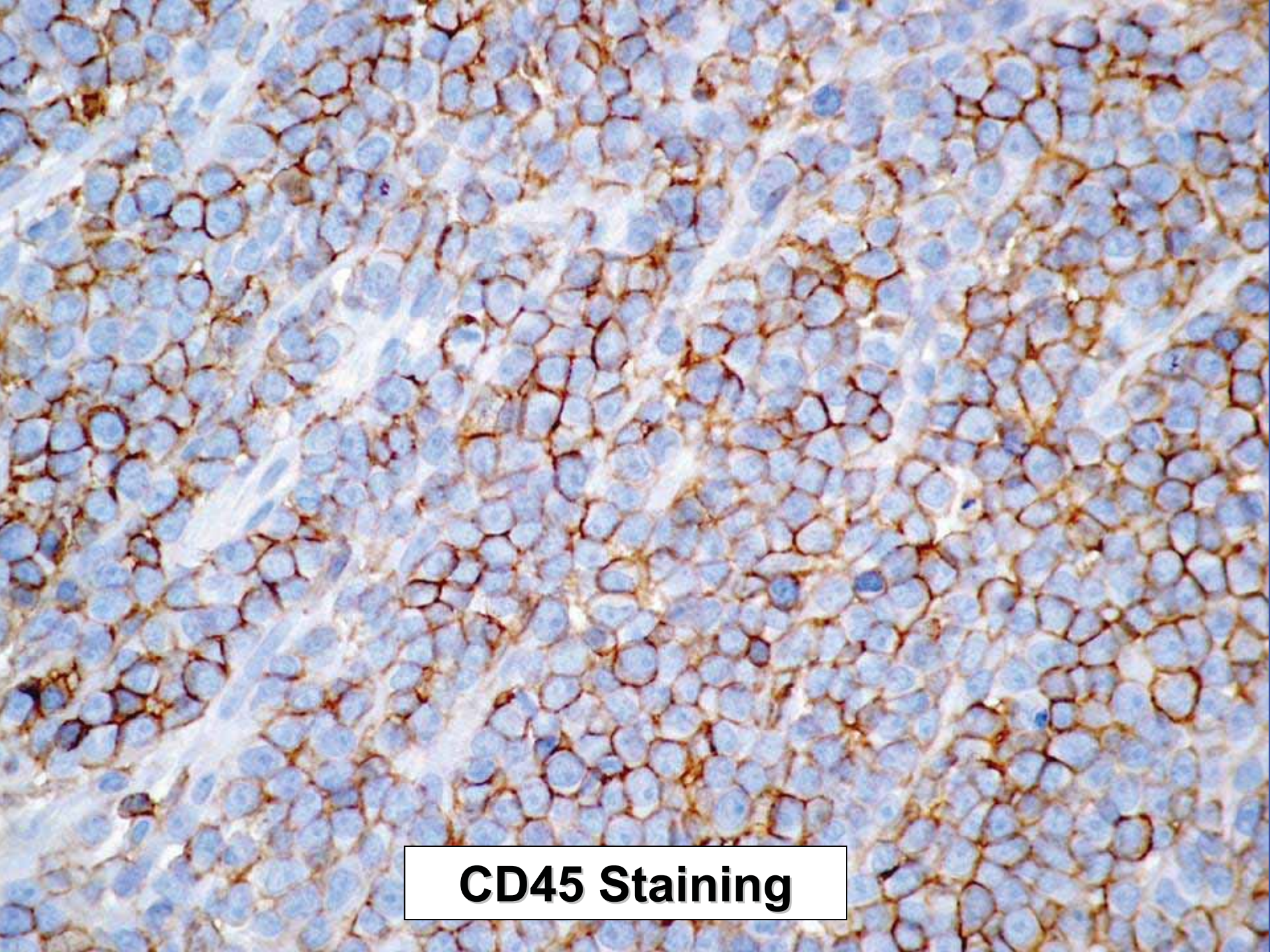
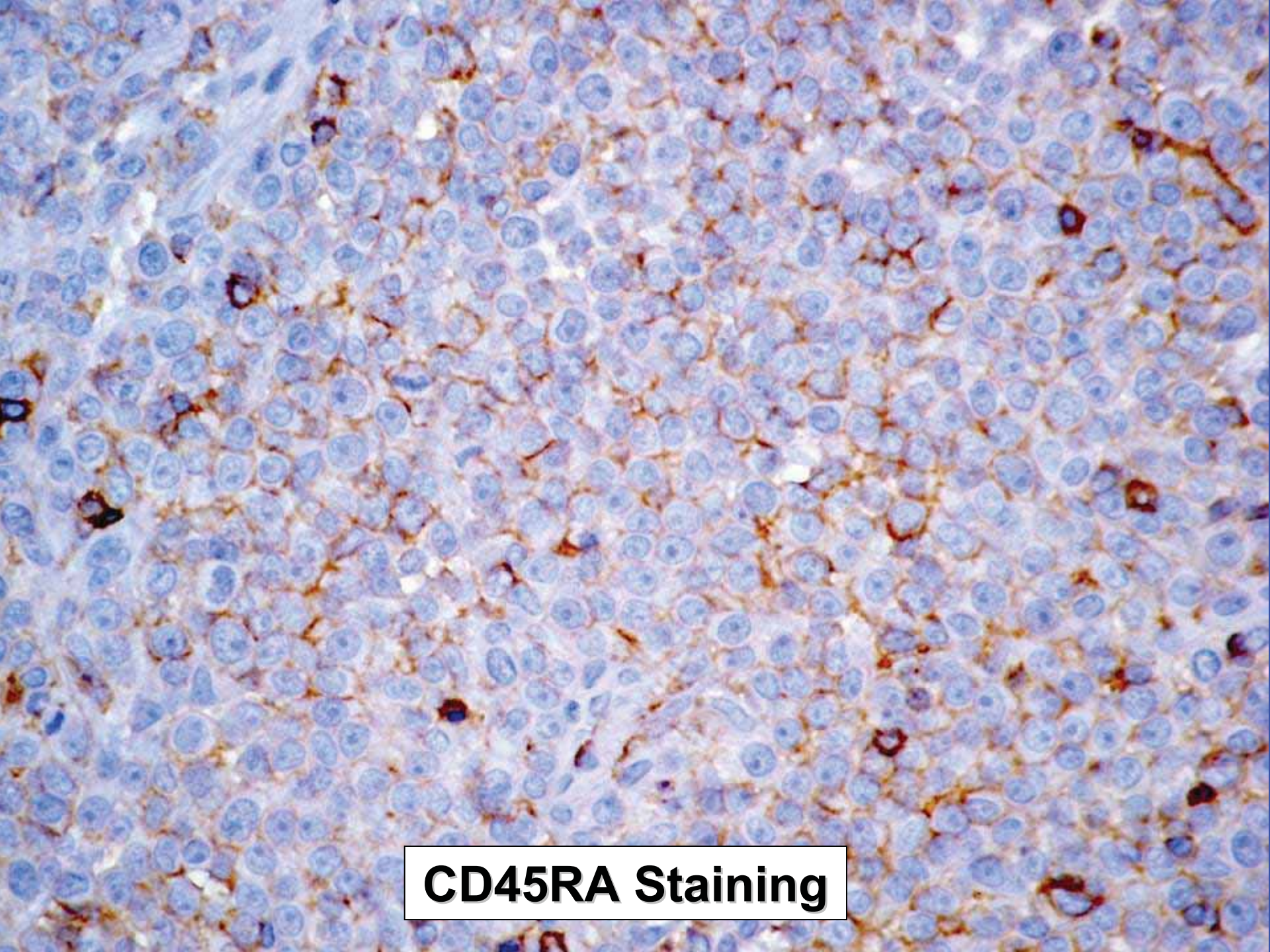


CD18 Staining



CD45 Staining

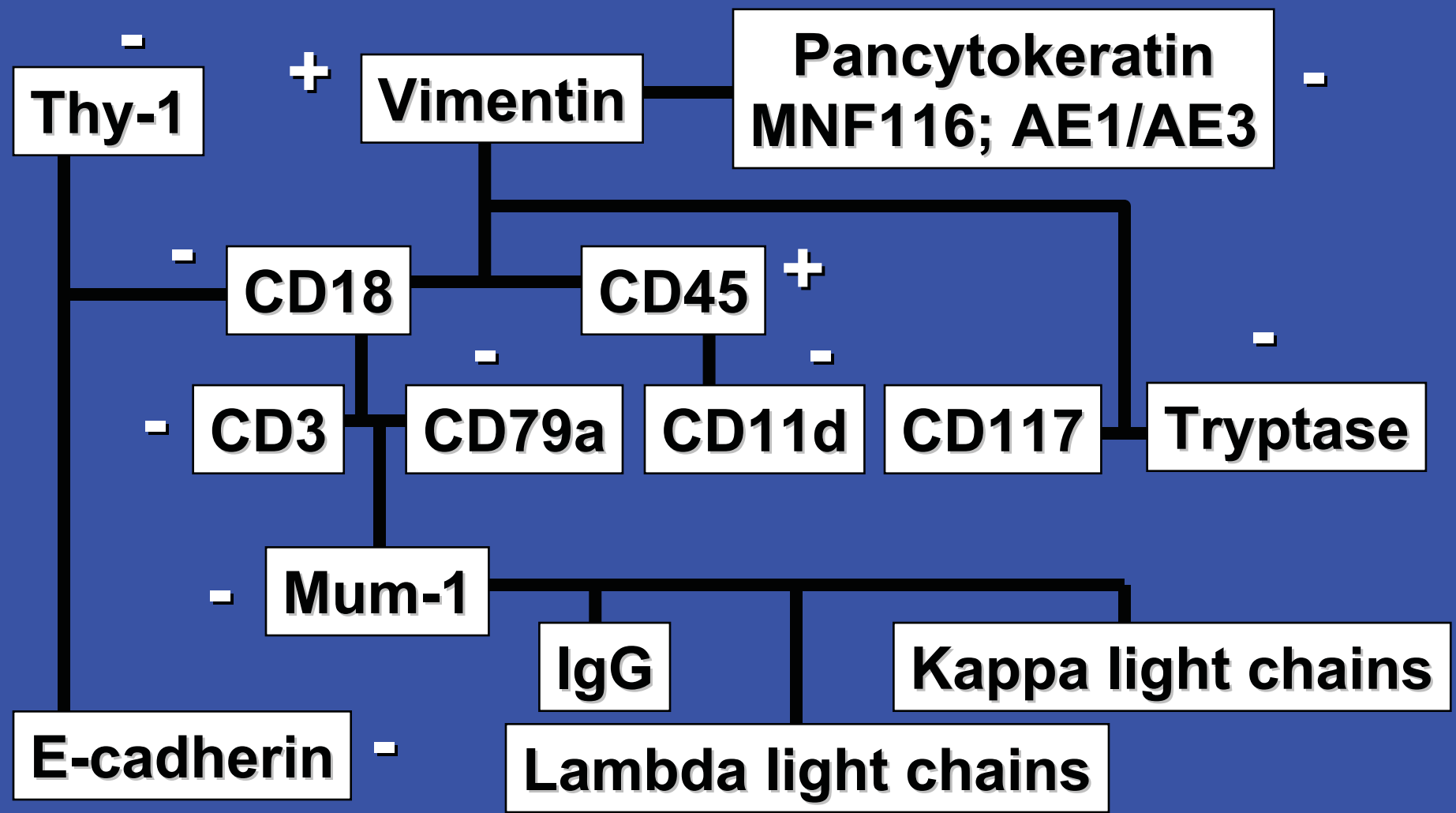


CD45RA Staining



What's your Diagnosis

Transmissible Venereal Tumor

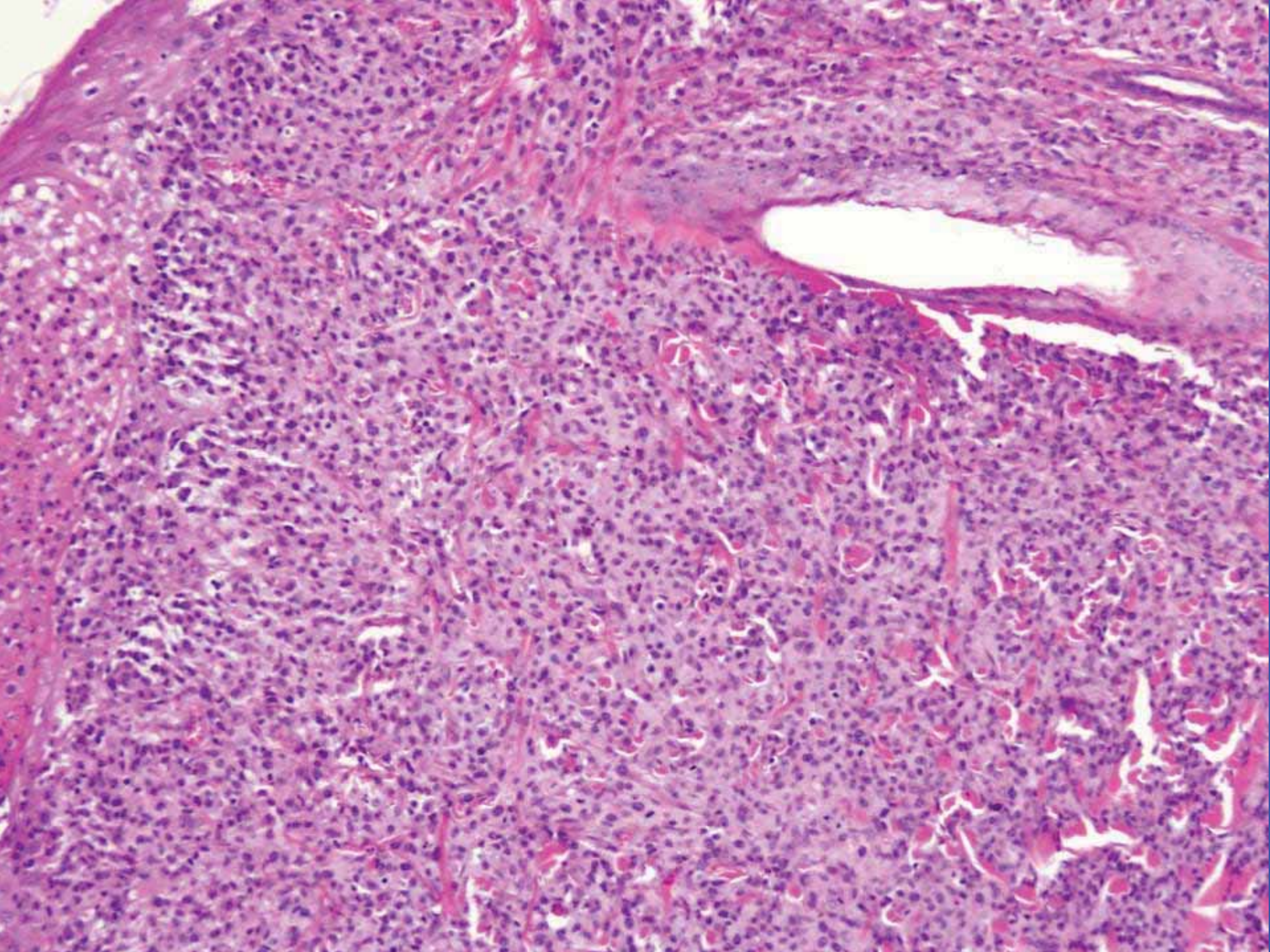


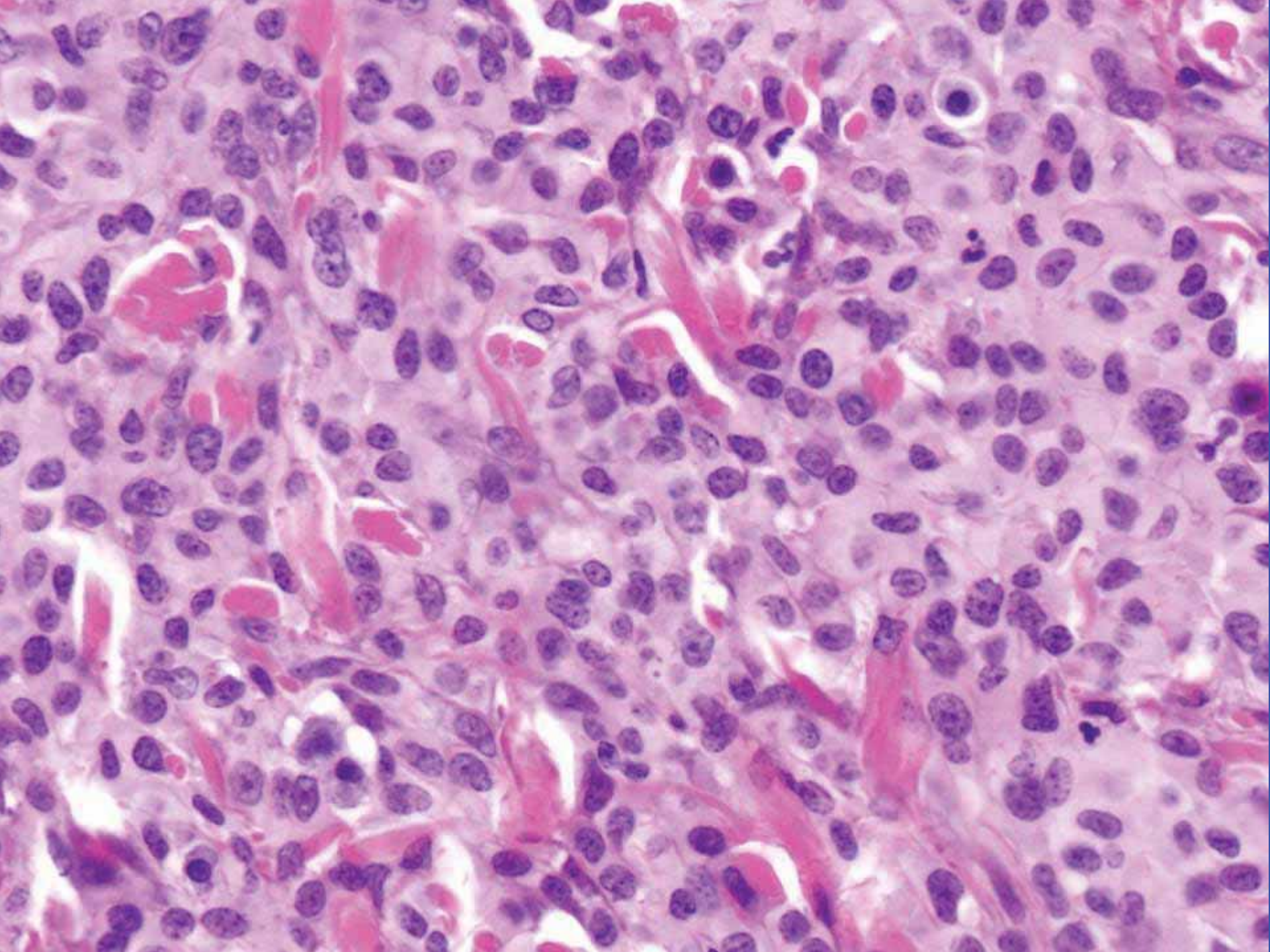
Transmissible Venereal Tumor

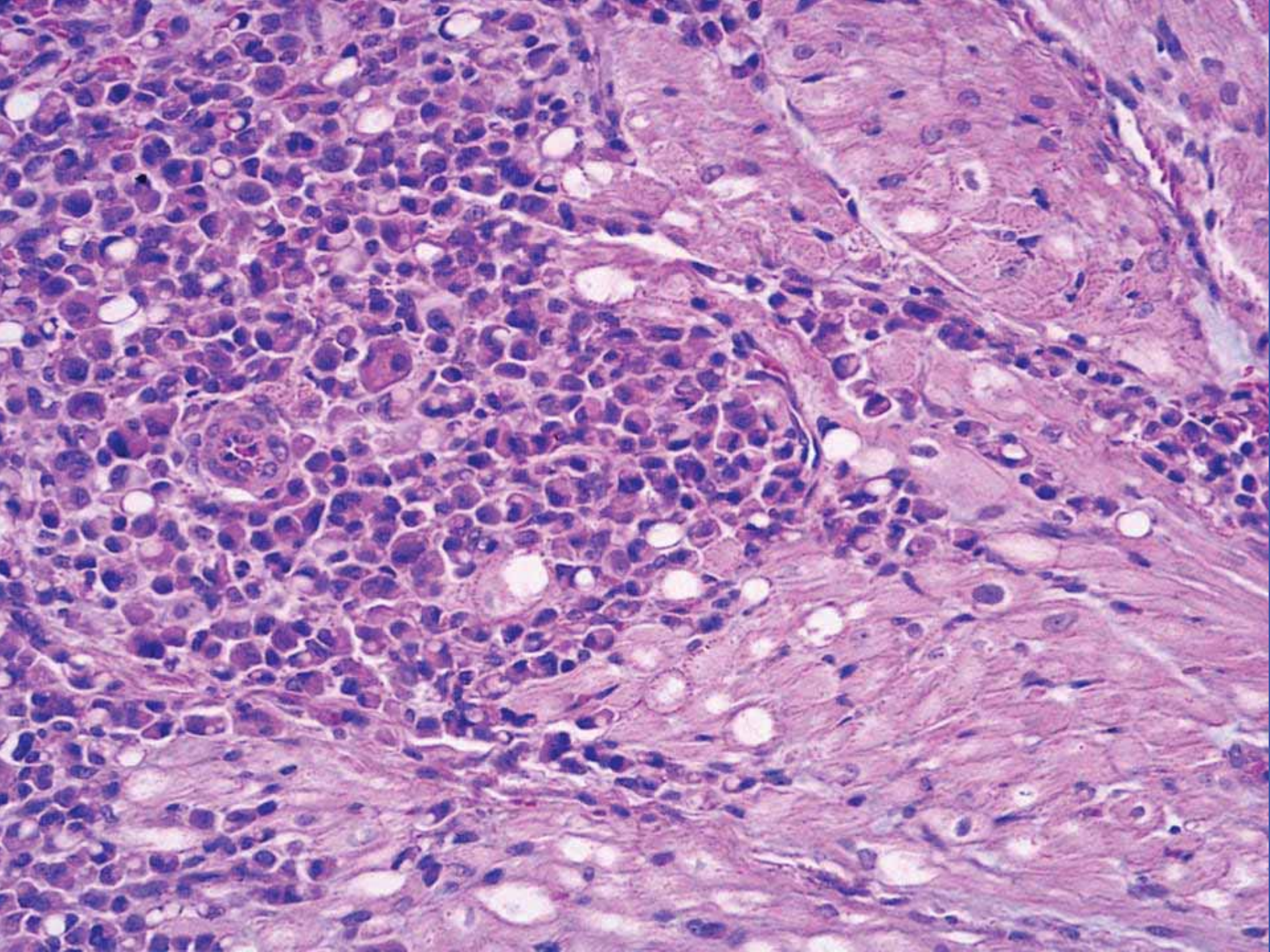
- Lineage of tumor cells is unknown; they have only 59 chromosomes (diploid canine = 78)
- **Inverted transposon** inserted 5' to exon-1 of c-myc enables molecular diagnosis of TVT by PCR
- **Immunohistology** - TVT lacks expression of lymphoid antigens and molecules expressed by **differentiated** macrophages and DC:
 - » **CD1, CD11/CD18, MHC-II**
- TVT cells express **CD45** (diffuse), **CD45RA** (variable), **CD49d** (variable)
- TVT is an **undifferentiated leukocytic tumor** - lacks expression of CD molecules of differentiated leukocytes; also lacks MPO

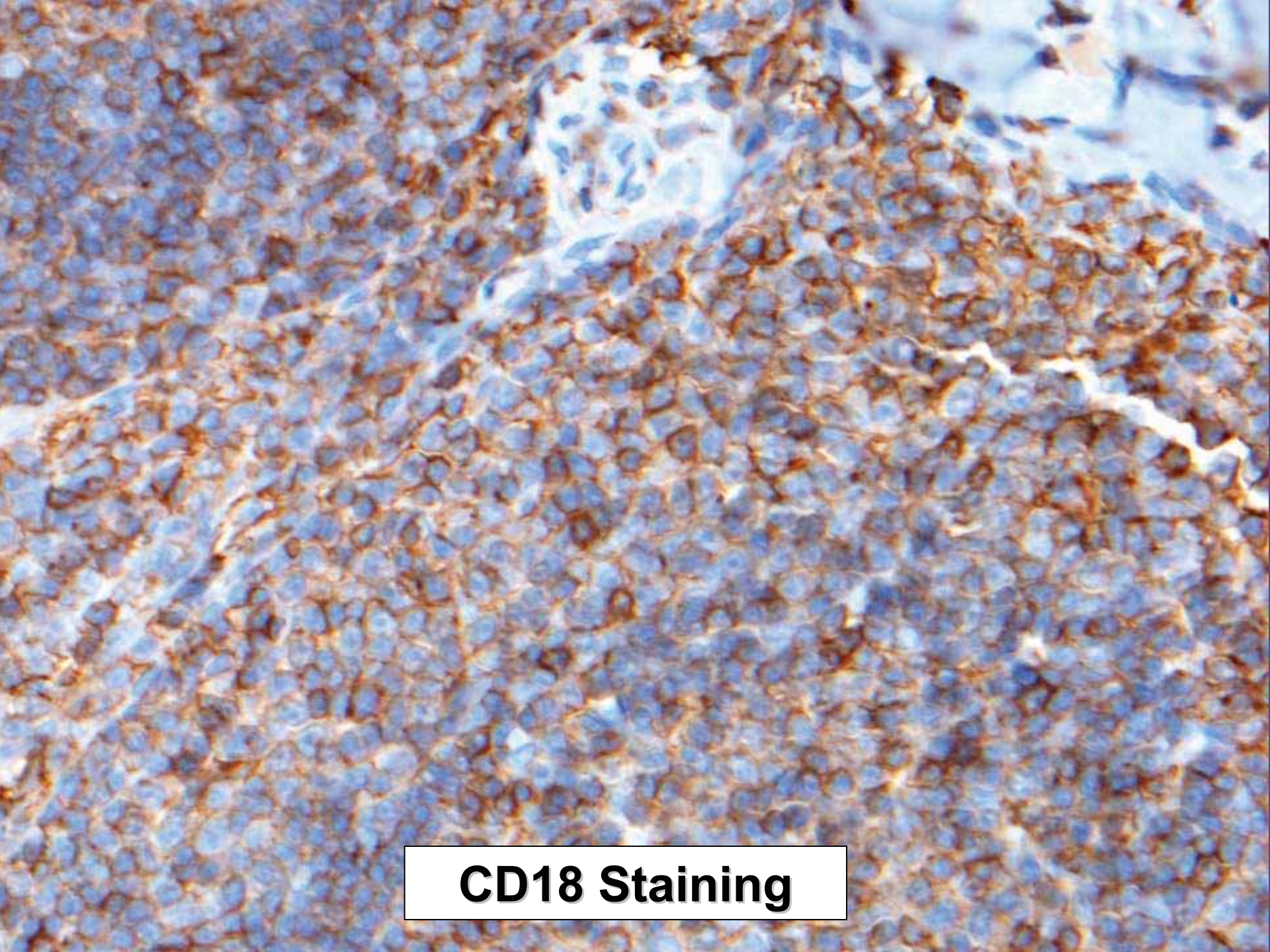
Facial Cutaneous Mass



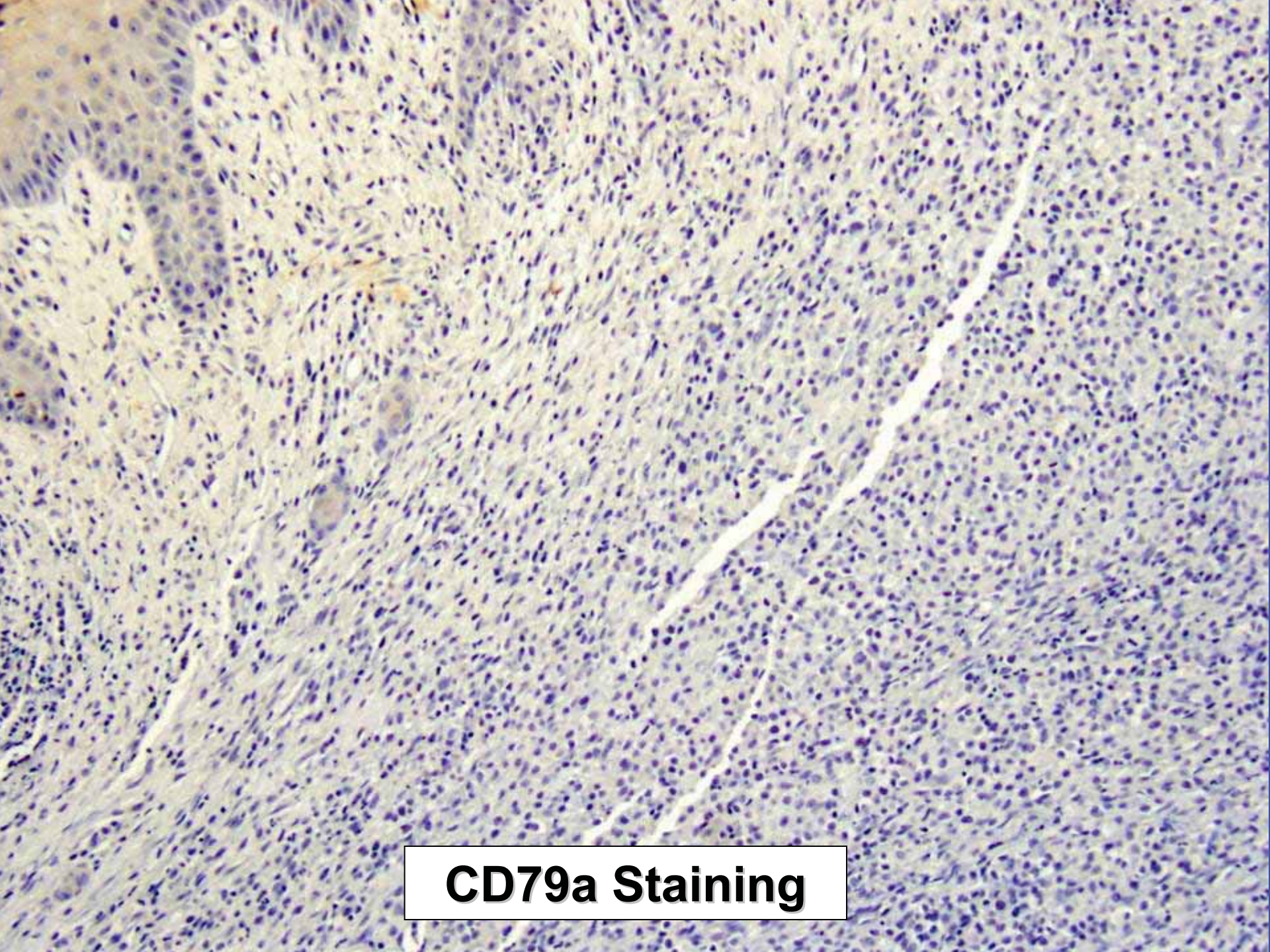




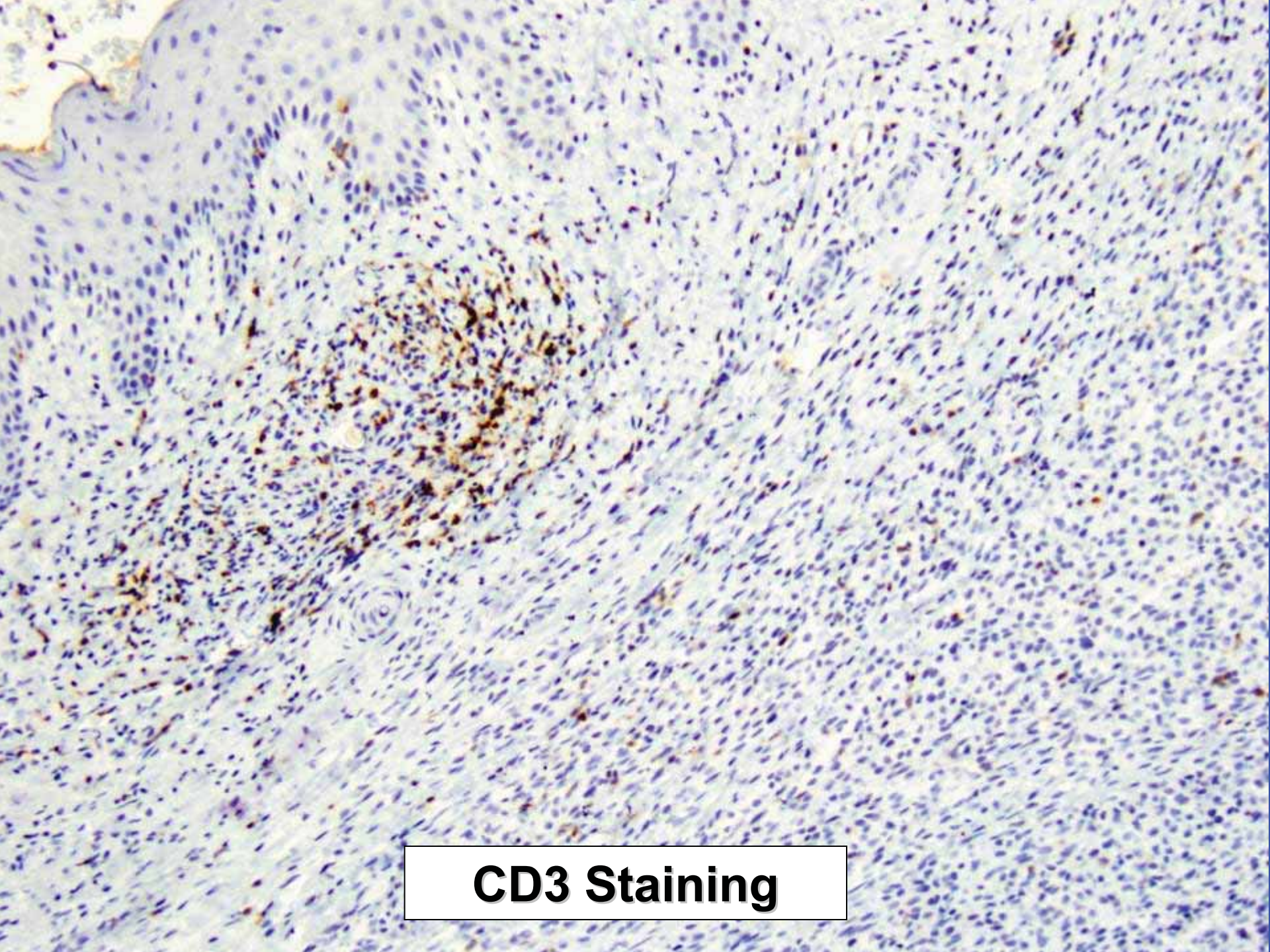




CD18 Staining



CD79a Staining

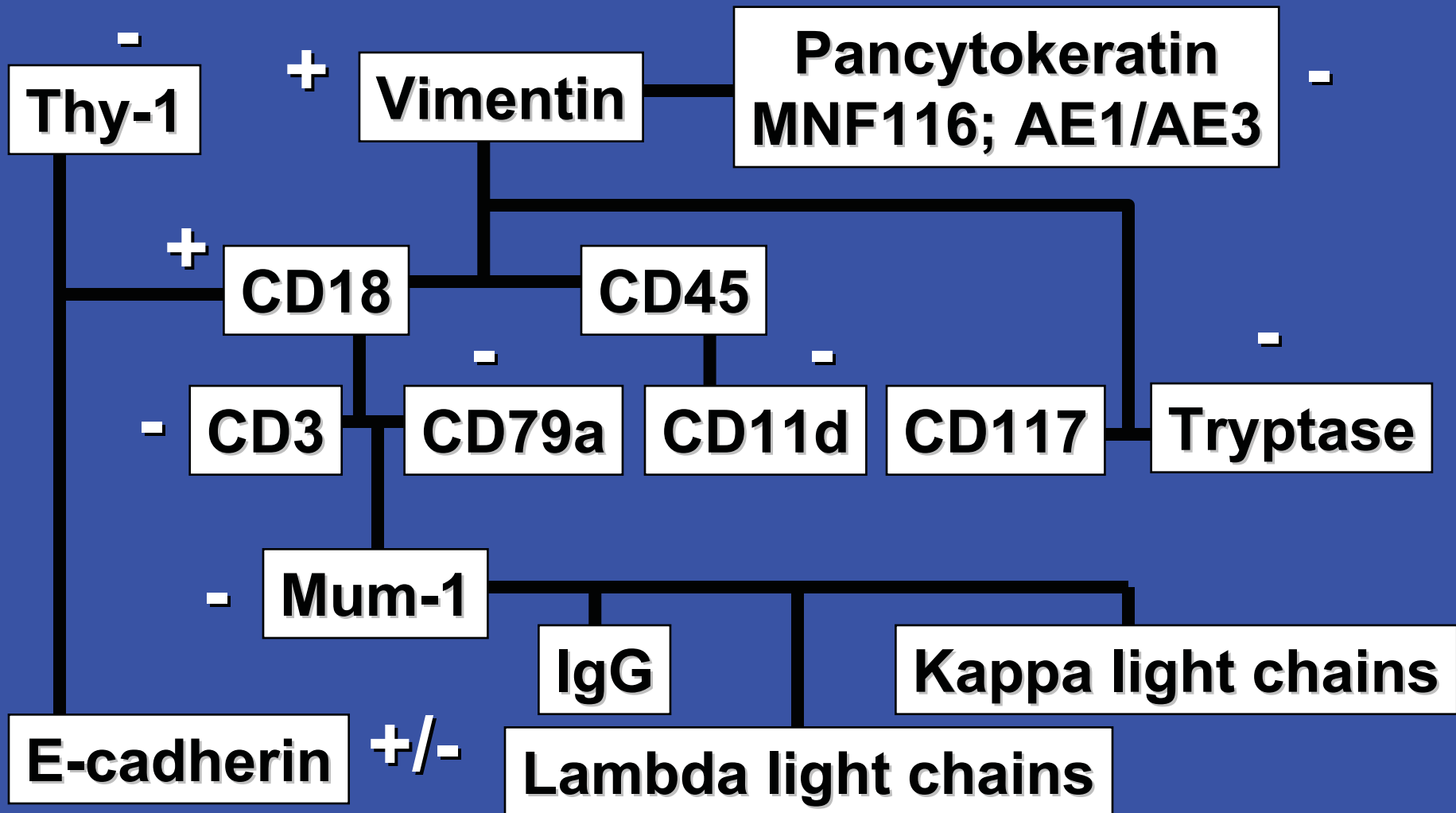


CD3 Staining



**What's
your
Diagnosis**

Feline Progressive Histiocytosis





E-cadherin Staining

Progressive Histiocytosis

- No breed or age predilection
- Females more often affected than males
- Solitary or multiple non-pruritic firm papules, nodules, and plaques
- Predilection for feet, legs, and face
- Systemic spread in 20% of cases
- Poorly circumscribed, epitheliotropic (45%), histiocytic infiltrates in superficial/deep dermis
- Monomorphous population early in disease
- With progression > cellular pleomorphism
- Histiocytes expressed CD1a, CD1c, CD18, MHCII
- No therapy response

Intestinal Infiltrate



Inflammatory Bowel Disease

- **Diagnosis of last resort, list the actual findings as diagnosis**
- **Idiopathic inflammation of small and large intestine and stomach (food sensitivity should be included in this group)**
- **Etiology undetermined: genetic, dietary, bacterial, immunologic, and mucosal permeability may play role**
- **Clinical Signs:**
 - » **Do not correlate with degree of inflammation**
 - » **SI: Vomiting and weight loss**
 - » **LI: Hematochezia and diarrhea**
 - » **Also: Lethargy, anorexia, ravenous appetite**
- **Microscopic lesions – 3 categories:**
 - » **Changes in mucosal architecture, epithelial alterations**
 - » **Fibrosis in lamina propria**
 - » **Increased numbers of leukocytes in lamina propria**
- **Patients diagnosed with IBD that fail to respond to treatment are often re-diagnosed with intestinal lymphoma at a later time**

Diagnosis of Intestinal Lymphoma versus IBD

- Currently based on clinical and morphological criteria
- Morphology:
 - » Marked cellular infiltration
 - » Monomorphic appearance of infiltrating cells
 - » Cellular immaturity of infiltrate
 - » Infiltration and destruction of the normal structures
 - » Marked contrast between very heavy cellularity in some segments yet virtually normal mucosa in immediately adjacent portions of the tract
- Mucosa associated lymphatic tissue (MALT):
 - » Populated by CD3+ cells in healthy animals
 - » Expansion of T cell populations in diffuse MALT in IBD and intestinal lymphoma

Intestinal Lymphoma



Intestinal Lymphoma

- **Types:**
 - Multicentric lymphoma → B-cell
 - Alimentary lymphoma → T-cell
- **Gross:** Diffuse or localized → Plaque, nodule, annular
- **Sites:**
 - Cat: Jejunum, ileocecolic junction >> others
 - Dog: small intestine > stomach > large intestine
- **Metastases:** Spleen, liver, kidney, LN, peritoneum
- **Cause:** FeLV (some cats)



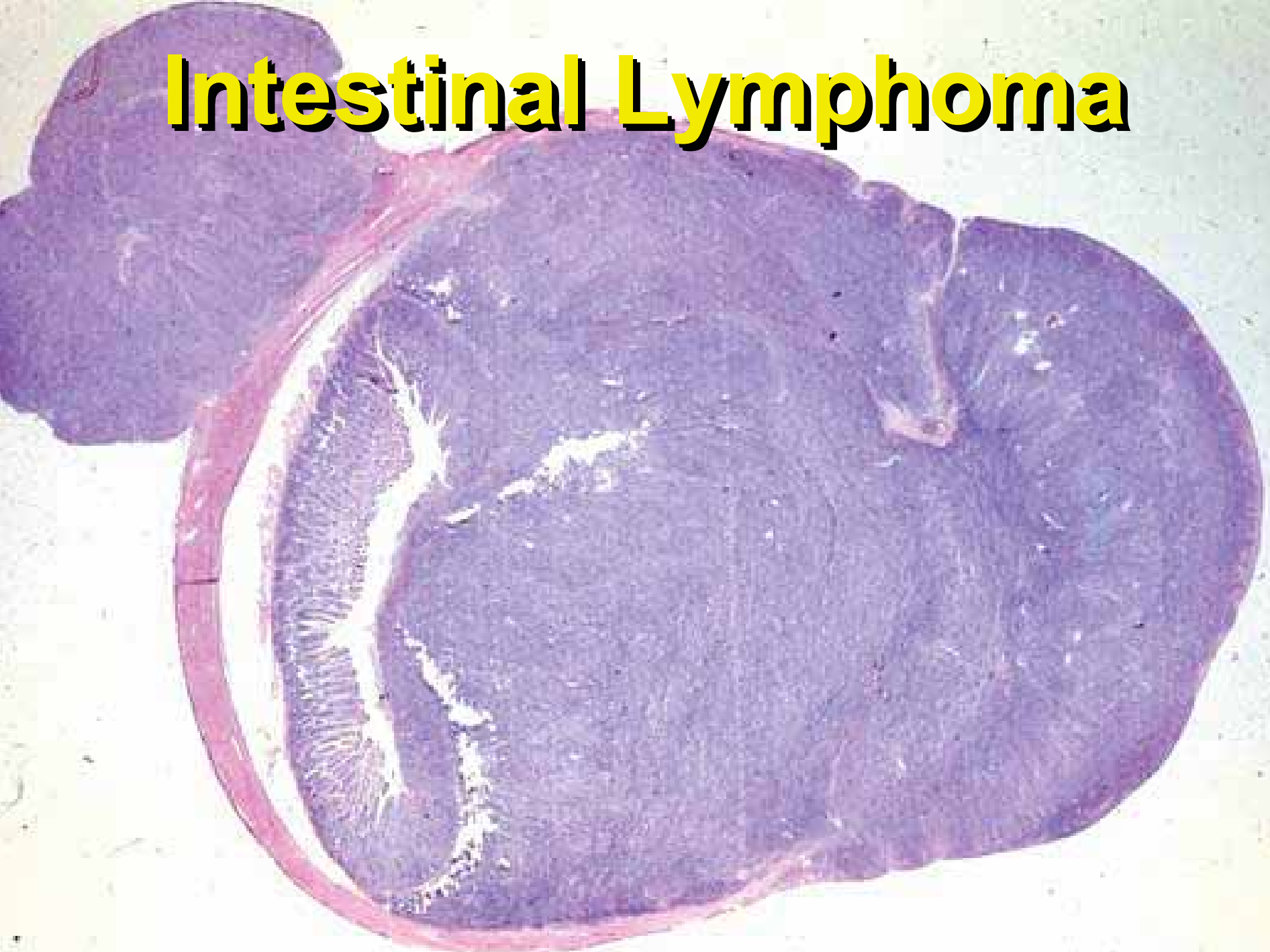
Intestinal Lymphoma



Intestinal Lymphoma



Intestinal Lymphoma



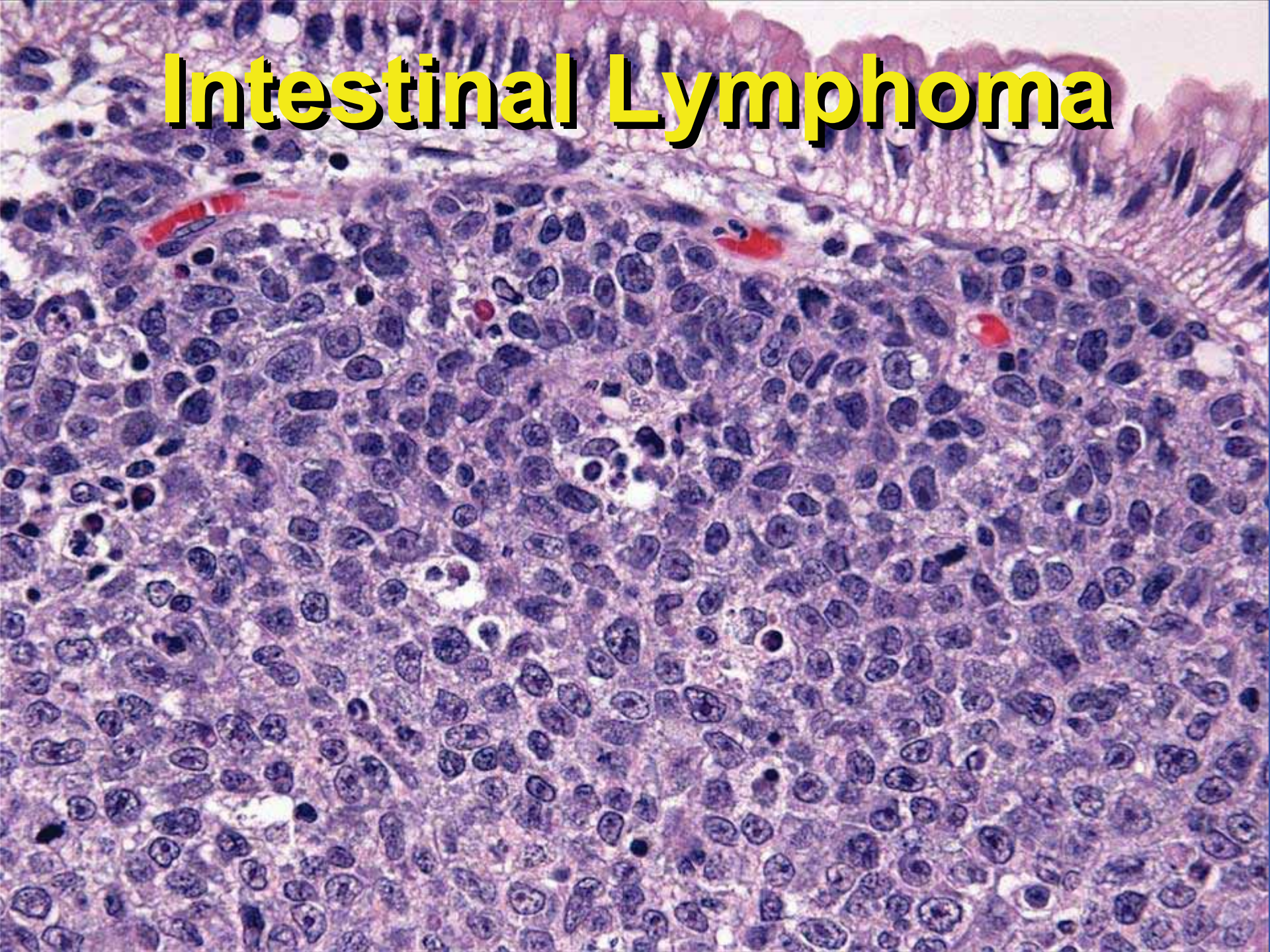
Intestinal Lymphoma



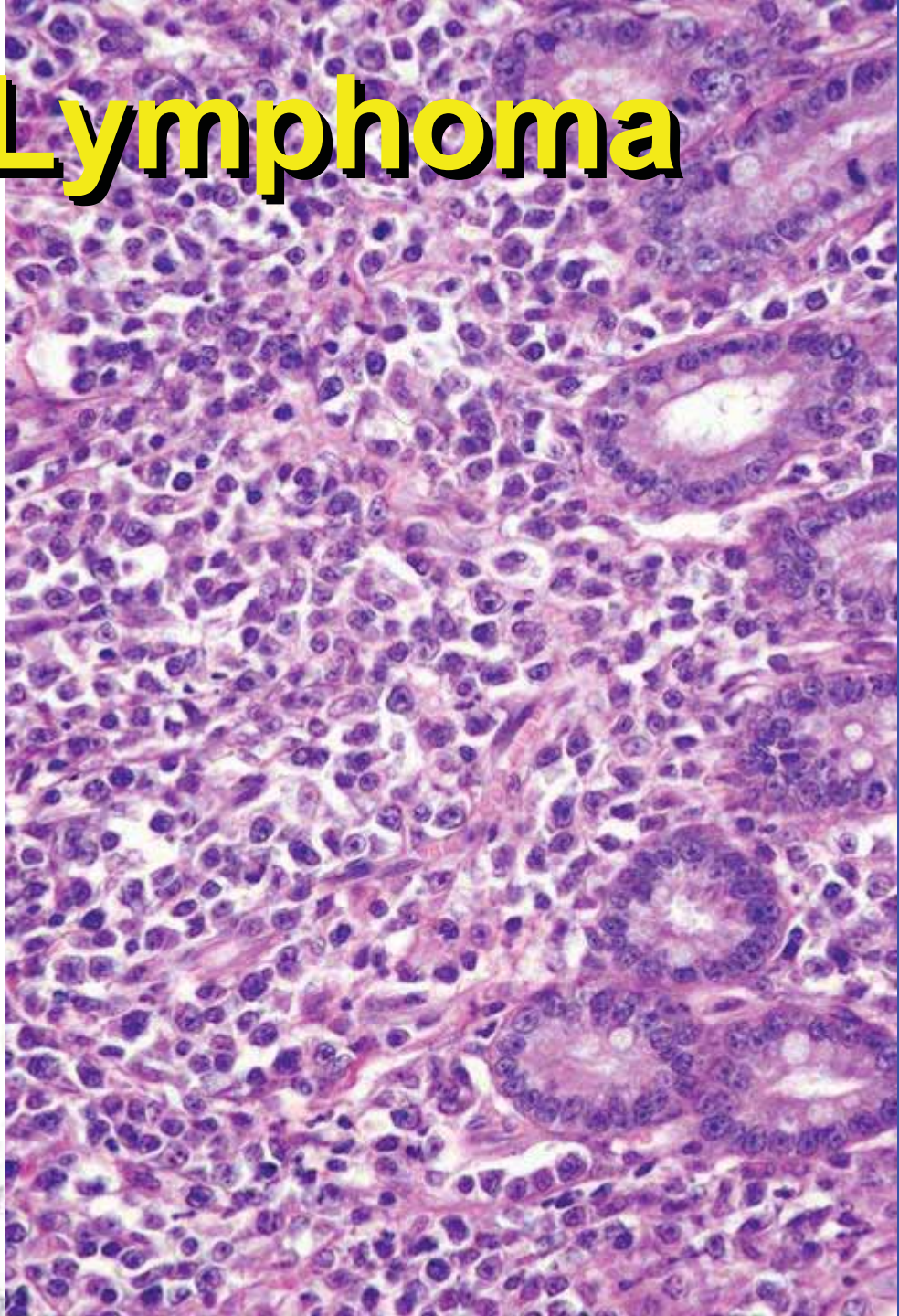
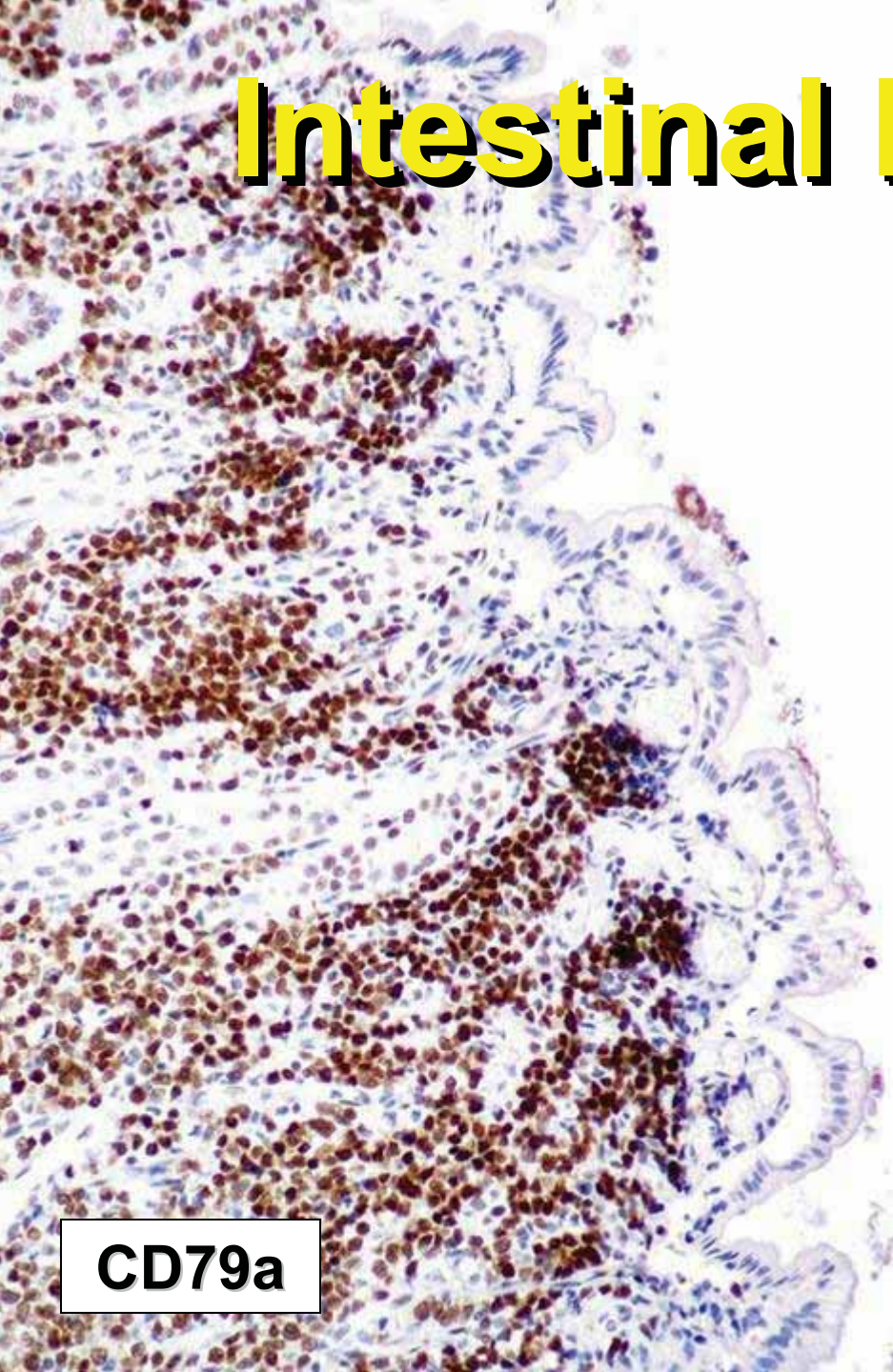


Feline GI B cell lymphoma

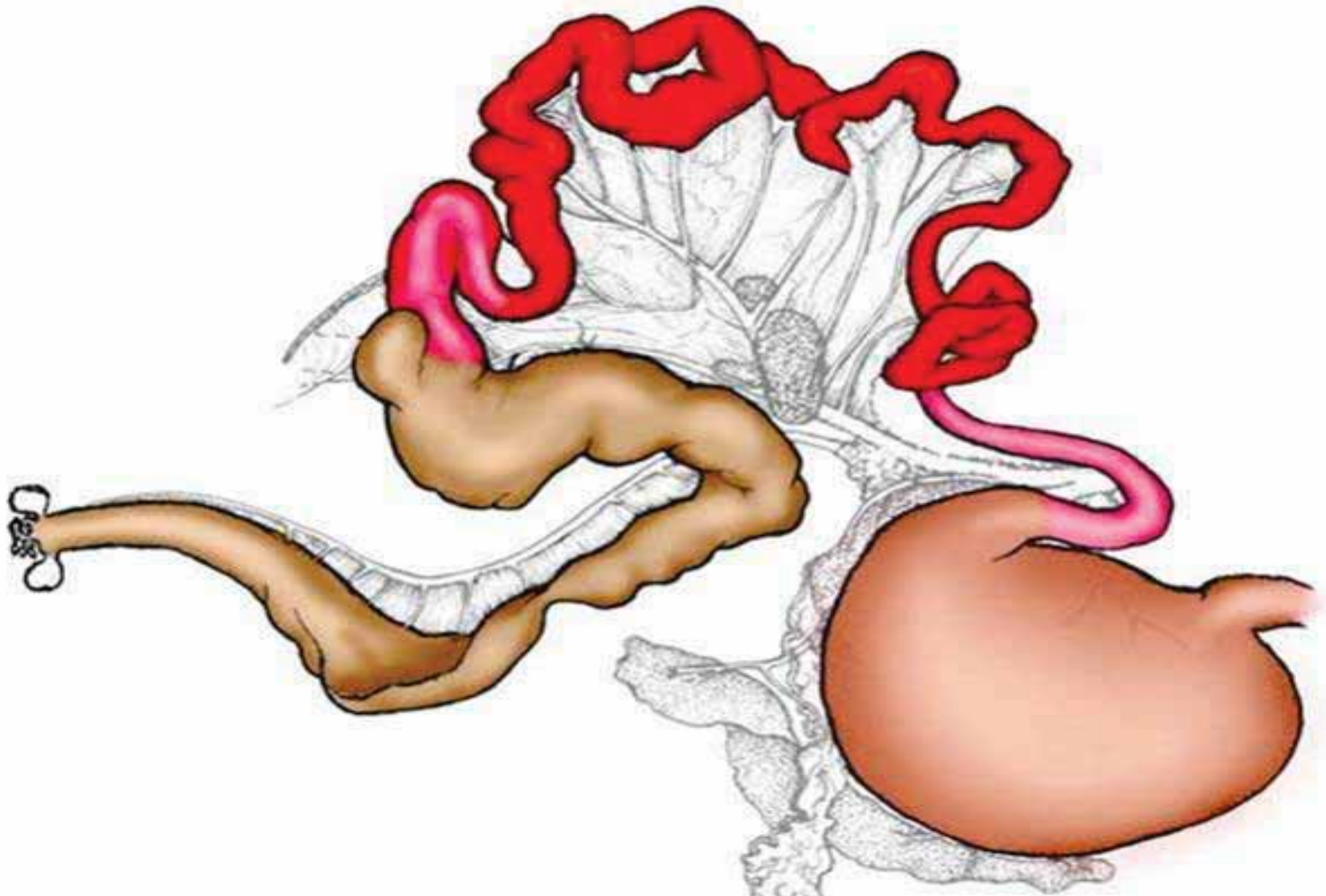
Intestinal Lymphoma



Intestinal Lymphoma



CD79a



Feline GI T cell lymphoma

Study Population

- **64 cats**
 - **Inclusion criteria:**
 - **Clinical history of chronic diarrhea**
 - **Small intestinal biopsies**
 - **Previously diagnosed as IBD or lymphoma**
 - **Sufficient formalin fixed material**
 - **Complete follow-up data and clinical history**
 - **Breed: 9 DLH, 3 DMH, 24 DSH, 28 Mixed Breed**
 - **Age: 1 - 27, mean: 11.5 years**
 - **Sex: 36 Neutered Males, 29 Spayed Females**
 - **Treatment: Diet change, Prednisone, Leukeran, Wisconsin protocol**
 - **Survival**
 - **Cause of death**

Methods

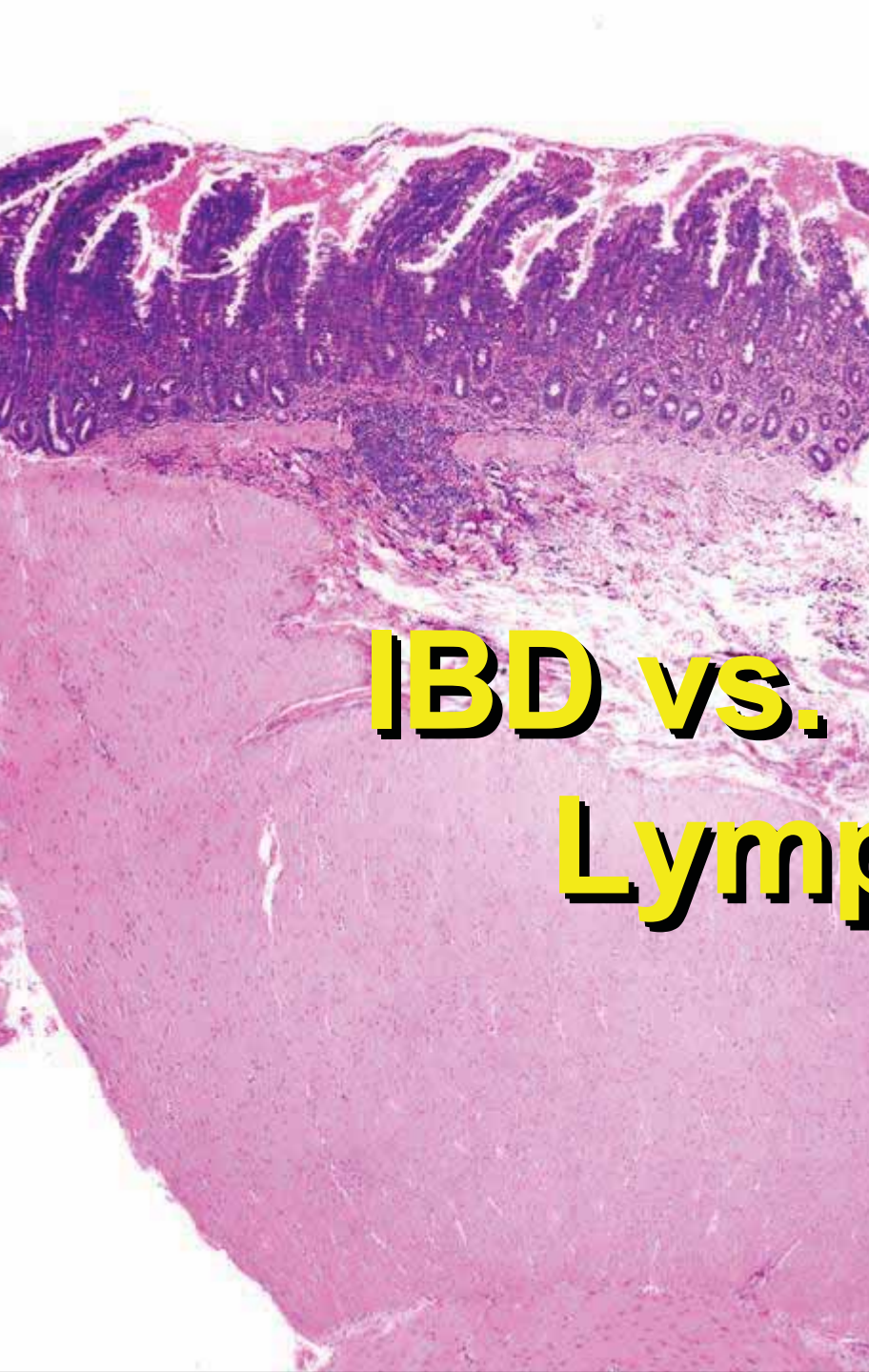


- **Microscopic Evaluation (H&E)**
 - **Location of lymphocytic infiltrate (intravascular, serosa, muscularis, submucosa, mucosa)**
 - **Mucosal infiltrates:**
 - **Distribution and density of cellular propria infiltrates**
 - **Epithelial involvement**
 - **Location (surface or crypts)**
 - **Aggregation (single cells, nests or plaques)**
 - **Cell morphology (monomorphism, mitotic index, cell size)**
- **Immunohistochemistry (IHC):**
 - **CD3 (T cells) and CD79a (B cells)**

Methods

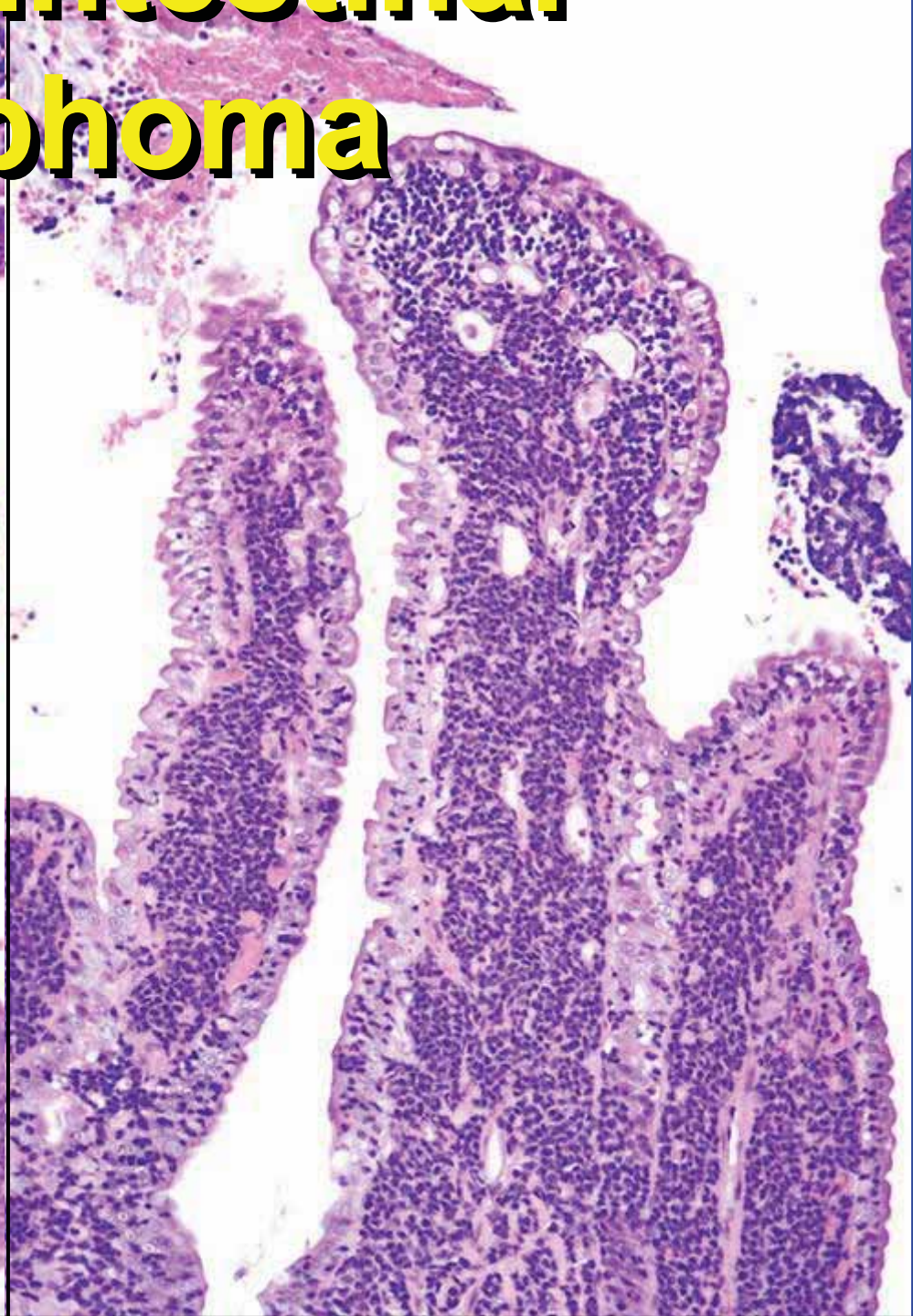
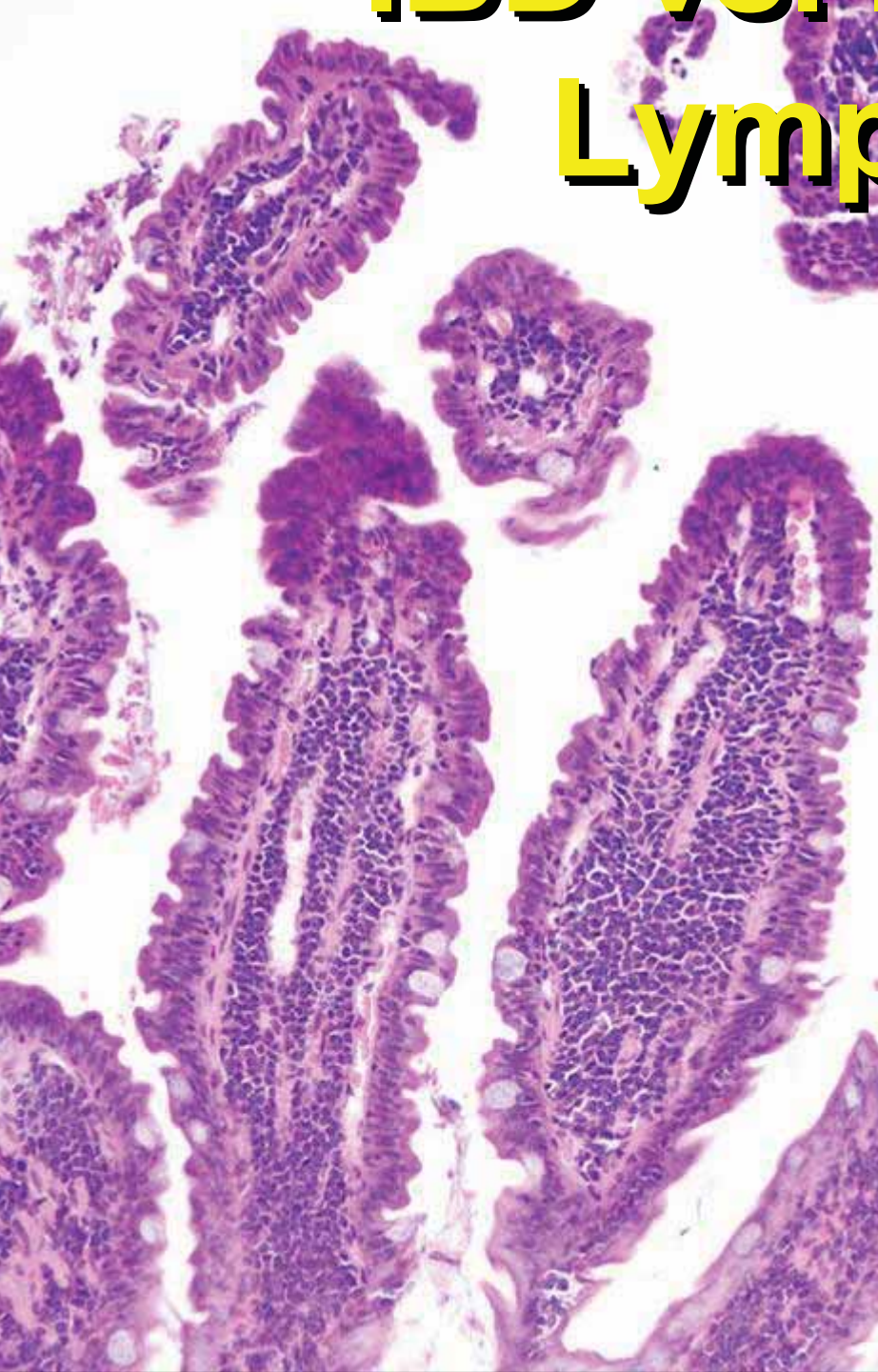


- **PCR for T and B cell clonality**
 - **T-cell PCR: TCRG V segment/TCRG J segment**
 - **B-cell PCR: CDR3 of the *IGH* V (2 multiplex PCR)**
- **Statistical analysis**
 - **Mantel-Haensel chi-square statistics**
 - **Associations between categorical risk factors (morphology, IHC, PCR) and study outcomes (survival times, mortality due to lymphoma versus inflammation or due to other causes)**



IBD vs. Intestinal Lymphoma

IBD vs. Infectious Lymphoma



IBD vs. Intestinal Lymphoma



CD79a

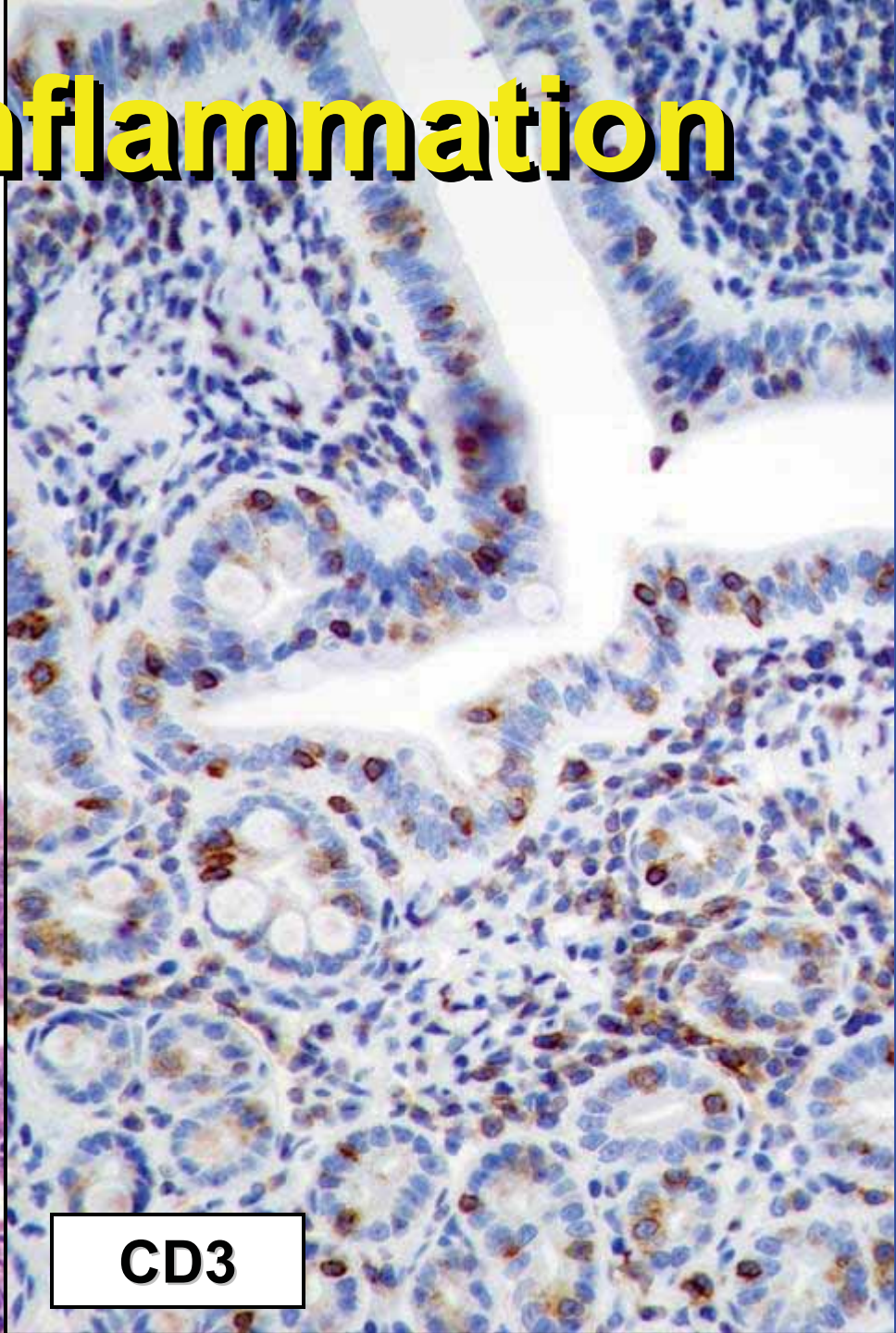
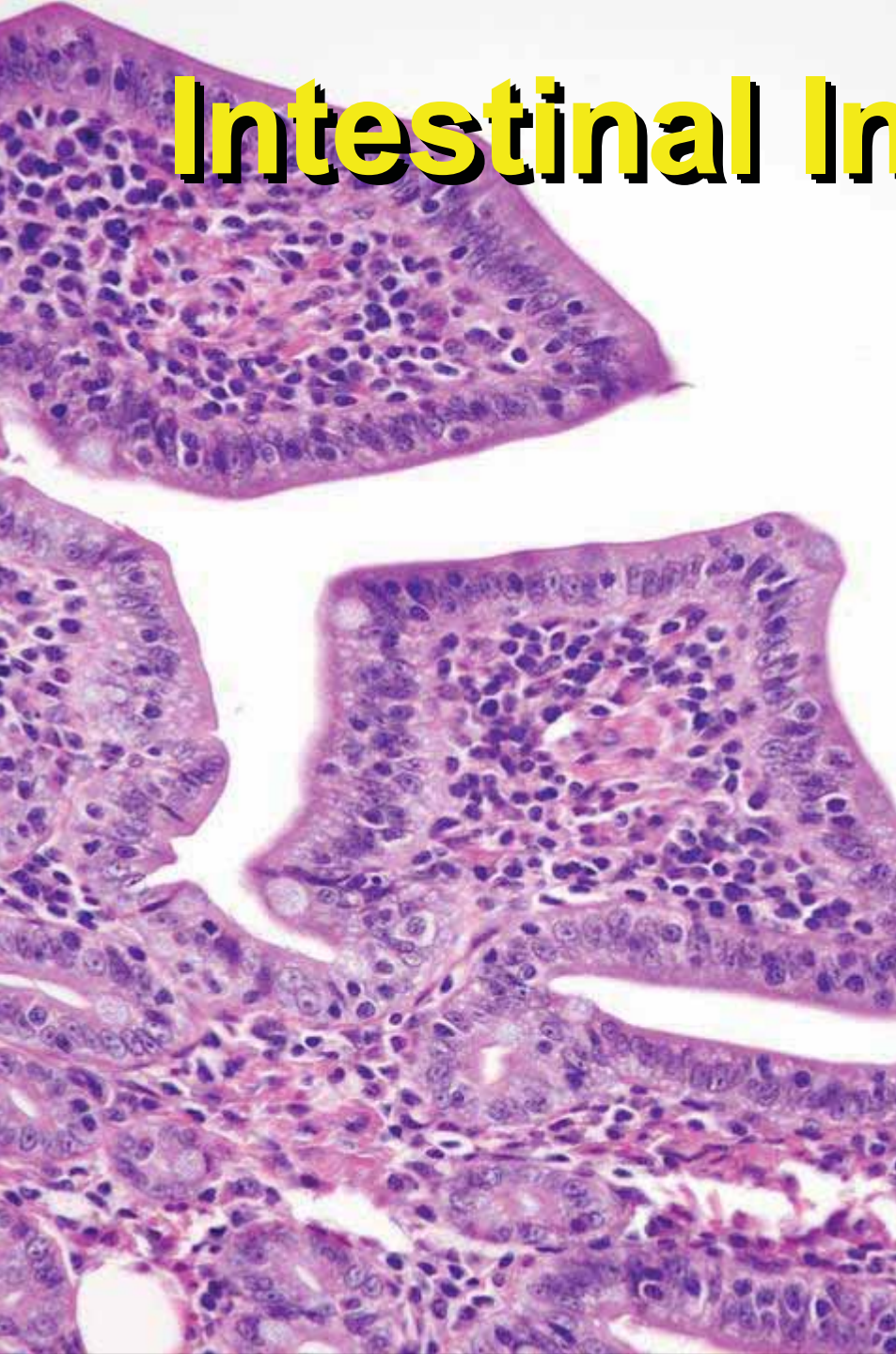


CD3



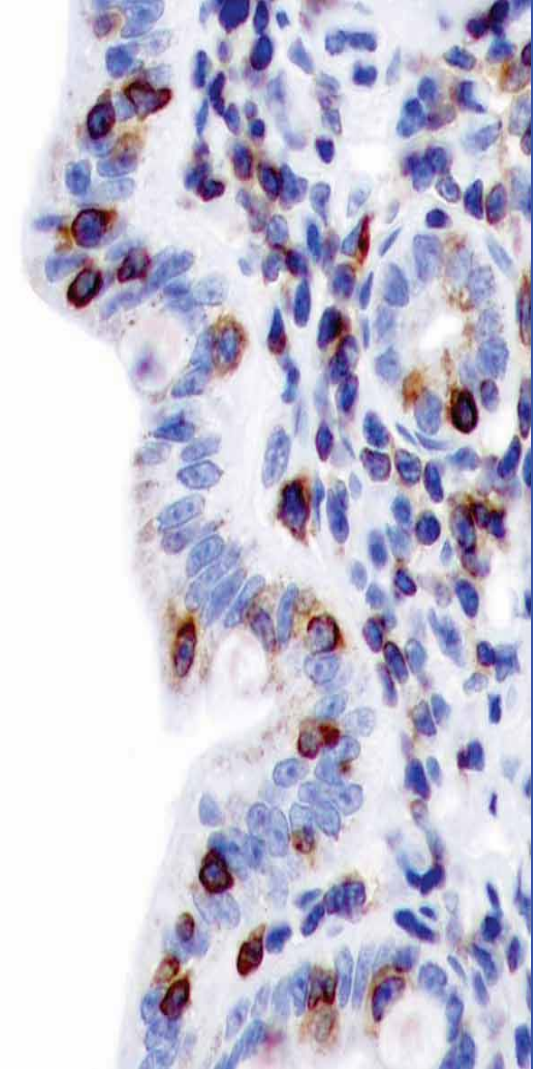
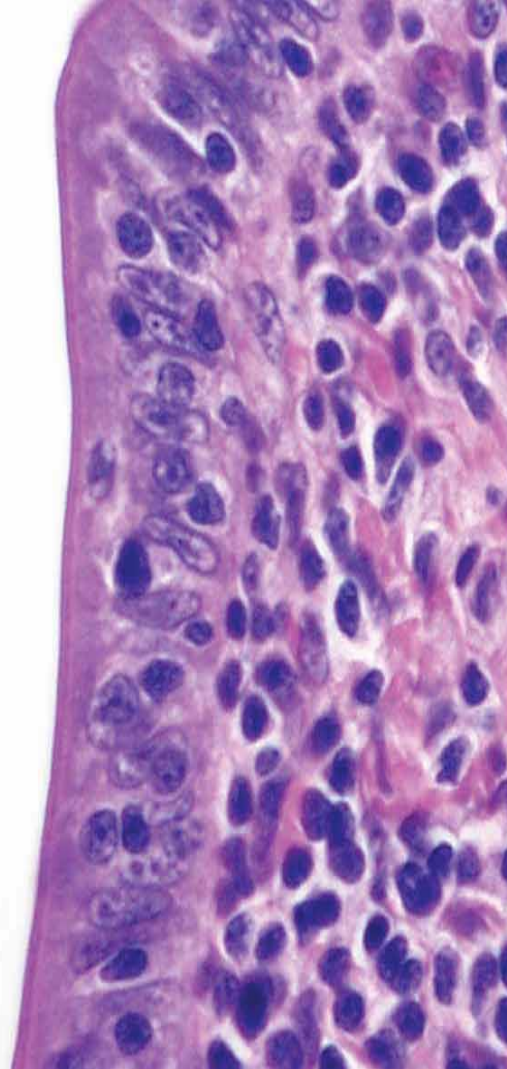
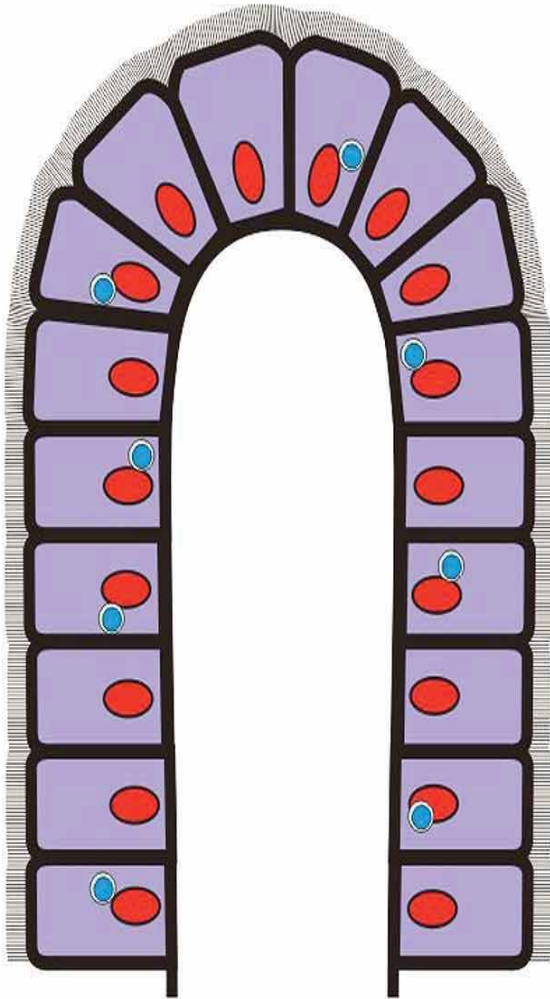
CD3

Intestinal Inflammation

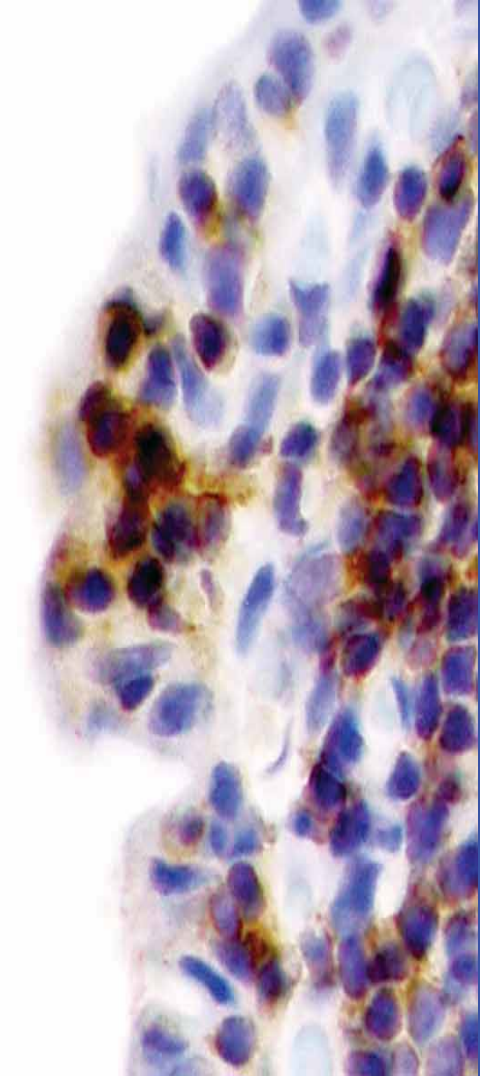
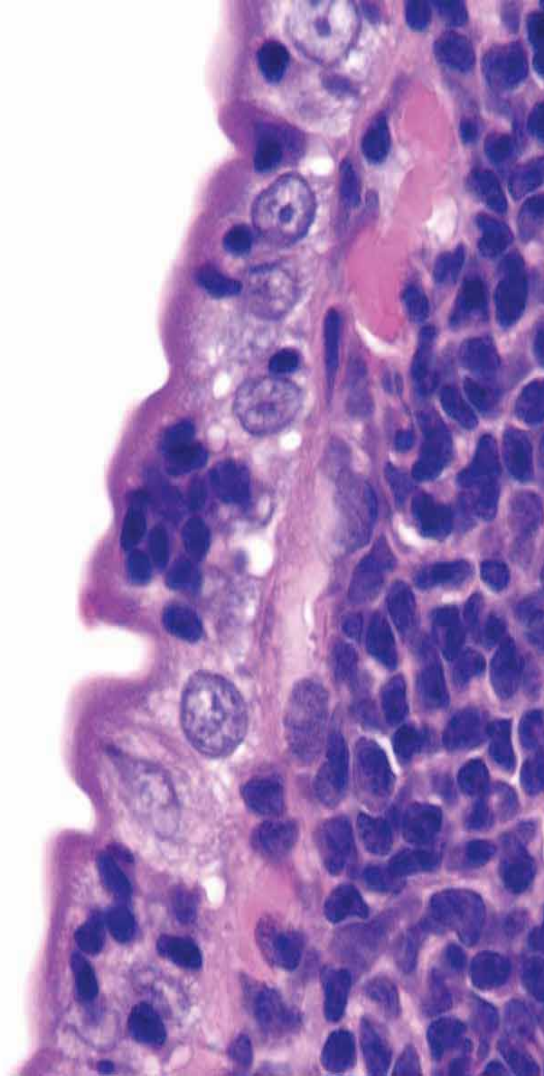
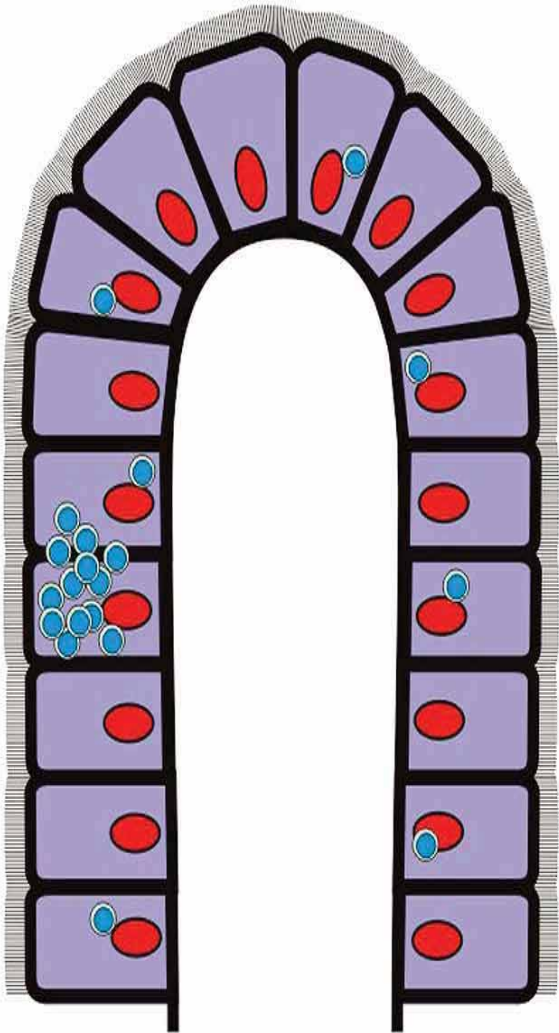


CD3

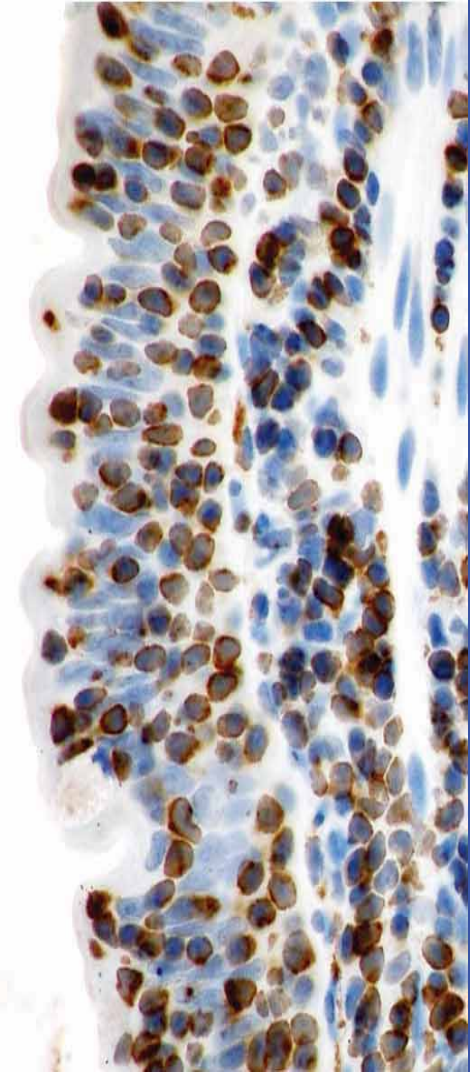
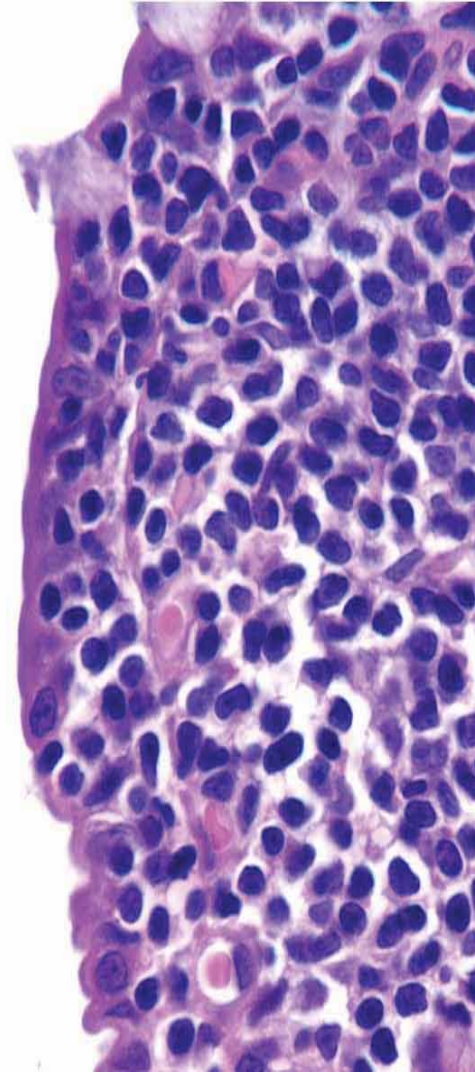
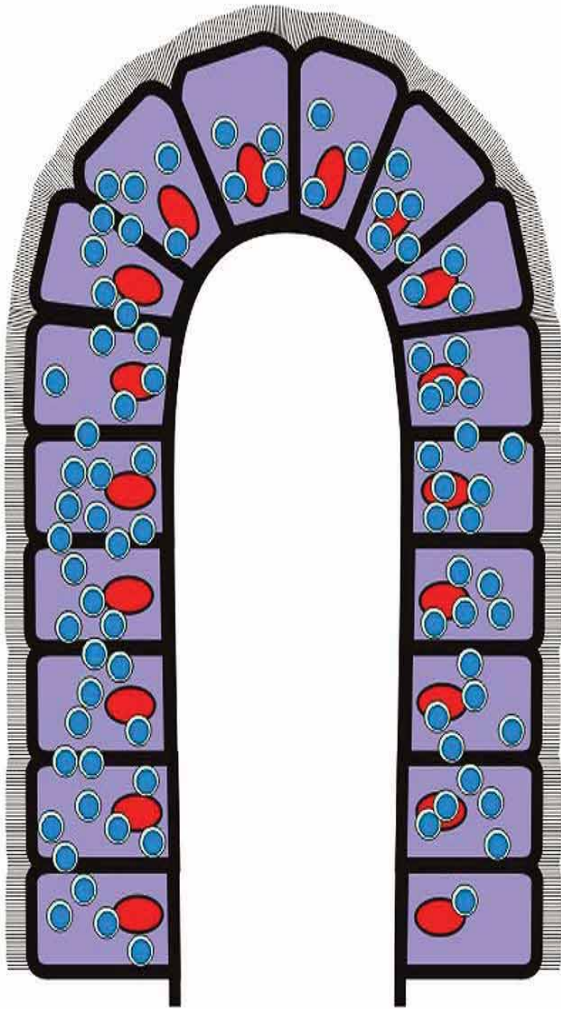
Mucosal Lymphoma - Epitheliotropism



Mucosal Lymphoma - Epitheliotropism

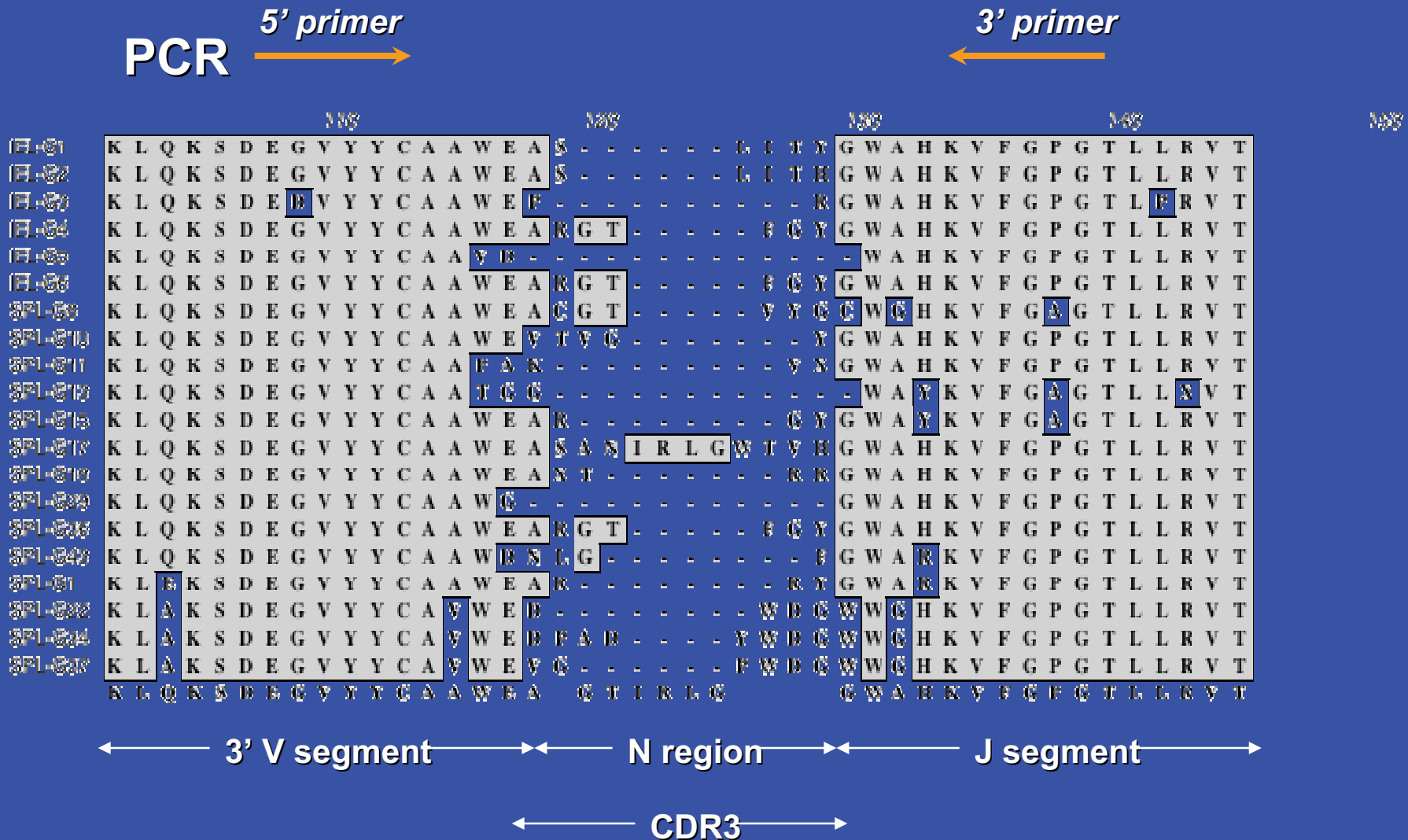


Mucosal Lymphoma - Epitheliotropism

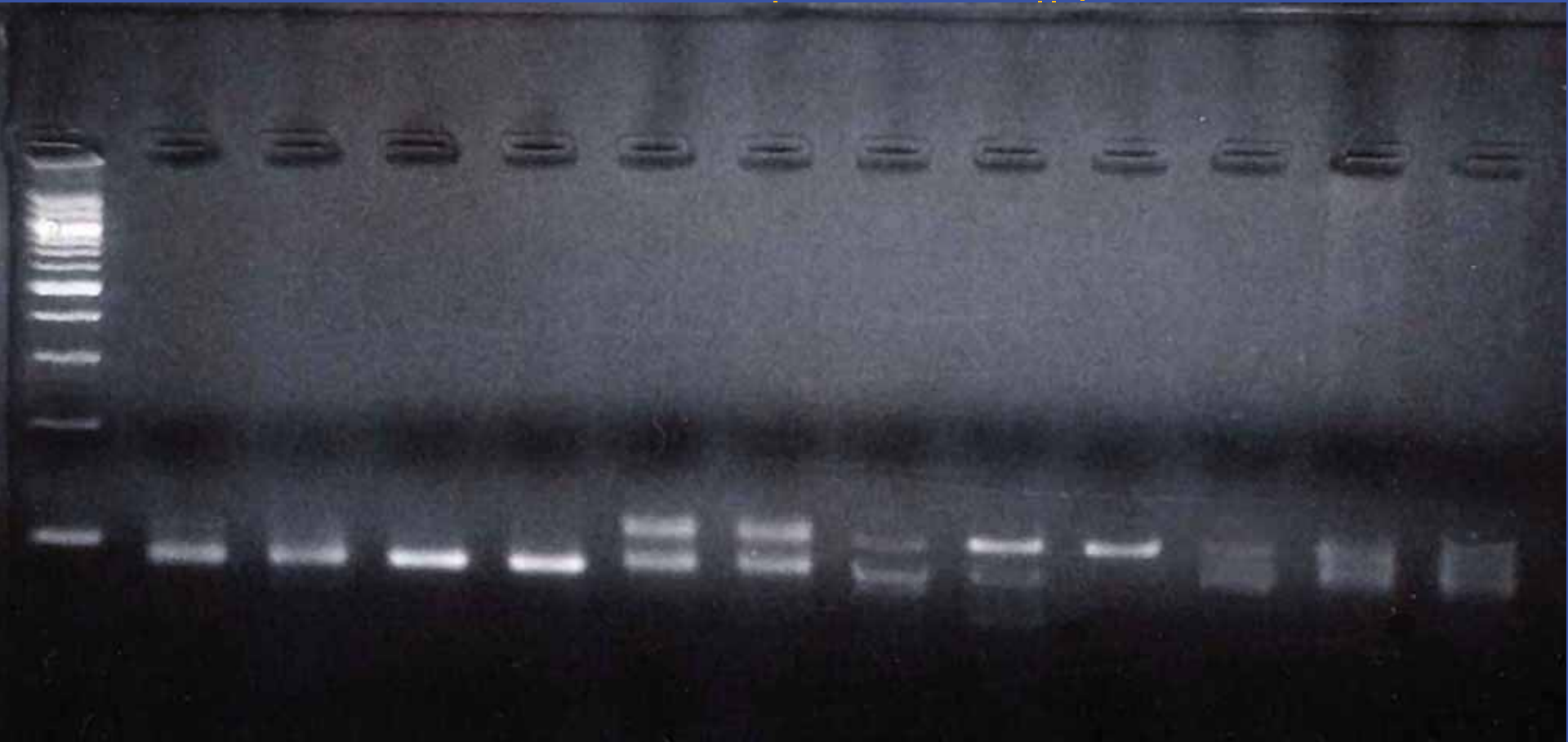


Feline TCRG V-N-J alignment CDR3 region

Moore et al., Vet Immunol Immunopathol 106: 167-178, 2005



PCR amplicons to demonstrate TCRG rearrangements



1 2 3 4 5 6

Histopathology



- **H & E Stain**
 - **92% were diagnosed by H & E**
- **Immunohistochemistry**
 - **Four cases were reclassified after IHC**
 - **23% still questionable after IHC (15/64)**
 - **52% T Cell Lymphoma (33/64)**
 - **9% B Cell Lymphoma (6/64)**
 - **16% IBD (10/64)**

		H&E Diagnosis			Total
		IBD	T Lym	B Lym	
IHC Diagnosis	IBD	14	1	0	15
	T Lym	5	35	1	41
	B Lym	0	2	6	8
Total		19	38	7	64

B Lym > T Lym	1
IBD > T Lym	5
T Lym > IBD	1
T Lym > B Lym	2

		H&E Diagnosis			Total
		IBD	T Lym	B Lym	
Final Diagnosis	IBD	9	4	0	13
	T Lym	10	32	1	43
	B Lym	0	2	6	8
Total		19	38	7	64

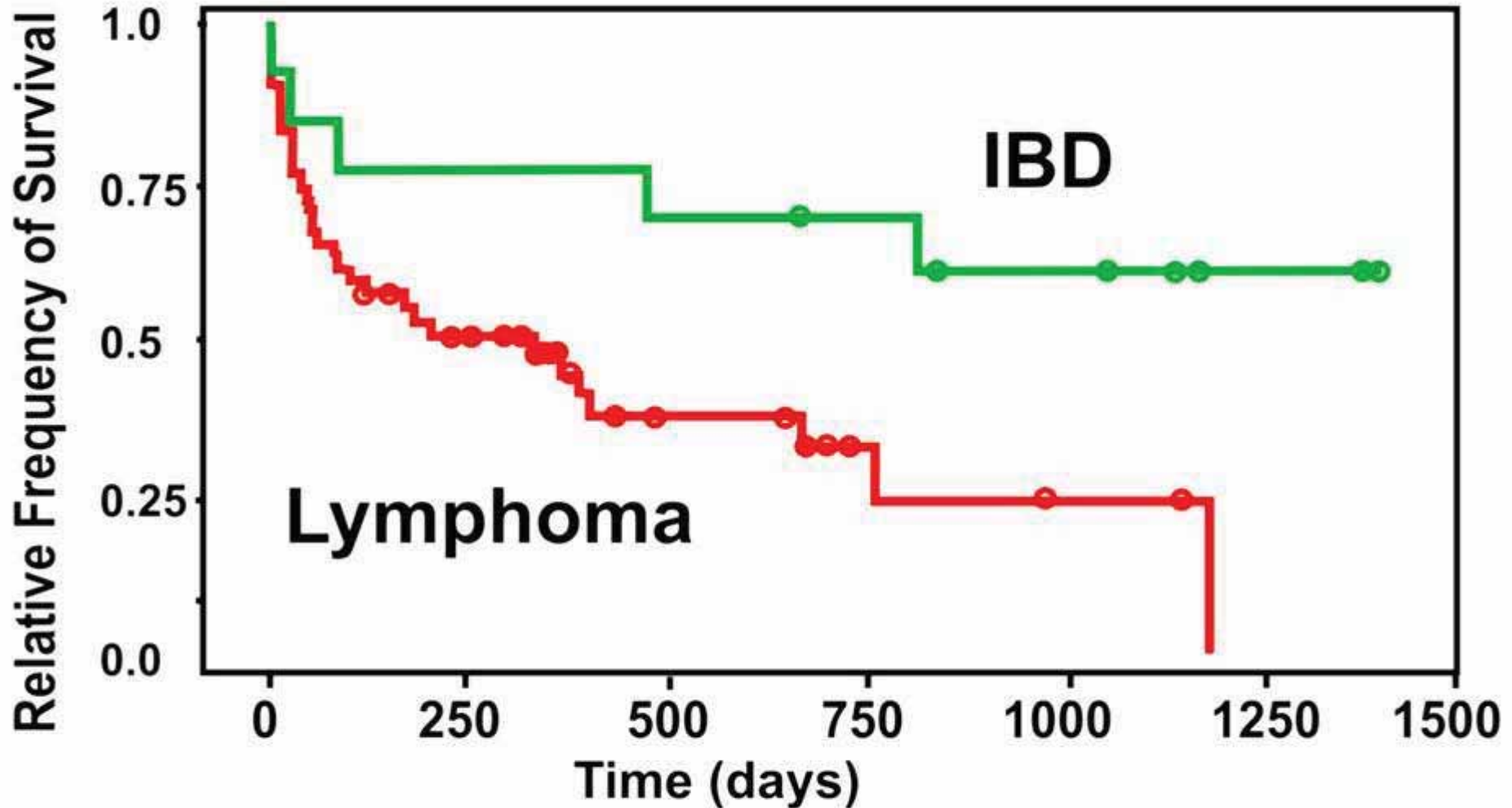
H&E > Final Diagnosis

B Lym > T Lym	1
IBD > T Lym	10
T Lym > IBD	4
T Lym > B Lym	2

IHC > Final Diagnosis

B Lym > T Lym	0
IBD > T Lym	5
T Lym > IBD	3
T Lym > B Lym	0

Days of Survival after Surgical Biopsy



Summary



- **Full thickness surgical biopsies are more diagnostic than endoscopic biopsies using H&E and IHC only**
- **Morphology and localization of lymphocytes, specifically epitheliotropism, in association with immunophenotyping improve the diagnostic accuracy**
- **Incorporation of clonality analysis by PCR will further improve the diagnostic accuracy**
- **PCR should always be performed in duplicate and denatured and re-annealed amplicons should be analyzed**
- **Microscopic examination, immunophenotyping and PCR should always be performed subsequent to each other and results should only be interpreted in context**

