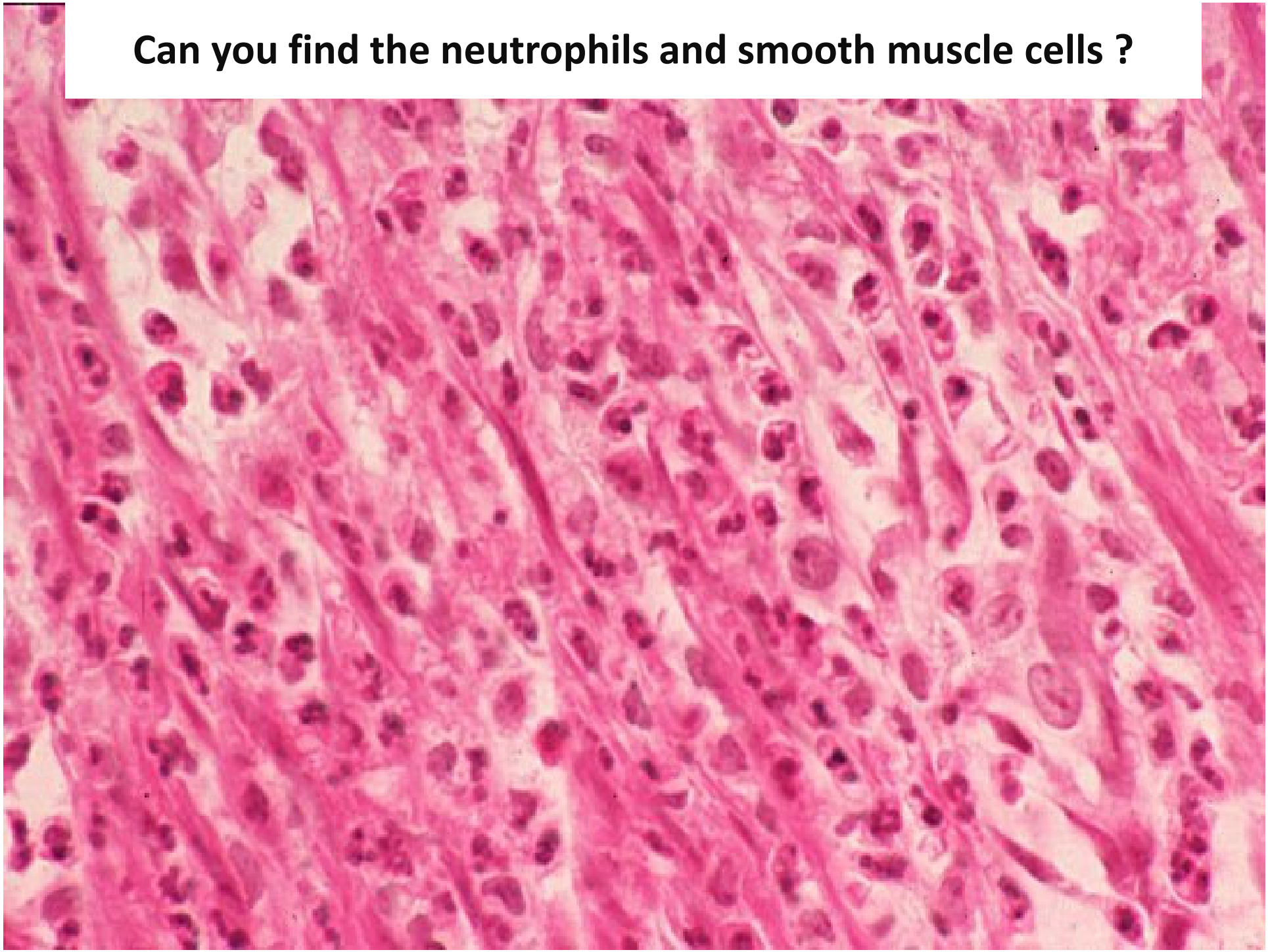
Identify neutrophils and red blood cells in hemorrhage



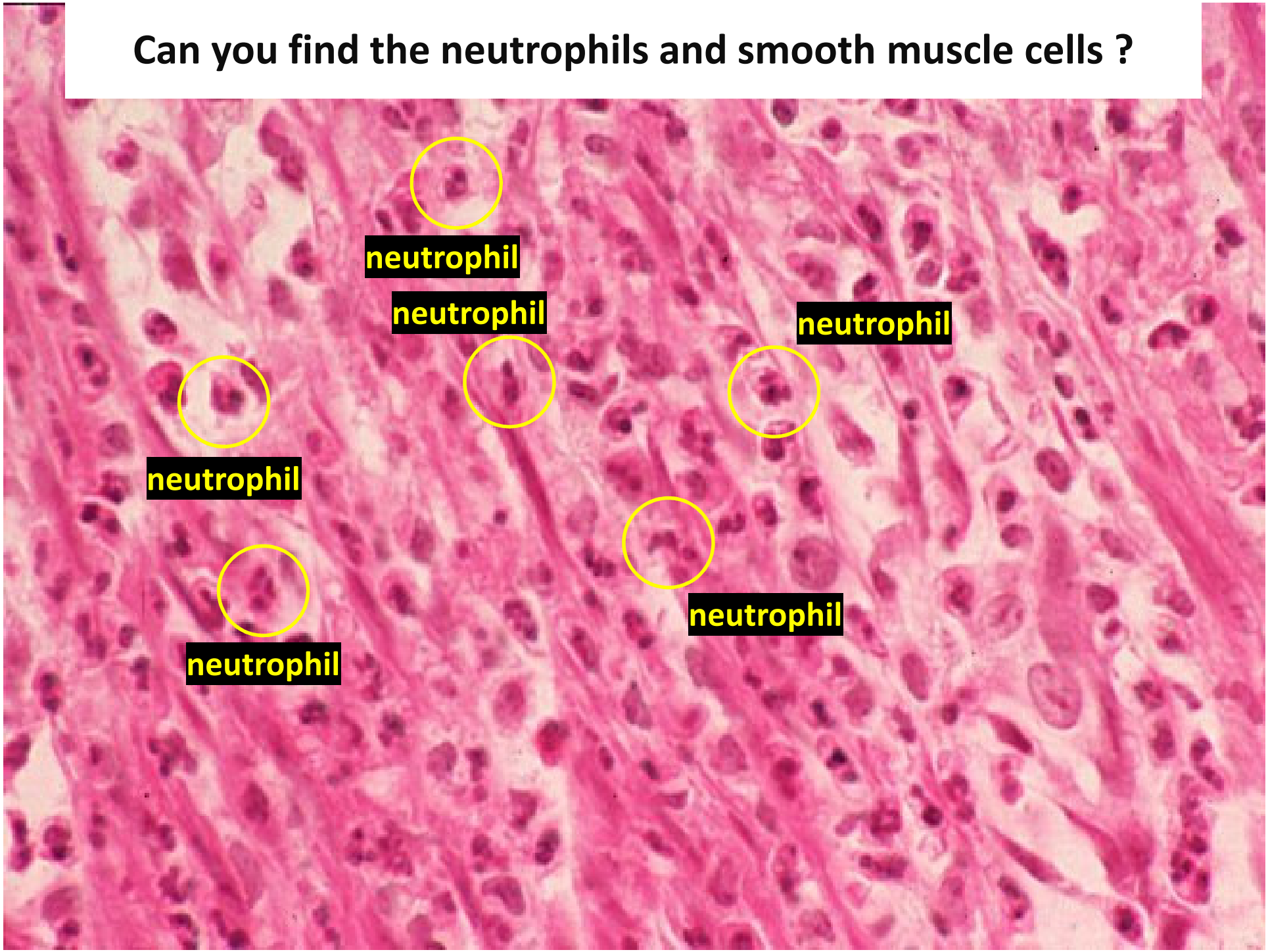

Identify neutrophils and red blood cells in hemorrhage
erythrocytes
erythrocytes


Identify neutrophils and red blood cells in hemorrhage
fibrin

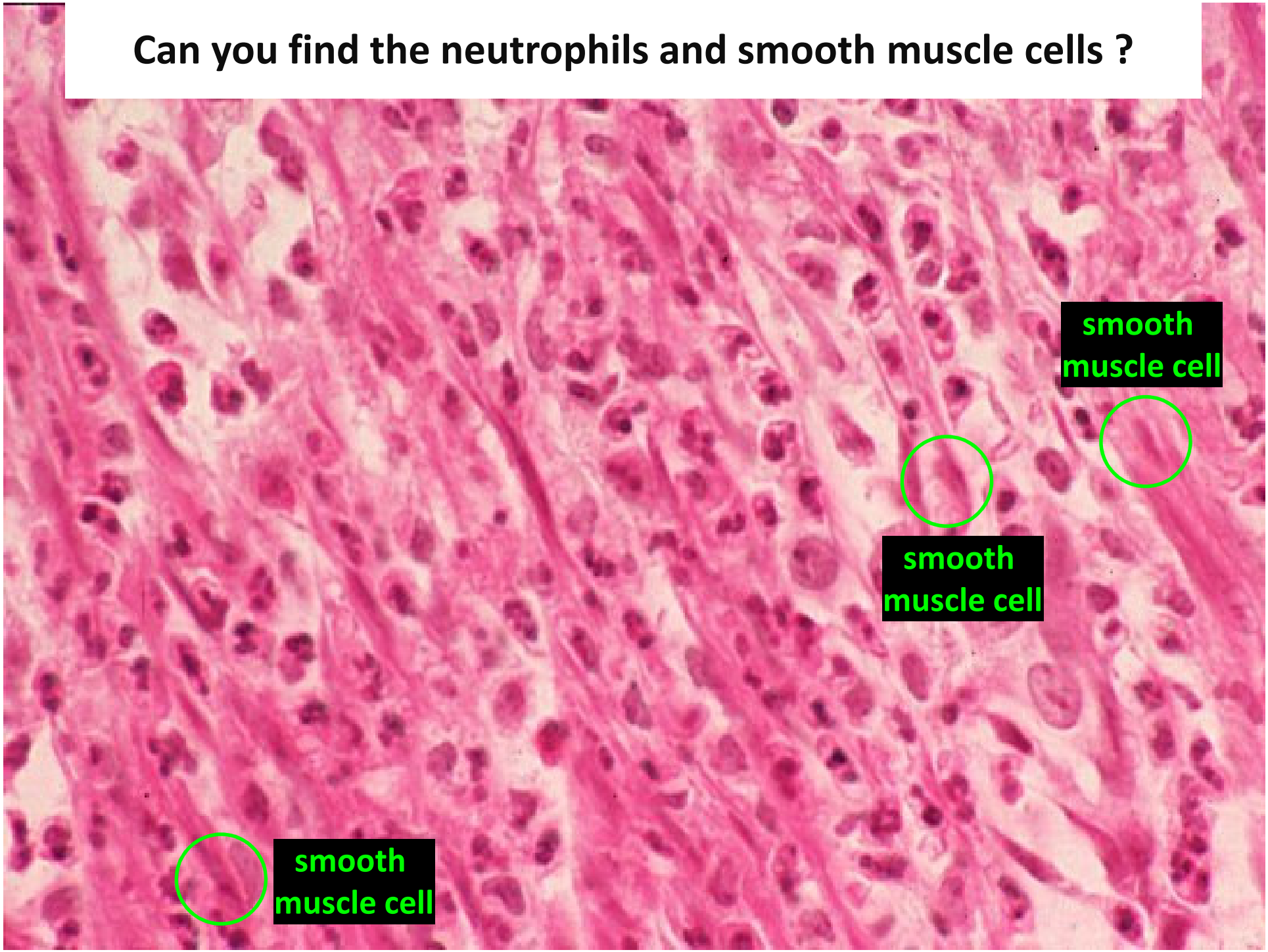
Can you find the neutrophils and smooth muscle cells ?



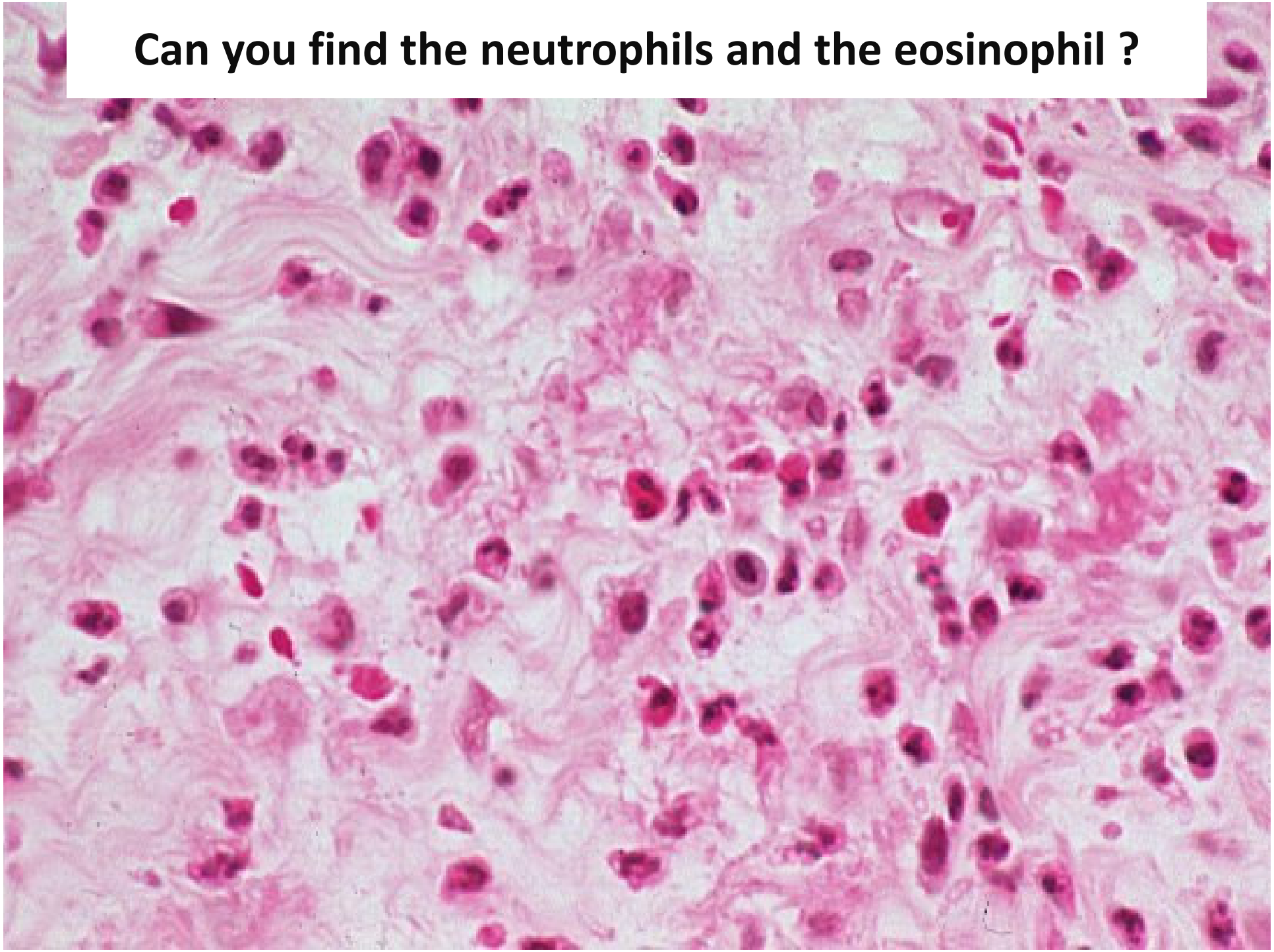
Can you find the neutrophils and smooth muscle cells ?



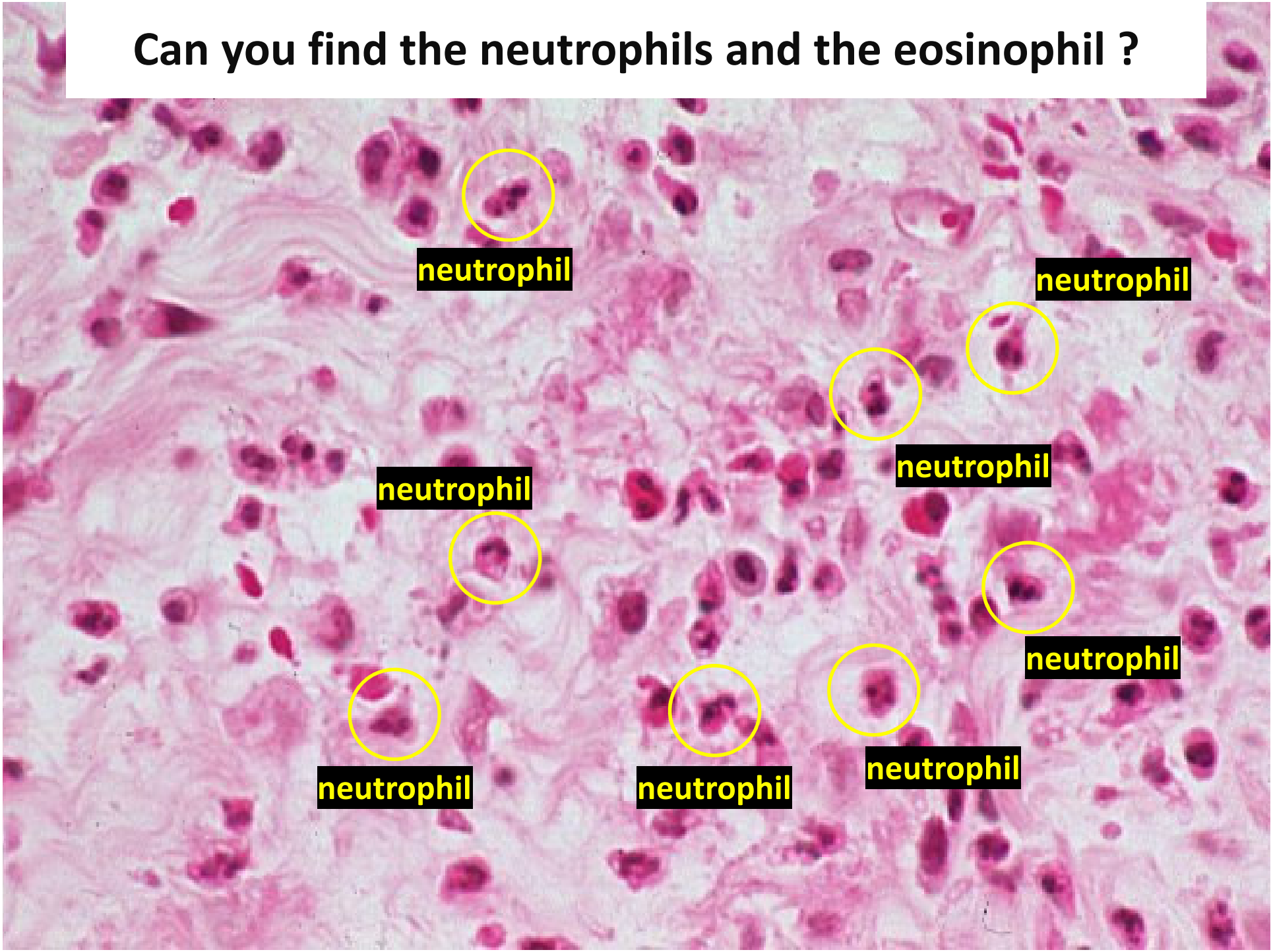
Can you find the neutrophils and smooth muscle cells ?



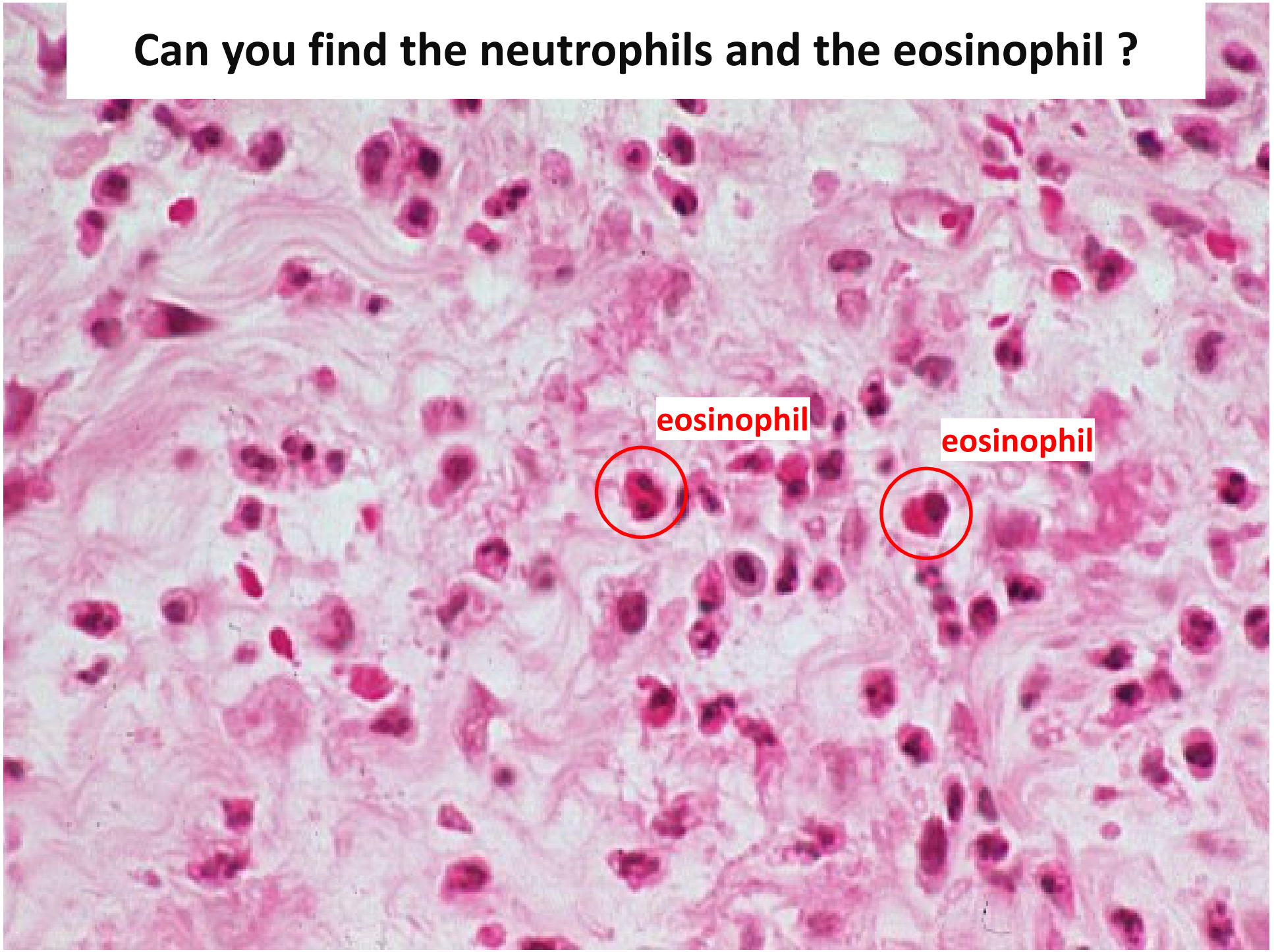
Can you find the neutrophils and the eosinophil ?



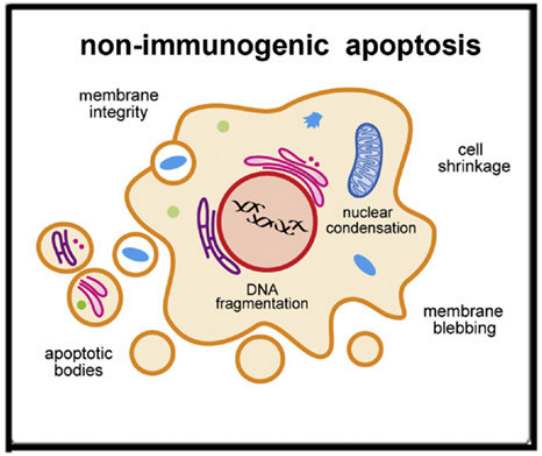
Can you find the neutrophils and the eosinophil ?



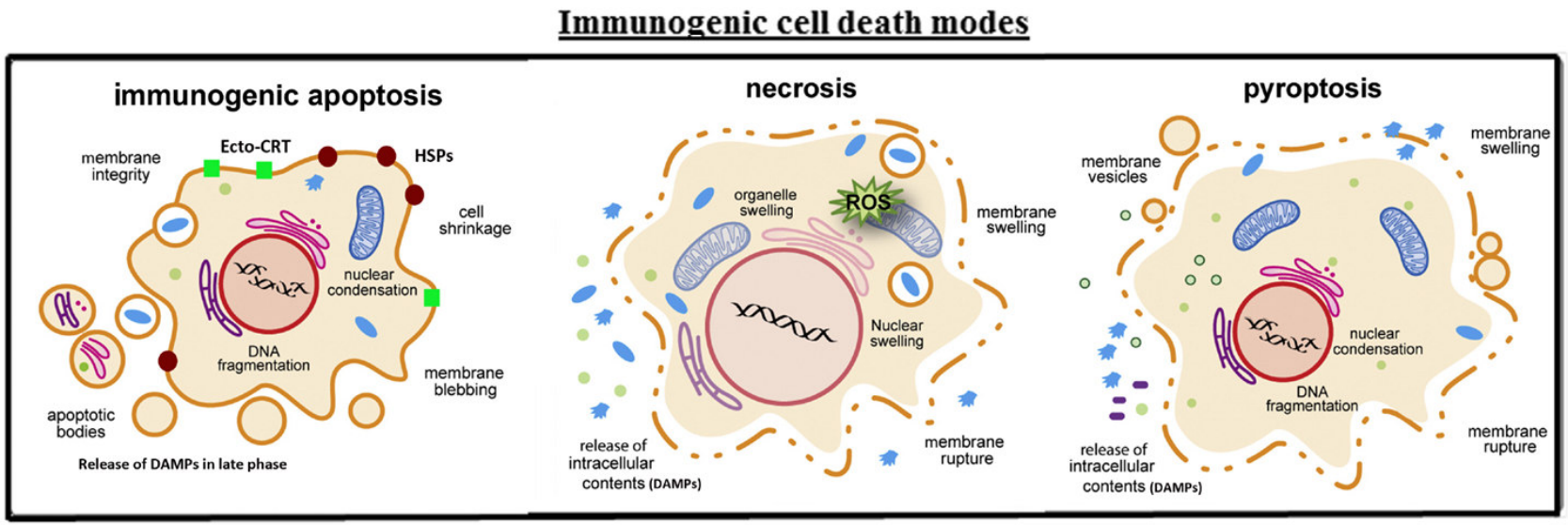
Can you find the neutrophils and the eosinophil ?

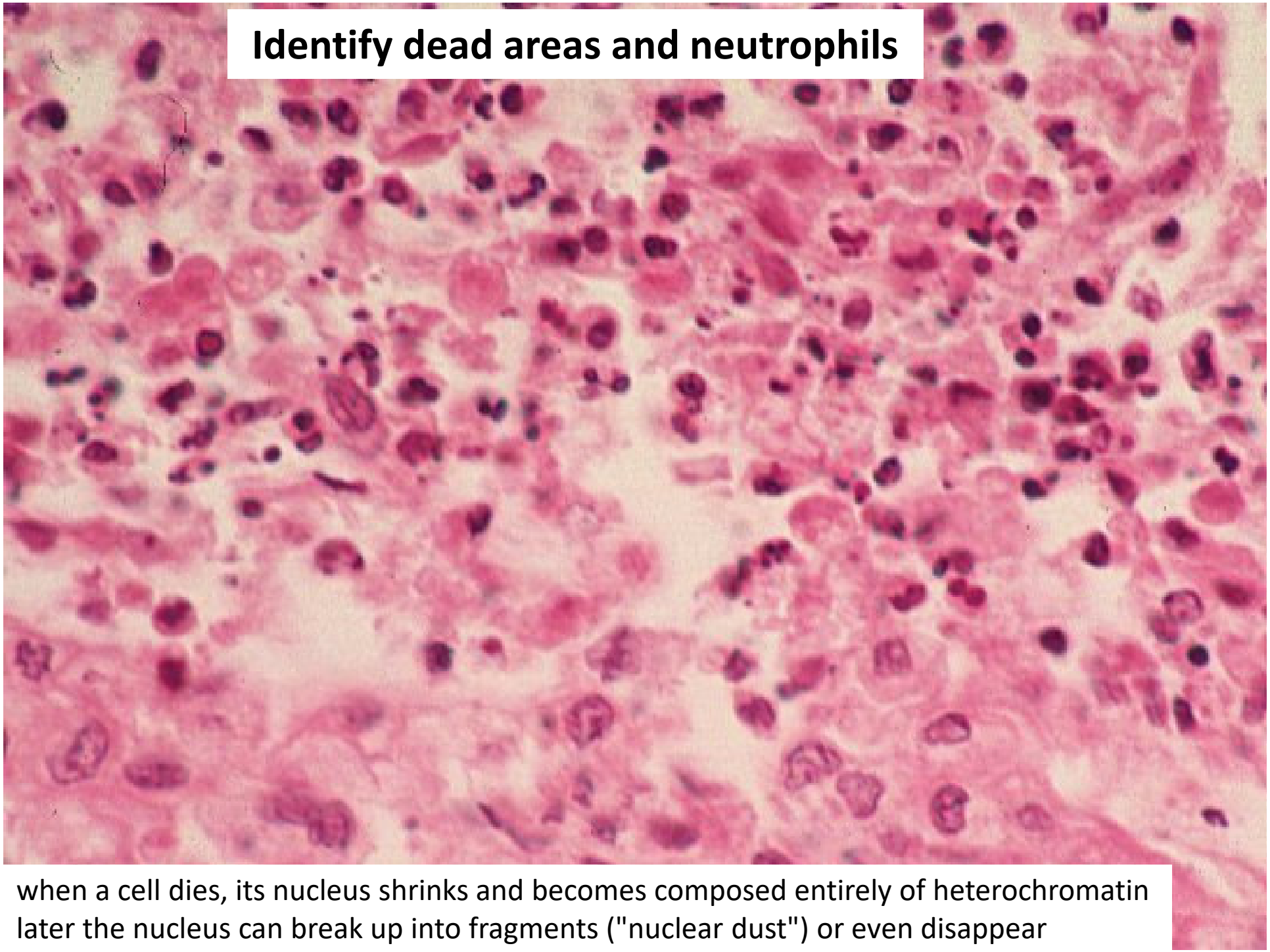


Immunogenic vs. non-immunogenic forms of cell death

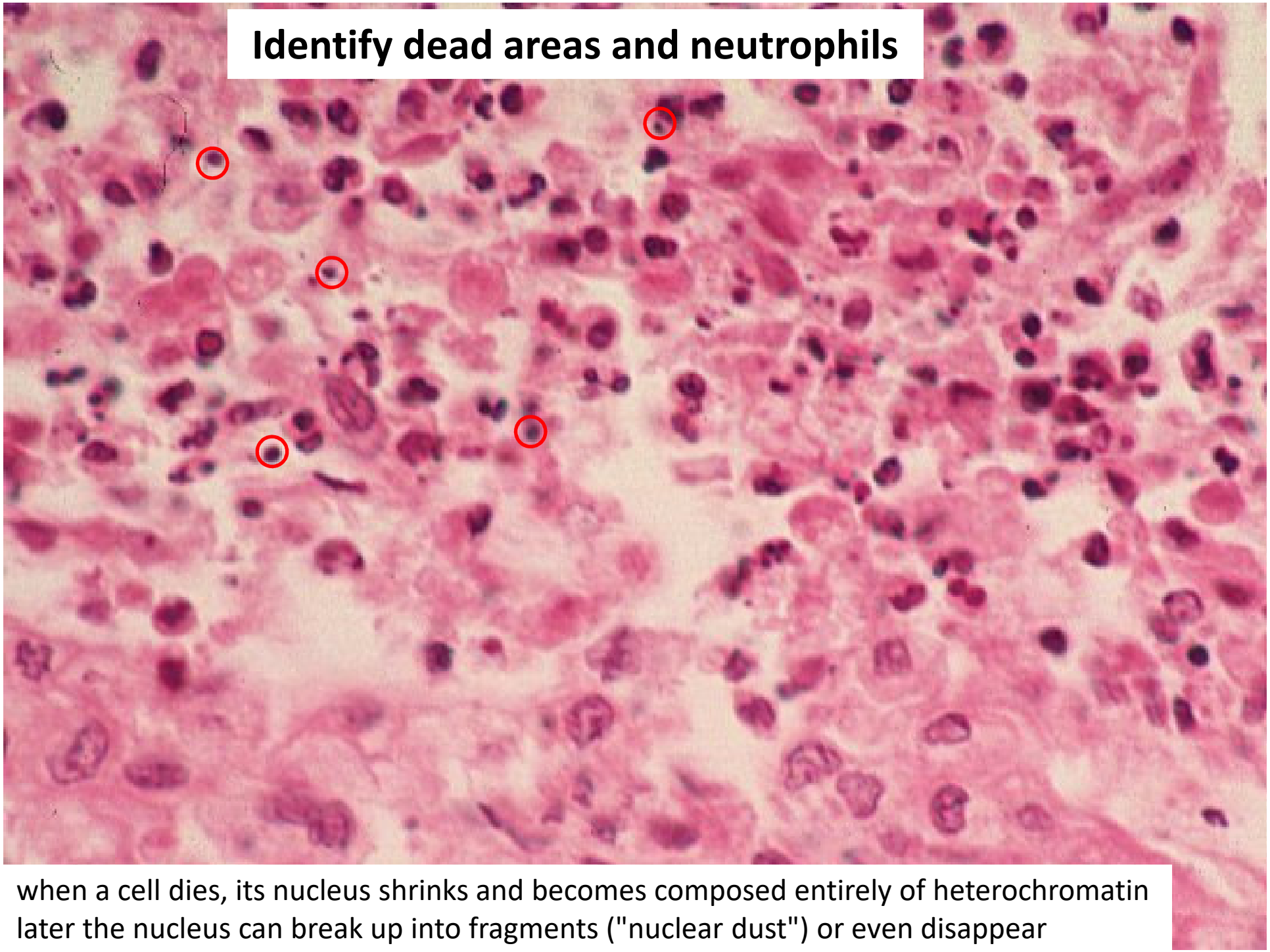


- in <u>apoptosis</u>, retention of plasma membrane integrity and formation of apoptotic bodies render it a non-immunogenic cell death
- certain chemotherapies lead to in <u>immunogenic apoptosis</u> with surface exposure of calreticulin (ecto-CRT) and heat-shock proteins (HSPs) prior to apoptosis, and other DAMPs released in the later phase
- in <u>necrosis</u>, secretion of pro-inflammatory cytokines and release of cytoplasmic content, including DAMPs (ATP, HMGB1, and uric acid, etc.) trigger inflammation



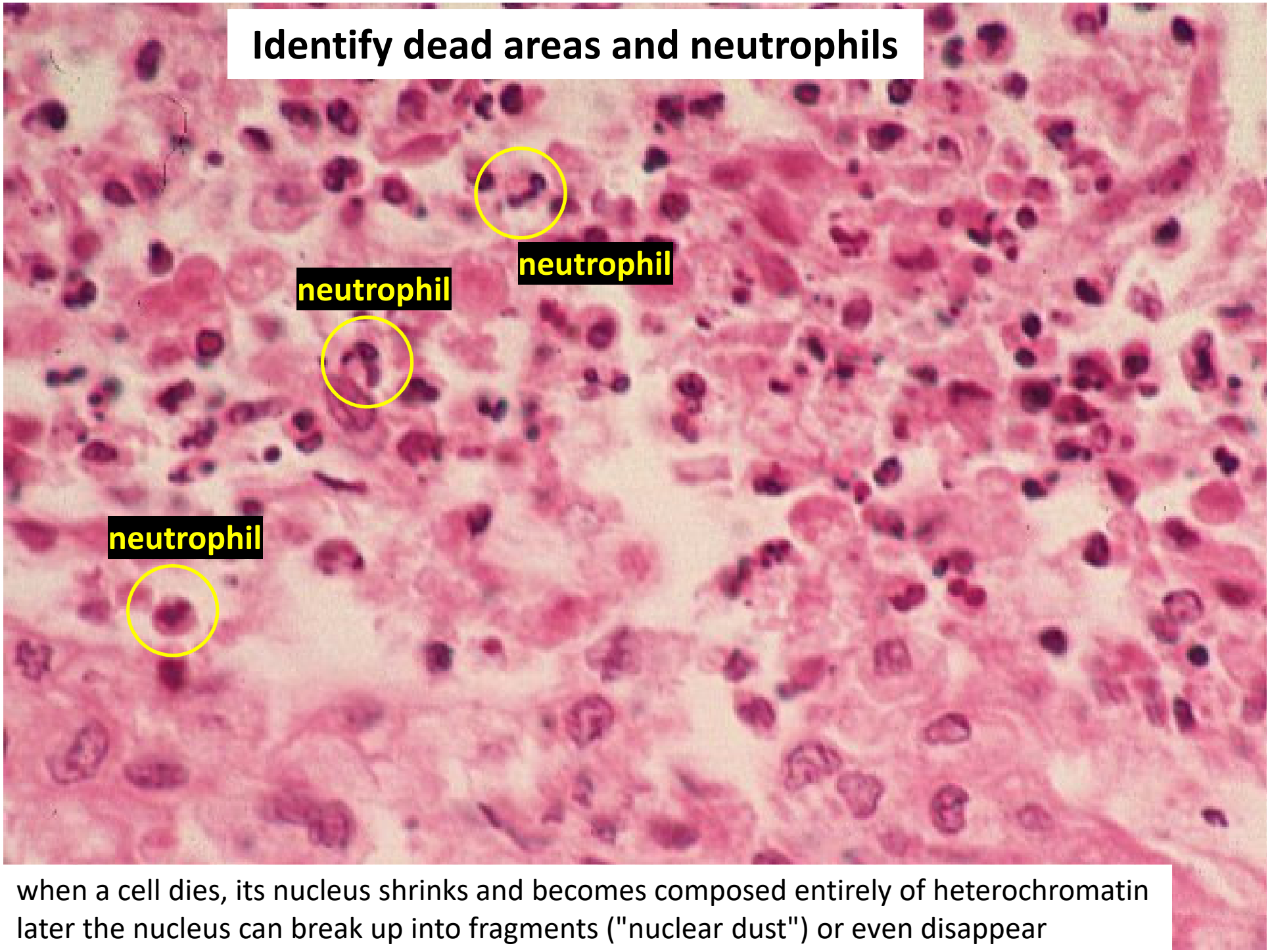


when a cell dies, its nucleus shrinks and becomes composed entirely of heterochromatin
later the nucleus can break up into fragments ("nuclear dust") or even disappear



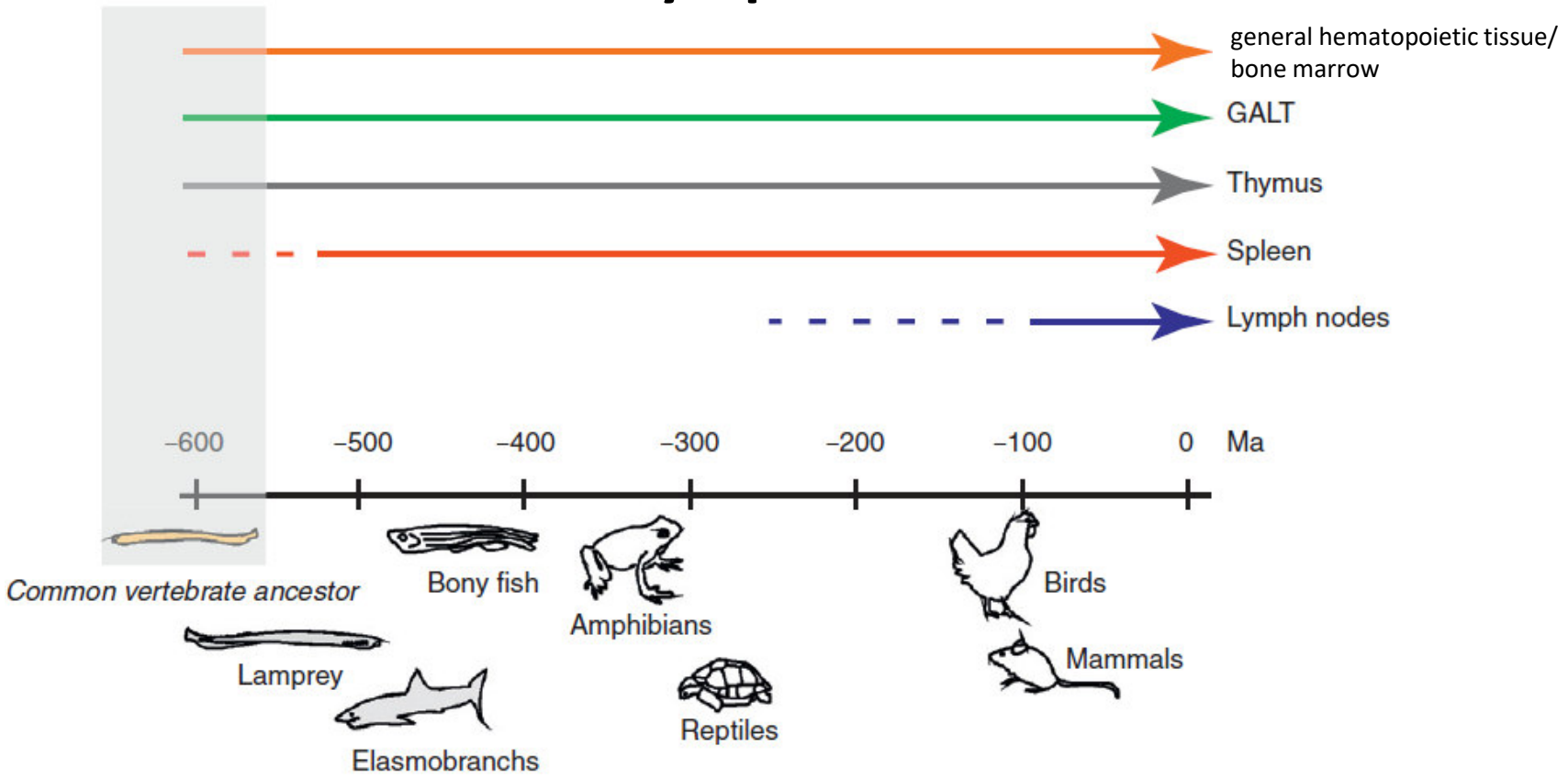
when a cell dies, its nucleus shrinks and becomes composed entirely of heterochromatin
later the nucleus can break up into fragments ("nuclear dust") or even disappear

Identify dead areas and neutrophils



when a cell dies, its nucleus shrinks and becomes composed entirely of heterochromatin
later the nucleus can break up into fragments ("nuclear dust") or even disappear

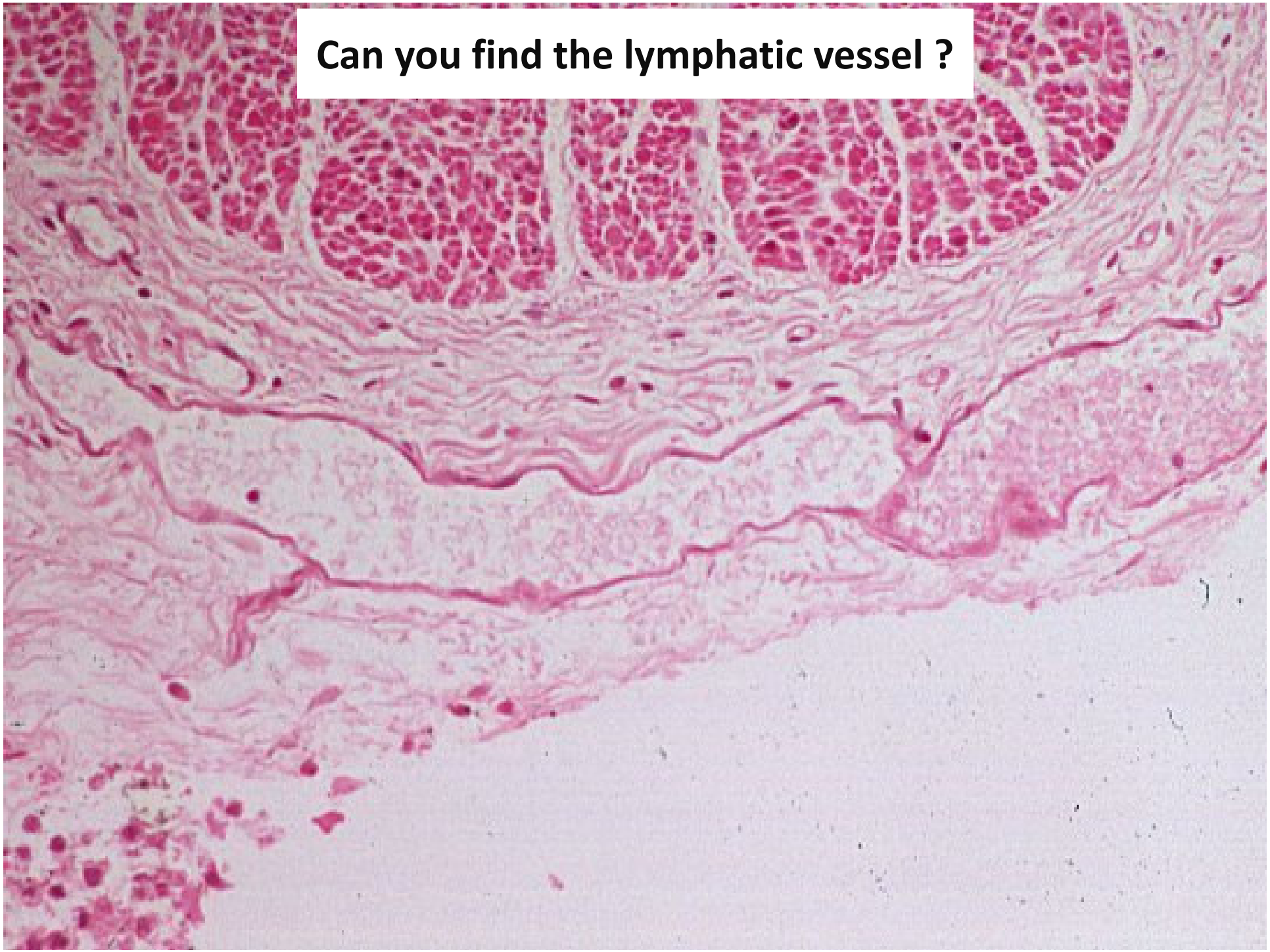
Evolution of lymphoid tissues

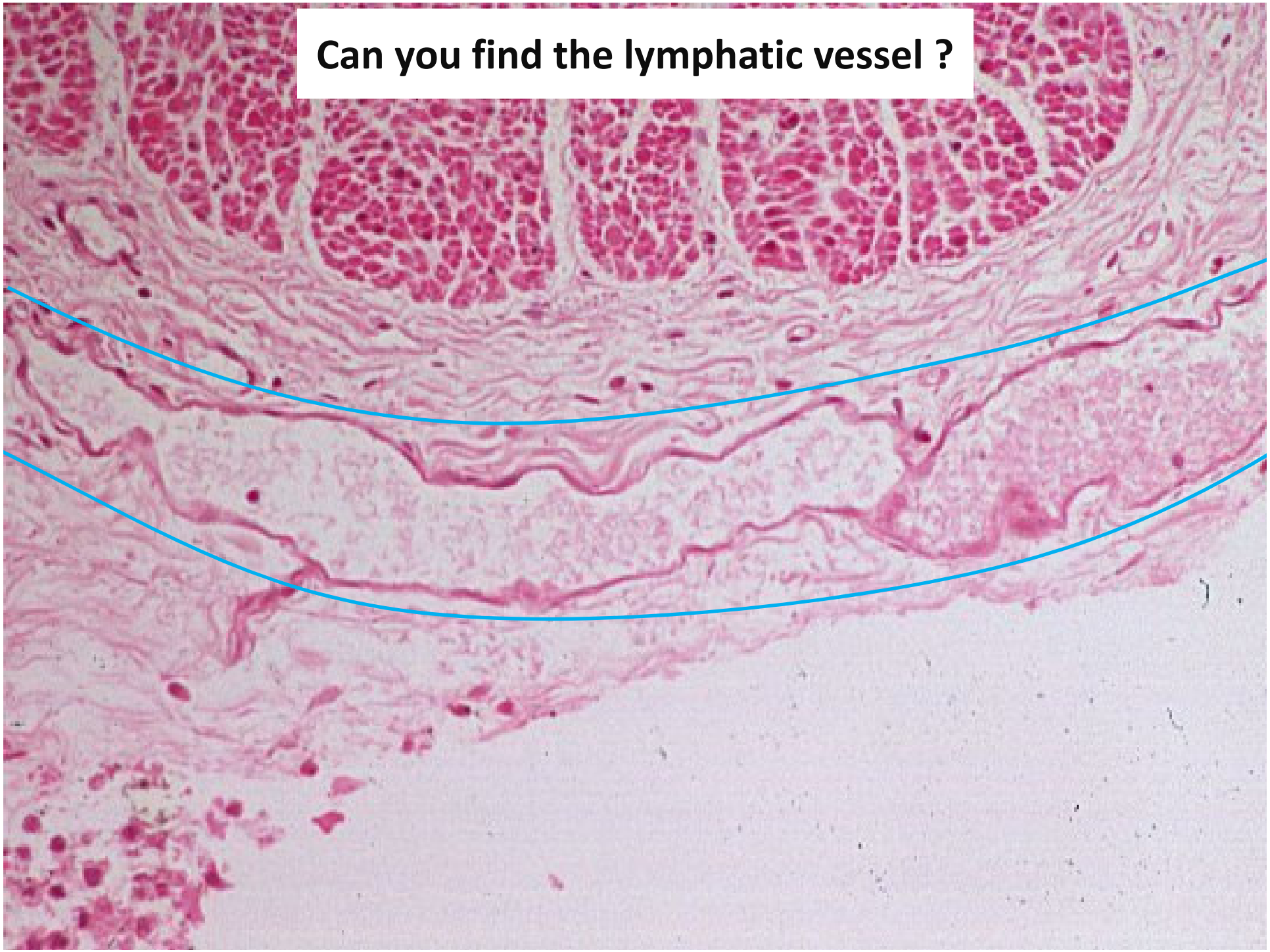


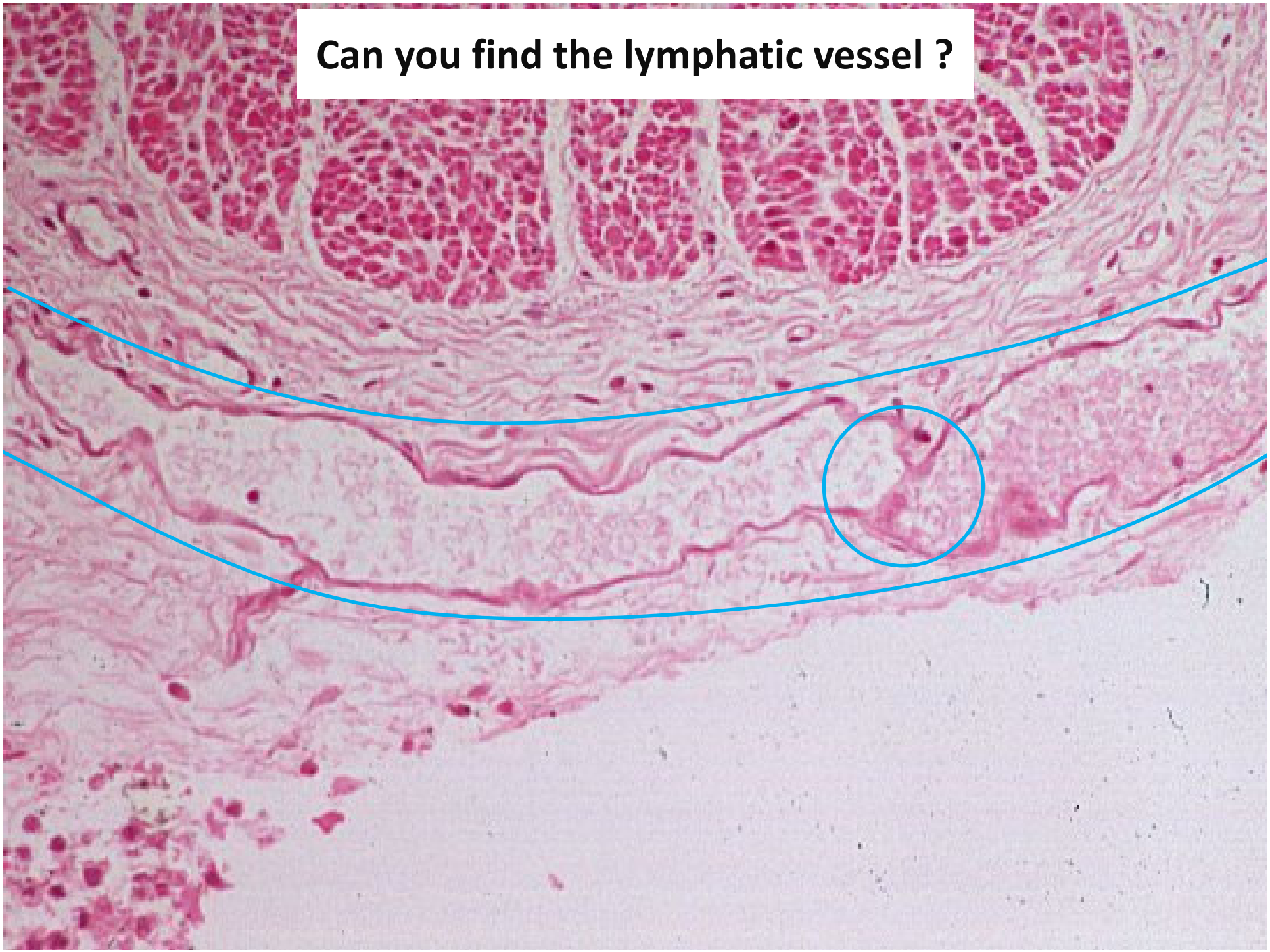
spleen is the most ancient secondary lymphoid organ, although its role in the removal of damaged or aged red blood cells possibly precedes its immune functions
contains white pulp that coordinates the interaction of different immune effector cells in which B cell areas enclose a central T cell zone that also contains dendritic cells
the marginal zone, a specialized antigen-trapping device, is populated by macrophages (only in mammals)

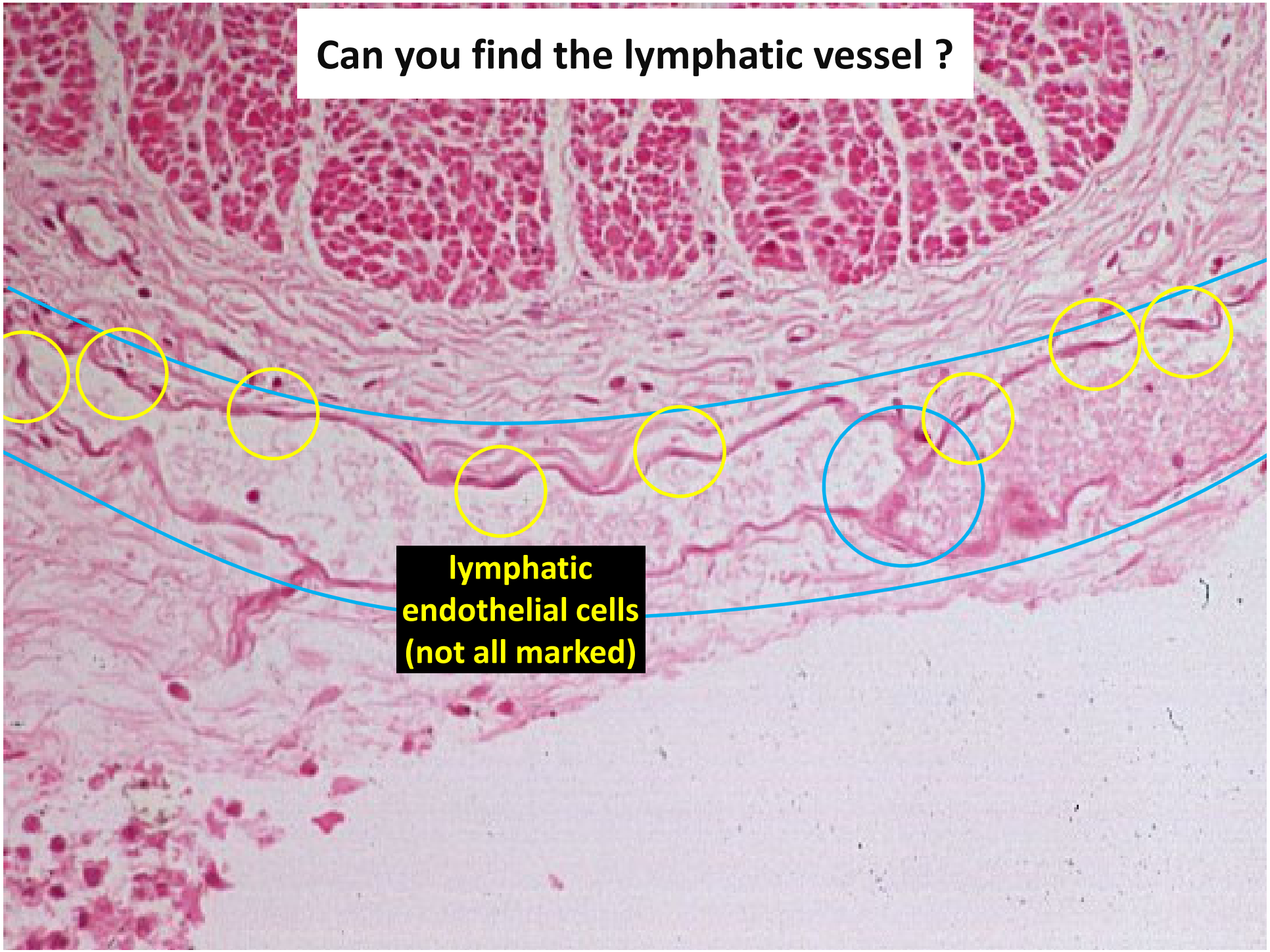
lymph nodes represent the only major recent innovation of the adaptive immune systems (restricted to birds and mammals) while the lymphatic system is evolutionarily more ancient
promote the cellular interactions that lead to T cell activation and the affinity maturation of antibodies, and consequently, efficient memory responses

Boehm et al. 2012 Trends Imm


Can you find the lymphatic vessel ?


Can you find the lymphatic vessel ?

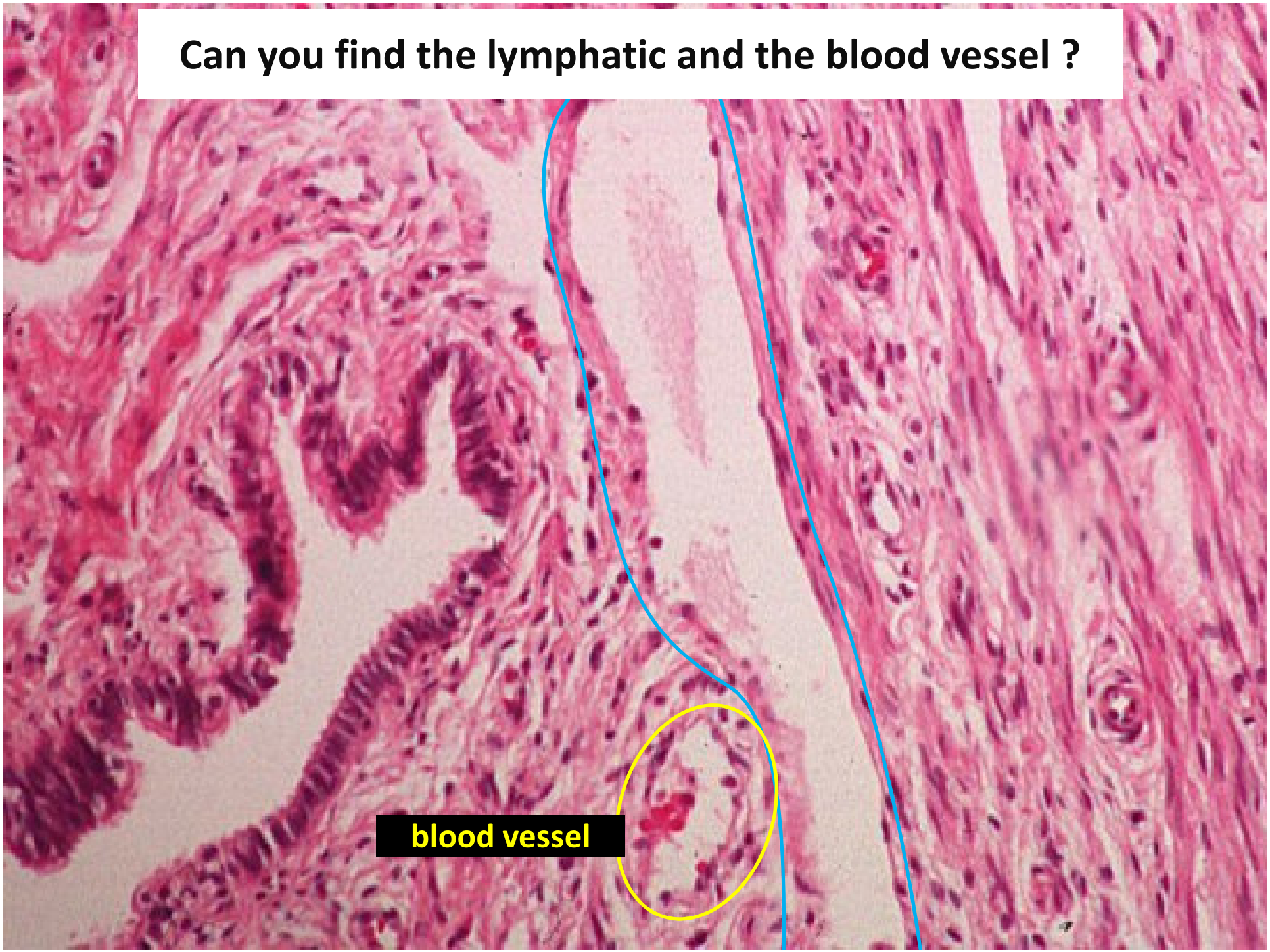

Can you find the lymphatic vessel ?


Can you find the lymphatic vessel ?
lymphatic endothelial cells (not all marked)

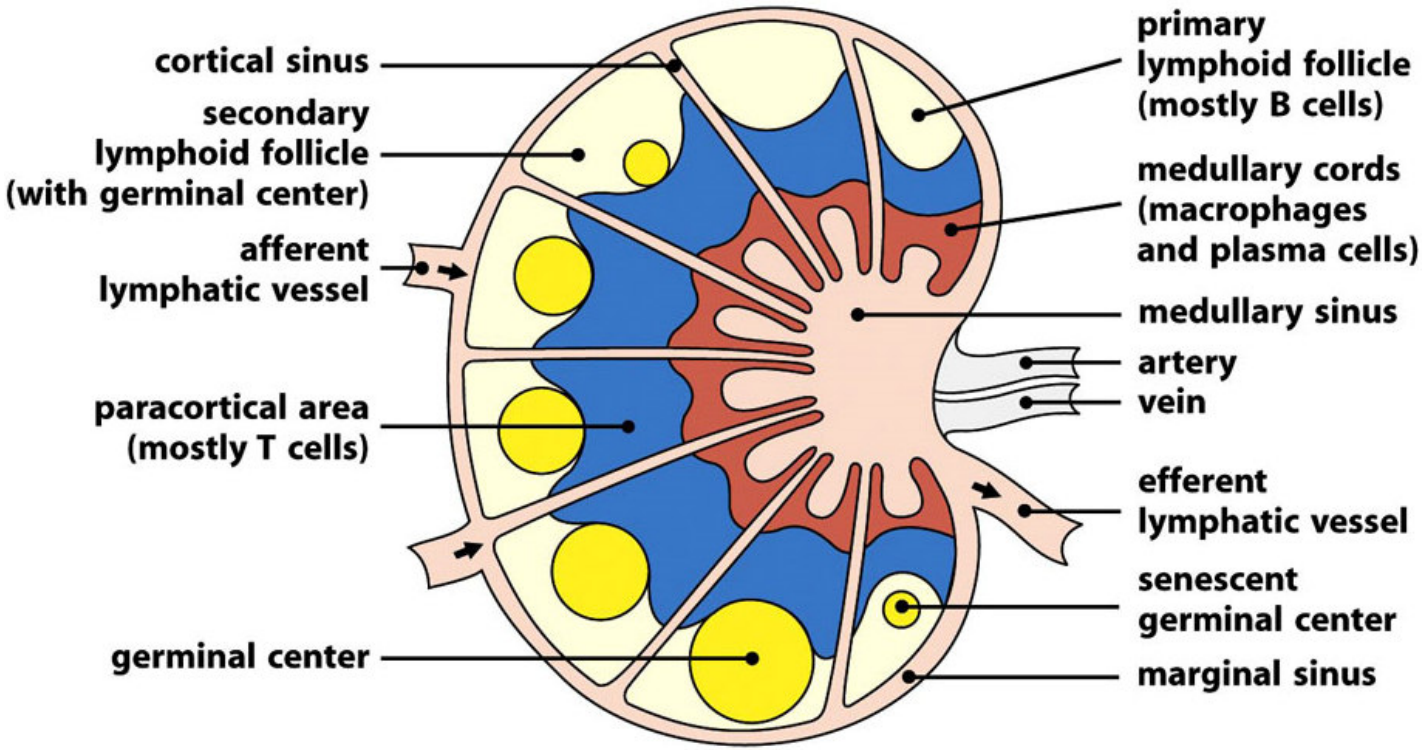
Can you find the lymphatic and the blood vessel ?


Can you find the lymphatic and the blood vessel ?

Can you find the lymphatic and the blood vessel ?

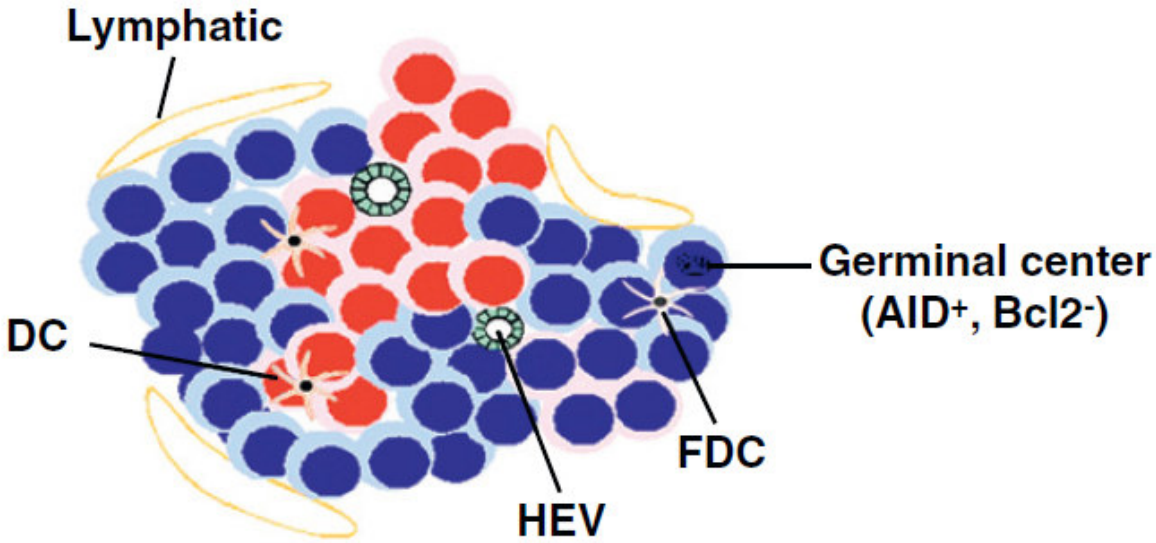


Lymph node structure and function



within LNs, B, and T cells are segregated into distinct functional areas: a **B cell area** mainly composed of densely packed B cells and **follicular DCs** (FDCs) forming **follicles**, and a **T-cell area** mainly composed of less dense accumulation of T cells and DCs. While DCs prime T cells through the presentation of antigen-derived peptides within major histocompatibility complex (MHC) class I and II molecules, FDCs shape the B-cell response by presenting unprocessed antigens in the form of antigen-antibody complexes that they trap through their complement or Fc receptors. After antigen encounter, activated B cells organize into a **germinal center** (GC), which is a site of active B-cell proliferation, class switch recombination (CSR), and somatic hypermutation (SHM), allowing the generation of B-cell clones highly specific for the antigen. A particular subset of T helper (Th) cells, namely **T follicular helper (Tfh) cells**, plays also a major role in GC responses, by delivering key signals for GC B-cell survival and differentiation

Tertiary lymphoid organs

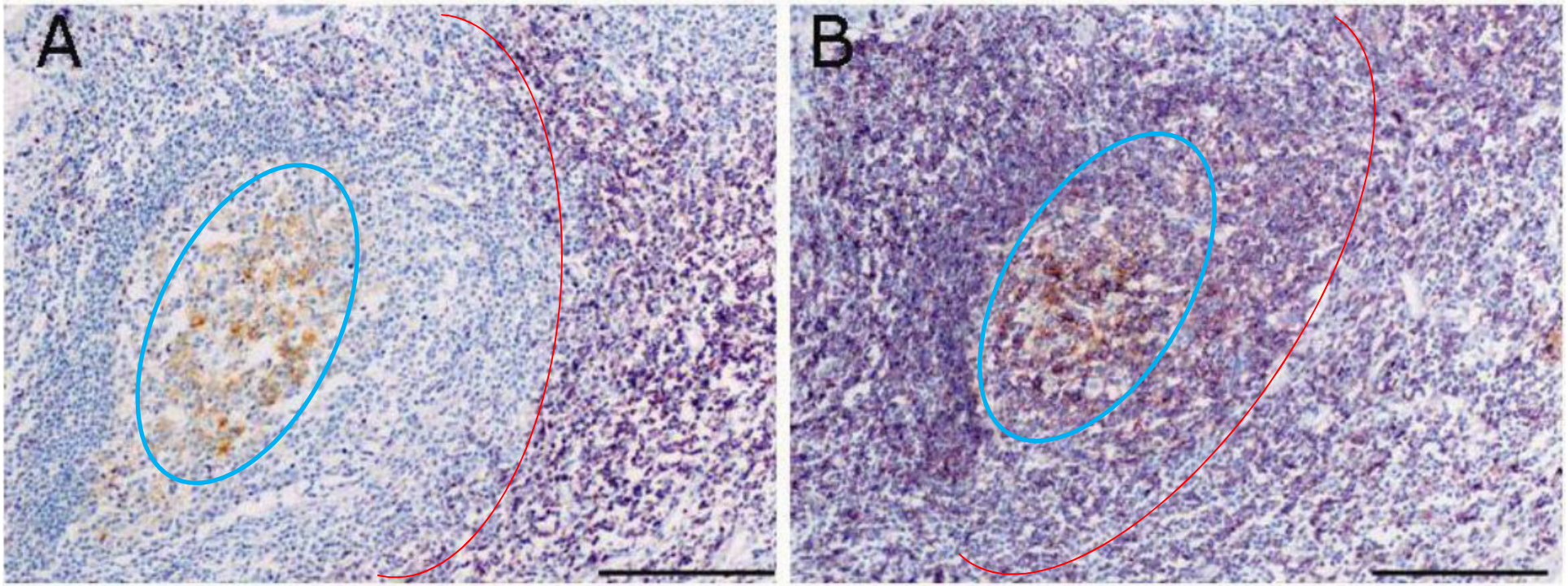


TLOs have been identified in cancer, chronic bacterial infections, autoimmune disease, transplant rejection and other diseases which involve long persistence of antigen stimuli

the difference between a chronic infiltrate and a TLOs resides in the degree of their internal organization:

- the organized infiltrate contains anatomically distinct yet adjacent T and B cell compartments
- the T cell area contains an extensive network of fibroblast reticular cells (FRCs)
- $PNAd^+$ or $MECA79^+$ high endothelial venules (HEVs) specialized in allowing extravasation of lymphocytes are present in the T cell area
- follicular dendritic cells (FDCs) are present
- there is evidence of B cell class switching and germinal center reactions in the B cell follicles
- activation-induced cytidine deaminase (AID) enzyme is present which generates somatic hypermutation in DNA for antibody maturation
- one difference between TLOs and LNs is the absence of NK cells in TLOs
- in addition to FDCs, B cells may act as APCs to activate CD4 and CD8 T cells

Tertiary lymphoid organs in cancer

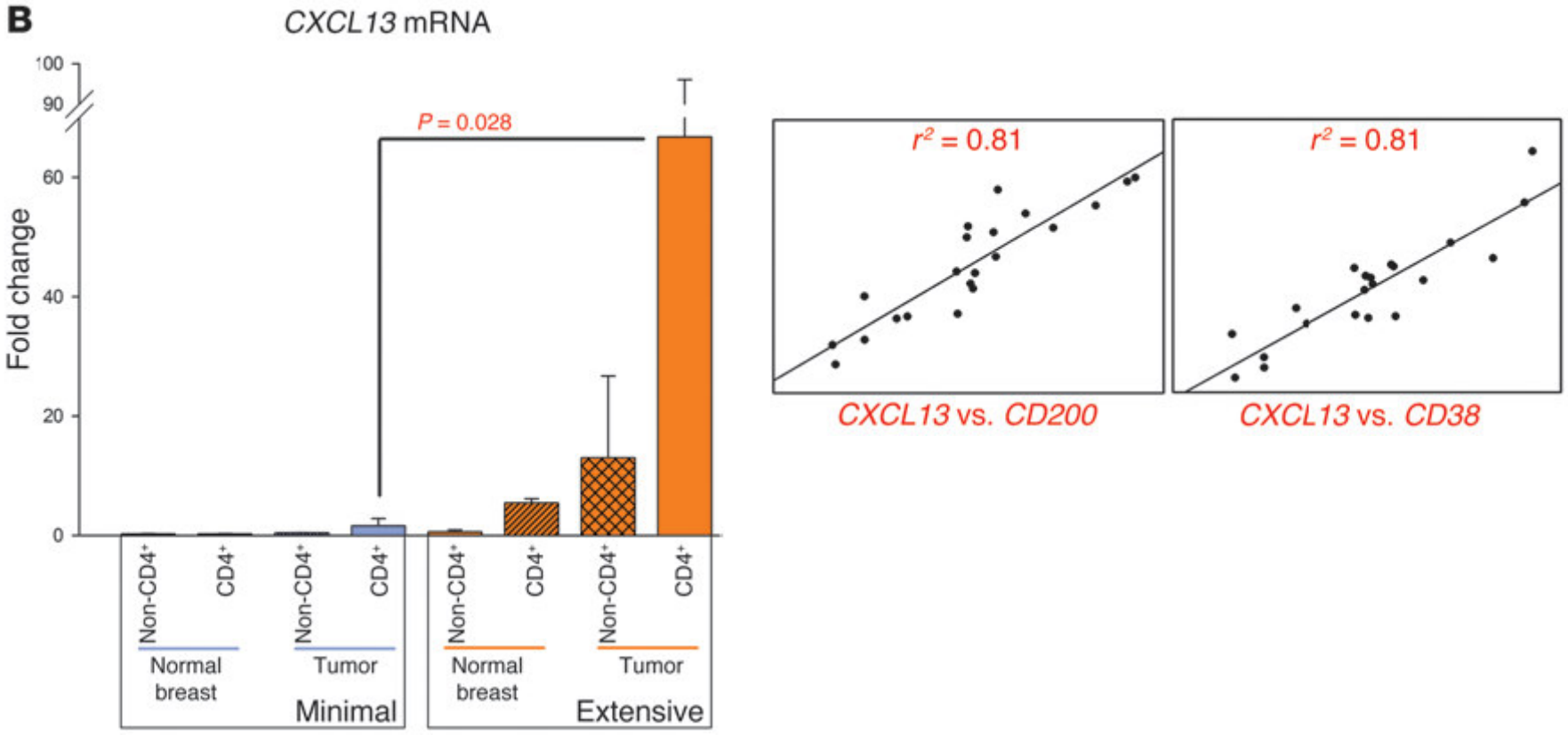


T cells (purple, A) and CD20^{+} B cells (purple, B) surround CD21^{+} follicular dendritic cells (brown, sections in A and B are consecutive and show the compartmentalization between B and T cells

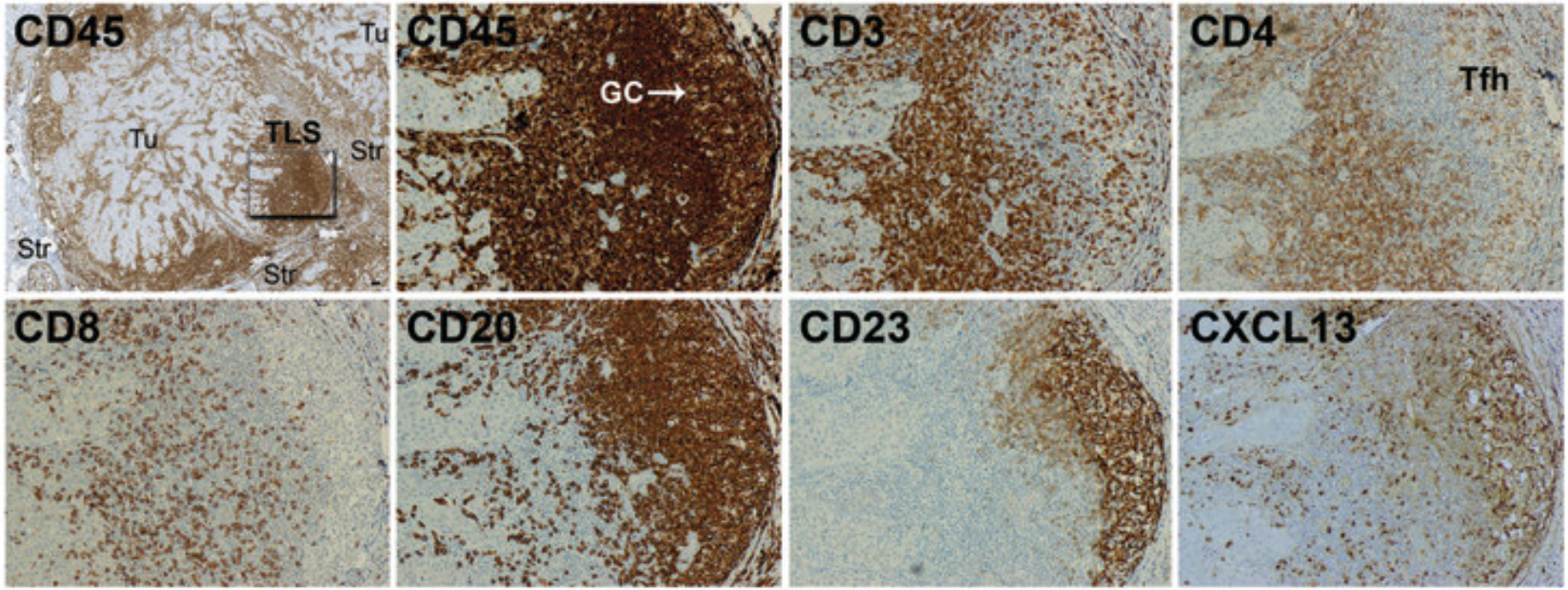
in tumors, a high density of memory T cells with a T helper cell 1 (Th1) and cytotoxic orientation, both in the center of the tumor and its invasive margin, correlates with longer patient's survival

anti-tumor immunity can develop at the tumor site in mouse models lacking lymph nodes demonstrating that effective T cell priming can occur locally in the tumor, possibly in TLOs

CXCL13 derived from T_{FH} is expressed at elevated levels in TILs from extensively infiltrated BC vs. minimal infiltrated BC



TLOs in breast cancer



CXCL13-producing T_{fh} cells and FDCs might catalyze the recruitment of other immune cells to the tumor, thereby initiating and/or enhancing TLS development and GC formation
increased frequency of TLS and GC in conjunction with increased T_{fh} and T_{h1} gene expression in CD4+ TIL from extensively infiltrated breast cancer, together with the combined activated/suppressed TIL profile, suggests that immune structures adjacent to the tumor bed may represent an important site for antitumor immune responses

Effects on survival