

Lymphoma: a heterogeneous disease

Application of the WHO Lymphoma Classification Scheme

Peter F. Moore

WHO - classification

- **Extension of REAL classification - 1994 ILSG**
- **Boadened to include myeloid, mast cell and histiocytic neoplasia**
- **Disease entities defined:**
 - **Lineage and postulated cell of origin**
 - **Morphology and Immunophenotype**
 - **Genetic features and Clinical features**

WHO - classification

- **Lymphoid neoplasia: B cell, T cell, NK cell and Hodgkins lymphoma**
- **Lymphomas and leukemias considered together - may be manifestations of the same tumor**
 - **B-CLL and B cell small lymphocytic lymphoma**
 - **Lymphoblastic lymphoma and lymphoblastic leukemia**

WHO - classification

- **B and T/NK lymphomas:**

 - Precursor cell lymphomas**

 - Mature cell lymphomas**

- **Non-Hodgkin lymphomas -**

 - Distinct diseases**

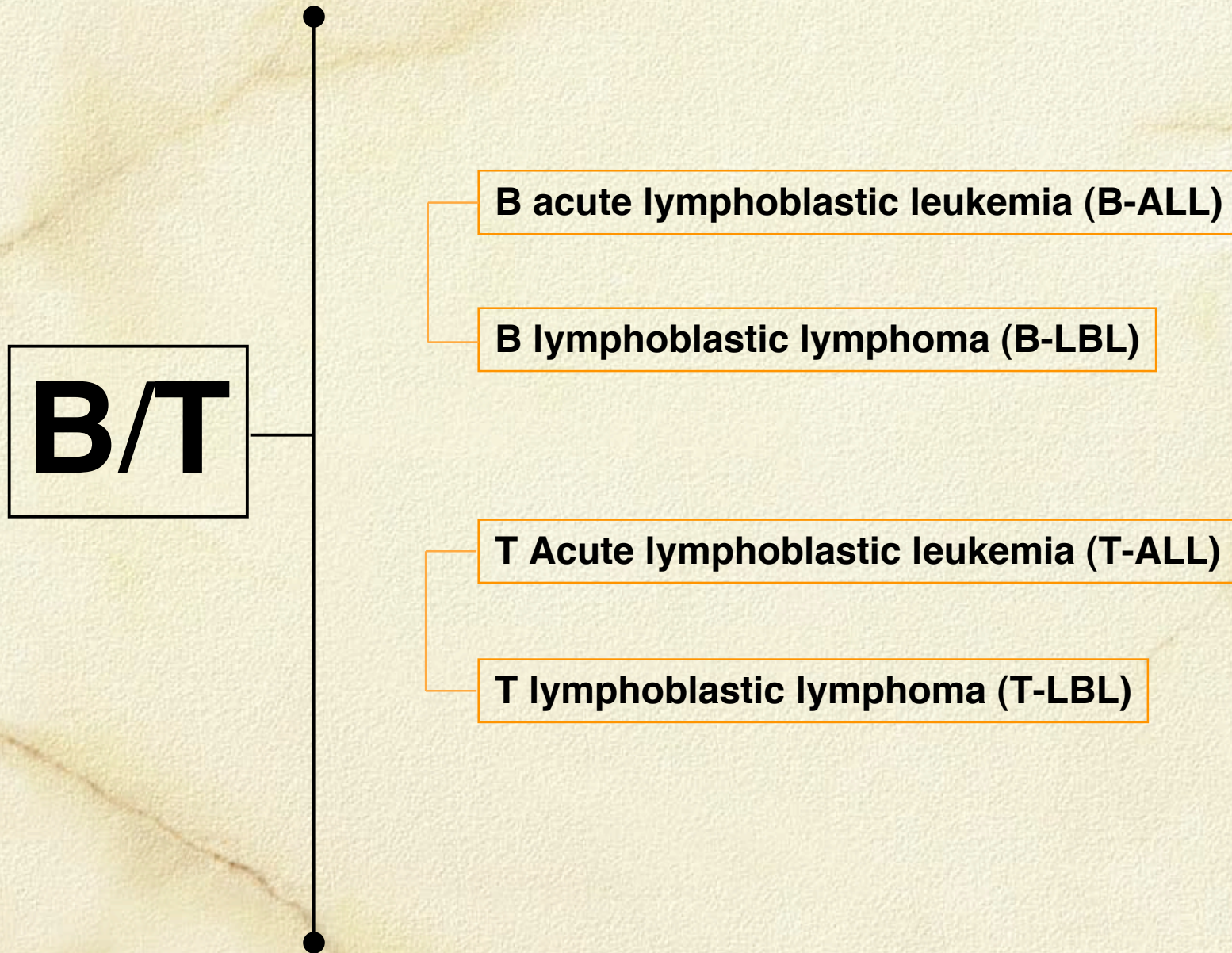
 - Distinctive clinical features/epidemiology**

 - Distinctive responses the therapy.**

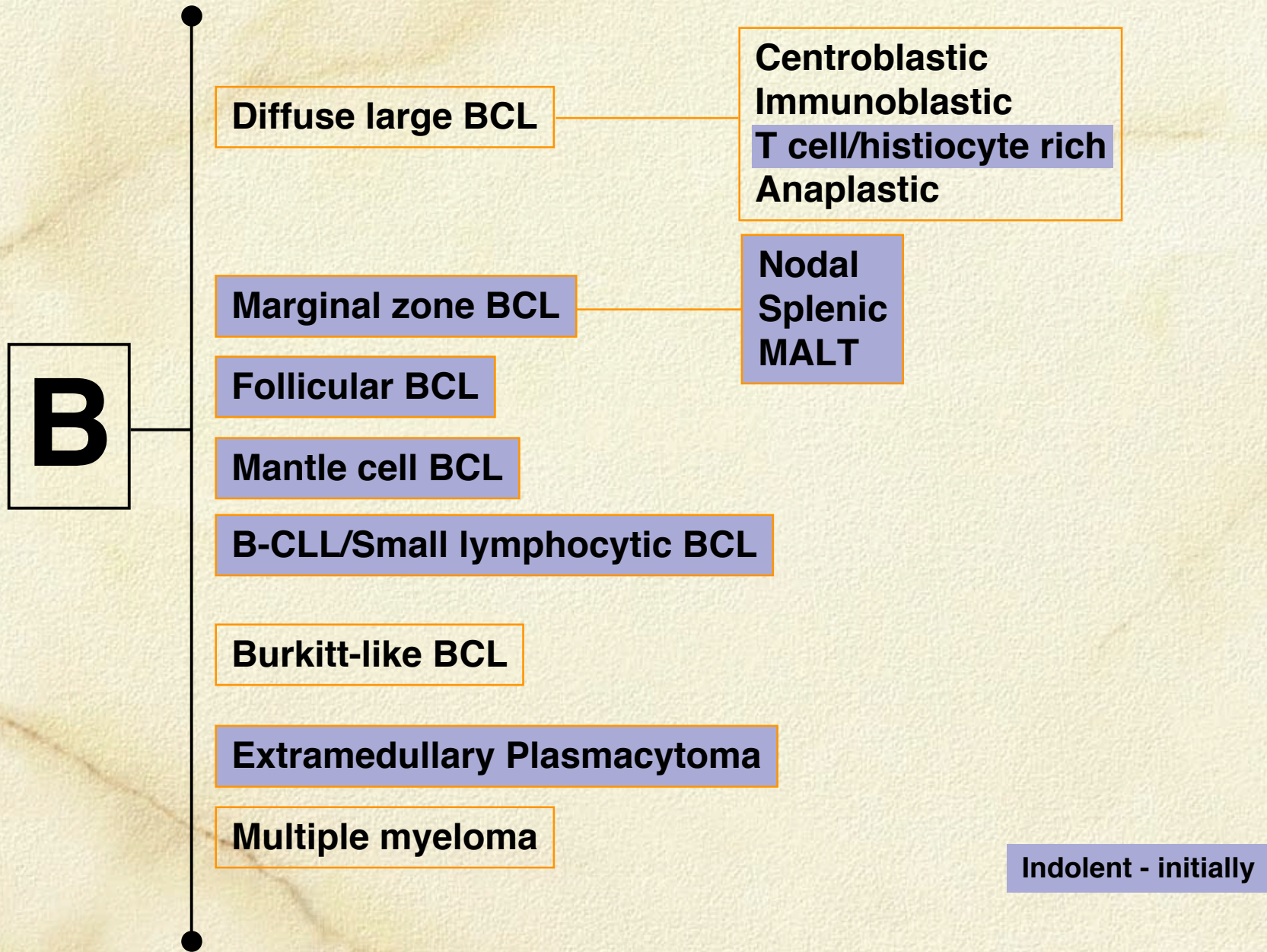
WHO classification of tumors of hematopoietic and lymphoid tissues

- **Proponents of other schemes have endorsed WHO classification**
- **First true world-wide consensus classification scheme for hematologic malignancies**
- **ACVP initiative - Lymphoma Study Group - investigated suitability of WHO scheme for animal lymphomas (led by Dr. Ted Valli)**

WHO lymphoma classification - Precursor B/T



WHO Lymphoma classification - Mature B cell



WHO Lymphoma classification - Mature T cell

T

Nodal TCL

Peripheral TCL - unspecified
T-zone TCL
Anaplastic large TCL
Angioimmunoblastic TCL

Cutaneous TCL

Mycosis fungoides
Pagetoid reticulosis
Sézary syndrome
Peripheral TCL - unspecified

Extranodal - other

Enteropathy associated TCL
Hepatosplenic TCL
Peripheral TCL - unspecified

LGL leukemia

T-LGL CLL
T-LGL ALL

LGL lymphoma

Indolent - initially

Indolent - some forms

Integrative diagnostics - Leukocytic diseases

- **Clinical and clinico-pathological data**
- **Morphological data - histology/cytology**
- **Immunophenotyping - reagent panels**
- **Molecular assessments - antigen receptor clonality for lymphoma**

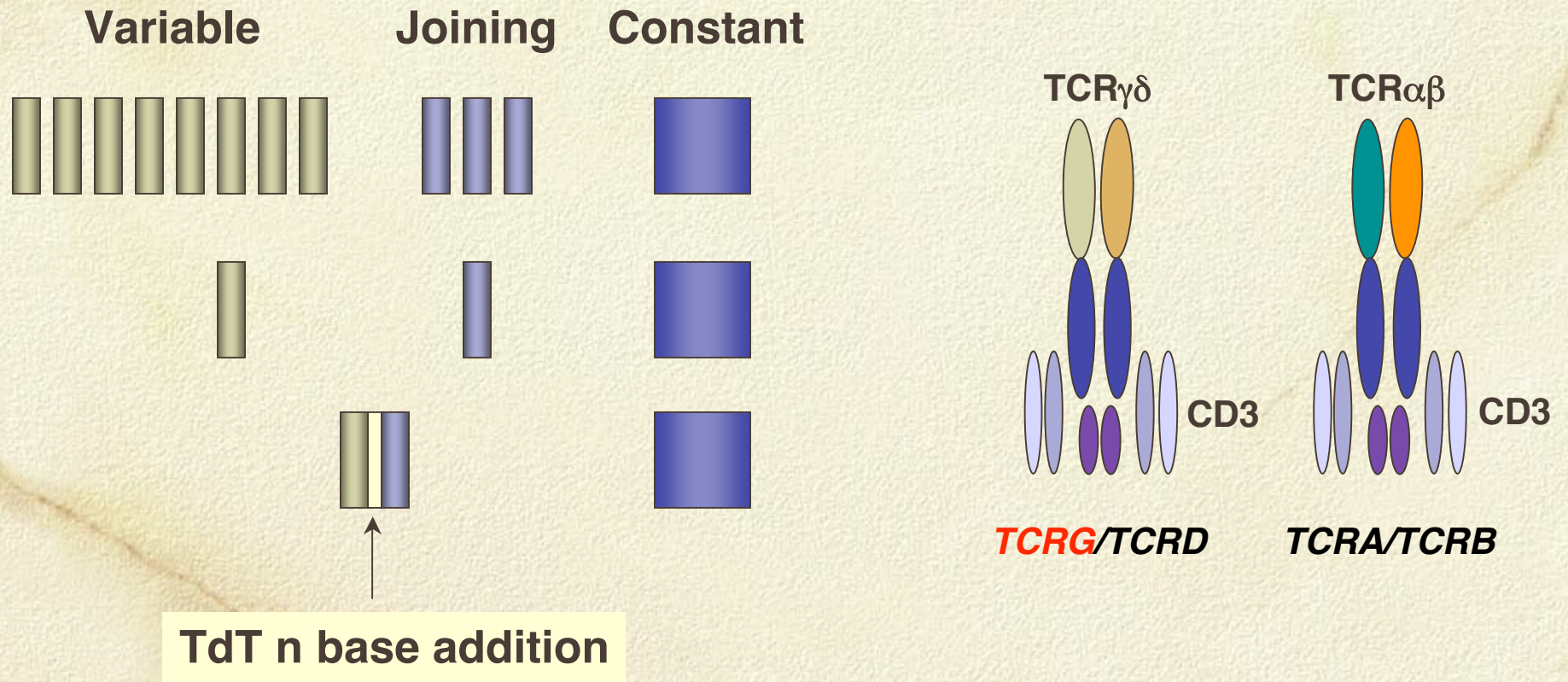
Lymphocyte Development

and

Antigen receptor gene rearrangement

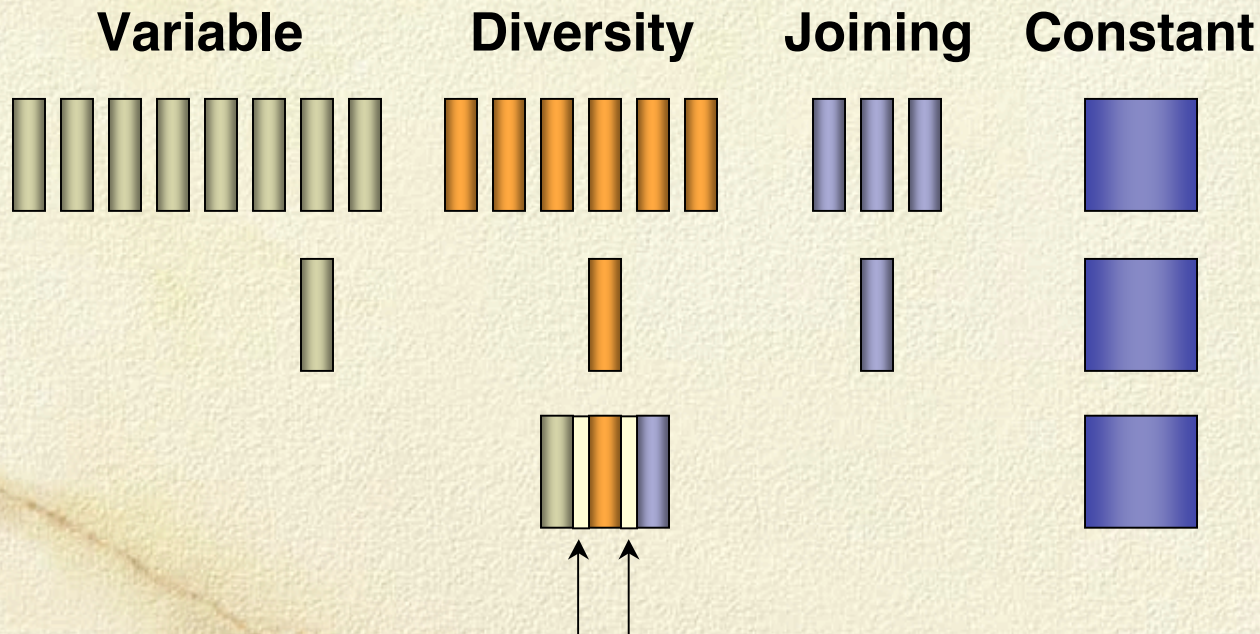
T cell receptor gene rearrangement

- **TCRG** - molecular clonality target (rearranged in $\gamma\delta$ and $\alpha\beta$ T cells)

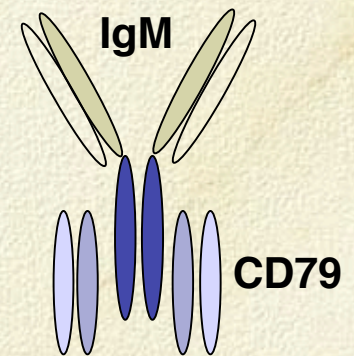


B cell receptor gene rearrangement

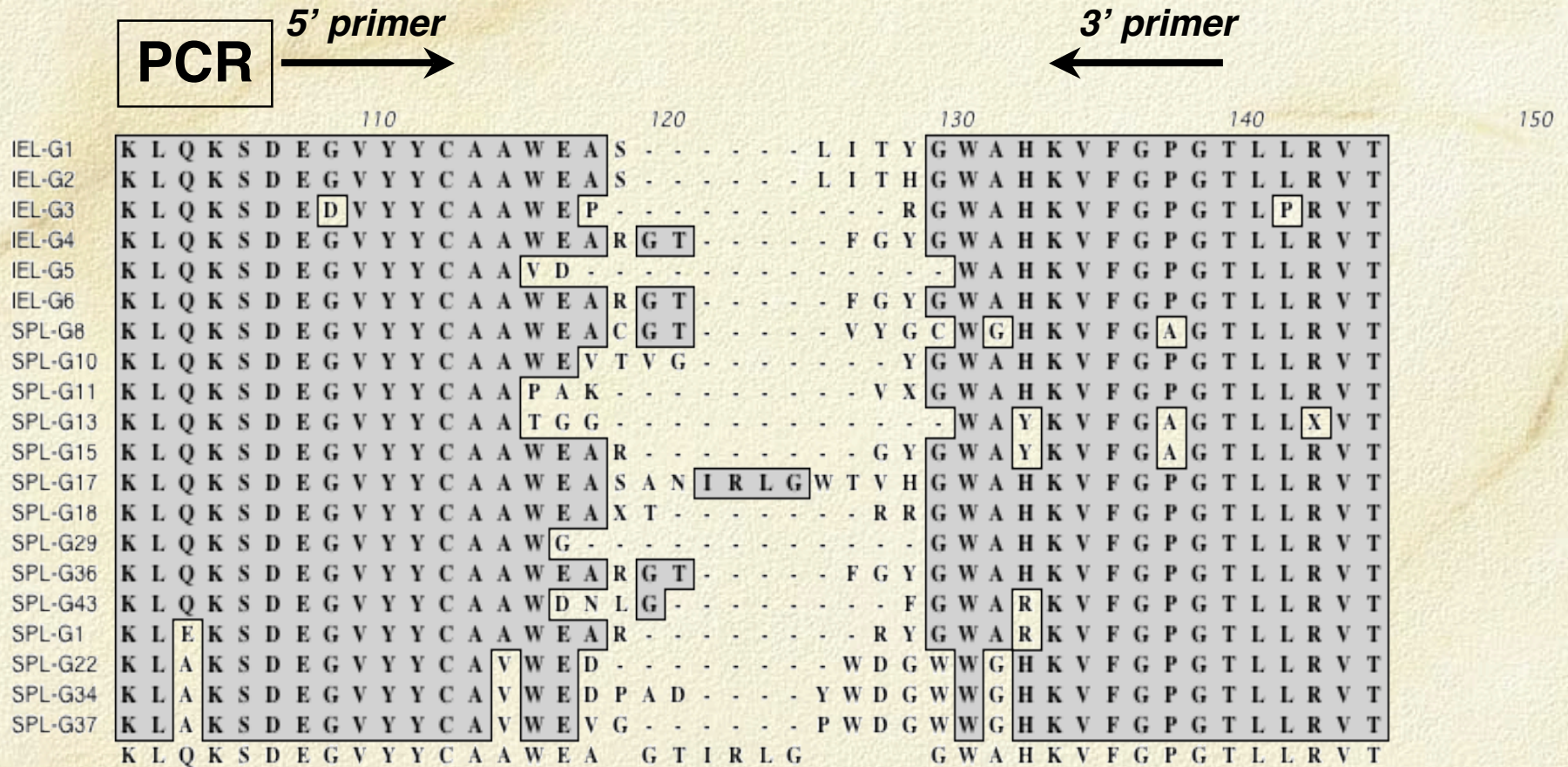
- Ig heavy chain (IGH) locus - molecular clonality target
- V segment mutation in germinal center B cells - reduces sensitivity



TdT n base addition



Feline TCRG V-N-J alignment CDR3 region



← **3' V segment** → ← **CDR3** → ← **J segment** →

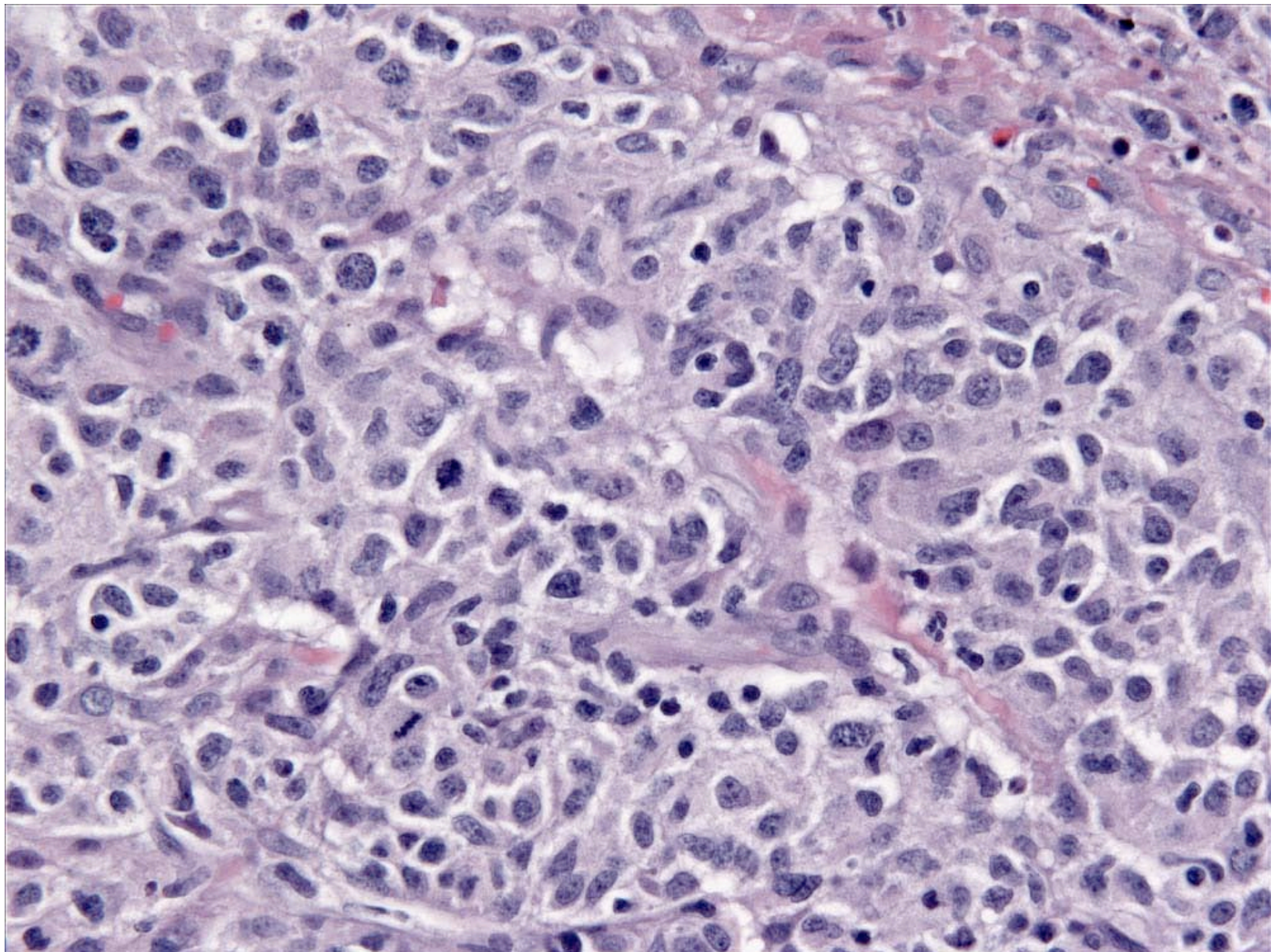


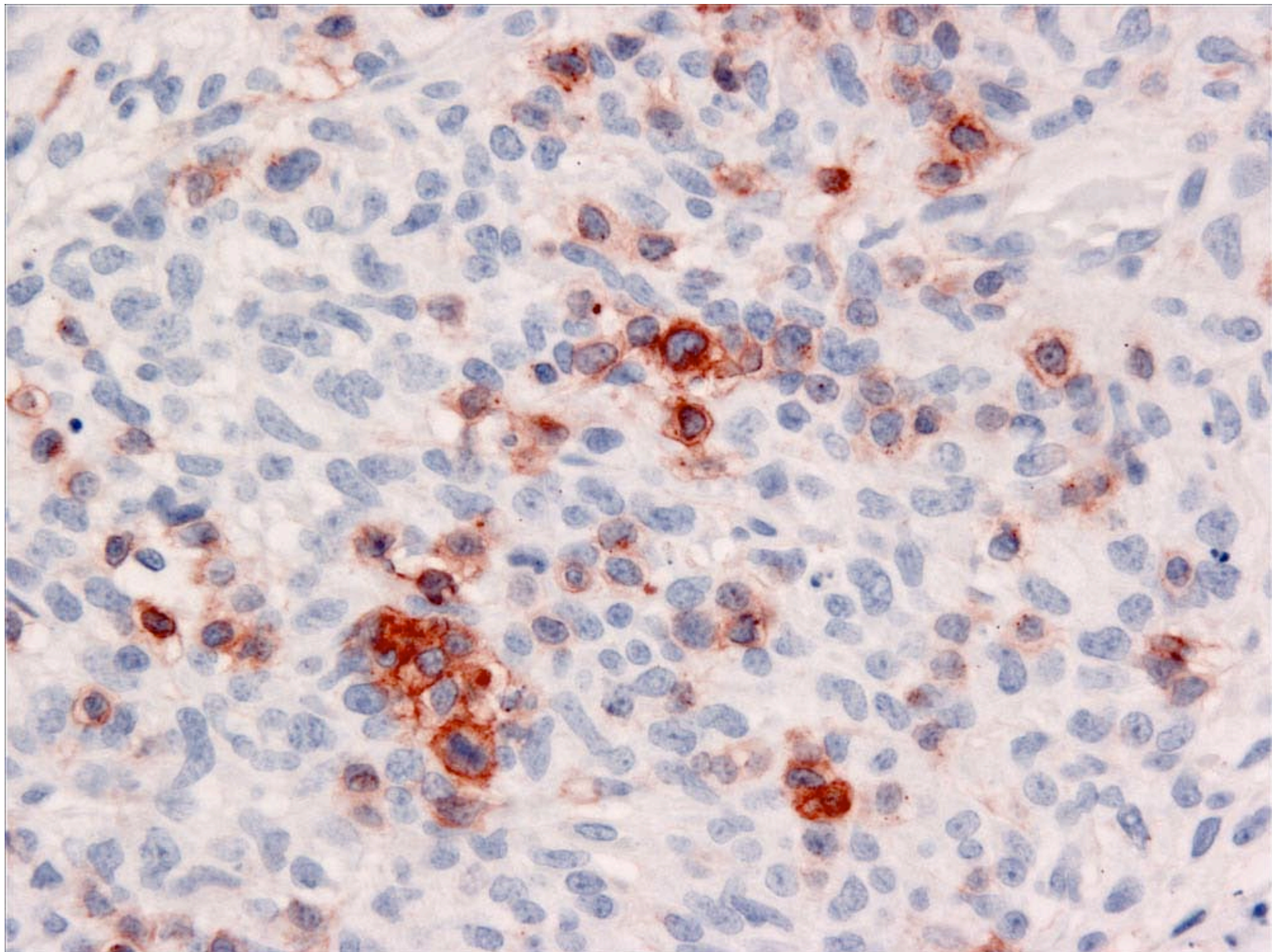
Ag receptor gene rearrangement - **indications**

- ❑ **Morphological, cytological, immunophenotypic properties inconclusive**
- ❑ **Lack of architectural effacement in organized lymphoid tissue - MZL or TZL**
- ❑ **Lamina proprial or intra-epithelial lymphocytosis in the small intestine**
- ❑ **Lympho-histiocytic proliferations in skin**

Canine “inflamed” T cell lymphoma (PTCL)

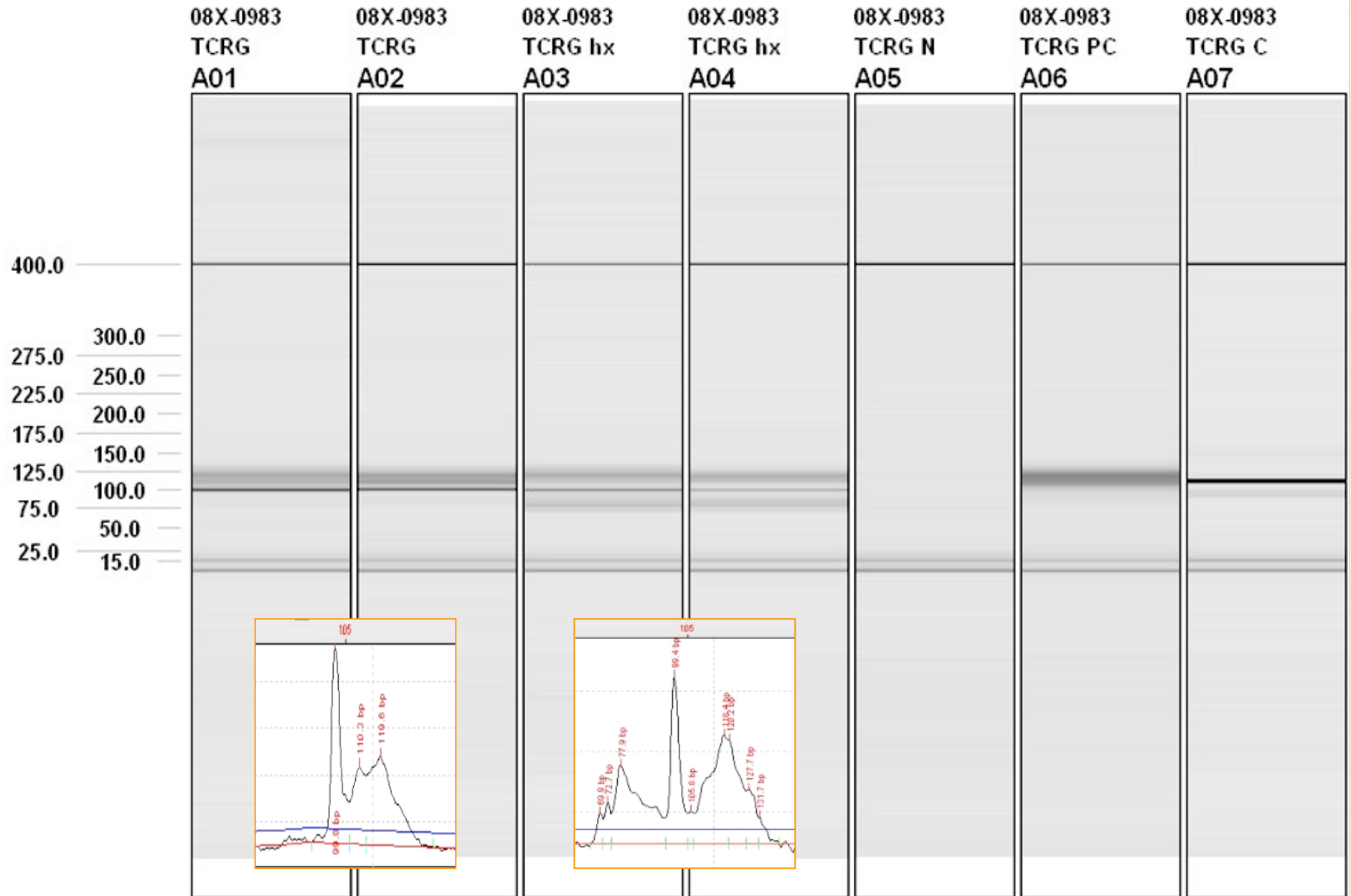
- **387403 - Bernese Mtn dog, MC, 6 yrs - masses on digit, carpus, mandible.**
- **Dec 07 - DX#1: Histiocytic dermatitis**
- **Mar 08 - DX#2: Histiocytic sarcoma**
- **May 08 - DX#3: Reactive histiocytosis**





387403 - Bernese mountain dog, MC, 6 years

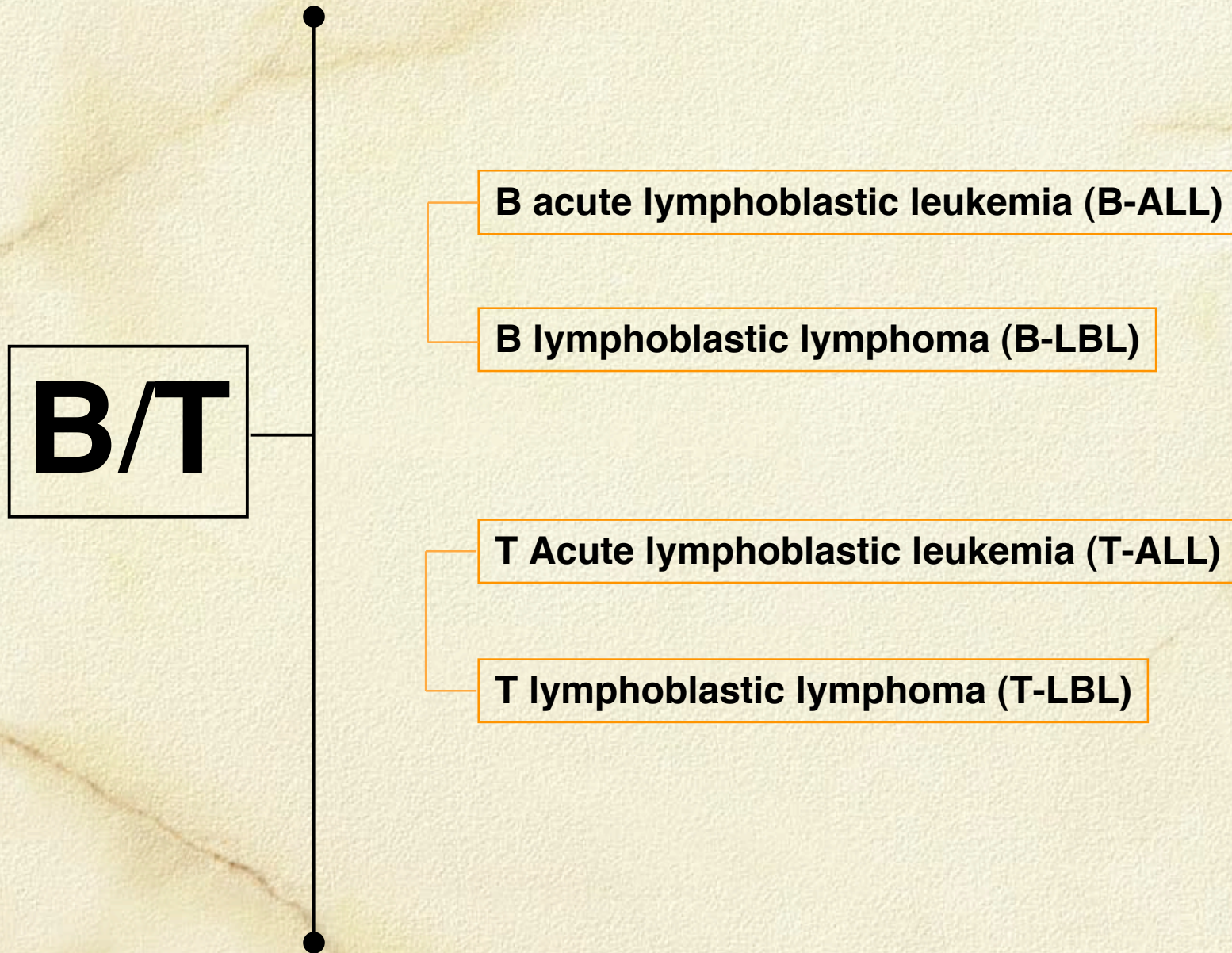
DX: Non-epitheliotropic T cell lymphoma (& lympho-histiocytic dermatitis)



Molecular clonality - limitations

- ❑ Sensitivity limited with high polyclonal background
 - ❑ Miss small clonal populations - e.g. inflamed lymphoma
- ❑ Sensitivity limited - B cell lymphoma - *IGH* V mutation
- ❑ Clonality is not equivalent to malignancy
 - ❑ Interpret results in appropriate context
- ❑ *IGH* and *TCRG* rearrangements are lineage associated -
- but not absolute markers of lineage
 - ❑ Cross lineage rearrangements in lymphoid and myeloid malignancies

WHO lymphoma classification - Precursor B/T



T-lymphoblastic lymphoma (T-LBL)

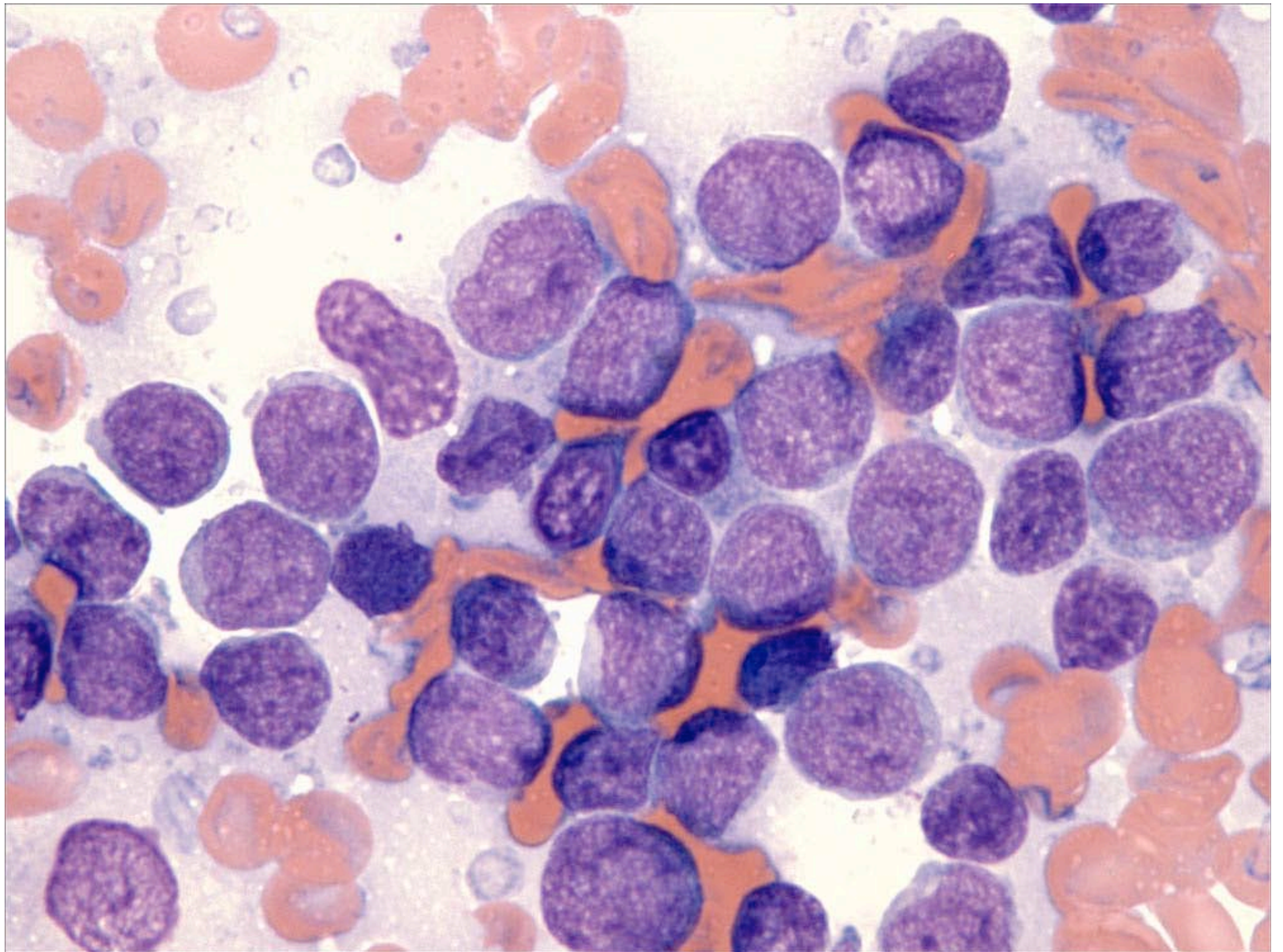
- **Mass lesion : T-LBL**

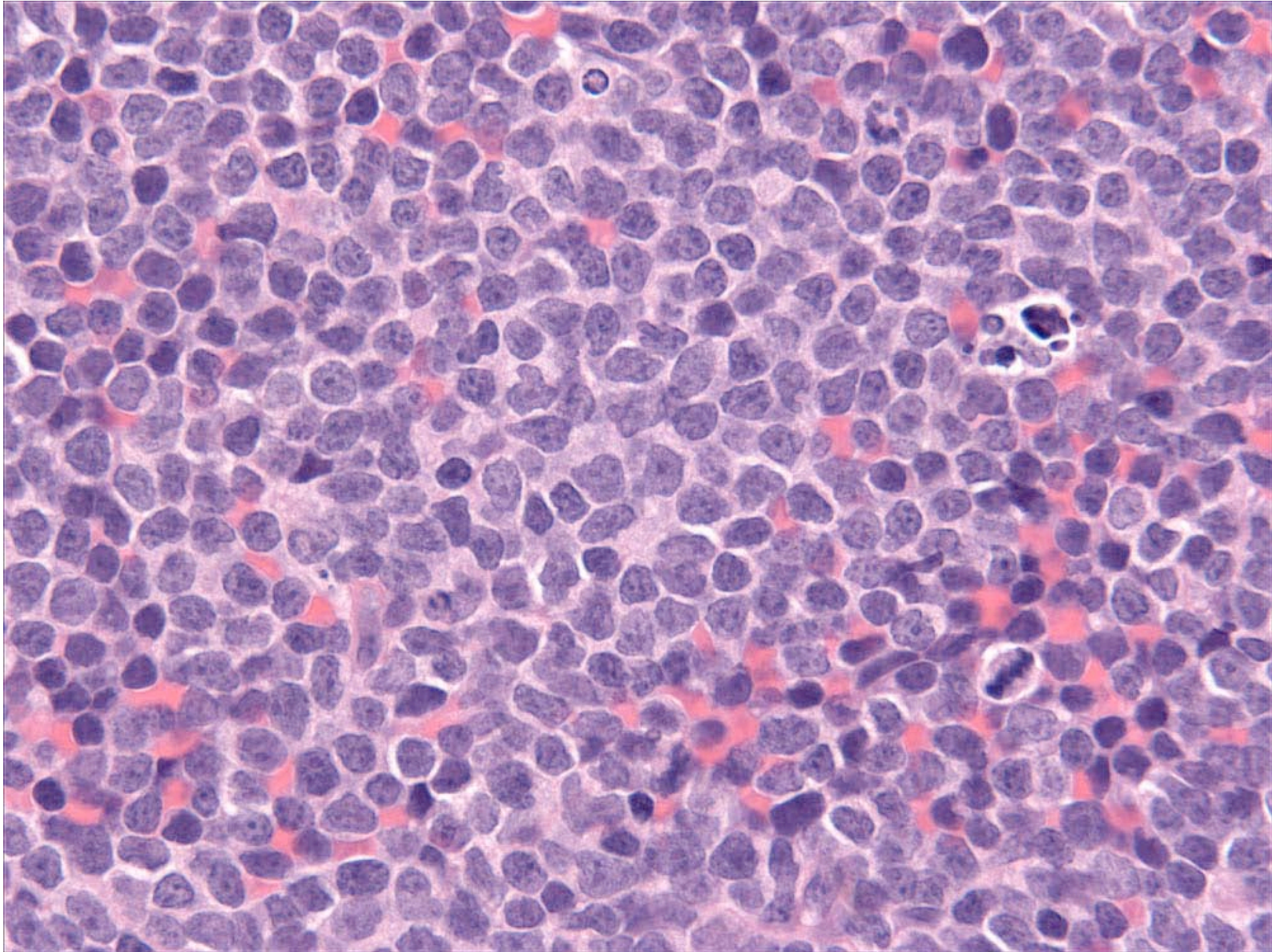
mediastinum, LNs, spleen, other sites

- **Predominance of blood/BM involvement: T-ALL**

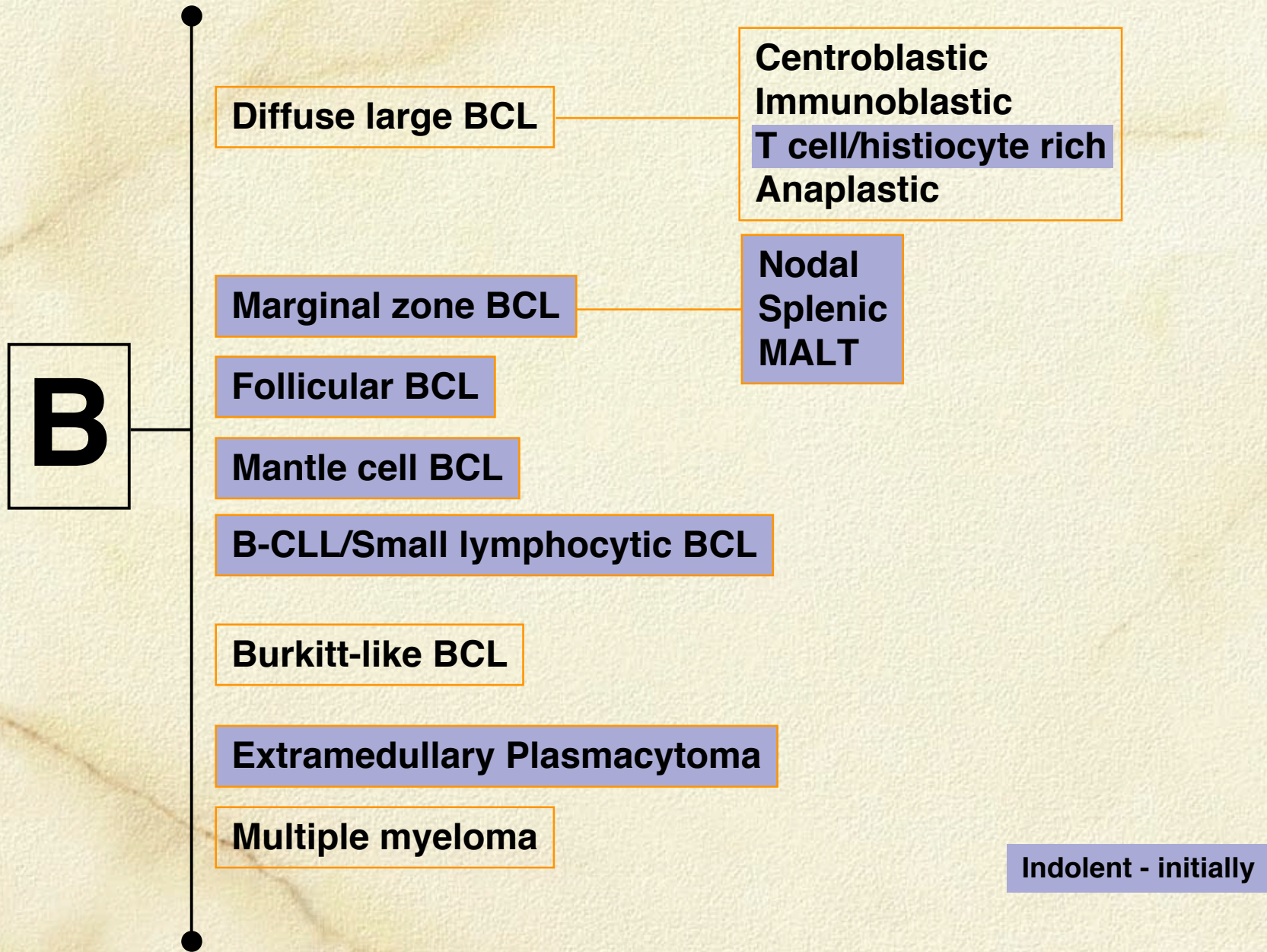
T-lymphoblastic lymphoma

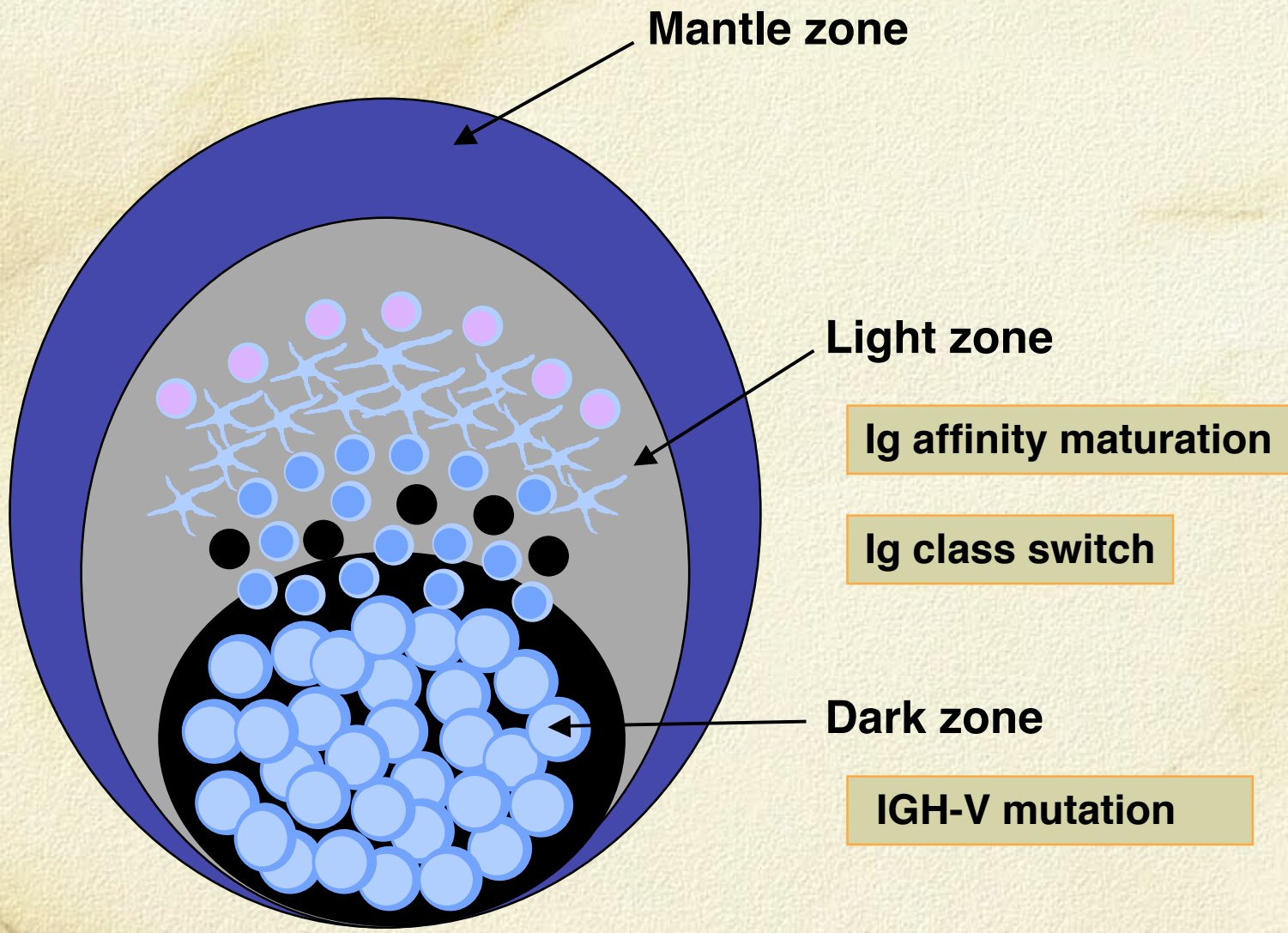
- ❑ **Origin:** Precursor T lymphoblast
- ❑ **Hypercalcemia a common feature**
- ❑ **High grade rapidly progressive**
- ❑ **Loss Cfa 11 in high- grade TCL**
- ❑ **P16 (Rb) deletion/inactivation in all cases**



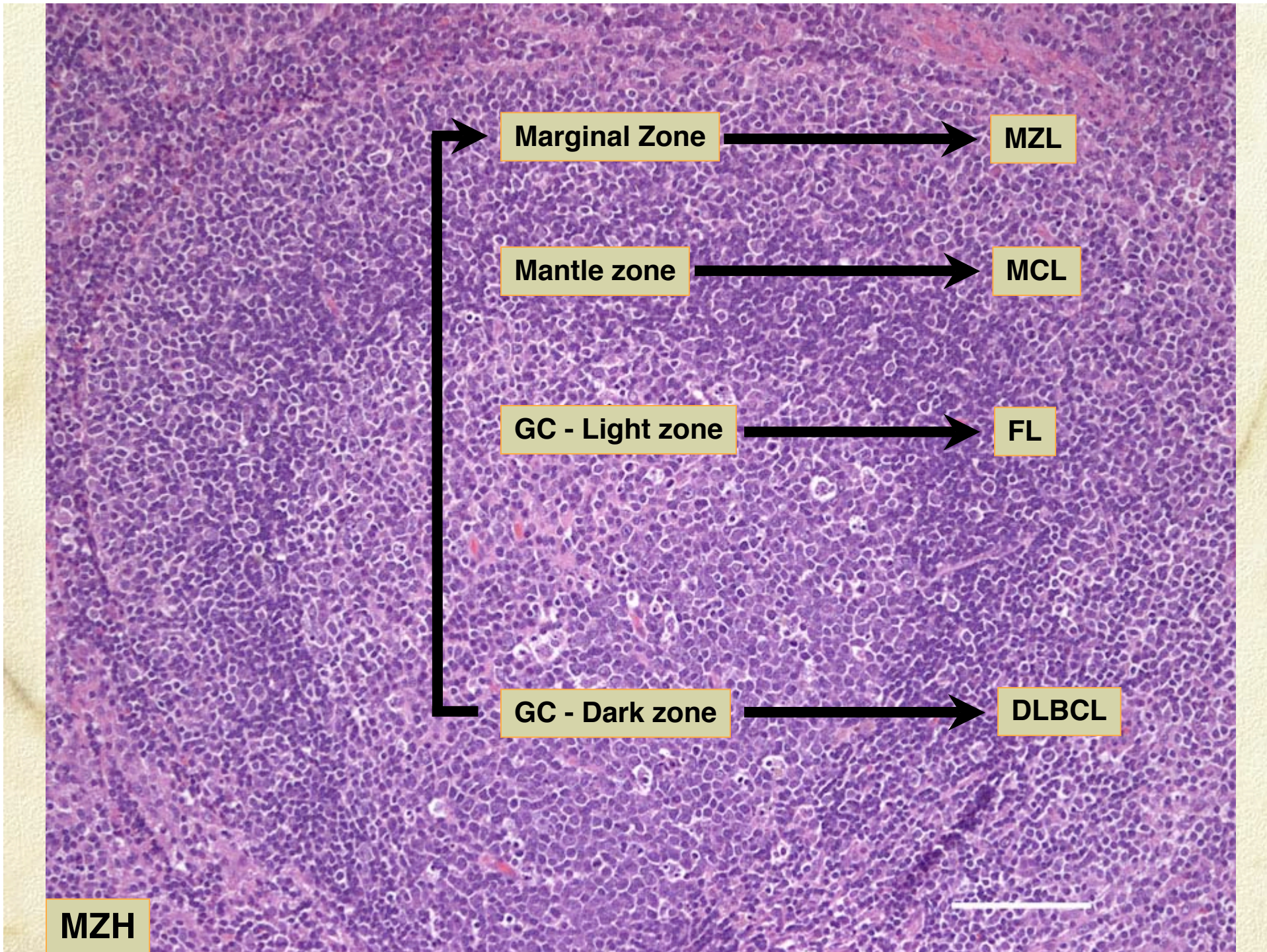


WHO Lymphoma classification - Mature B cell





Germinal Center Responses



MZH

Diffuse Large B cell Lymphoma (DLBCL)

- **Centroblastic**
- **Immunoblastic**
- **T cell/histiocyte rich**
- **Anaplastic**

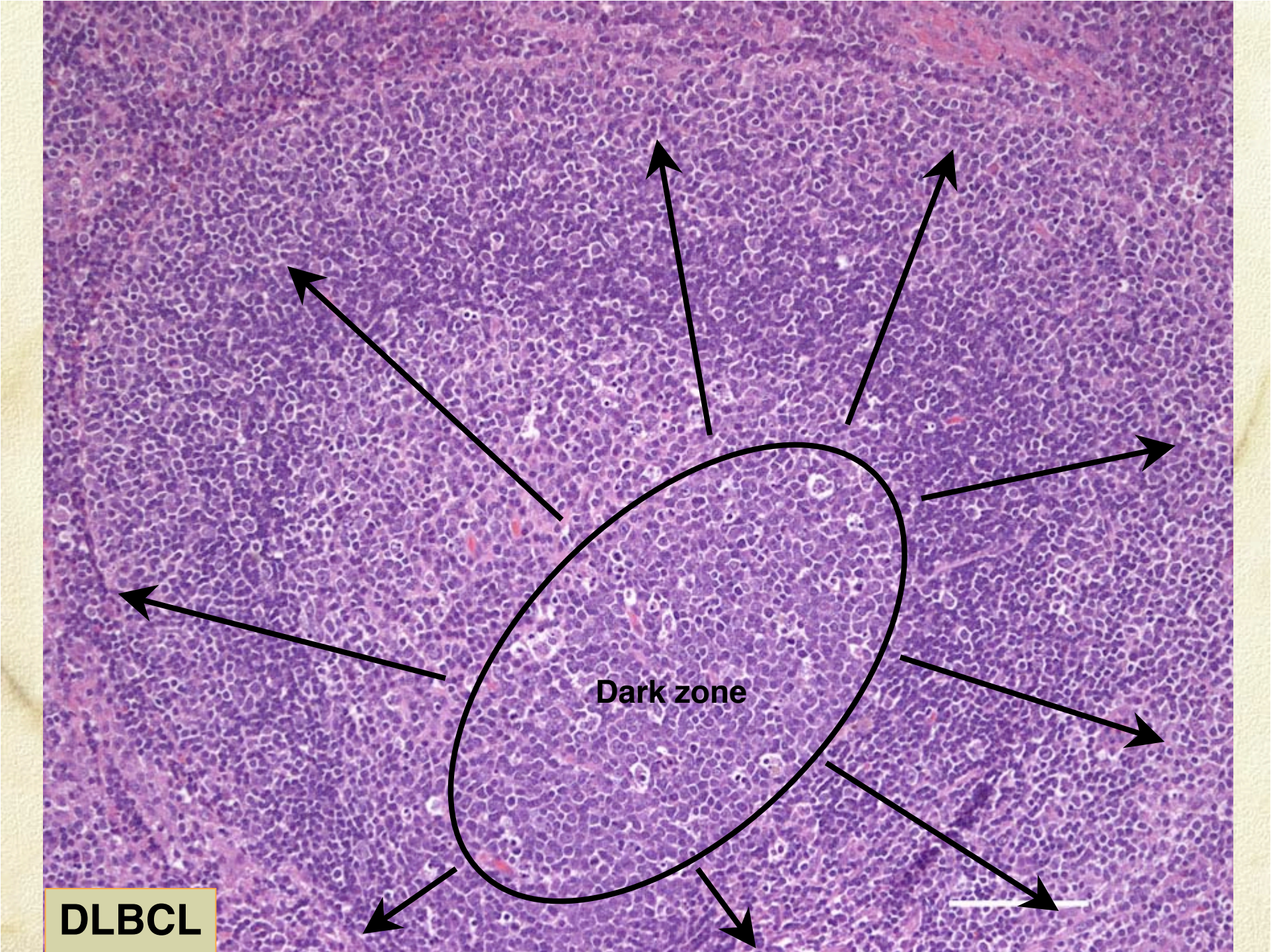
Diffuse Large B cell Lymphoma

- ❑ **Origin:** centroblasts in GC dark zone
- ❑ **Lymph nodes; spleen; extranodal**
- ❑ **Most prevalent lymphoma in dogs**
- ❑ **High grade lymphoma - high proliferative fraction**

Spleen - diffuse large B cell lymphoma

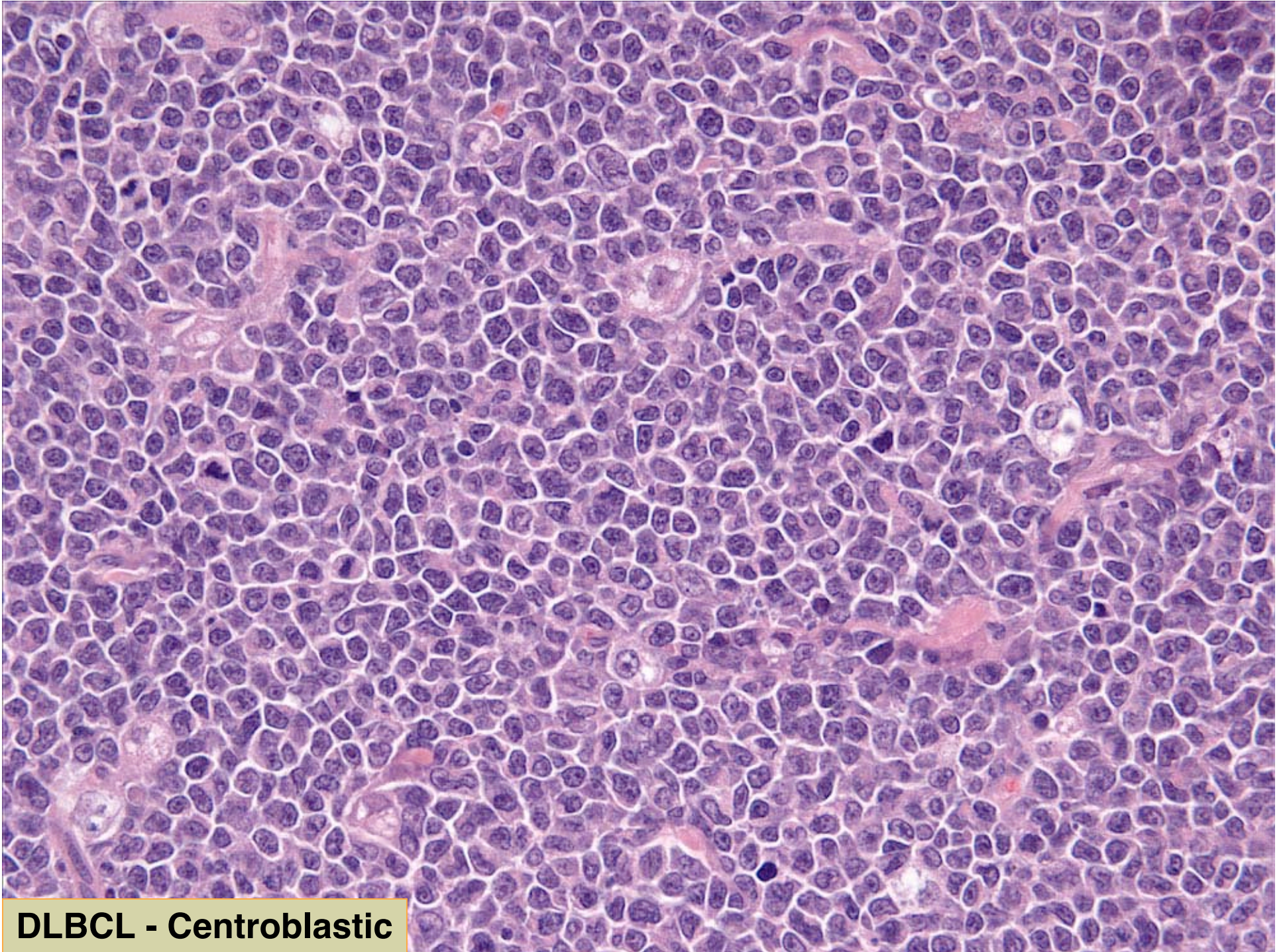


Canine spleen - splenomegaly due to white pulp infiltration/obliteration

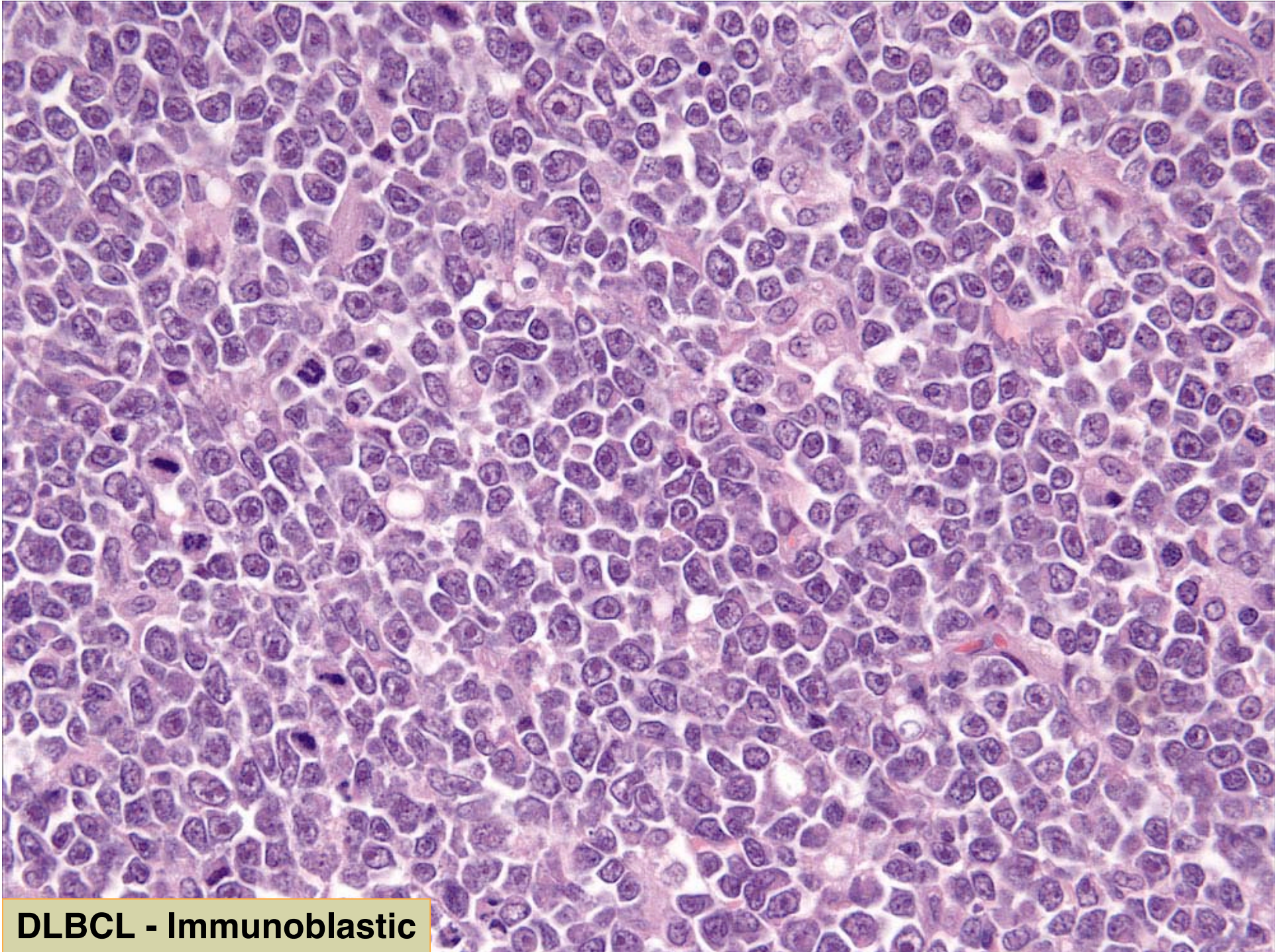


DLBCL

Dark zone

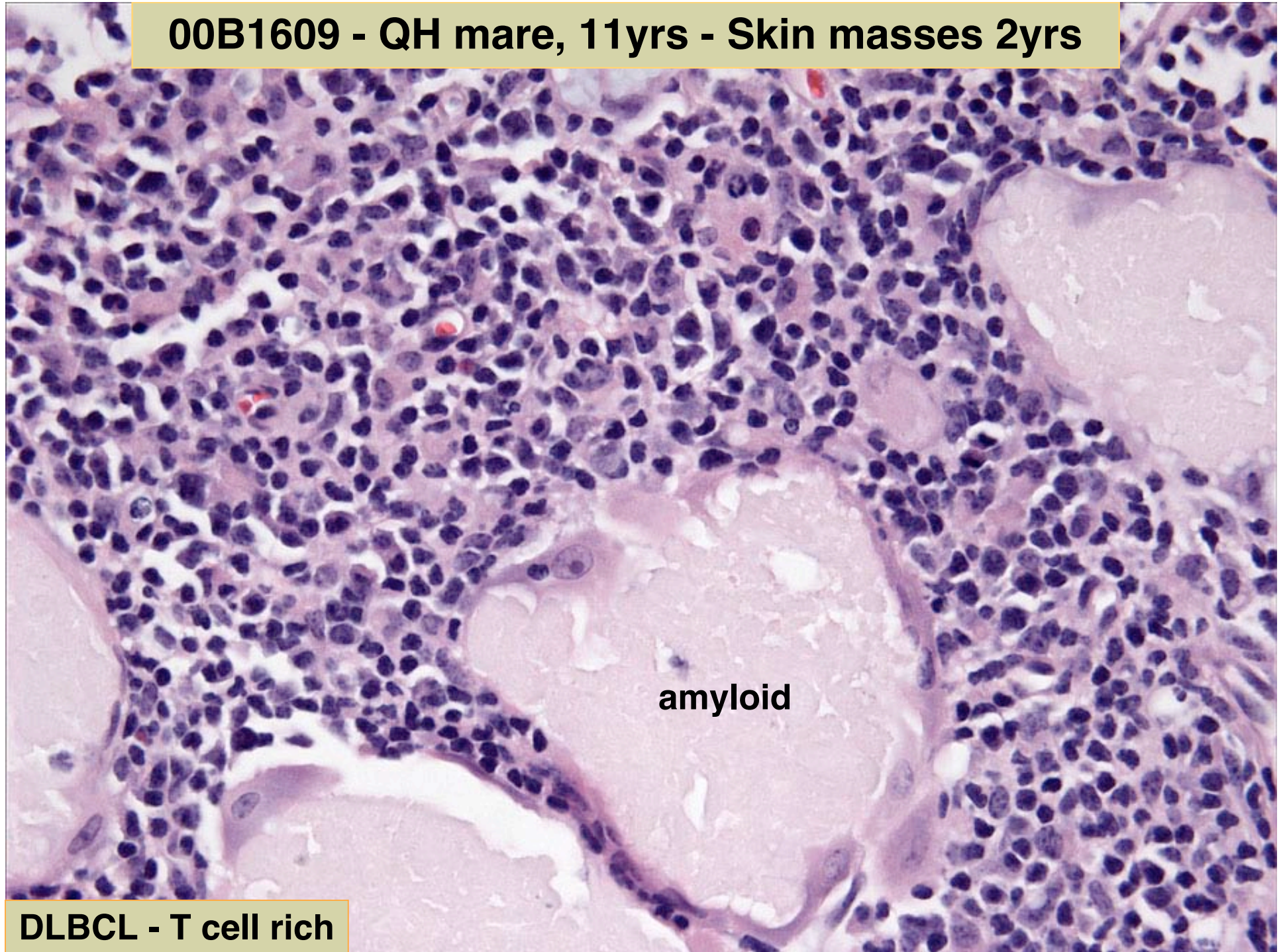


DLBCL - Centroblastic



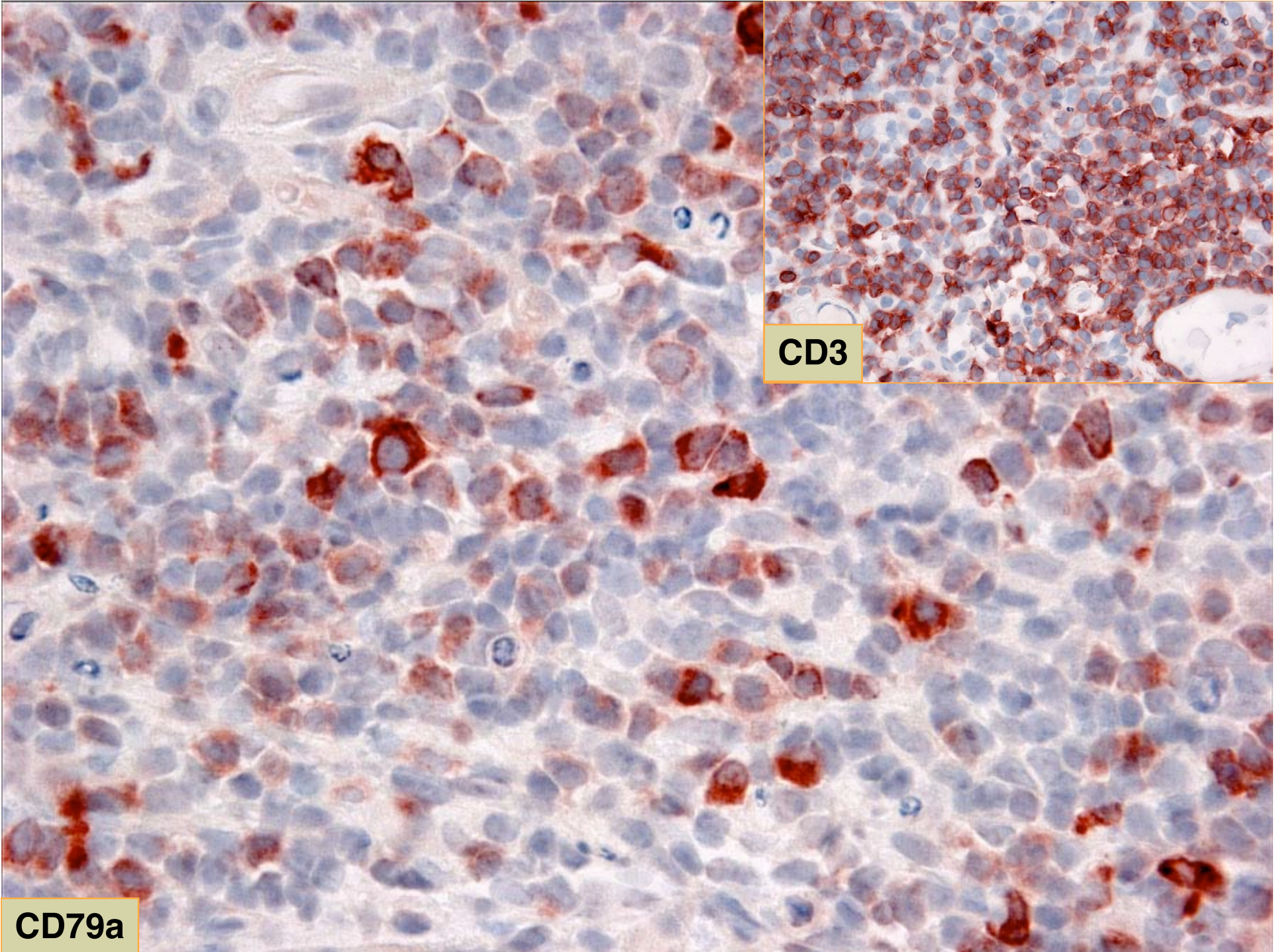
DLBCL - Immunoblastic

00B1609 - QH mare, 11yrs - Skin masses 2yrs



amyloid

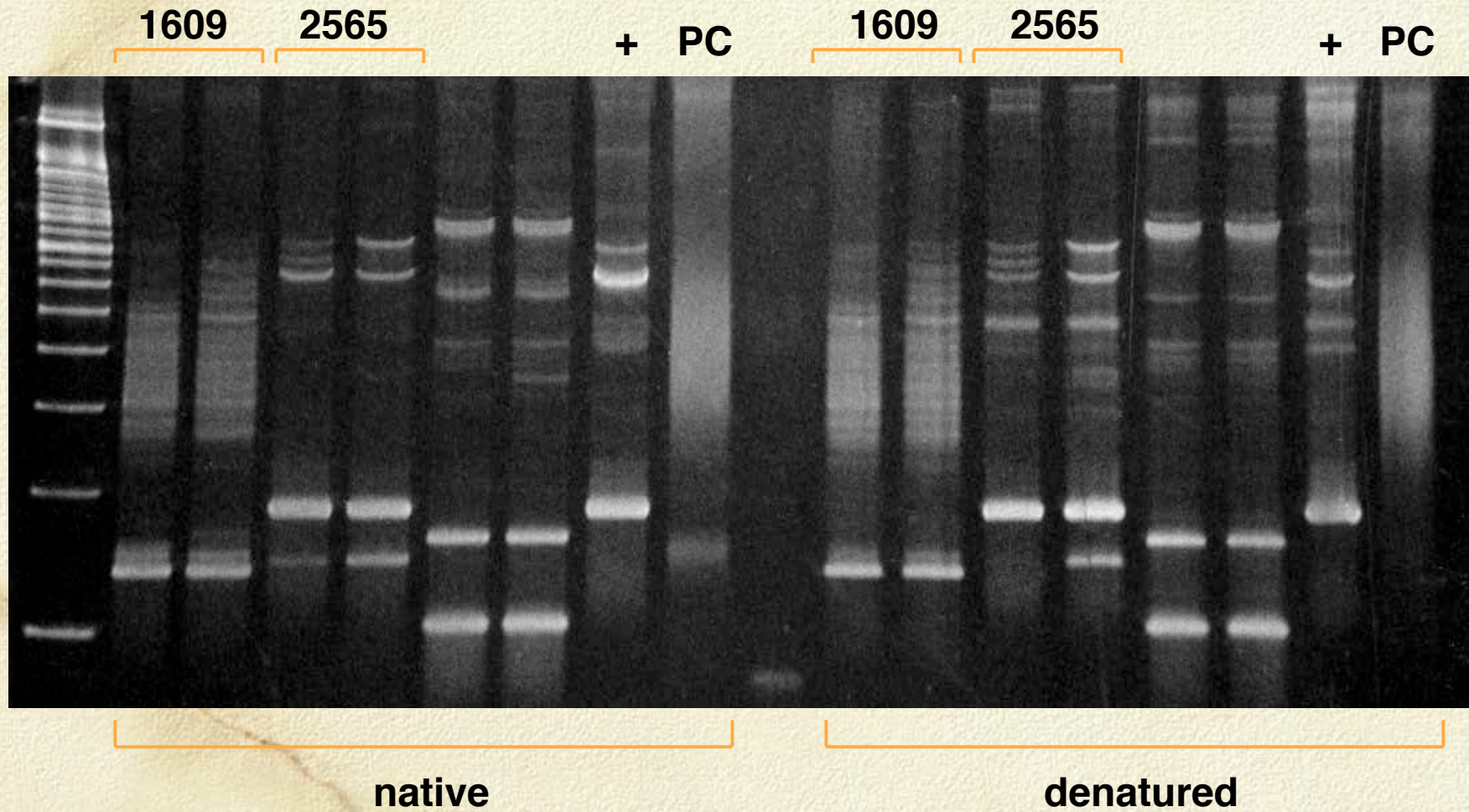
DLBCL - T cell rich

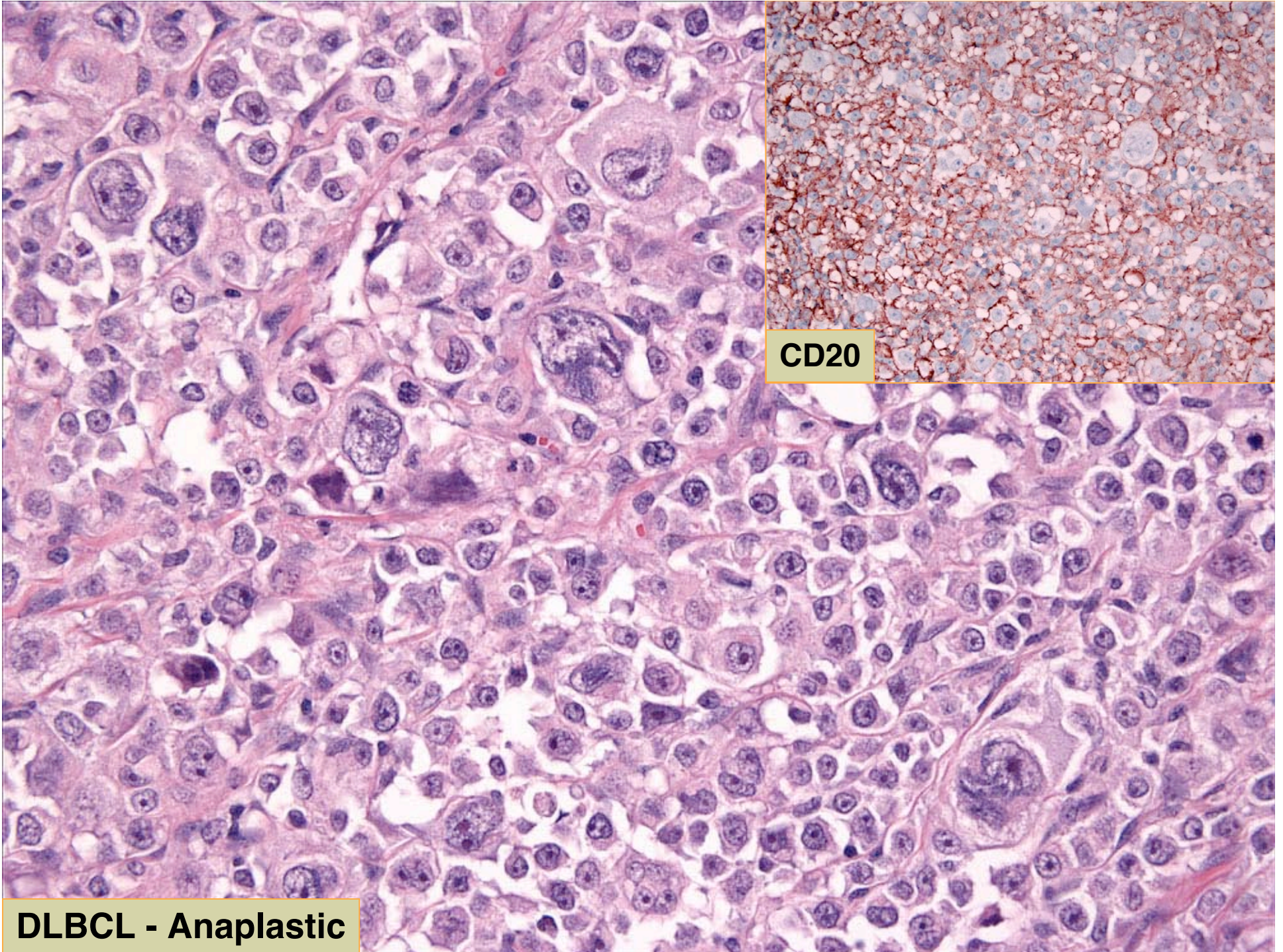


CD3

CD79a

T cell rich B cell lymphoma - equine skin





CD20

DLBCL - Anaplastic

Marginal zone lymphoma (MZL)

- ❑ **Nodal - most common**
- ❑ **Spleen - solitary mass and/or diffuse**
- ❑ **Extranodal - MALT lymphoma - rare**

Marginal zone lymphoma

- **Origin:** LN - perifollicular MZ B cells (chronic follicular hyperplasia) (dogs)

Splenic MZ B cells (dogs)

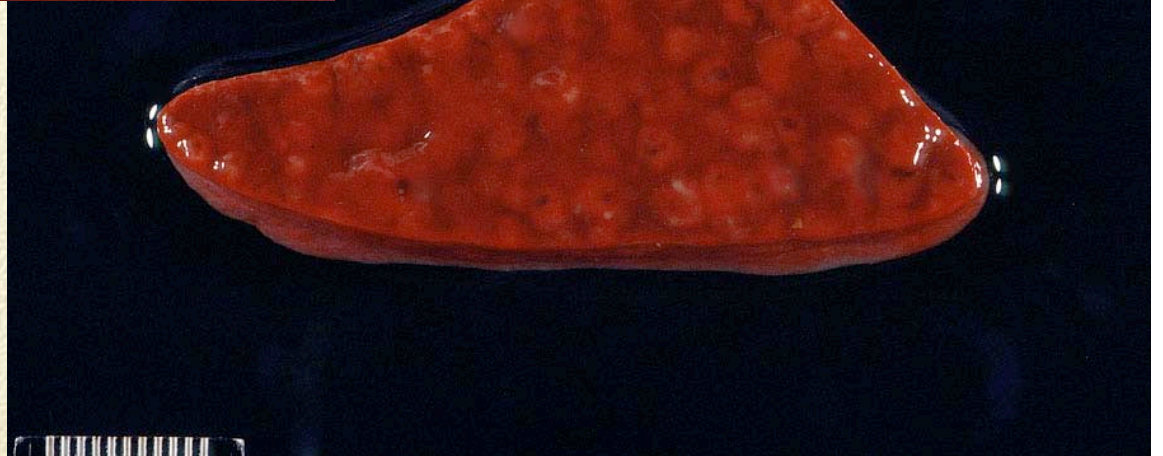
BALT and NALT - cats - rare

- **DX: architecture + cytologic characteristics**
- **DDX - nodular hyperplasia when spleen involved**
- **Indolent lymphoma - low proliferative fraction**
- **May evolve into DLBCL**

Spleen - marginal zone lymphoma

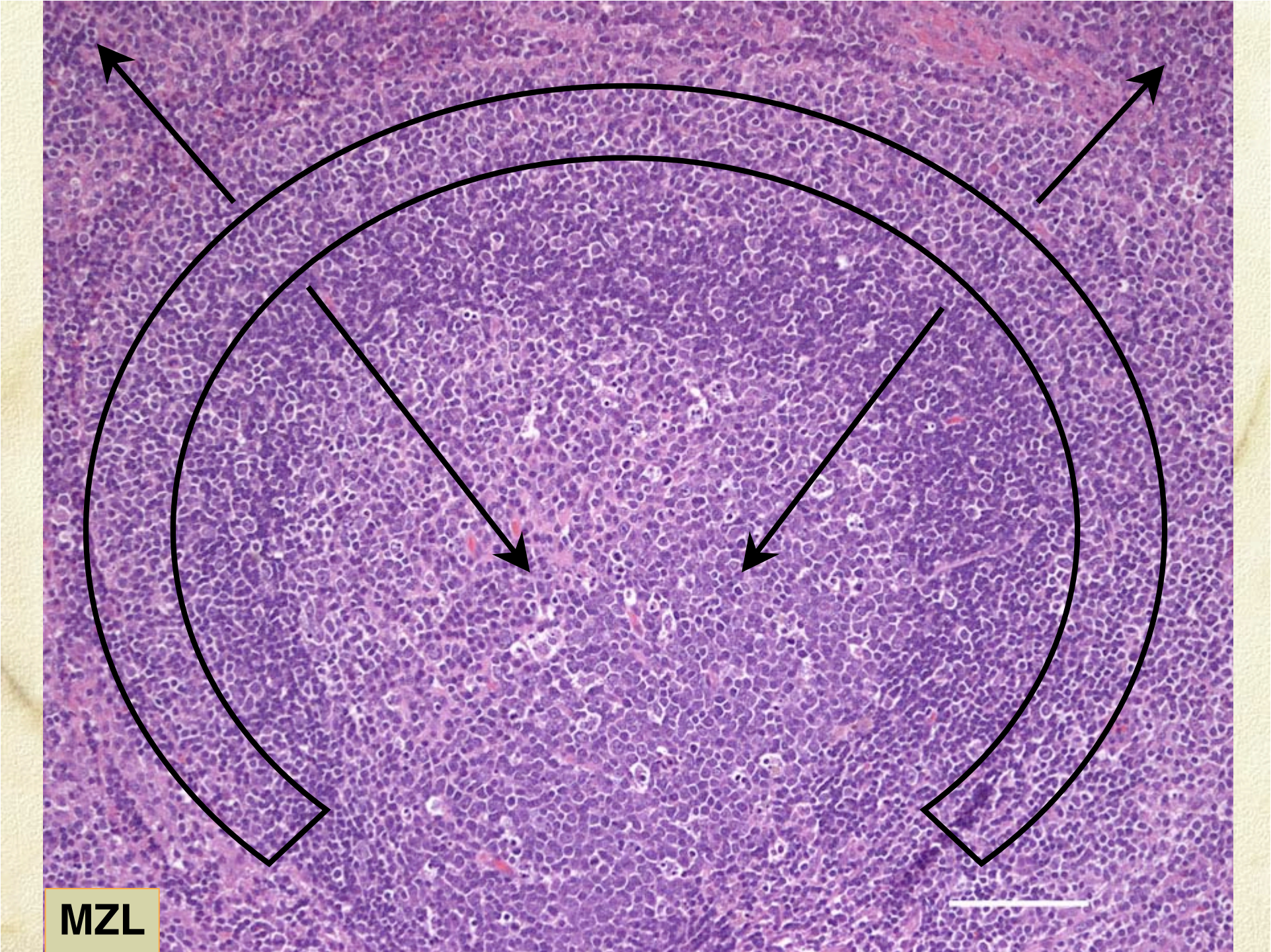


MZL- solitary mass and diffuse involvement. Perifollicular marginal zones slowly coalesce.

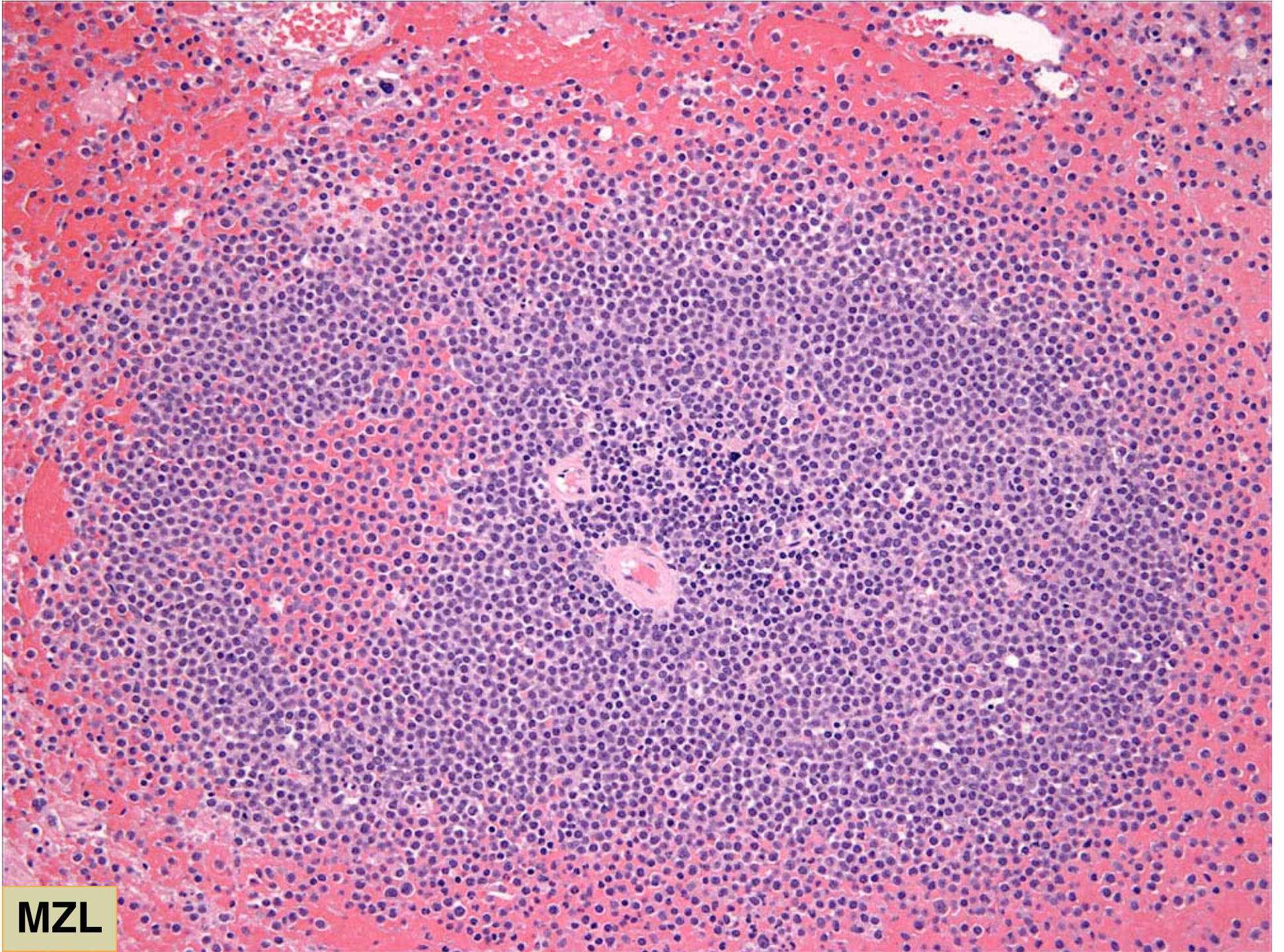




Marginal zone hyperplasia - lymph node

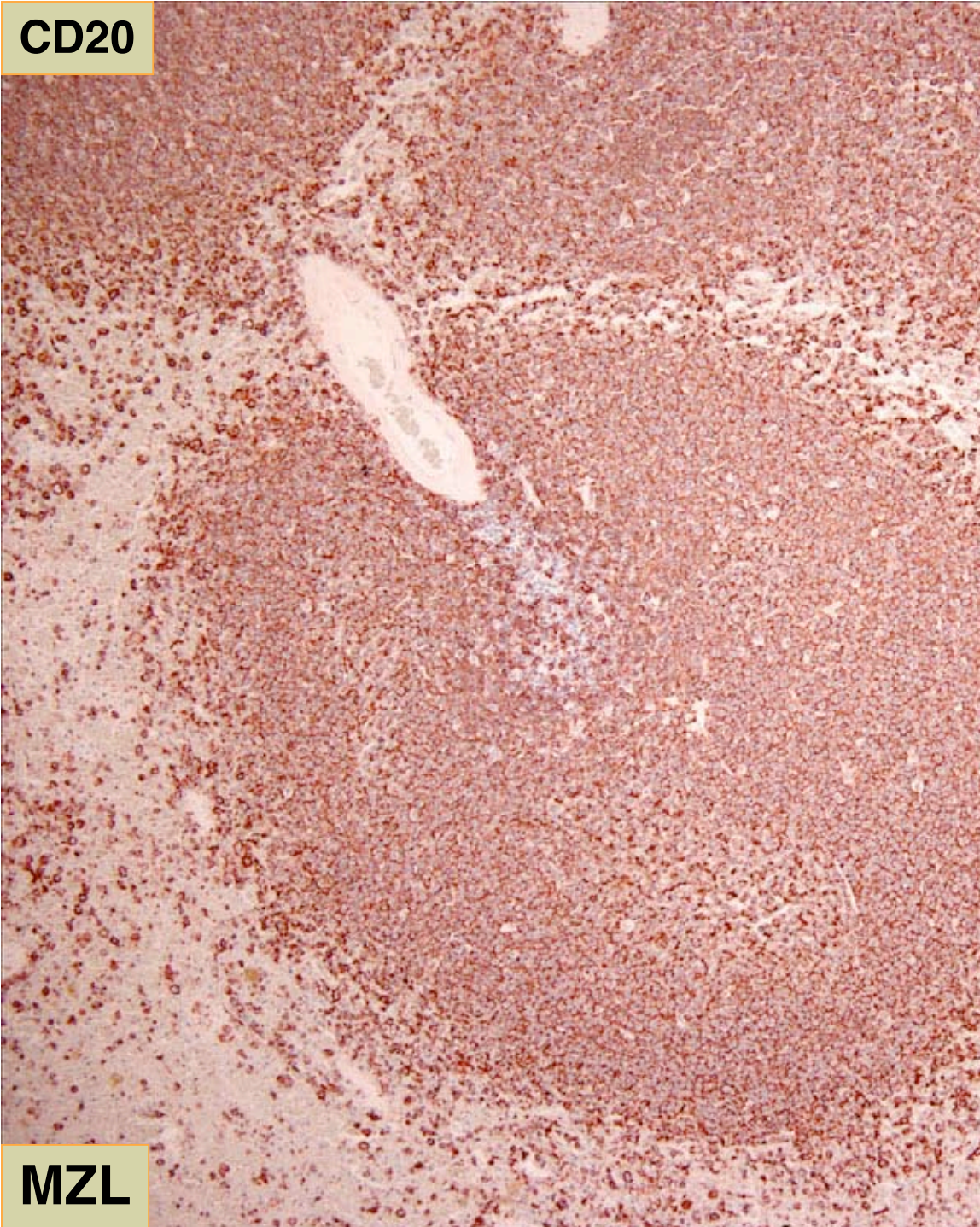


MZL

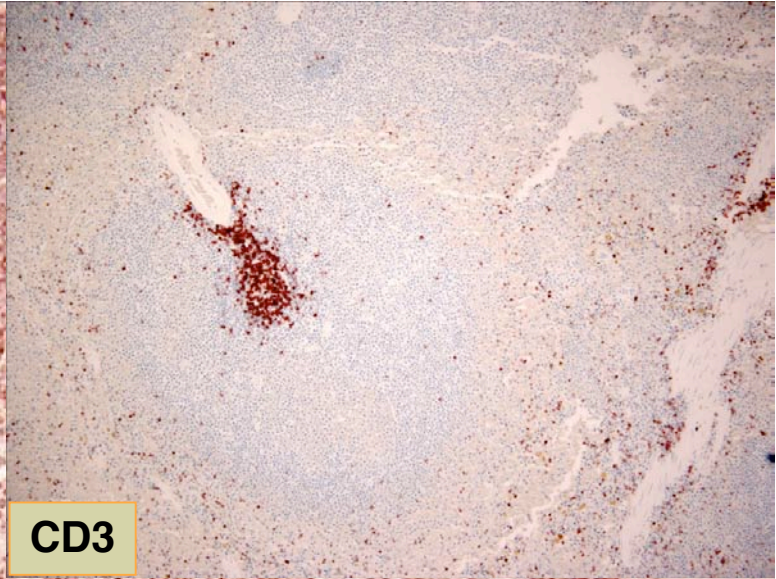


MZL

CD20

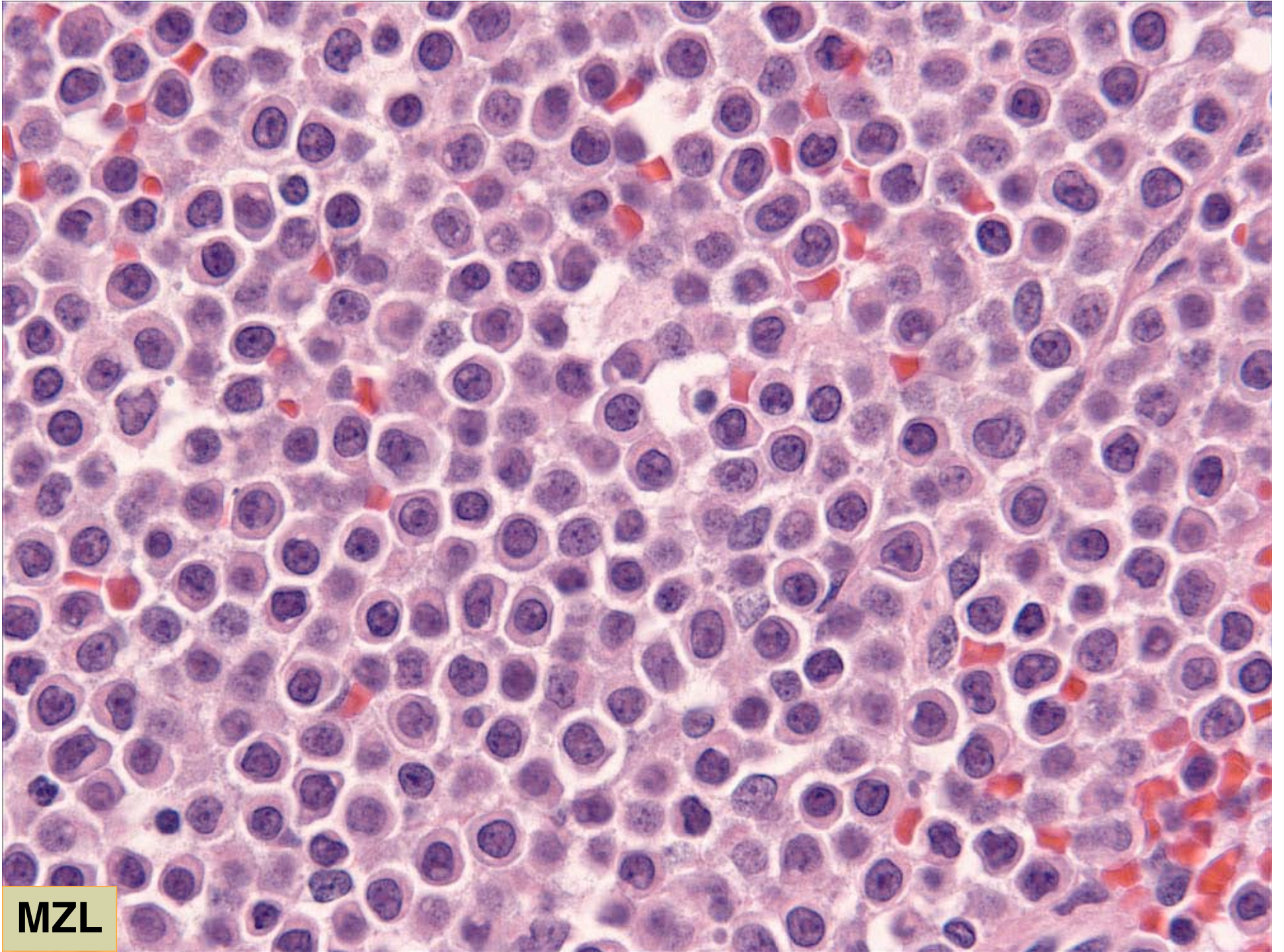


CD3



MZL





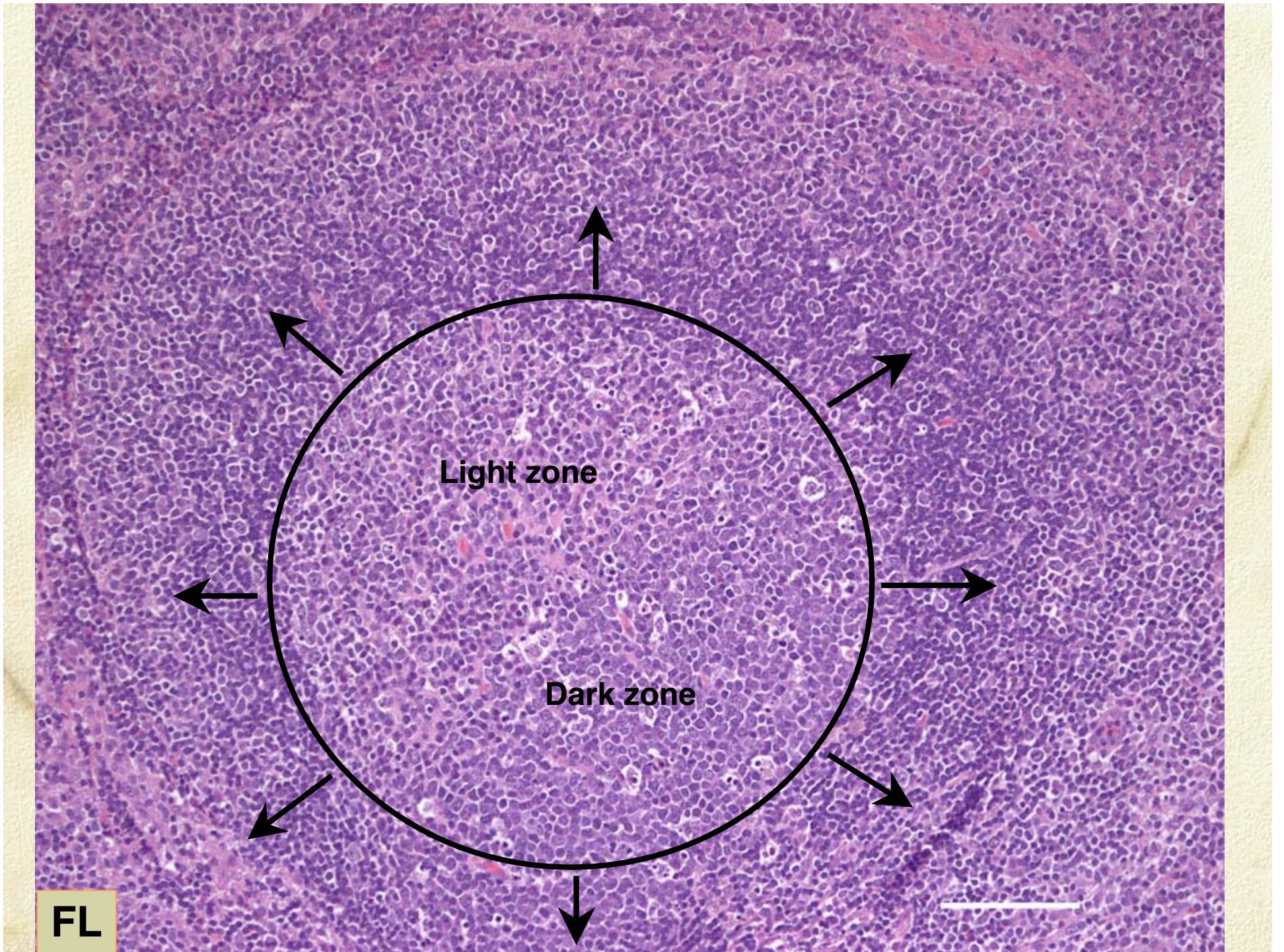
MZL

Follicular lymphoma (FL)

- **Nodal - most common**
- **Splenic**
- **Extranodal**

Follicular lymphoma

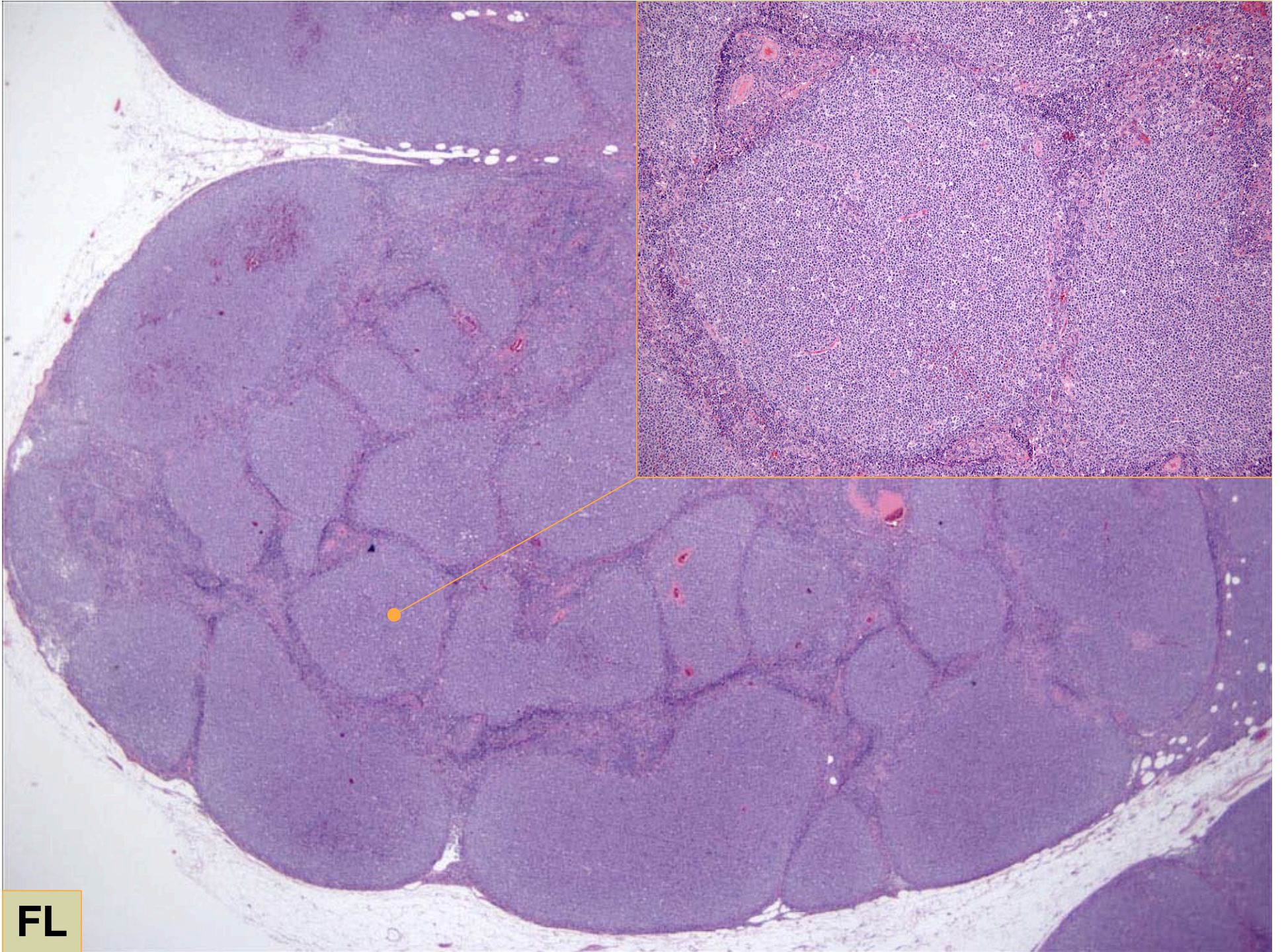
- ❑ **Origin:** Centrocytes in GC light zone
- ❑ **DX:** architecture + cytologic characteristics
- ❑ **Indolent B cell lymphoma - low proliferative fraction**
- ❑ **May evolve into DLBCL**
- ❑ **Human: t(14:18) - BCL2 gene rearranged**



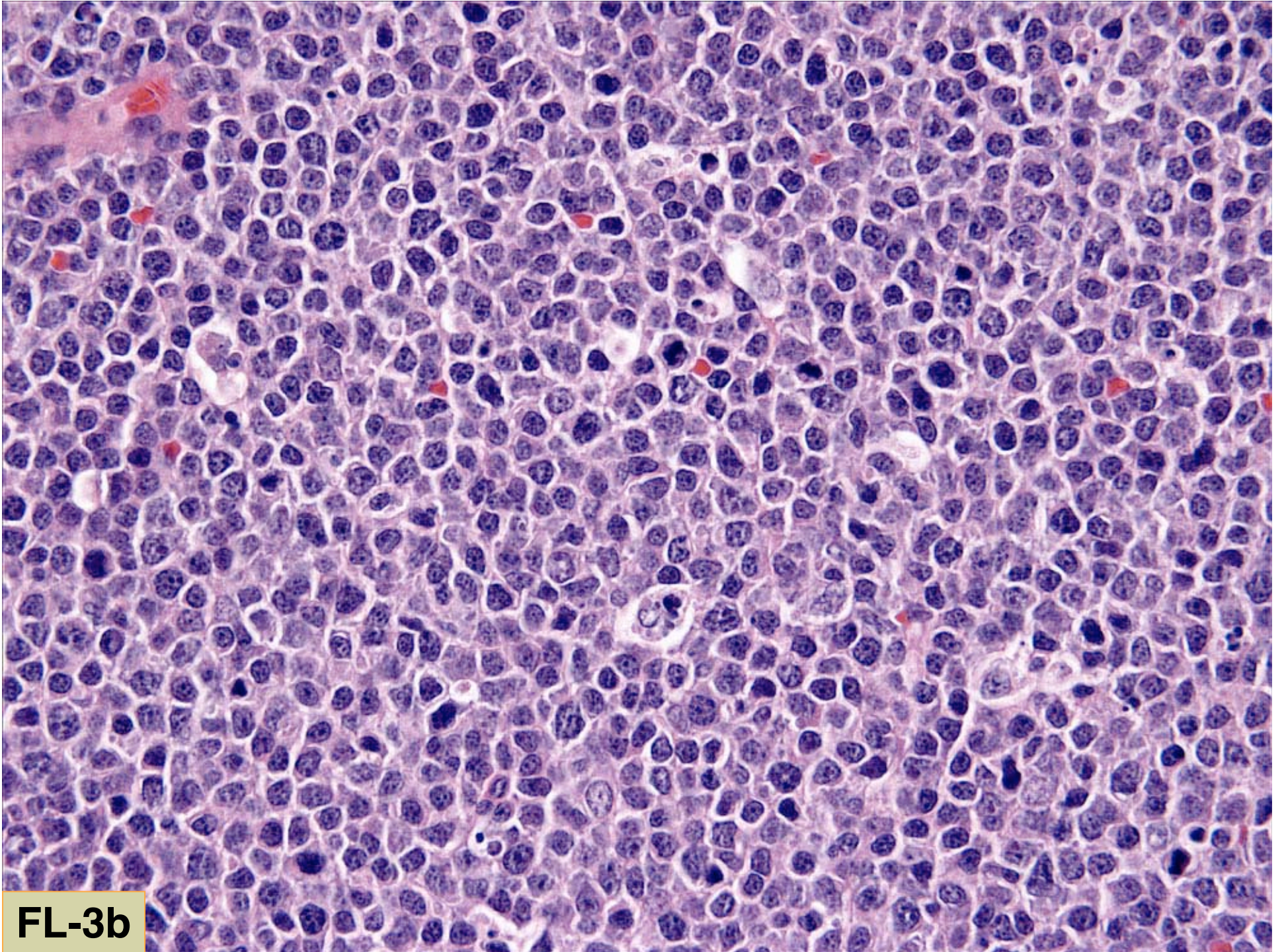
Light zone

Dark zone

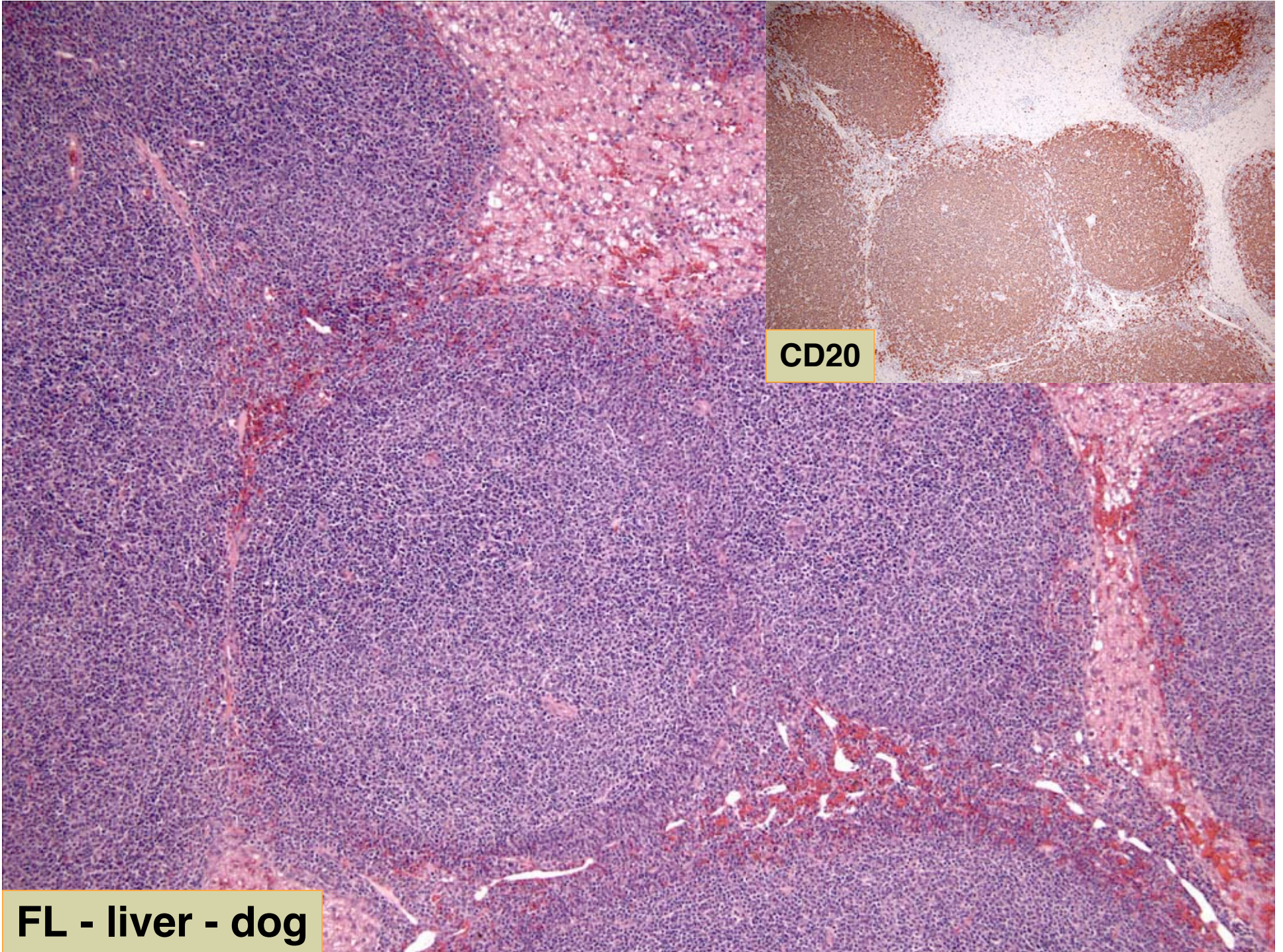
FL



FL

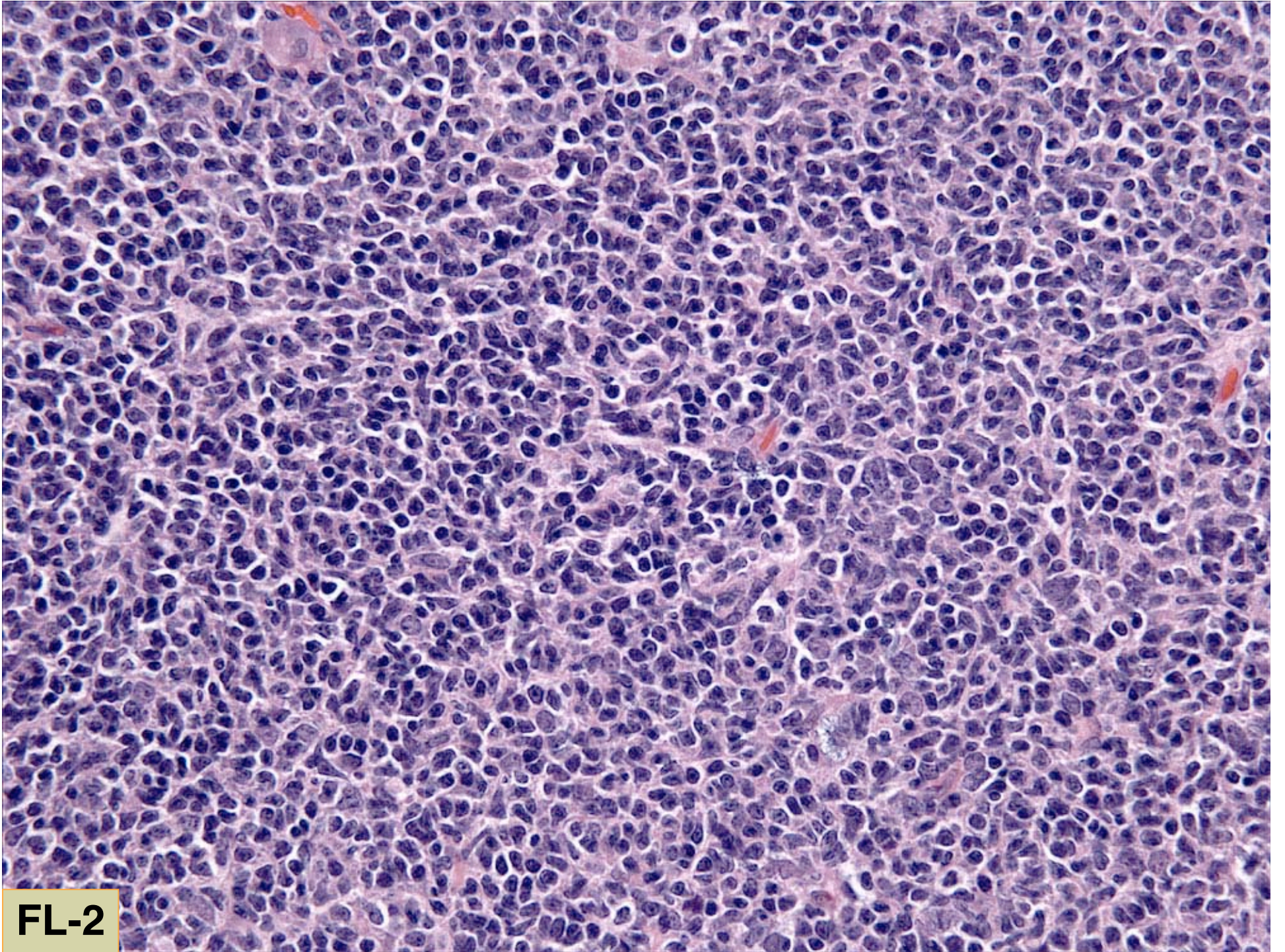


FL-3b



CD20

FL - liver - dog



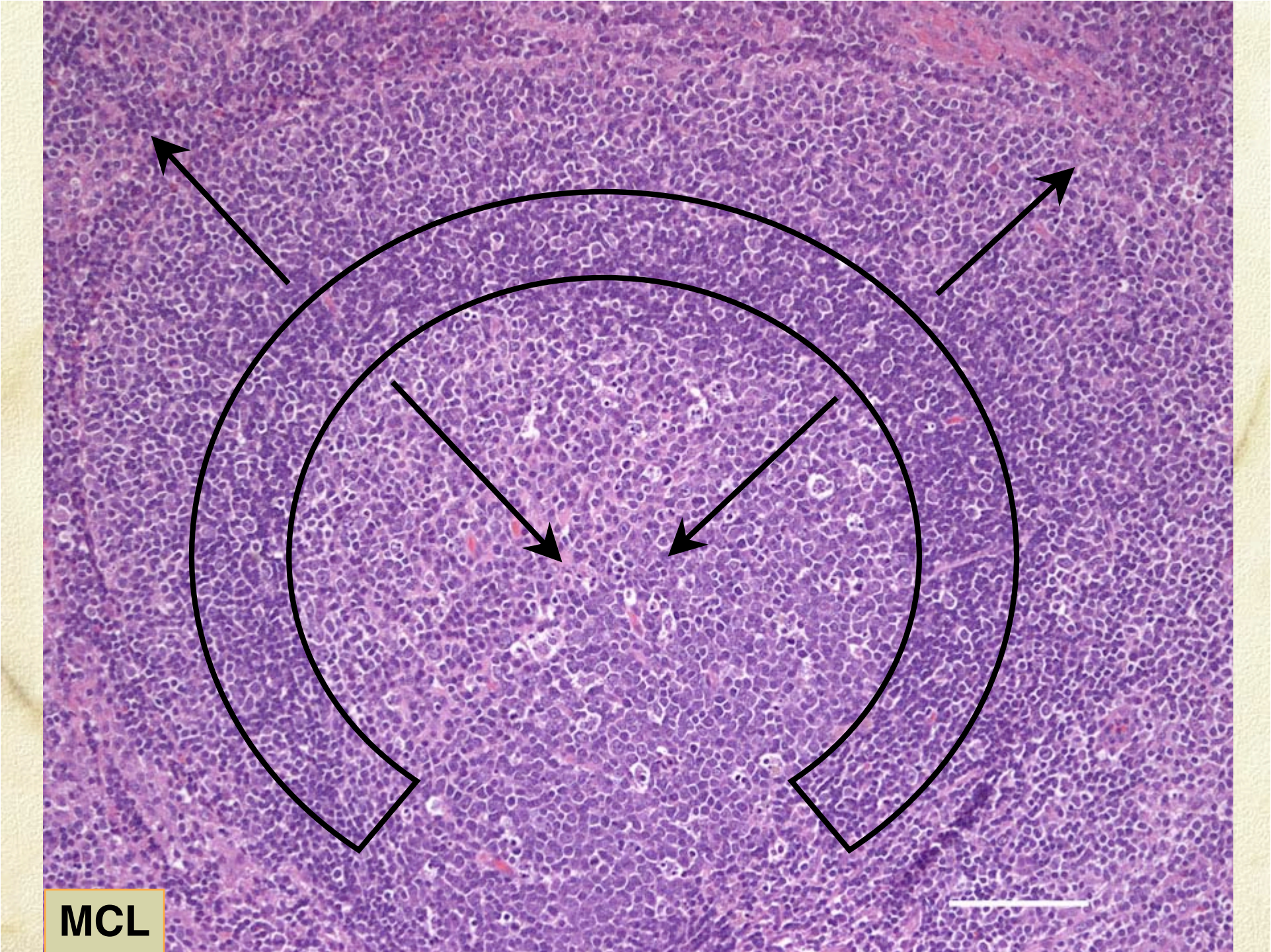
FL-2

Mantle cell lymphoma (MCL)

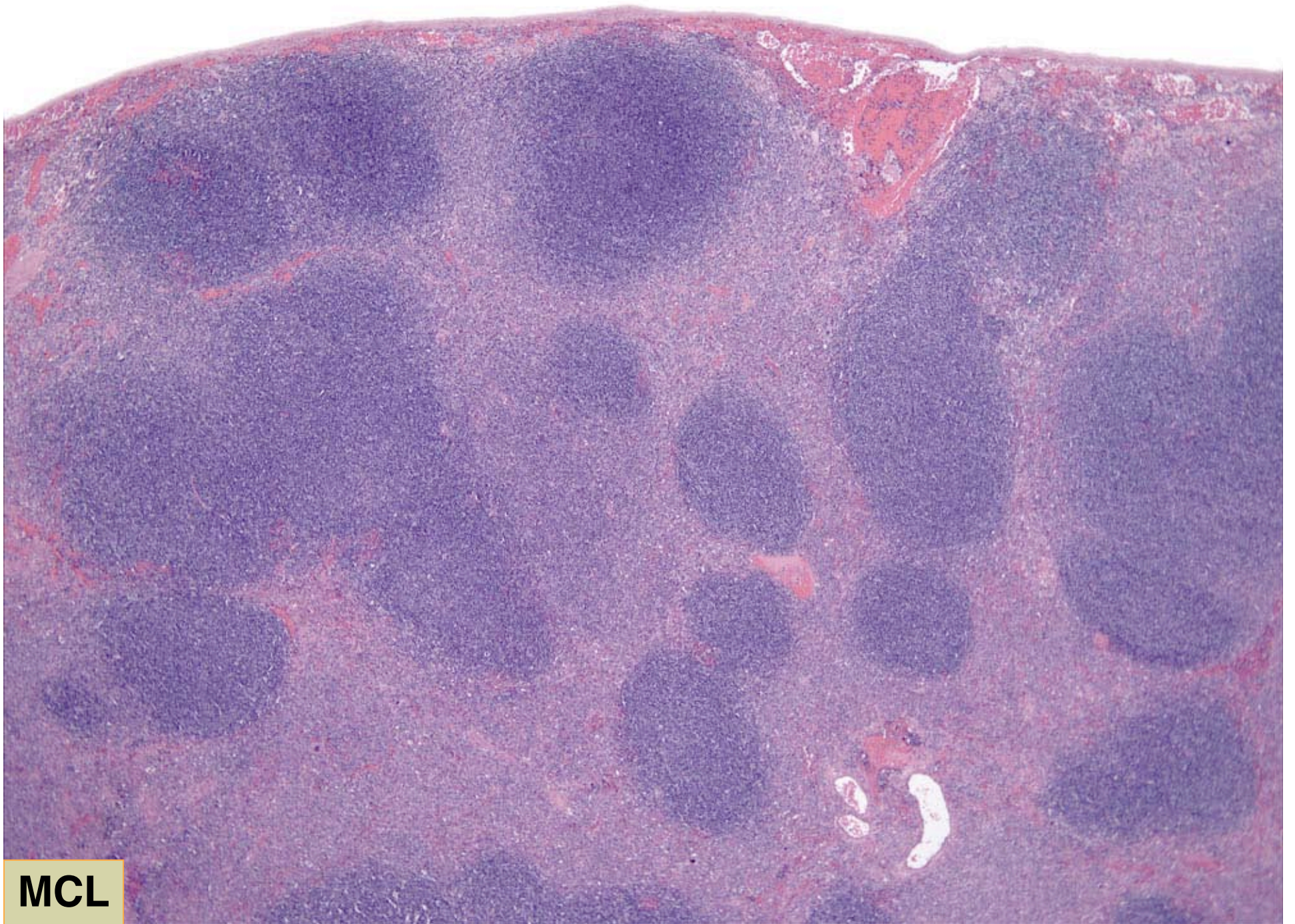
- **Nodal**
- **Spleen - 3 dogs (clonal IGH)**
- **Bone marrow**
- **Extranodal - GI tract**

Mantle cell lymphoma

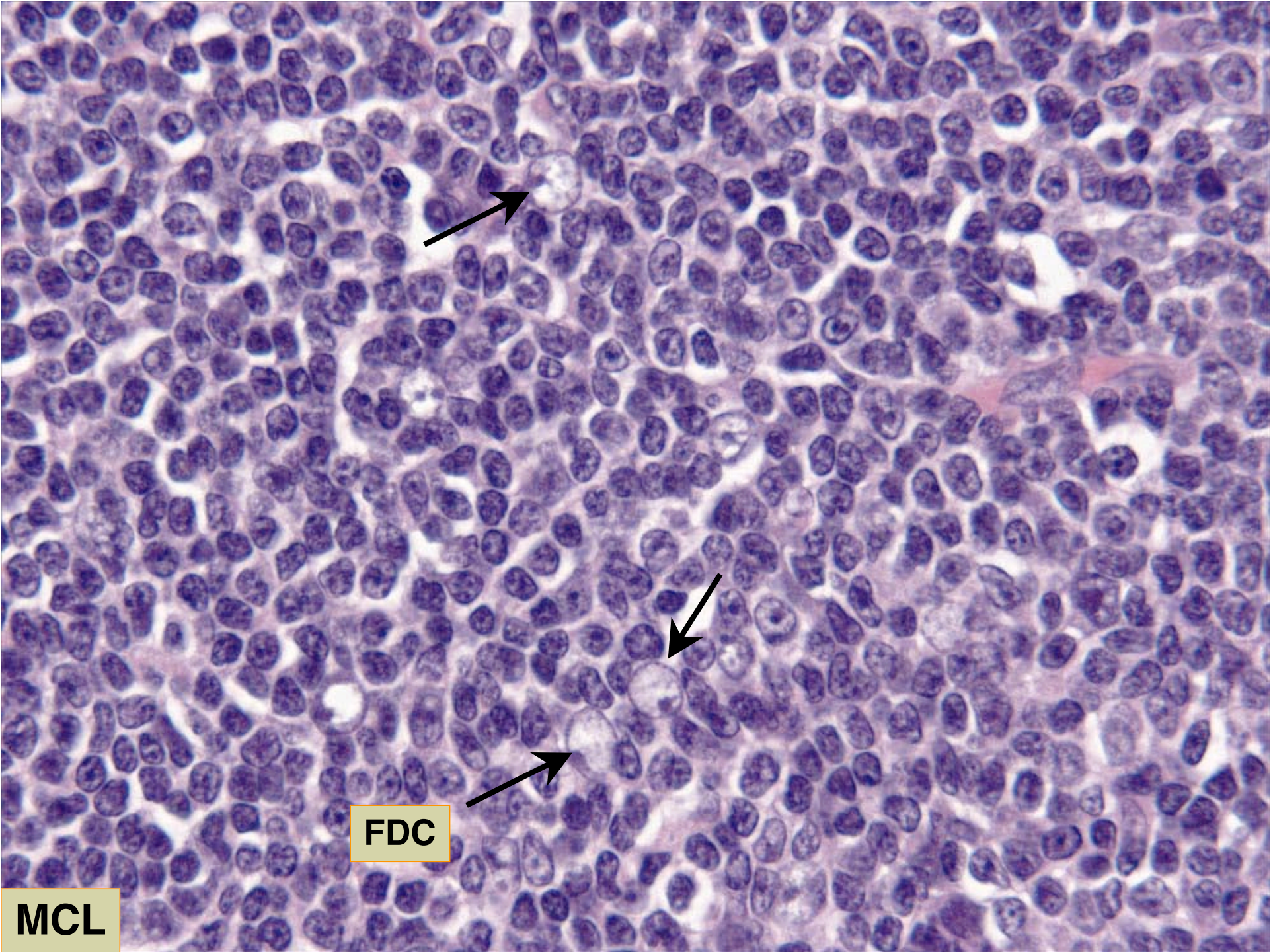
- **Origin:** B cell from inner mantle zone
- **DX:** architecture + cytologic characteristics
- **Solitary nodular mass in the spleen of dogs**
- **DDX:** splenic nodular hyperplasia in dogs
- **Indolent B cell lymphoma - low proliferative fraction - dogs; more aggressive in humans - esp. blastoid variant**
- **Human: CD5+, BCL2+, Cyclin D1+**



MCL



MCL



MCL

FDC

WHO Lymphoma classification - Mature T cell

T

Nodal TCL

Peripheral TCL - unspecified
T-zone TCL
Anaplastic large TCL
Angioimmunoblastic TCL

Cutaneous TCL

Mycosis fungoides
Pagetoid reticulosis
Sézary syndrome
Peripheral TCL - unspecified

Extranodal - other

Enteropathy associated TCL
Hepatosplenic TCL
Peripheral TCL - unspecified

LGL leukemia

T-LGL CLL
T-LGL ALL

LGL lymphoma

Indolent - initially

Indolent - some forms

Peripheral T cell lymphoma - PTCL

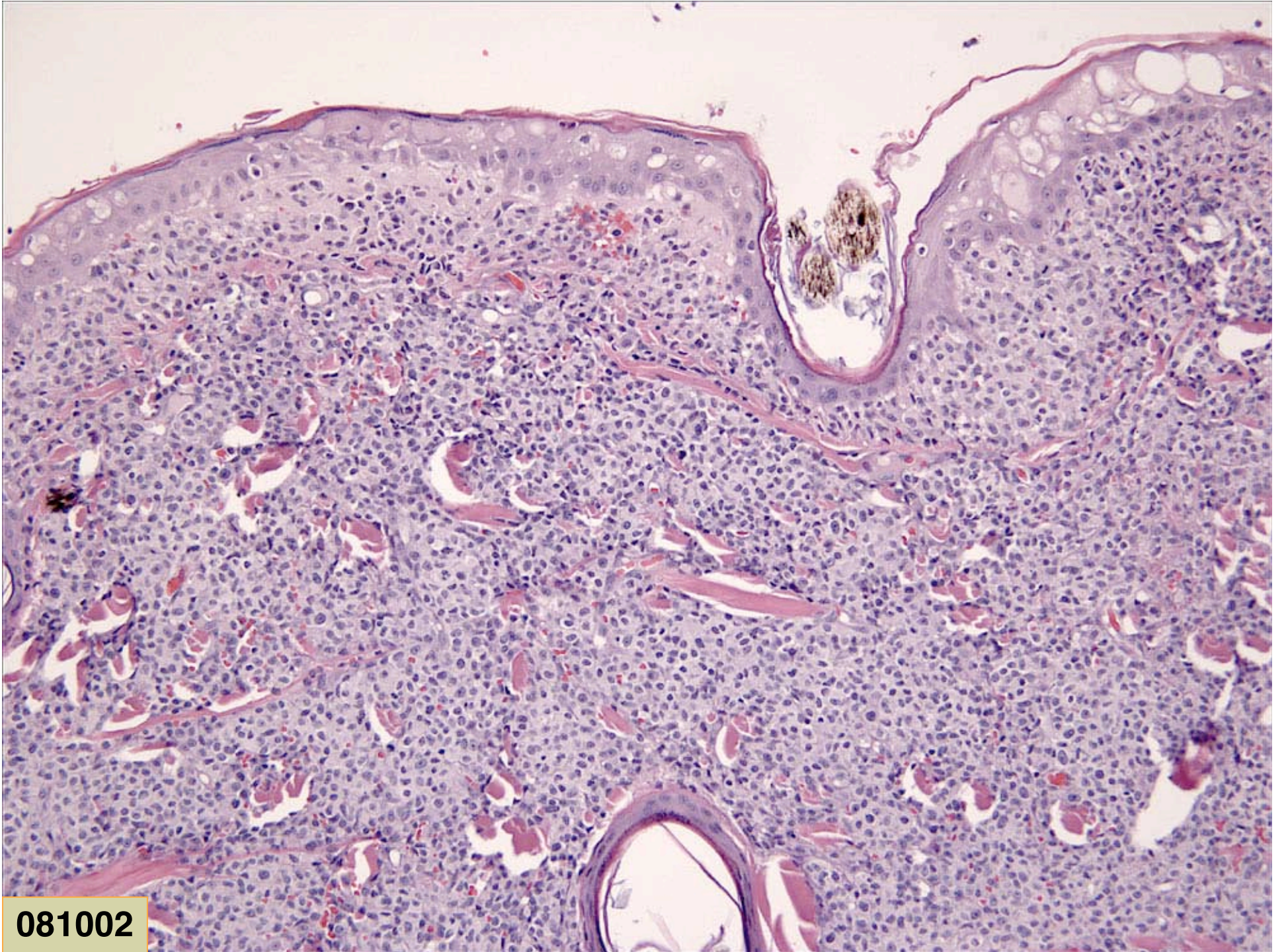
- **Heterogeneous group**
- **Nodal**
- **Skin (non-epitheliotropic TCL)**
- **Generalized (2° leukemia common)**

Peripheral T cell lymphoma - PTCL

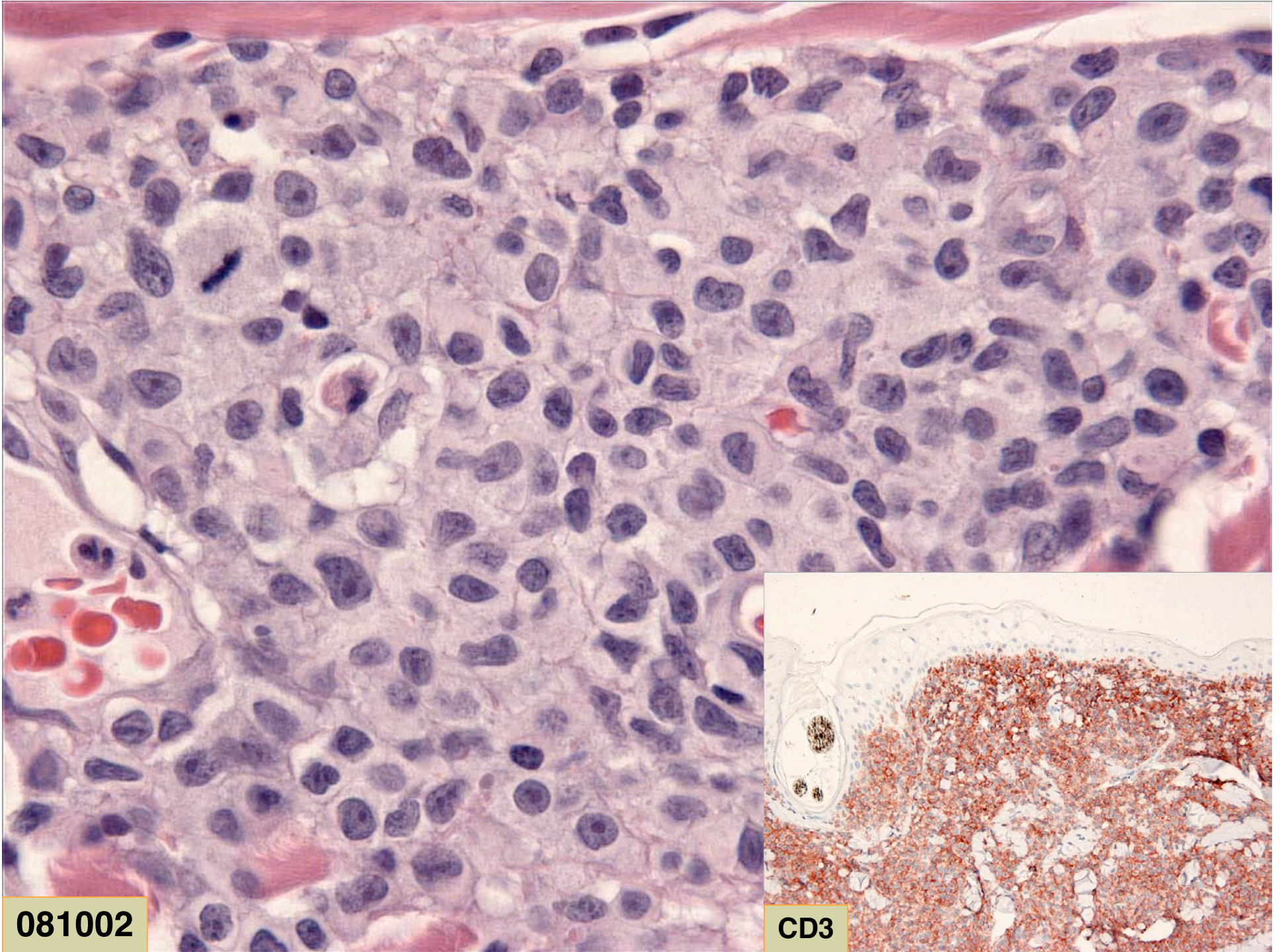
- **Origin:** Peripheral T cells
- **High-grade lymphoma - high proliferative fraction**
- **Cytology extremely variable**
- **Inflamed lymphoma - esp. cutaneous PTCL**
- **DDX: Reactive (cutaneous) histiocytosis**
- **P16 (Rb) deletion/inactivation in all cases**

NE-CTCL

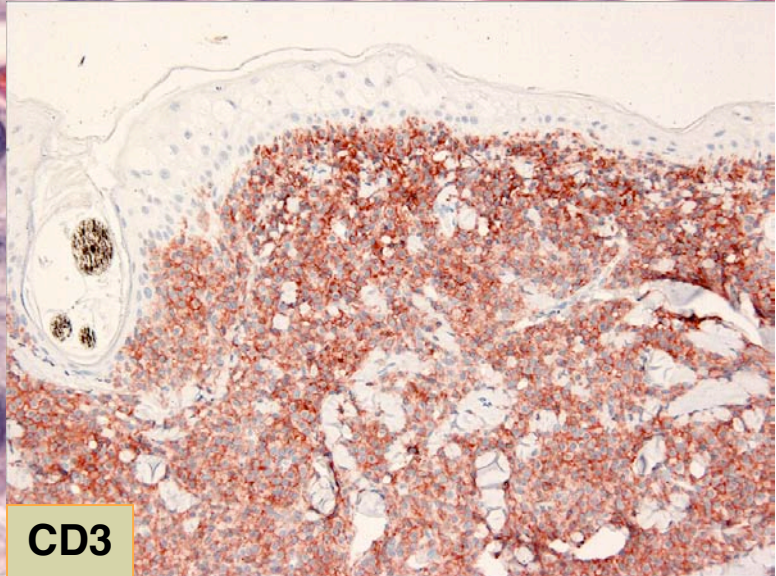




081002



081002



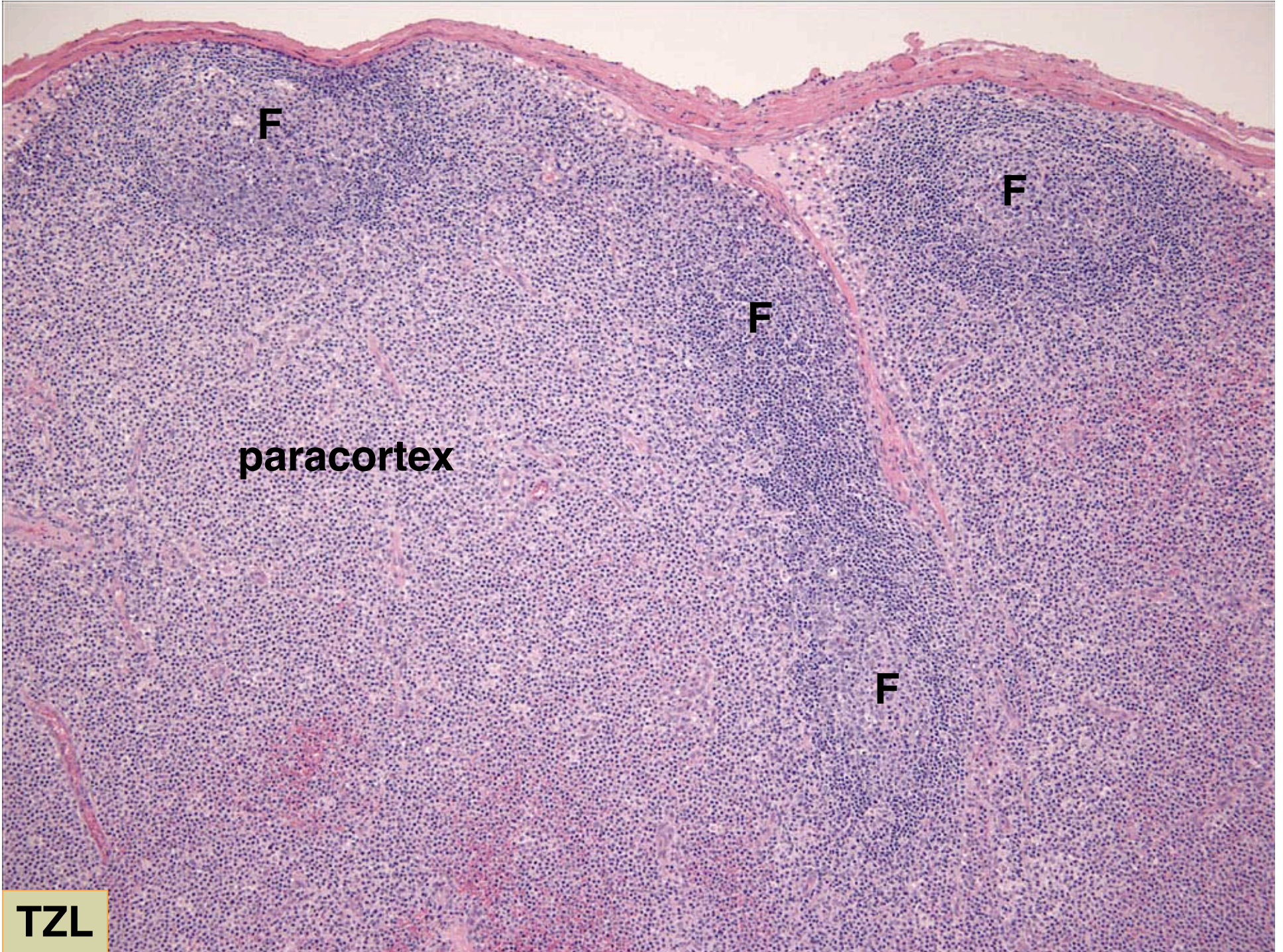
CD3

T-zone lymphoma (TZL)

- **Nodal**
- **Human - variant of PTCL - i.e. high-grade lymphoma**

T-zone lymphoma

- **Origin:** Peripheral T cells
- **Variable LN involvement (1, 2 or generalized)**
- **Indolent lymphoma (dogs) - years**
- **Low proliferative fraction - mitotic rate low - (if not - PTCL)**
- **2° leukemia observed - prognosis unaffected**
- **DDX: paracortical hyperplasia (TCRG clonality)
Marginal zone BCL (MZL) - requires IHC**



F

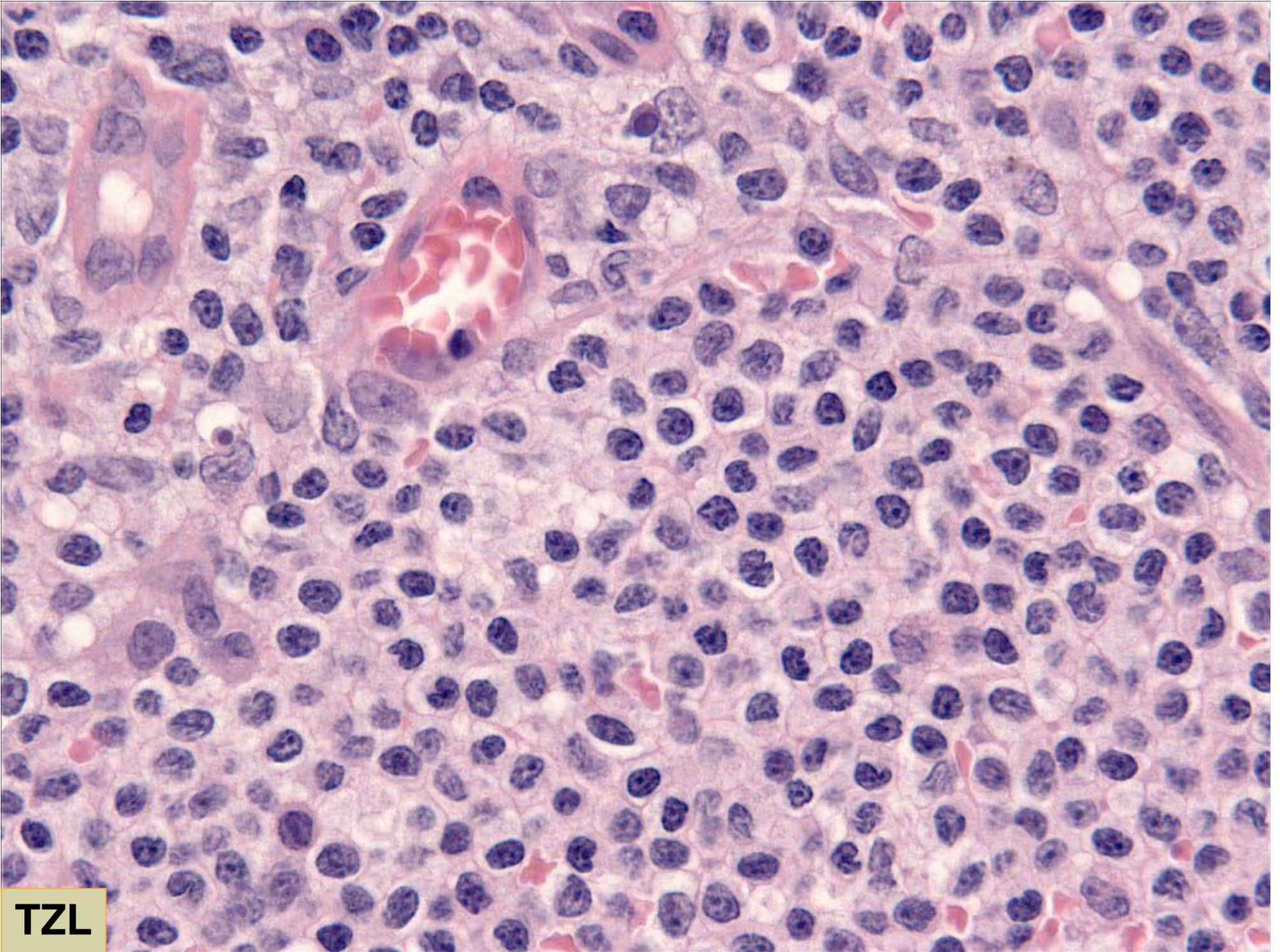
F

F

paracortex

F

TZL



TZL

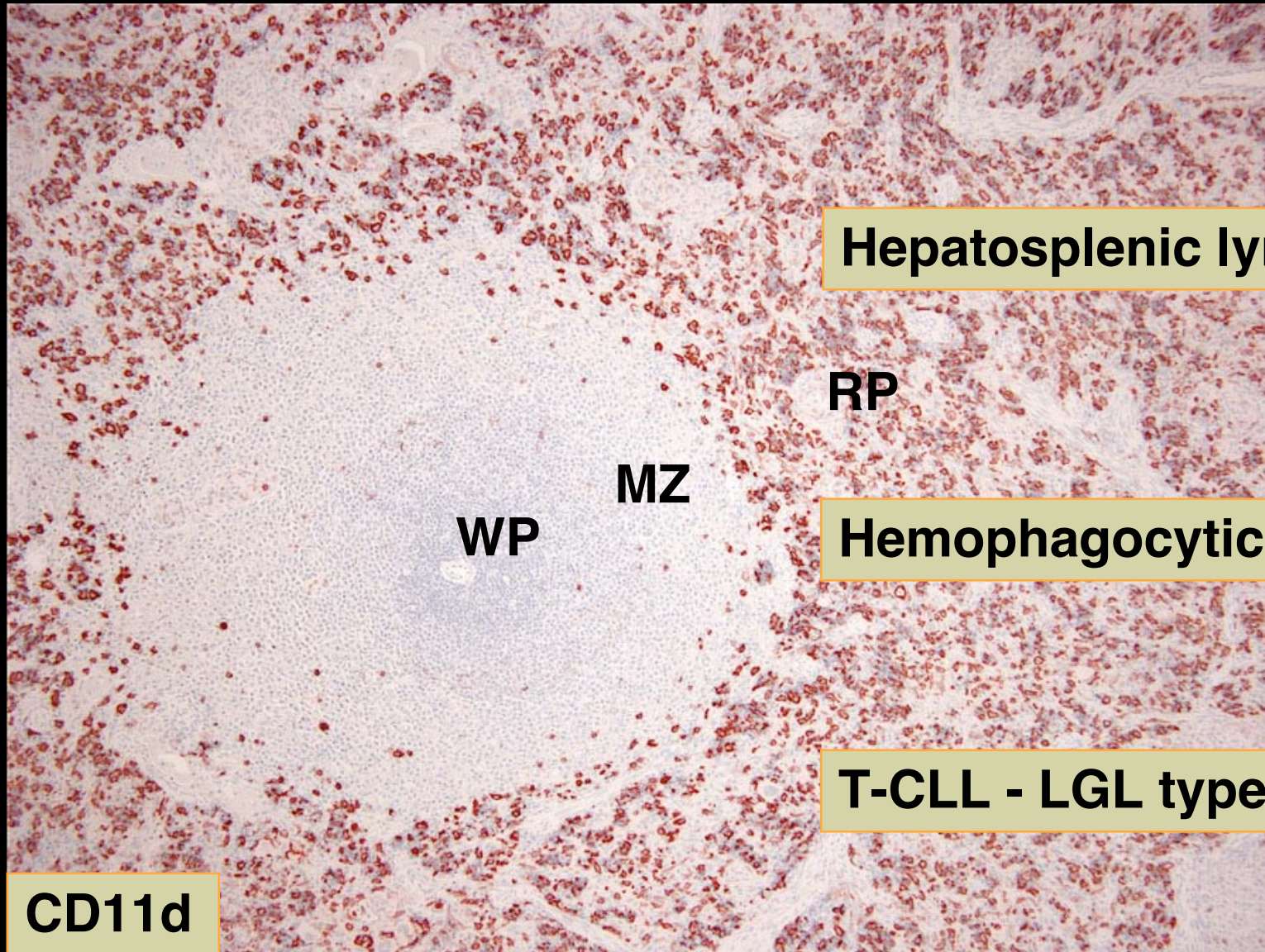
Hepatosplenic lymphoma (HS-TCL)

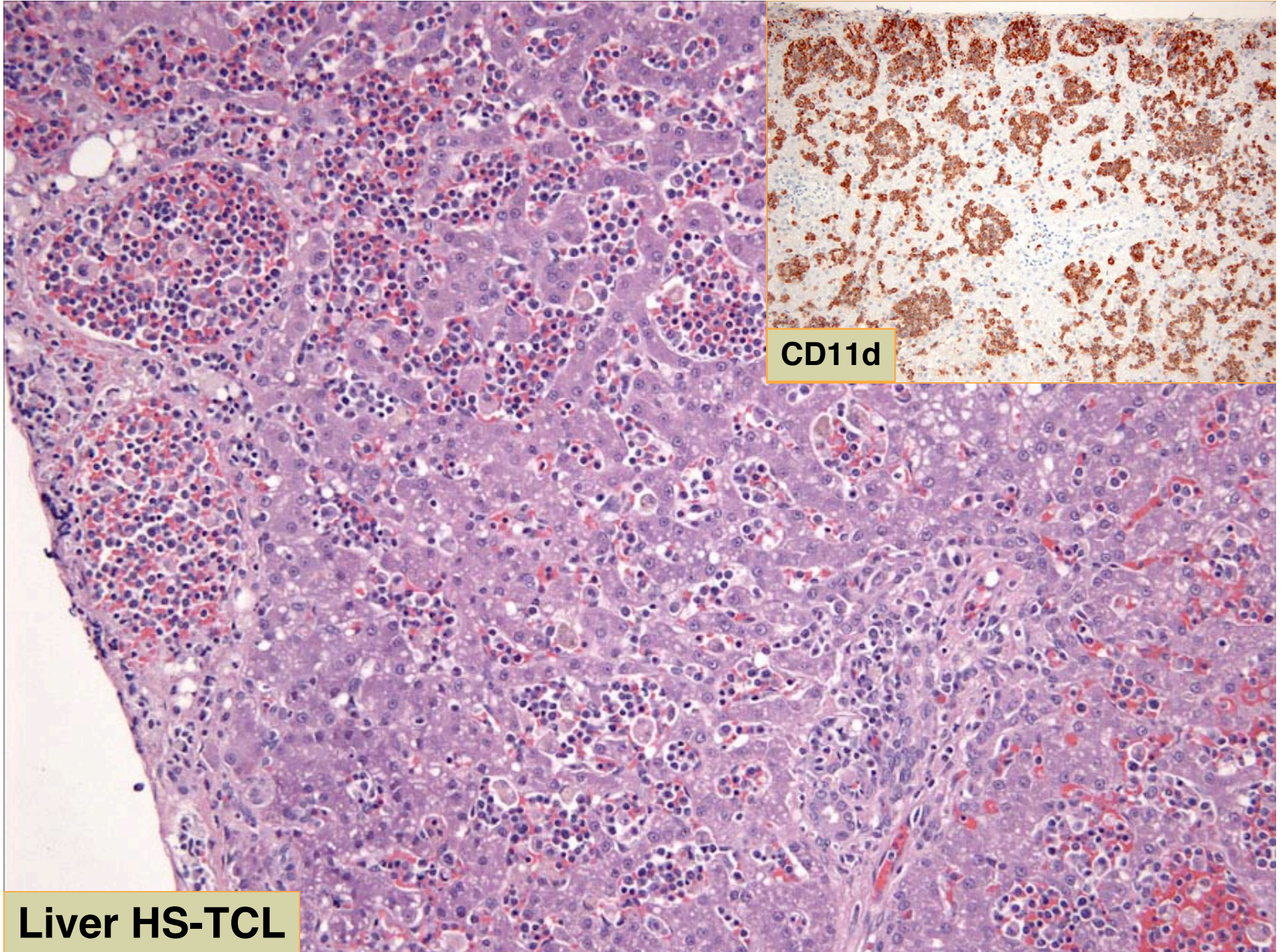
- **Spleen**
- **Liver**
- **Bone marrow**
- **Generalized lymphadenopathy lacking**

Hepatosplenic T cell lymphoma

- **Origin:** splenic red pulp $\gamma\delta$ T cell
- **Cytology** - LGL. Usually $\text{TCR}\gamma\delta+$ $\text{CD11d}+$
- **2° hemophagocytic syndrome common (CD11d+ macrophages activated); malignant T cells erythrophagocytic**
- **Clinical** - aggressive course, anemia, thrombocytopenia (immune mediated??)
- **DDX:** hemophagocytic histiocytic sarcoma

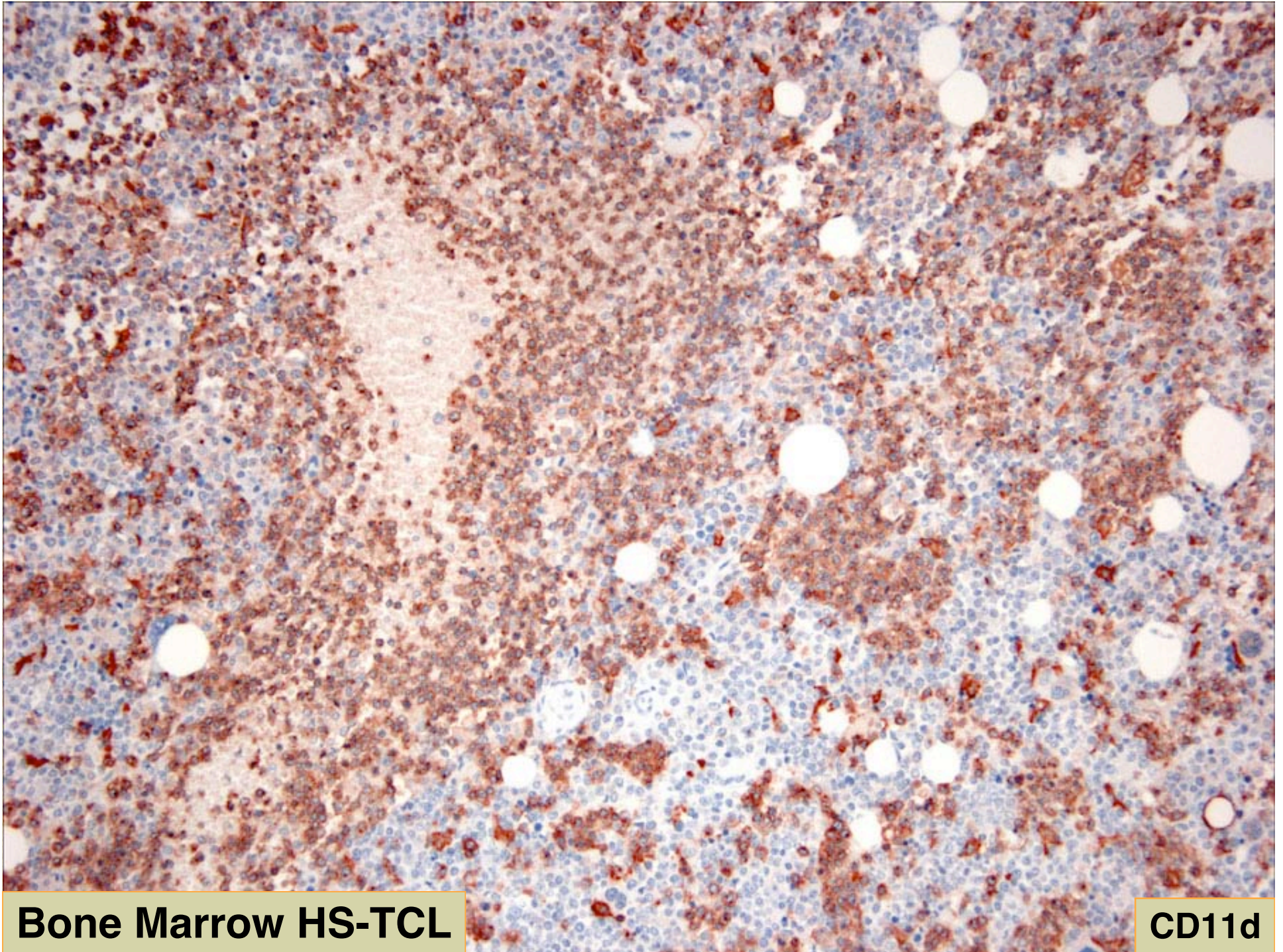
Splenic red pulp - CD11d+ diseases





CD11d

Liver HS-TCL



Bone Marrow HS-TCL

CD11d

Lymphocyte Trafficking
and
Tissue Localization of Disease

Lymphomas of skin and gut

T cell lymphomas of skin and gut

Marked species differences

incidence

behavior

immunophenotype

$\alpha\beta$ T cells

- **Naïve T cells - exported from the thymus**
 - **Recirculate between blood and lymph nodes**
- **Effector memory T cells - wide migratory range**
 - **Recirculate between blood and cutaneous or mucosal sites**
- **Central memory T cells - retain migratory path of naïve T cells**

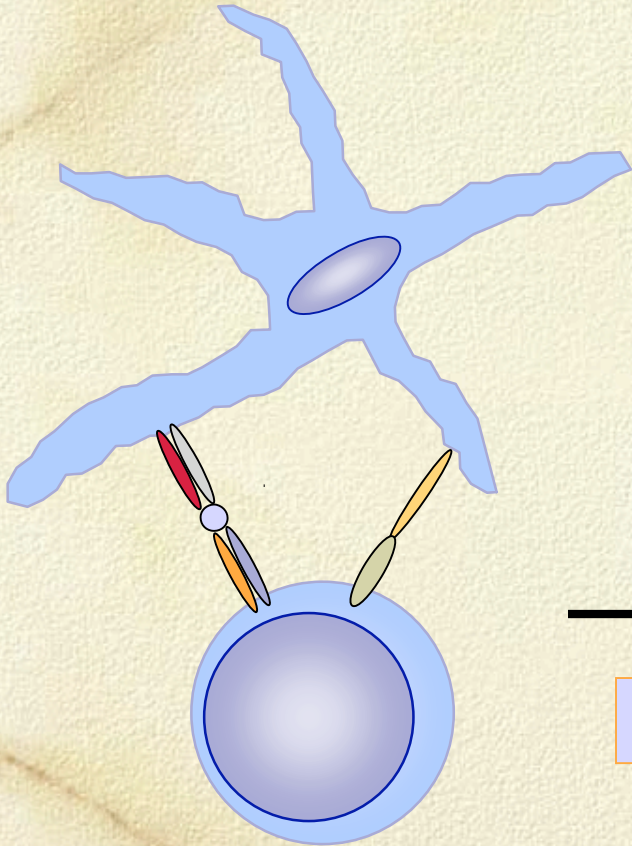
Lymphocyte recruitment - to skin

How are the migratory pathways of naïve lymphocytes redirected to skin?

Dendritic Cell Imprinting

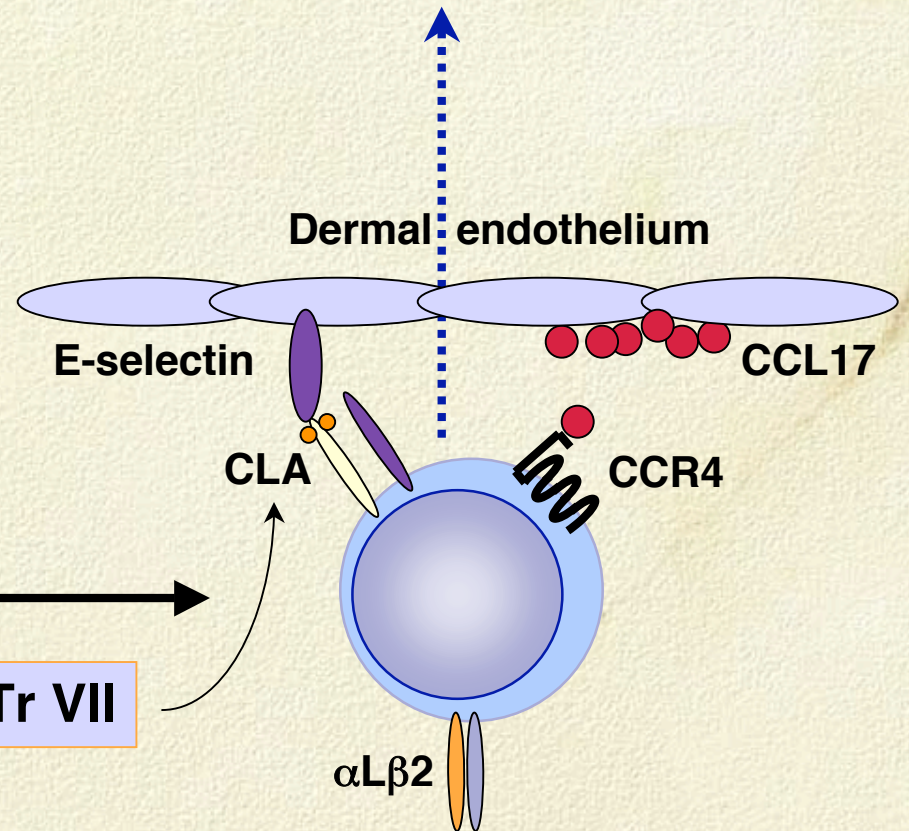
LN Paracortex

Dendritic Cell



Naïve T cell

Home to SKIN



Fucosyl Tr VII

Memory T cell

WHO Lymphoma classification - Mature T cell

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LGL leukemia

T-LGL CLL
T-LGL ALL

LGL lymphoma

Indolent - initially

Indolent - some forms

Cutaneous Lymphoma

- **Epitheliotropic TCL**

 - Mycosis fungoides**

 - Pagetoid reticulosis**

 - Sézary syndrome**

- **Non-epitheliotropic PTCL**

- **Non-T non-B lymphoma**

- **B cell lymphoma (Diffuse large BCL)**

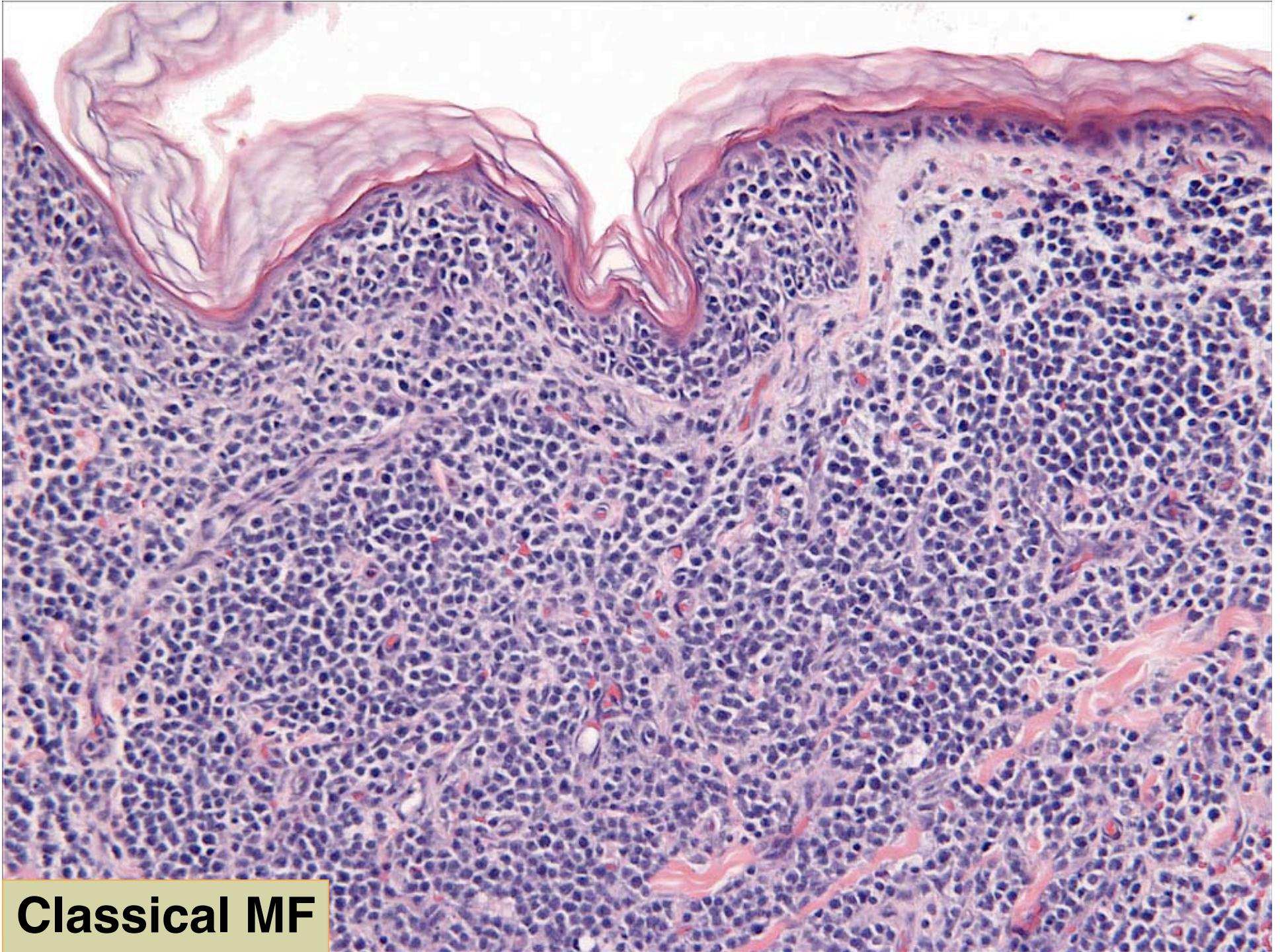
- **Plasmacytoma**

Skin homing T cell lymphoma

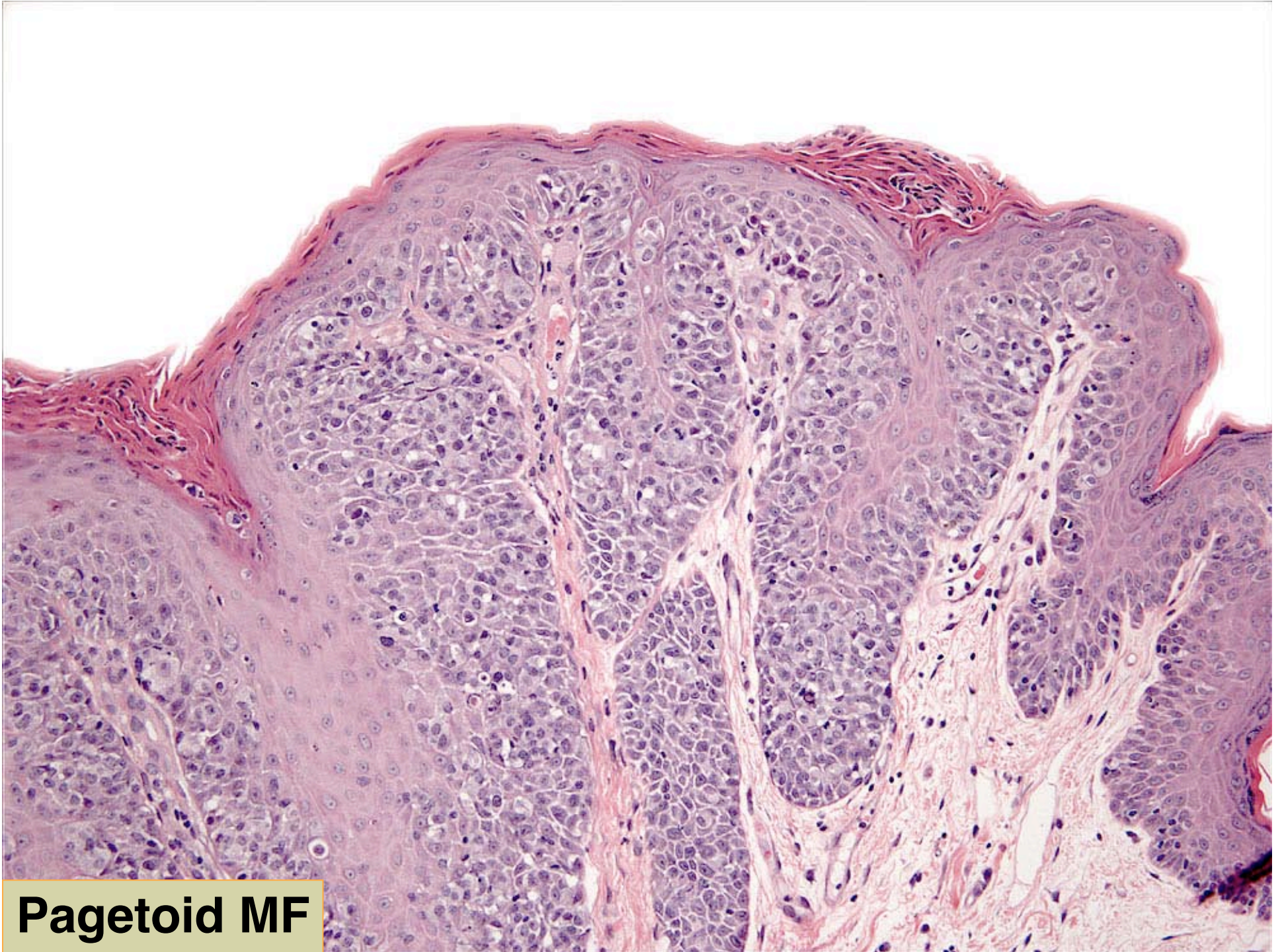


Epitheliotropic T cell lymphoma - skin

- **Mycosis fungoides (MF) - lesions confined to skin for **extended period** - clinical course up to 4 yrs.**
- **MF is a disease of skin homing memory T cells**
- **Dissemination initially occurs within the skin and skin draining lymph nodes**
- **Evidence of dissemination - identical T cell clone found in multiple skin sites**

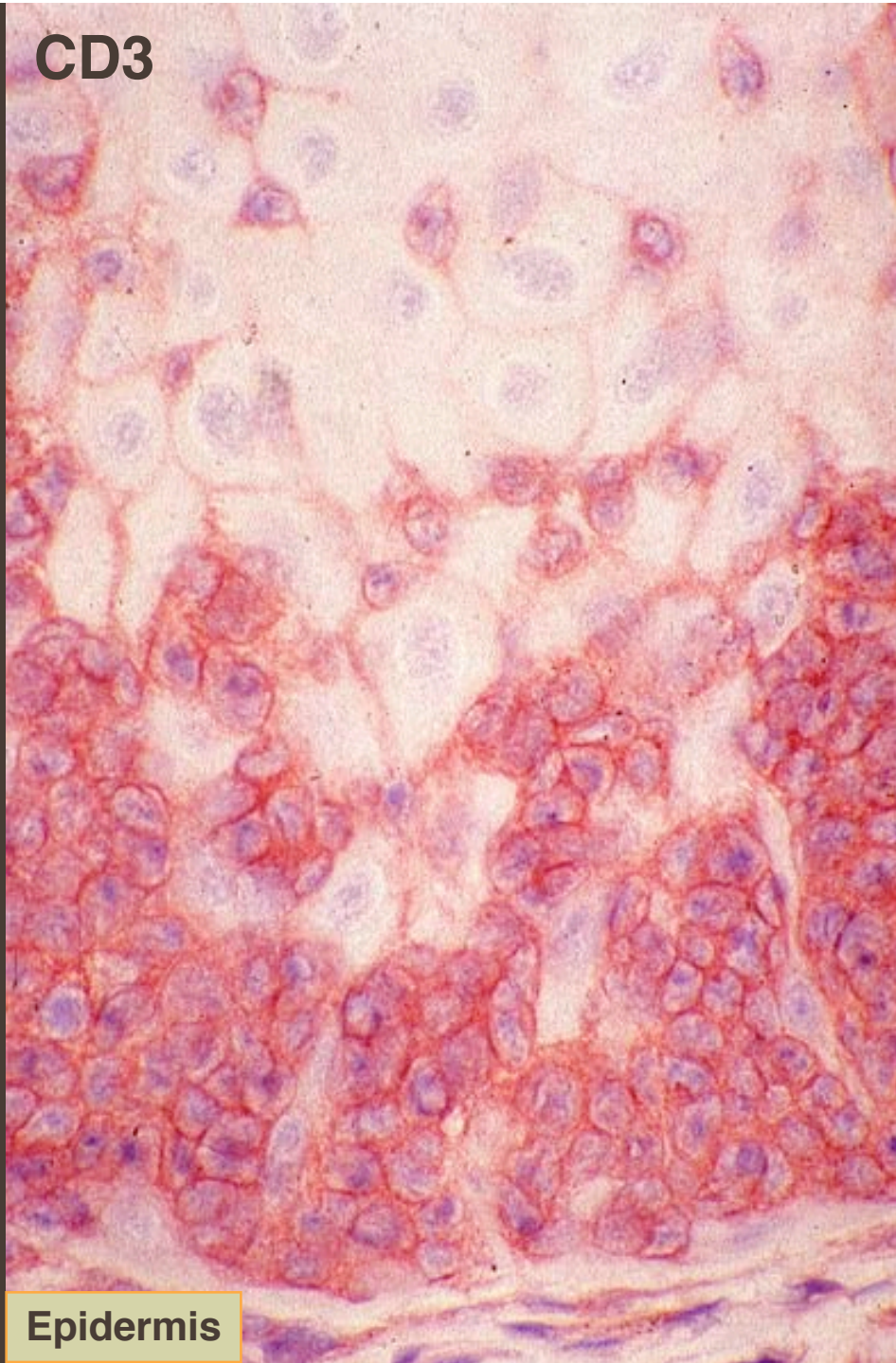


Classical MF



Pagetoid MF

CD3



Epidermis

CD3



Hair follicle

Canine Mycosis Fungoides

Immunophenotype

- Consistent expression of CD3 (n = 56)
- **CD8+** (80% cases) or **CD4-CD8-** (20% cases)
- Memory cell phenotype (CD45+CD45RA-CD49d+)
- **Marked contrast to human MF - TCR $\alpha\beta$ +CD4+**

Canine Mycosis Fungoides

- **T CELL RECEPTOR USAGE?**
- **Development program for TCR specific probes**
- **Mab specific for TCR $\alpha\beta$ and TCR $\gamma\delta$ developed**

Canine MF - TCR Expression

- TCR immunophenotype in MF all forms

TCR $\alpha\beta$ + **21 cases (40%)**

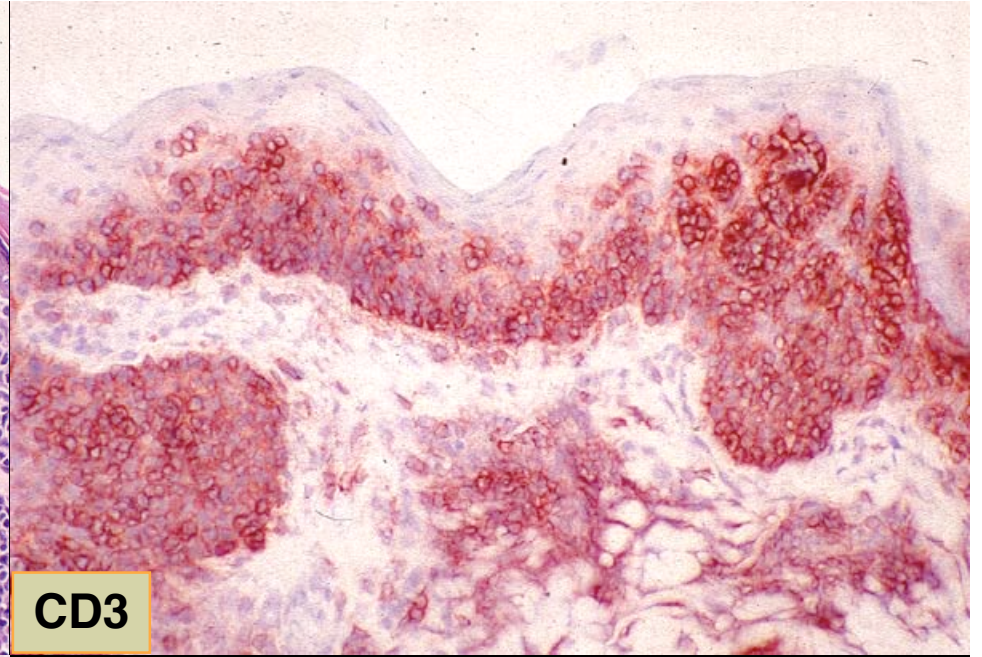
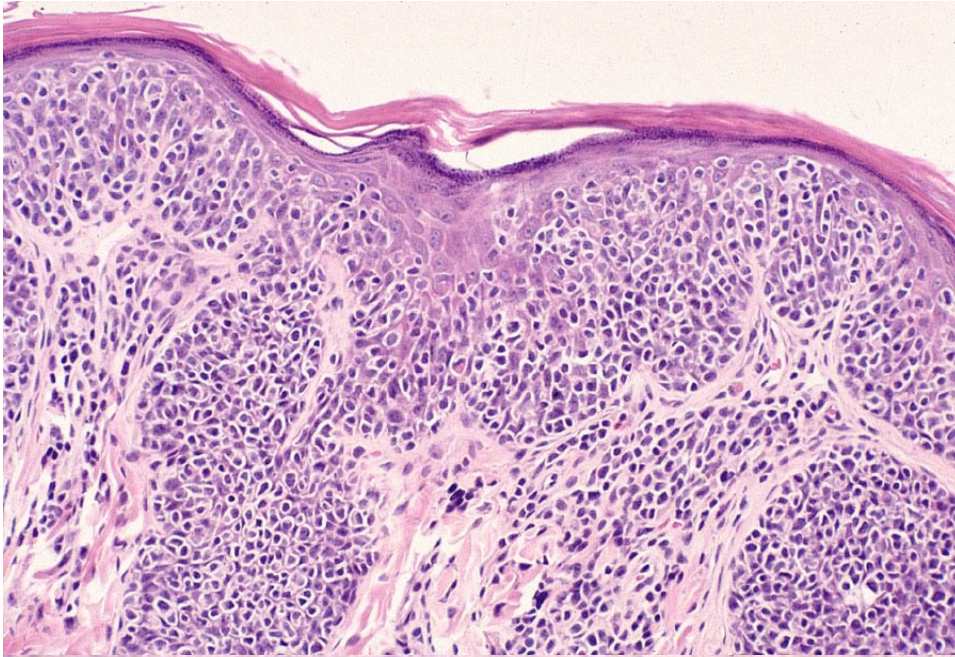
TCR $\gamma\delta$ + **32 cases (60%)**

- Canine MF involves $\gamma\delta$ T cells at much higher incidence than human MF

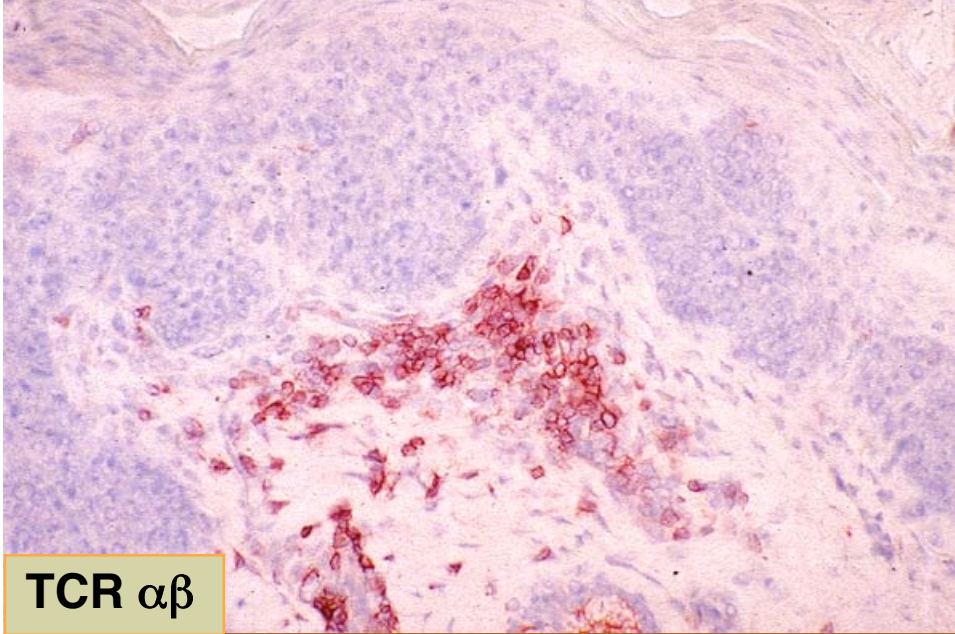
Canine MF - TCR Expression

Classical MF: $\text{TCR}\alpha\beta+$ \approx $\text{TCR}\gamma\delta+$ (n=38)

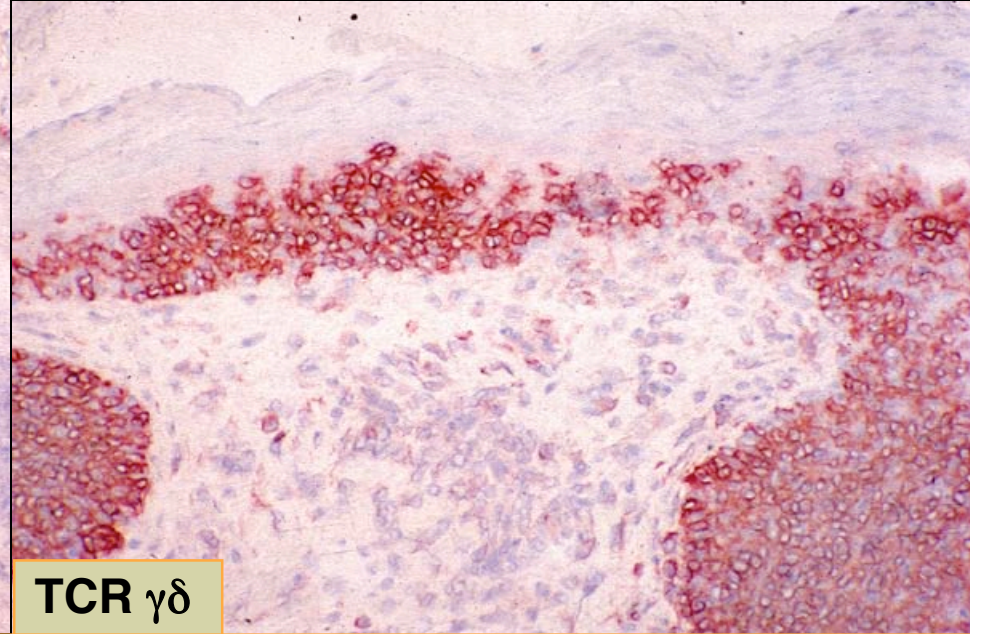
Paquetoid MF: $\text{TCR}\gamma\delta+$ (n=15)



CD3



TCR $\alpha\beta$



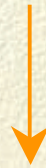
TCR $\gamma\delta$

Pagetoid MF: a lymphoma of $\gamma\delta$ T cells

Canine MF - pagetoid reticulosis

Exclusive expression of TCR $\gamma\delta$

Clonal origin from resident epidermal $\gamma\delta$ T cells



Prolonged expansion entirely within the epidermis

WHO Lymphoma classification - Mature T cell

T

Nodal TCL

Peripheral TCL - unspecified
T-zone TCL
Anaplastic large TCL
Angioimmunoblastic TCL

Cutaneous TCL

Mycosis fungoides
Pagetoid reticulosis
Sézary syndrome
Peripheral TCL - unspecified

Extranodal - other

Enteropathy associated TCL
Hepatosplenic TCL
Peripheral TCL - unspecified

LGL leukemia

T-LGL CLL
T-LGL ALL

LGL lymphoma

Indolent - initially

Indolent - some forms

Gastrointestinal lymphoma

- **Enteropathy associated TCL (EATCL)**
 - **small cell**
 - **large cell**
 - **LGL**
- **Diffuse large BCL**

Enteropathy associated TCL

- **Origin:** intestinal homing T cell (IEL or LPL)
- **Small intestine - high prevalence in cats**
- **IBD: precursor lesion in most cats - distinction (TCRG clonality)**
- **Small cell - indolent**
- **Large cell (LGL) - aggressive high grade**
- **Architecture - mucosal or transmural**

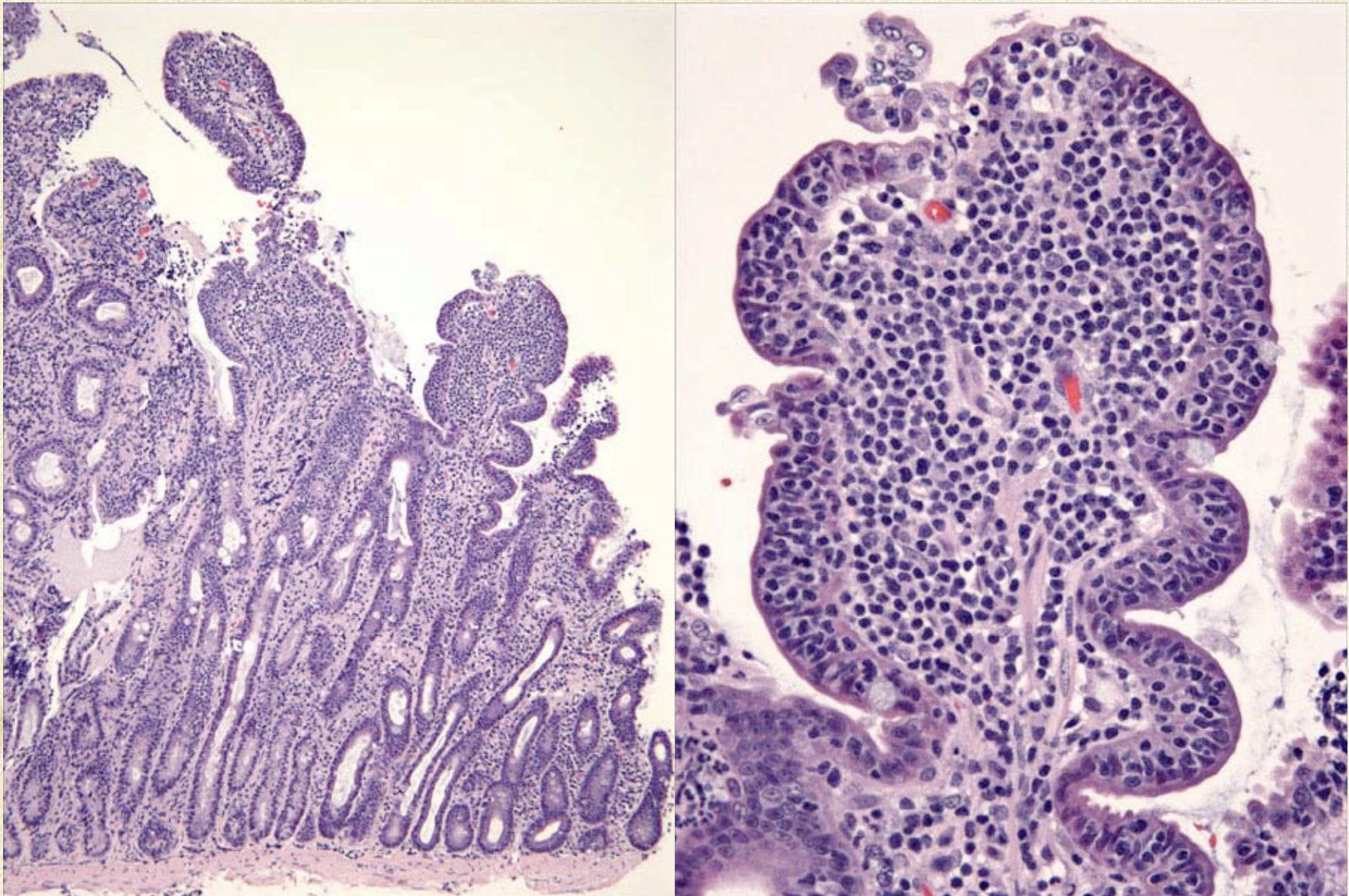
Mucosal homing T cell lymphoma



Feline small intestine: diffuse MALT

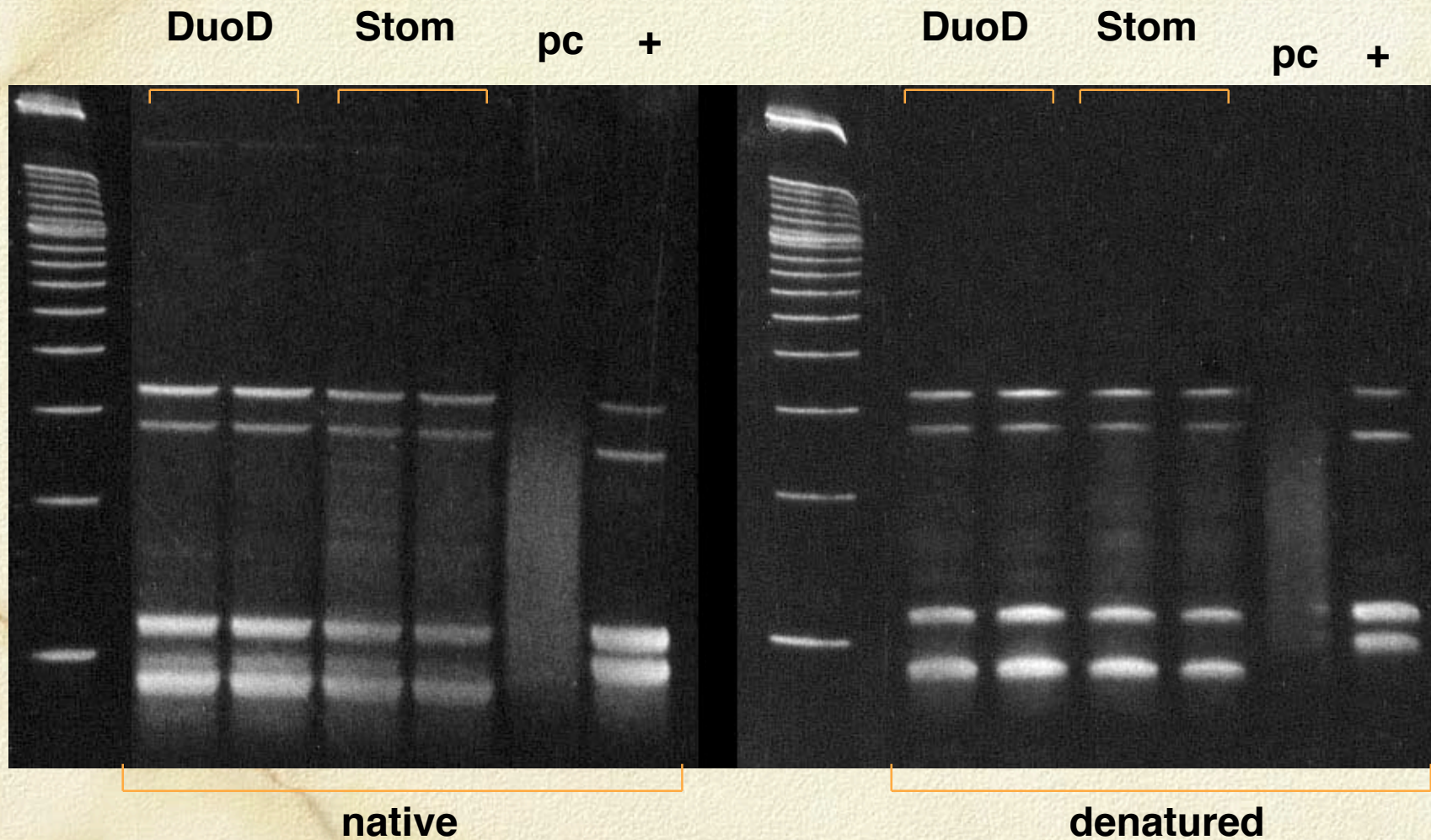
- IELs - distinctive phenotypic subsets versus PBL
- Expression of $\beta 7$ integrins ($\alpha 4\beta 7$) linked to mucosal homing
- **Feline IEL (30%) granulated** - perforin, granzymes
- **CD8 $\alpha\alpha$ T cells predominate** - role in immune surveillance
- **Feline IEL (70%+) express the mucosal integrin - CD103 ($\alpha E\beta 7$)**

04B0314 -Feline, DLH, FS, 13 yrs



Mucosal epitheliotropic T cell lymphoma - duodenum - endoscopic

***TCRG* clonality - 04B0314 -Feline, DLH, FS, 13 yrs
Endoscopic biopsy - duodenum and stomach**



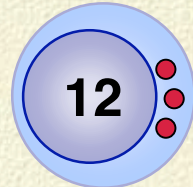


eTCL small cell

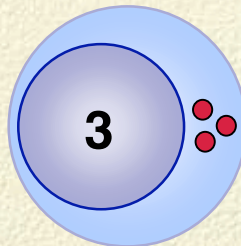
Mucosal lymphoma - cytology



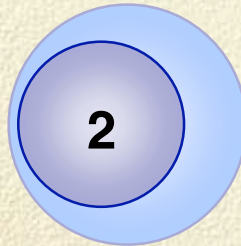
Small lymph



S-GL



L-GL



Large lymph

85

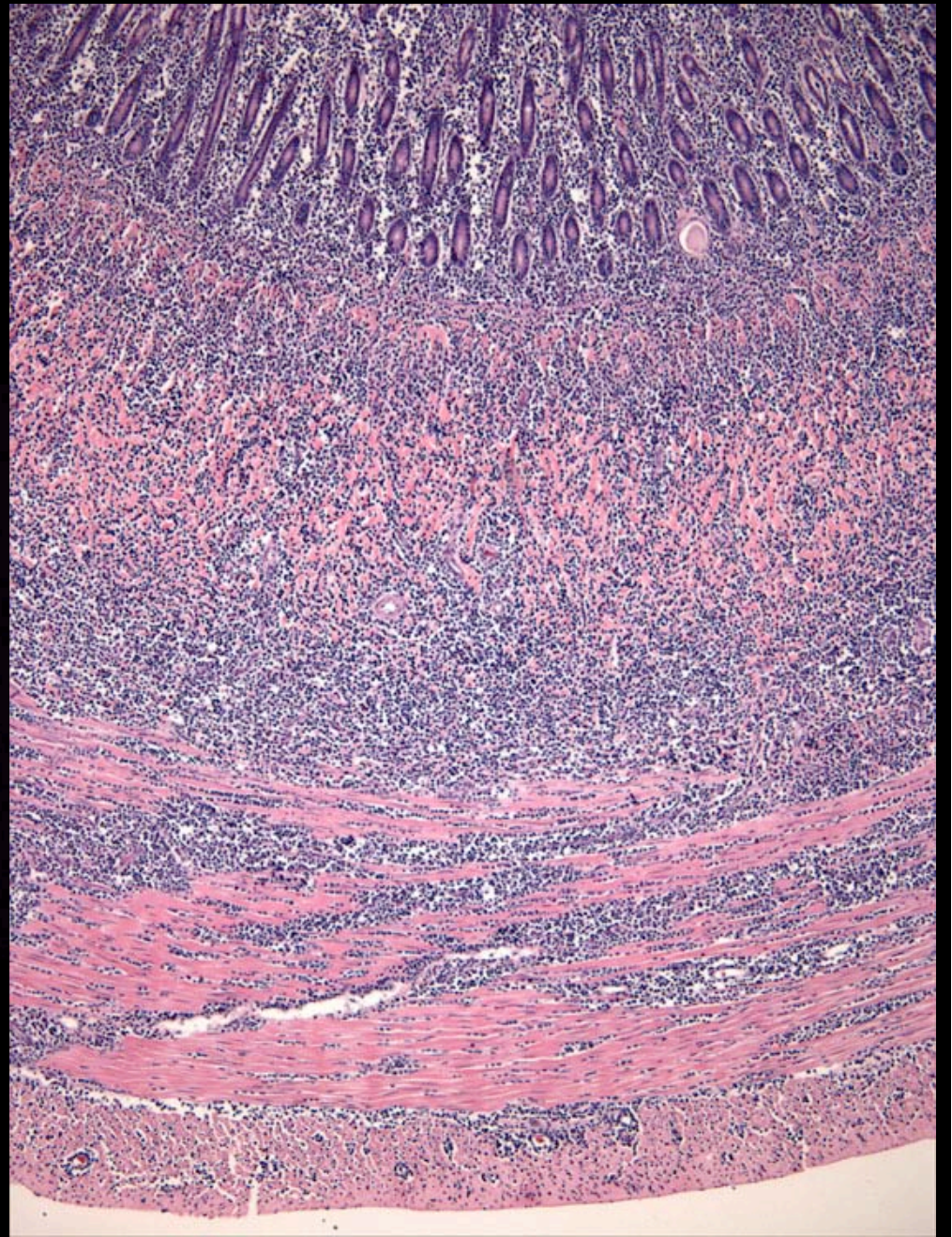
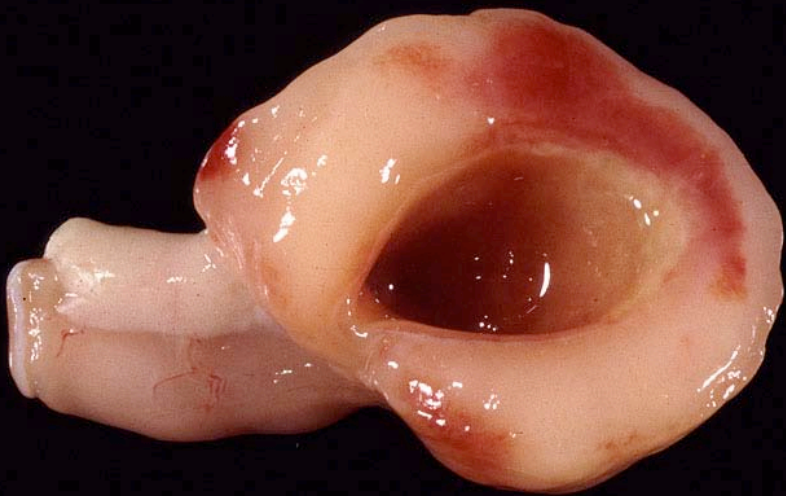
Mucosal lymphoma - survival

Dead

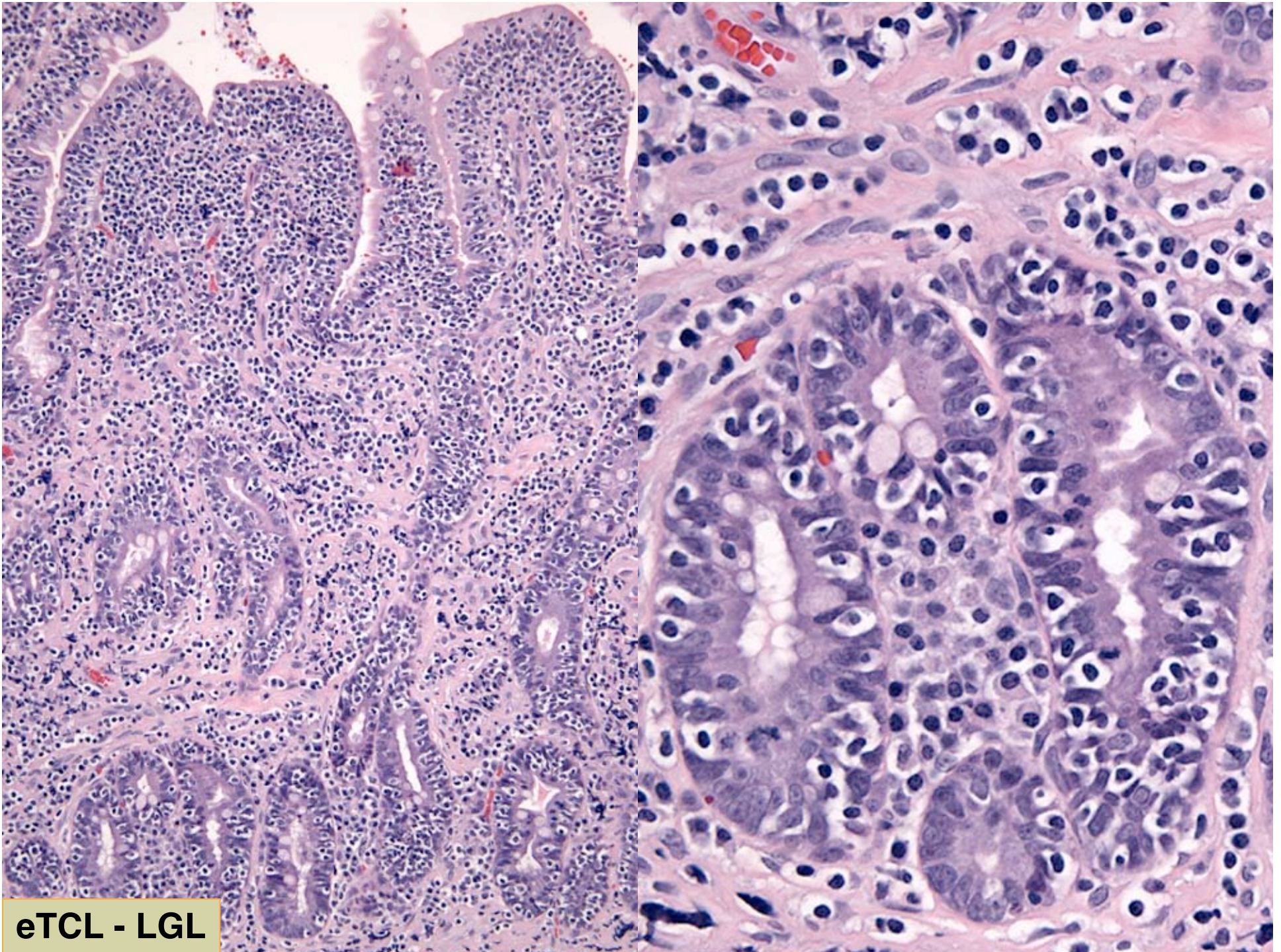
13.9 ± 13.1m (0.5 - 46m, n=29)

Alive

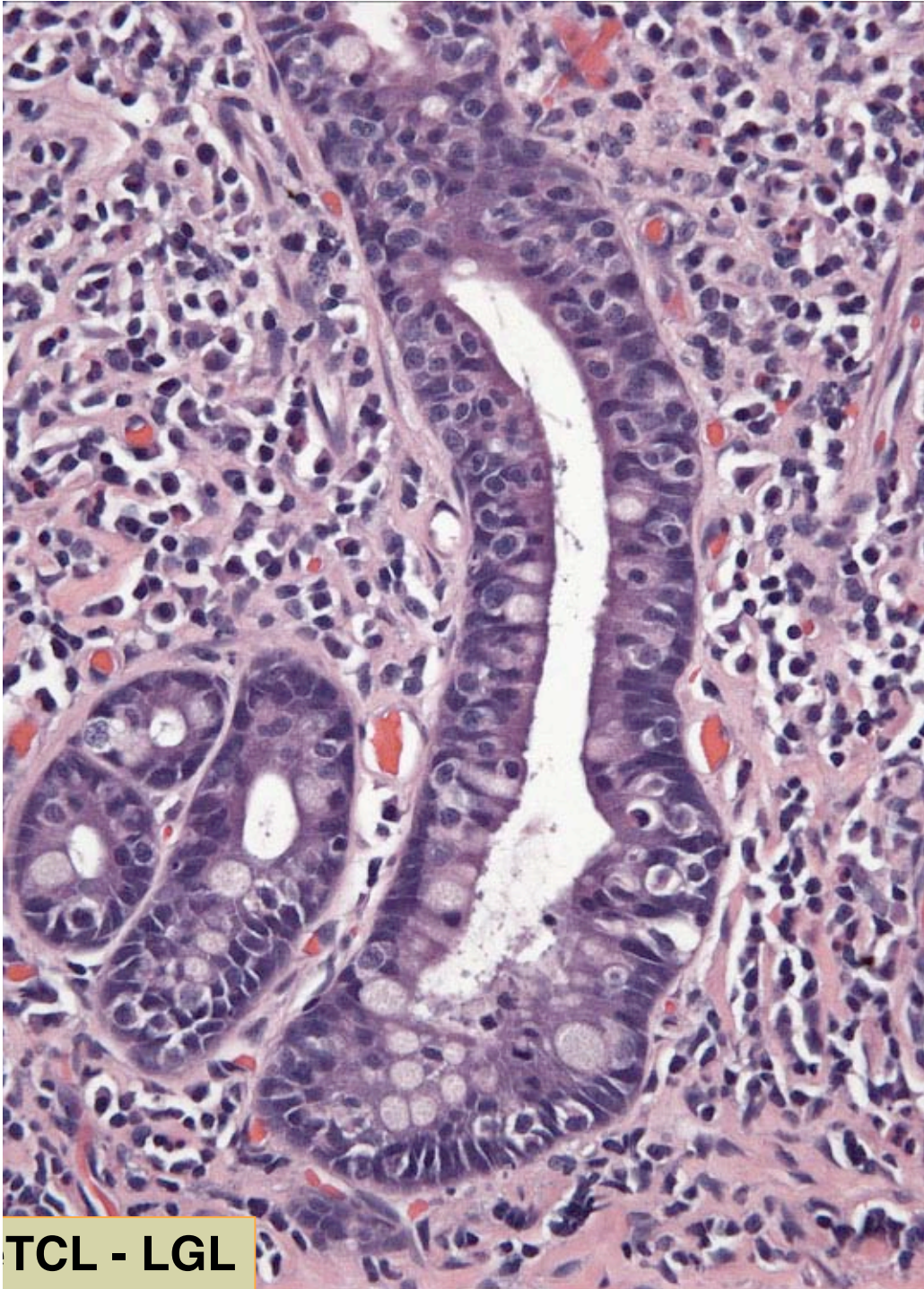
24.2 ± 17.1m (4 - 51m, n=16)



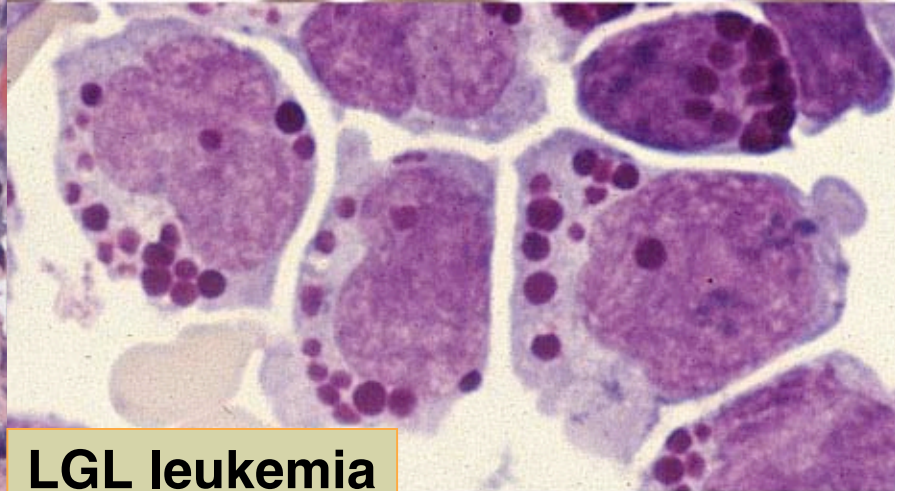
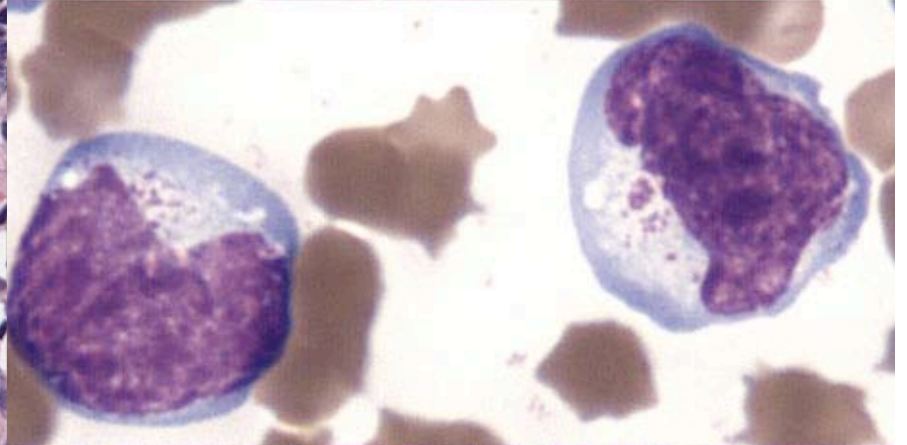
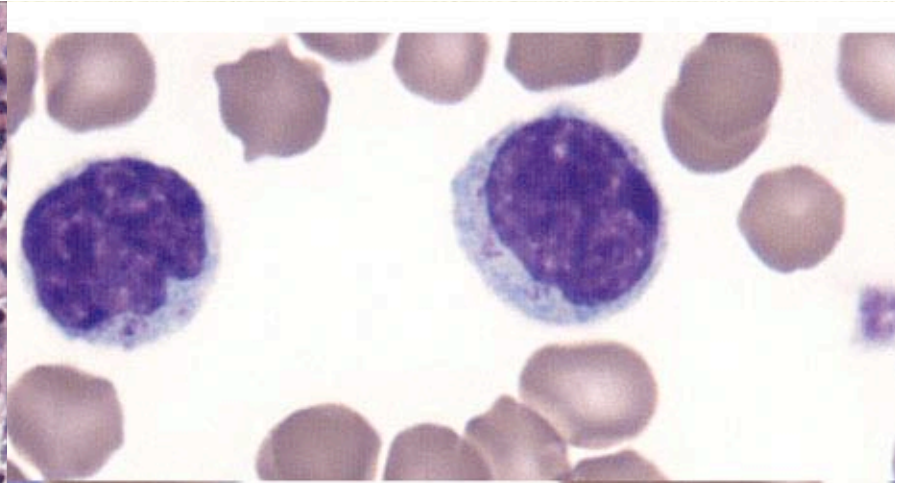
Jejunal mass: transmural LGL T cell lymphoma



eTCL - LGL



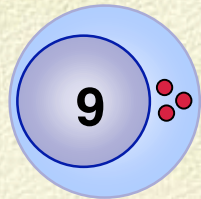
TCL - LGL



LGL leukemia

Transmural T cell lymphoma - survival

Dead



L-GL

2.9 ± 4.8m (0.1 - 15m)

2



L-lymph

2m & 60m

Alive

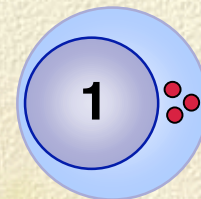
1



S-lymph

28m

1



L-GL

2m

Lymphoma

- WHO classification scheme is applicable to canine (feline) lymphoma
- Basic immunophenotyping often needed (B/T)
- Molecular clonality necessary in some instances (TZL, MZL, MCL, FL and T cell/histiocyte rich BCL)
- Recognition of homogeneous lymphoma groups will lead to **tailored therapies** and discovery of underlying **molecular defects**

Thanks

- **Japanese Society of Veterinary Pathology**
- **Colleagues** - Ted Valli, Bill Vernau, Paola Roccabianca, Verena Affolter, Barbara Hirt, Jenny Woo, Dimitri Danilenko, Sean McDonough, Thierry Olivry, Paul Rossitto, Petra Graham, Sandra Kosten, Eric Cavanaugh
- **Funding** - LABL, Center for Companion Animal Health, Center for Equine Health, Morris Animal Foundation, MacDonald-Rivas grant, ICOS Corporation.