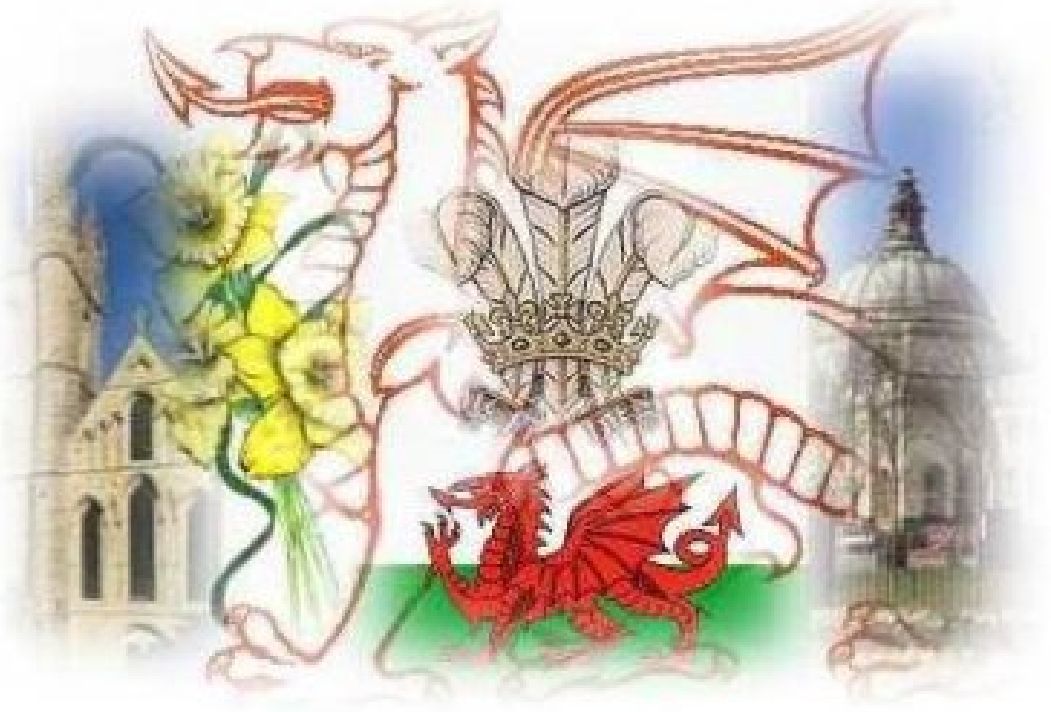


EBV is Everywhere – Why is EBV so Important in Lymphoma Biology?

Dr Stefan Dojcinov

University Hospital of Wales, Cardiff

VIRUS PARTICLES IN CULTURED LYMPHOBLASTS FROM BURKITT'S LYMPHOMA

INTEREST in Burkitt's malignant lymphoma [1] has centred largely on the climatic and geographical factors which determine its distribution,[2 3] since these can be taken to suggest that a transmissible vector-borne agent may be involved in causation.[4 5] As part of an investigation into this possibility a line of lymphoblasts from a Burkitt tumour has been established in tissue culture [6] for various types of study; this communication gives a preliminary account of virus particles in cells of this line from the first two cultures examined by electron microscopy.

1. Burkitt, D. *Brit. J. Surg.* 1958, **46**, 218.
2. Burkitt, D. *Brit. med. J.* 1962, ii, 1019.
3. Burkitt, D. *Nature, Lond.* 1962, **194**, 232.
4. Burkitt, D. *Postgrad. med. J.* 1962, **38**, 71.
5. Burkitt, D. *in* International Review of Experimental Pathology (edited by G. W. Richter and M. A. Epstein); vol. 2, p. 67. New York and London, 1963.

METHODS

Collection of cells.—The cells were taken from two separate stationary cultures after 75 and 82 days in vitro respectively; they were collected in suspension by drawing the culture fluid, in which they grow as free-floating individuals,[6] into a syringe pre-warmed to 37°C.

Preparation for electron microscopy.—The cells were fixed by

6. Epstein, M. A., Barr, Y. M. *Lancet*, 1964, i, 252.

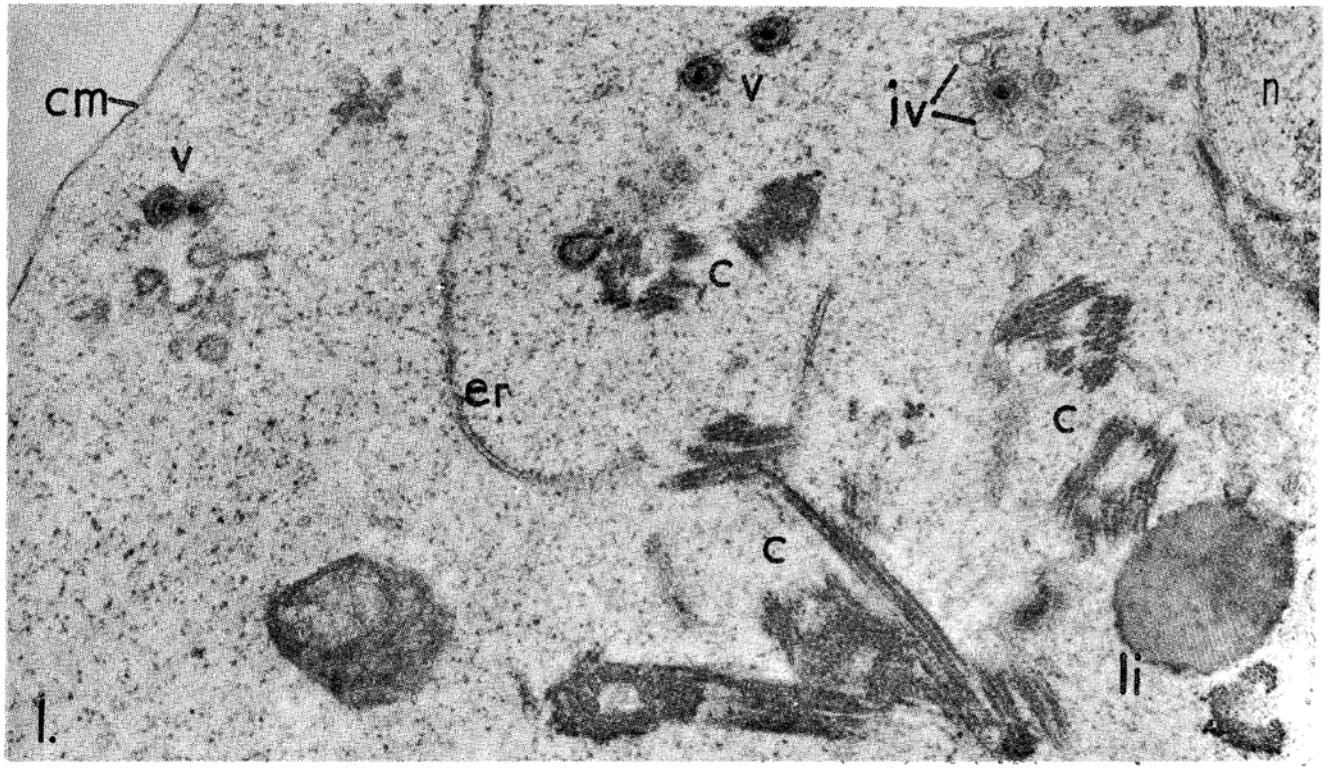


Fig. 1—Part of a cultured lymphoblast derived from a Burkitt lymphoma. The cell membrane (cm) crosses the top left corner and the nucleus (n), bounded by its double membrane, lies in the upper right portion of the field. The intervening cytoplasm contains several mature virus particles (v) within spaces enclosed by fine membranes, some immature particles (iv), and crystals (c) cut in various planes; a large lipid body (li) and endoplasmic reticulum (er) can also be seen. In addition profuse free ribosomes lie scattered throughout the cytoplasmic matrix. Electronmicrograph × 42,500.

EBV
Transforming Capacity

"…It has not so far been demonstrated that the virus in fact induces cell proliferation, but two pieces of evidence at least hint that this could be the case and that the virus might even be of significance in the aetiology of the tumour…"

Epstein MA, Henle G, Achong BG and Barr YM (1965) J Exp Med 121: 761-770.

- Lymphomas (B, T, NK)
- Nasopharyngeal Ca
- Gastric Ca
- Sarcoma

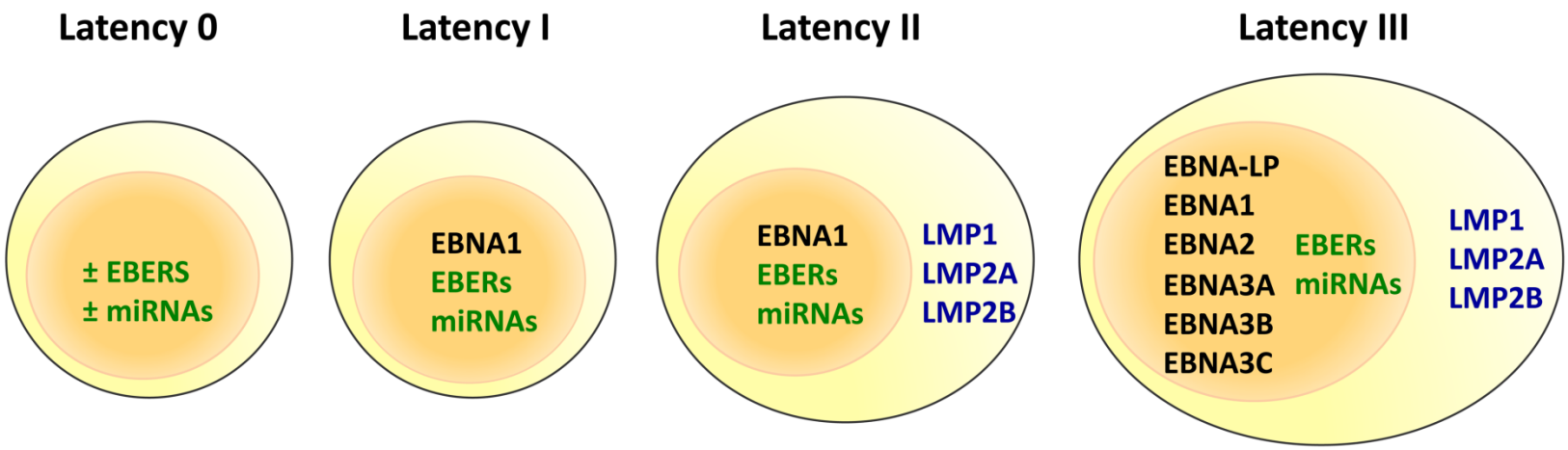
EBV

Majority of population infected

Asymptomatic lifelong B-cell infection - Latent cycle

Under control of EBV specific T-cells

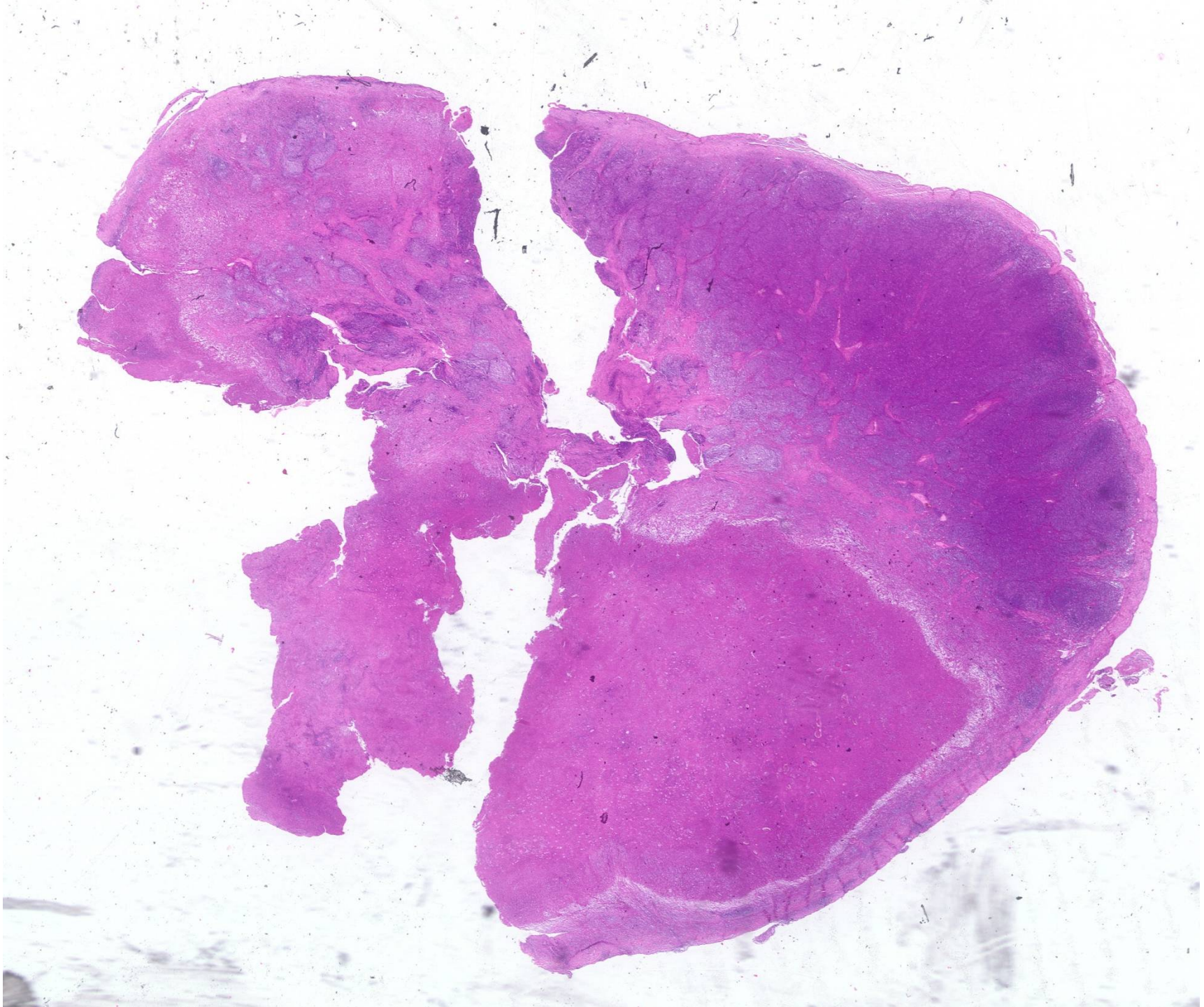
EBV can adopt different latency states

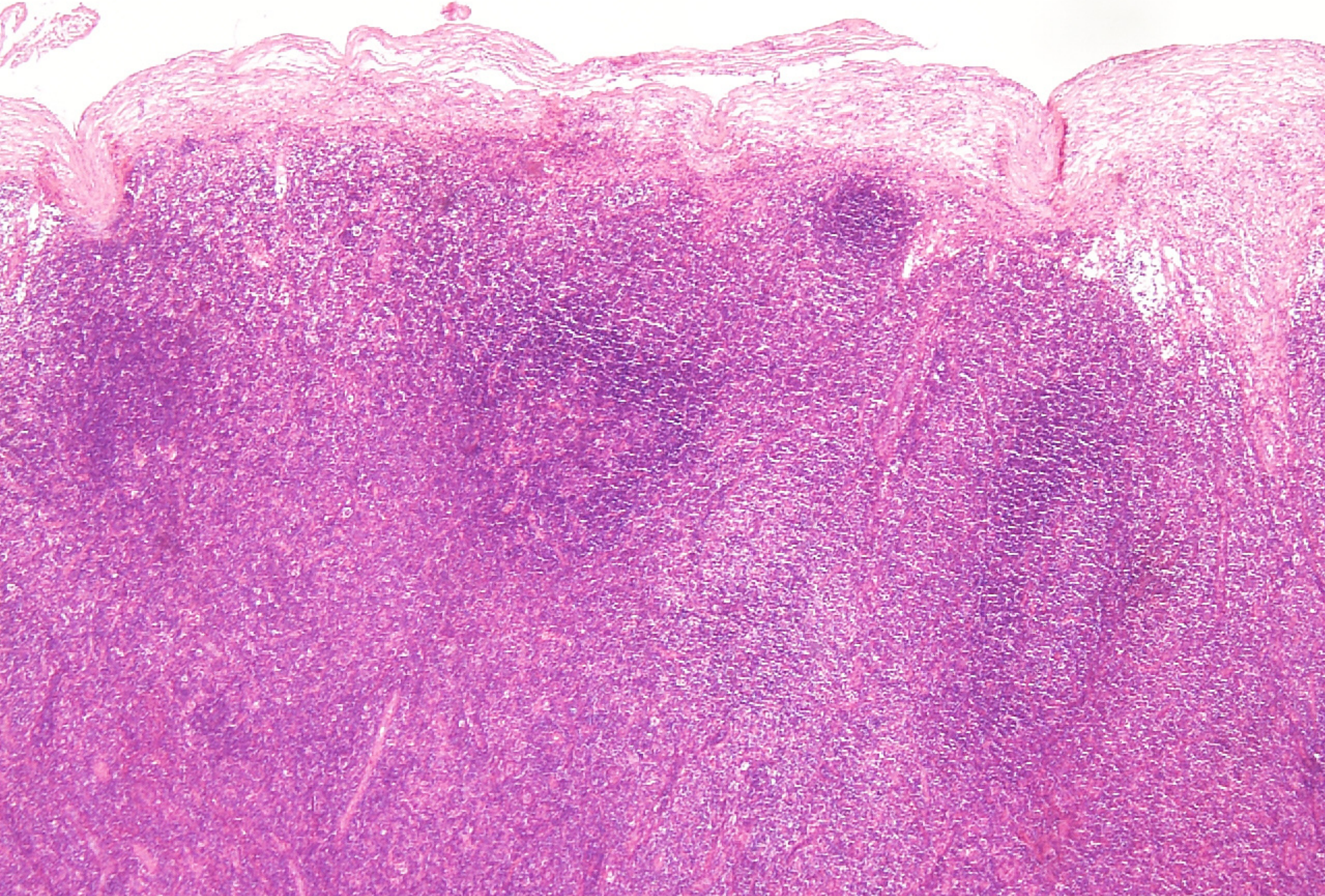


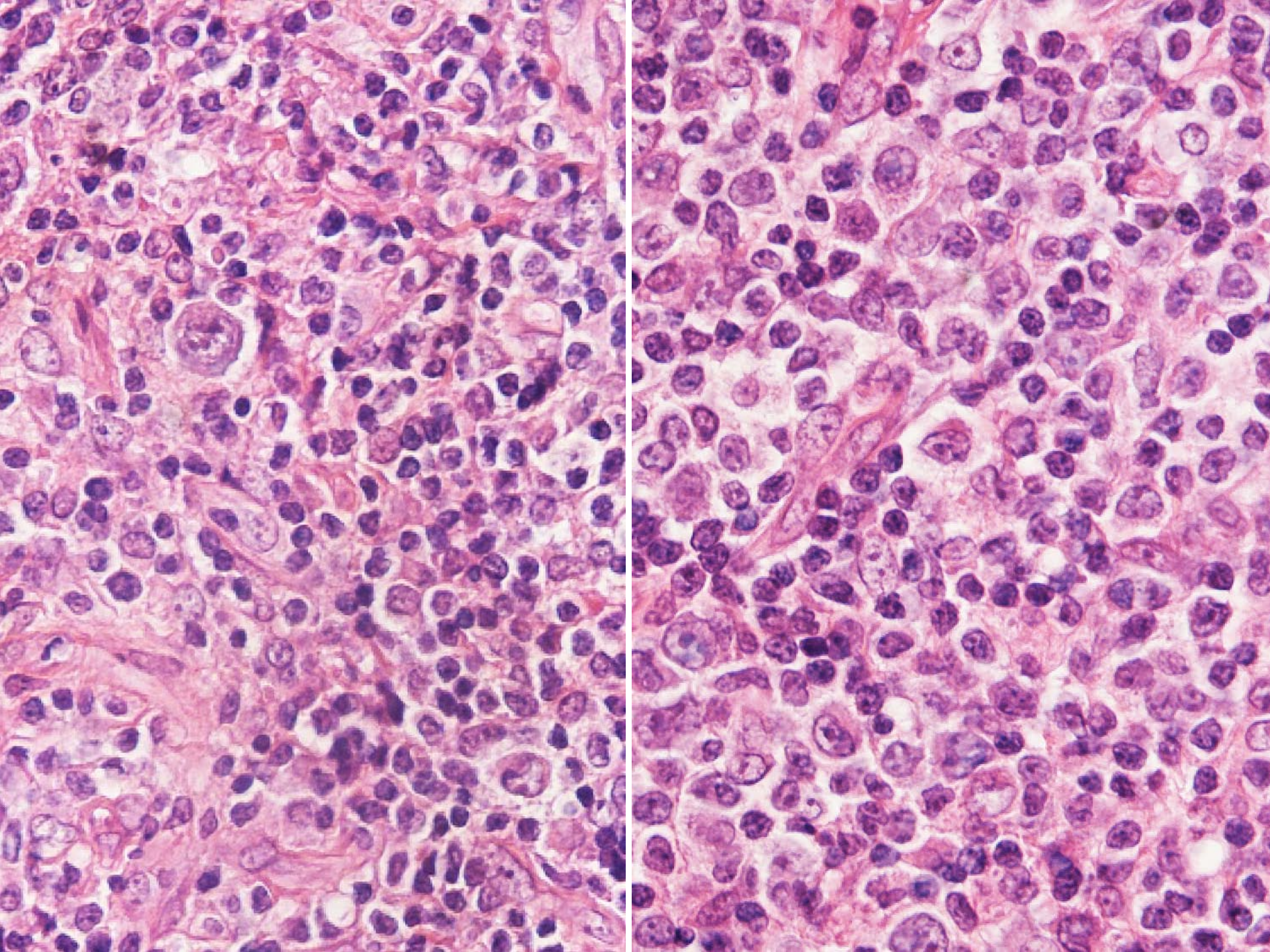
memory B cells in healthy persistence	BL LYG	HL NPC	IS associated LPDs (PTLD)

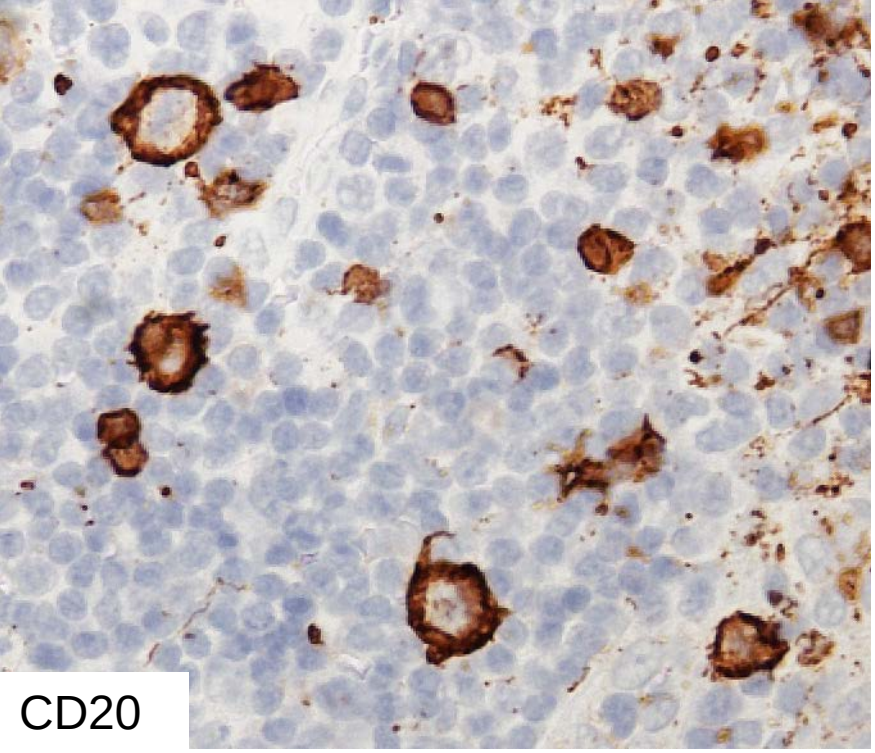
Scope
Post WHO 2008 state of affairs in EBV lymphoproliferations

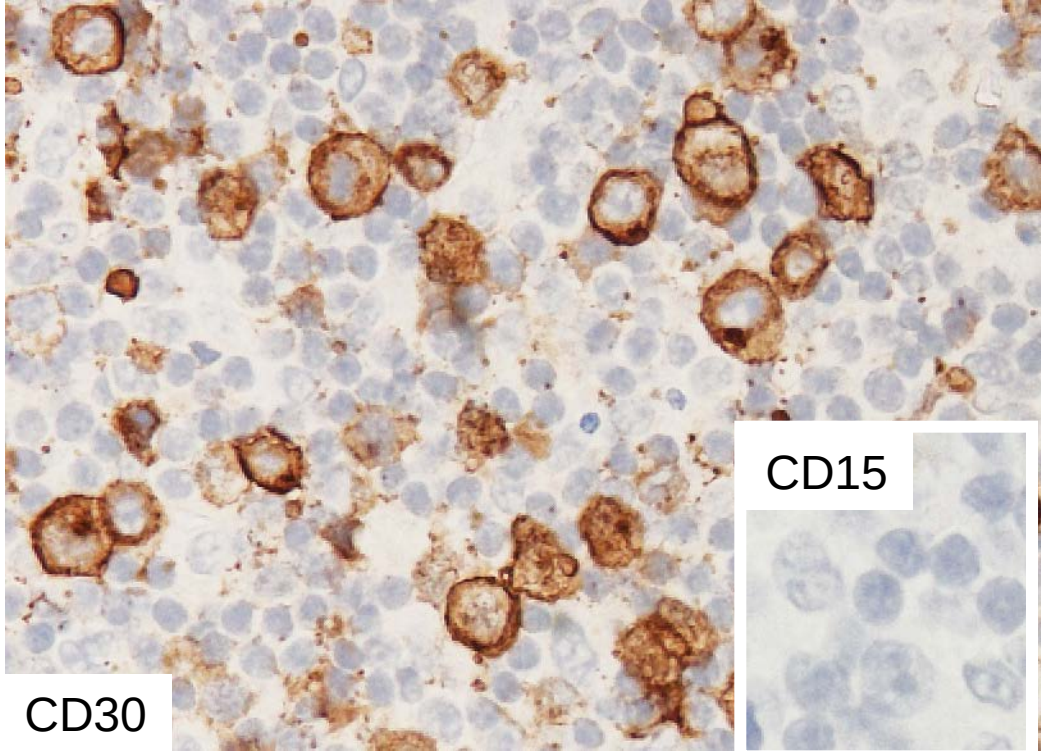
- Some “Old Friends”
- Newly recognised EBV associated entities
- Important differential diagnoses
- A review of common EBV associated entities



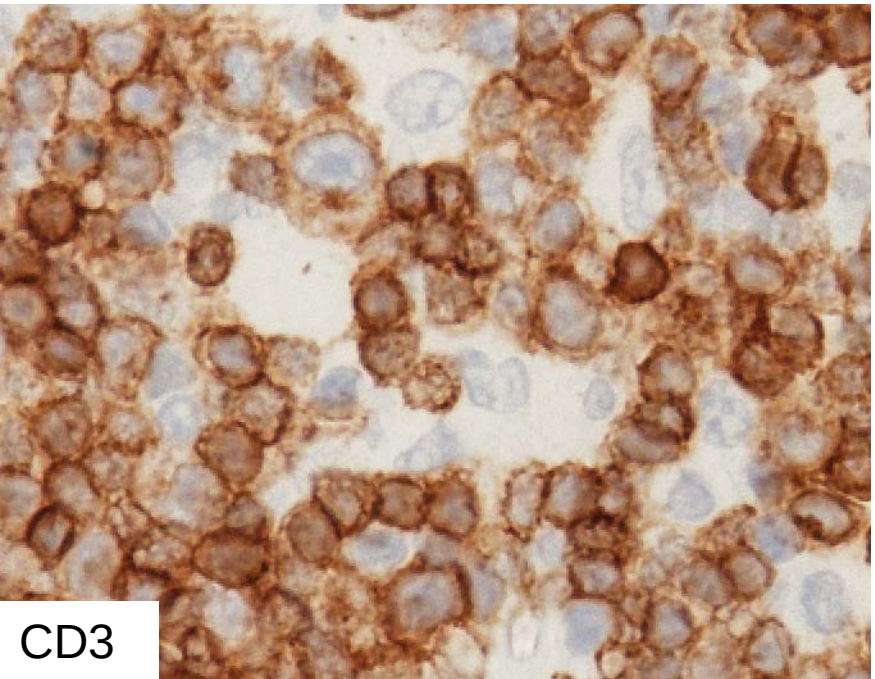





CD20


CD30

CD15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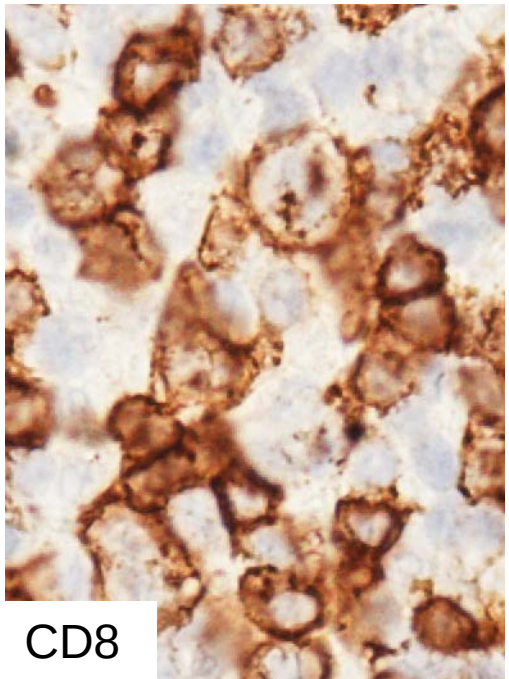
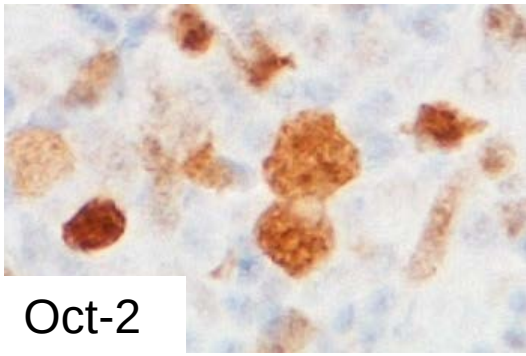
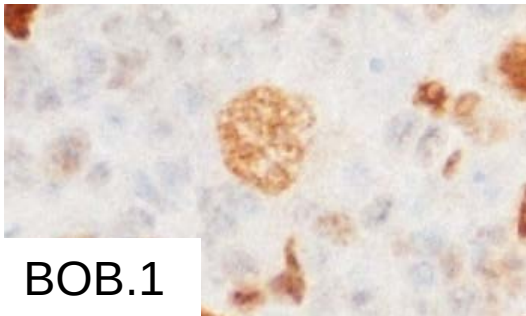
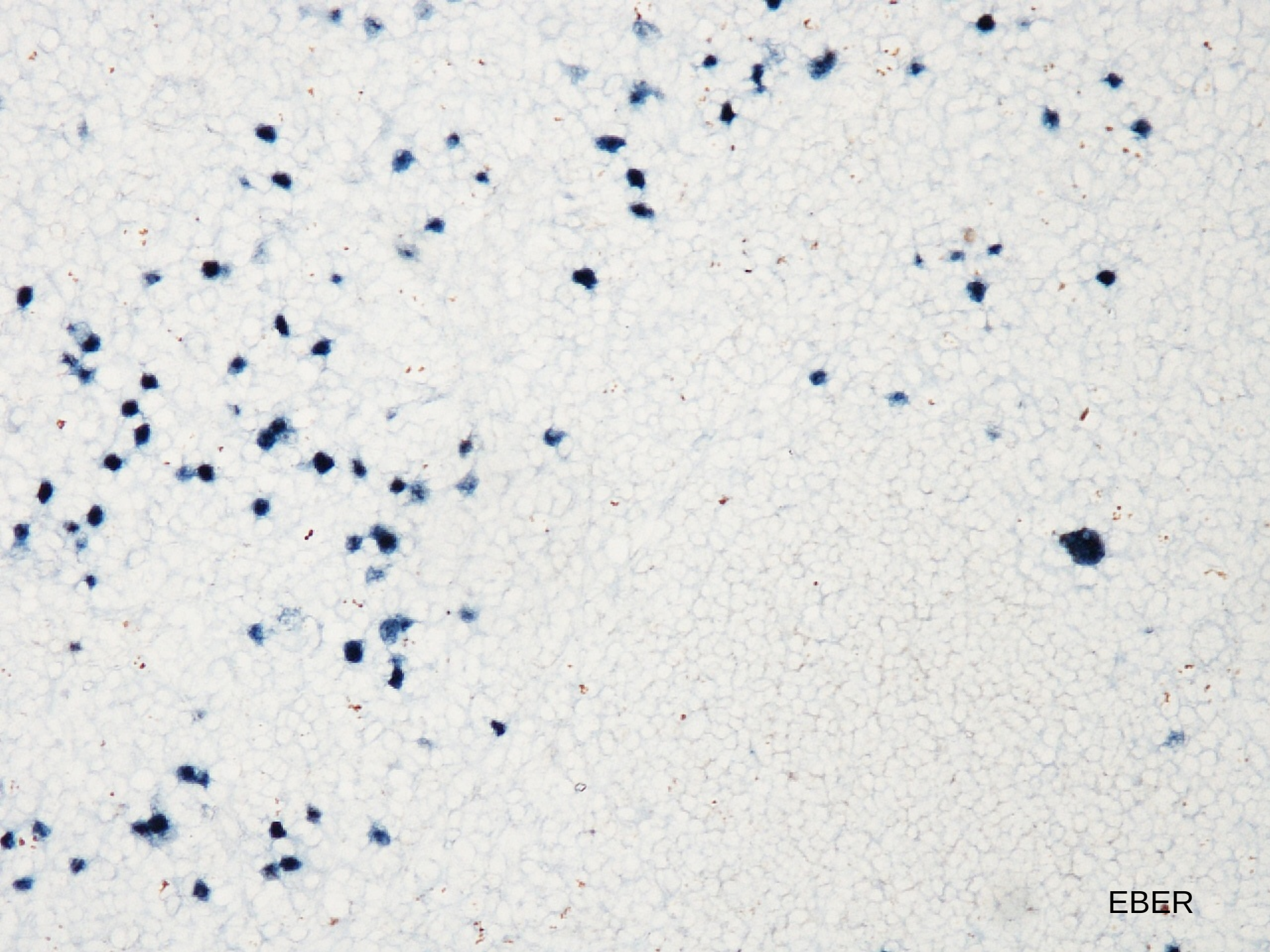

CD8


Oct-2


BOB.1


EBER

Infectious Mononucleosis

Monospot Test

- Specificity 96-100%
- Sensitivity 70-92%
- Some young individuals fail to produce heterophil Abs

Infectious Mononucleosis

Differential diagnosis

- Other viral infections
- Drug reaction
- Vaccinal reaction

- Hodgkin
- T-cell rich B-cell lymphoma

Infectious Mononucleosis

Pathological features

- Variable degree of follicular hyperplasia
- Striking paracortical hyperplasia
- Immunoblasts, may form clusters
- RS-like cells
- High mitotic activity
- Lymphocytes and plasma cells in background
- Necrosis common

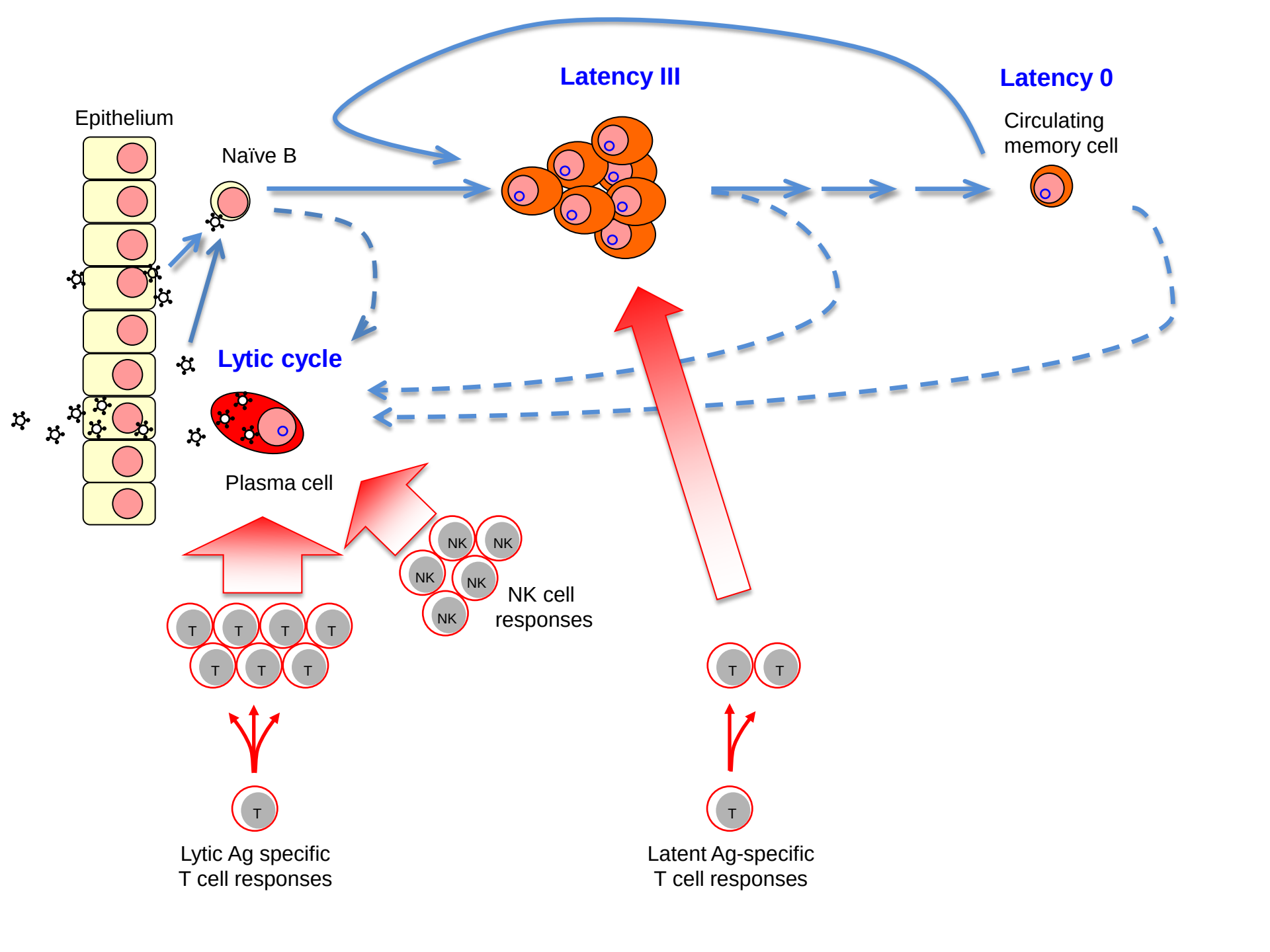
Simple key distinctive feature

- Retention
- Retention
- Retention (of architecture)

- Polymorphism
- Polymorphism
- Polymorphism (T-cell + B-cell blasts)

EBV associated LPD

B-cell:	IS (PTLD, Iatrogenic, HIV, PID, Immunosenecence)
	Burkitt lymphoma
	Lymphomatoid granulomatosis
	Classical Hodgkin lymphoma
	Primary effusion lymphoma
	Plasmablastic lymphoma
	Pyothorax associated lymphoma
	B-cell LPD in angioimmunoblastic lymphoma
T/NK cell:	Extranodal NK/T-cell lymphoma, nasal type
	NK/T-cell leukaemia
	Chronic Active EBV infection
	HV, HV-like lymphoma
	Aggressive EVB+ LPD of childhood


Latency III
Latency 0
Epithelium
Naïve B
Circulating
memory cell
Lytic cycle
Plasma cell
NK
NK
NK
NK
NK
NK cell
responses
T
T
T
T
T
T
T
T
T
T
Lytic Ag specific
T cell responses
Latent Ag-specific
T cell responses

Immunosenescence

Natural decay of the immune system taking place with ageing

Immunosuppression due to Immunosenescence

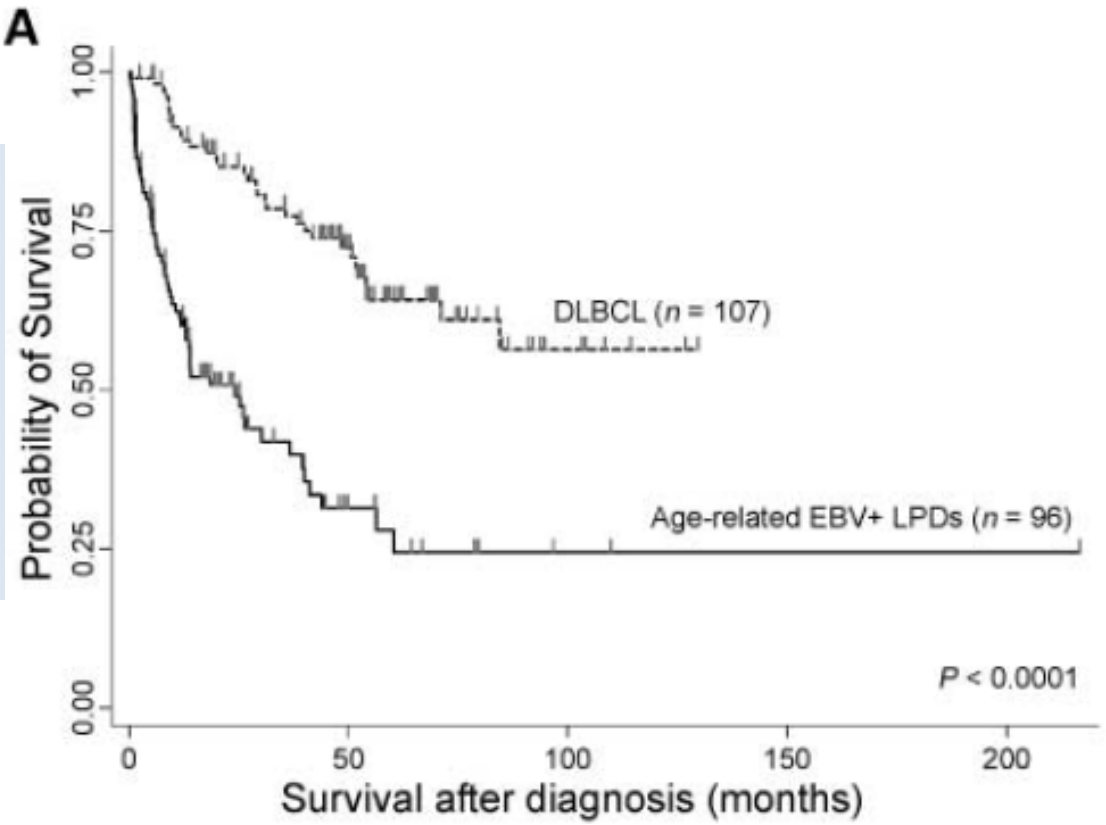
EBV+ Diffuse Large (and "polymorphous") B-cell Lymphoma of the Elderly

Senile EBV+ B-Cell Lymphoproliferative Disorders

A Clinicopathologic Study of 22 Patients

Oyama et al. Am J Surg Path 27(1): 16-26, 2003

- >60
- Extranodal presentation
- Poor prognosis



Oyama, T., et al. Clin.Cancer Res. 13.17 (2007): 5124-32.

Shimoyama, Y. J.Clin.Exp.Hematop. 46.1 (2006): 1-4.

T-cell Competence

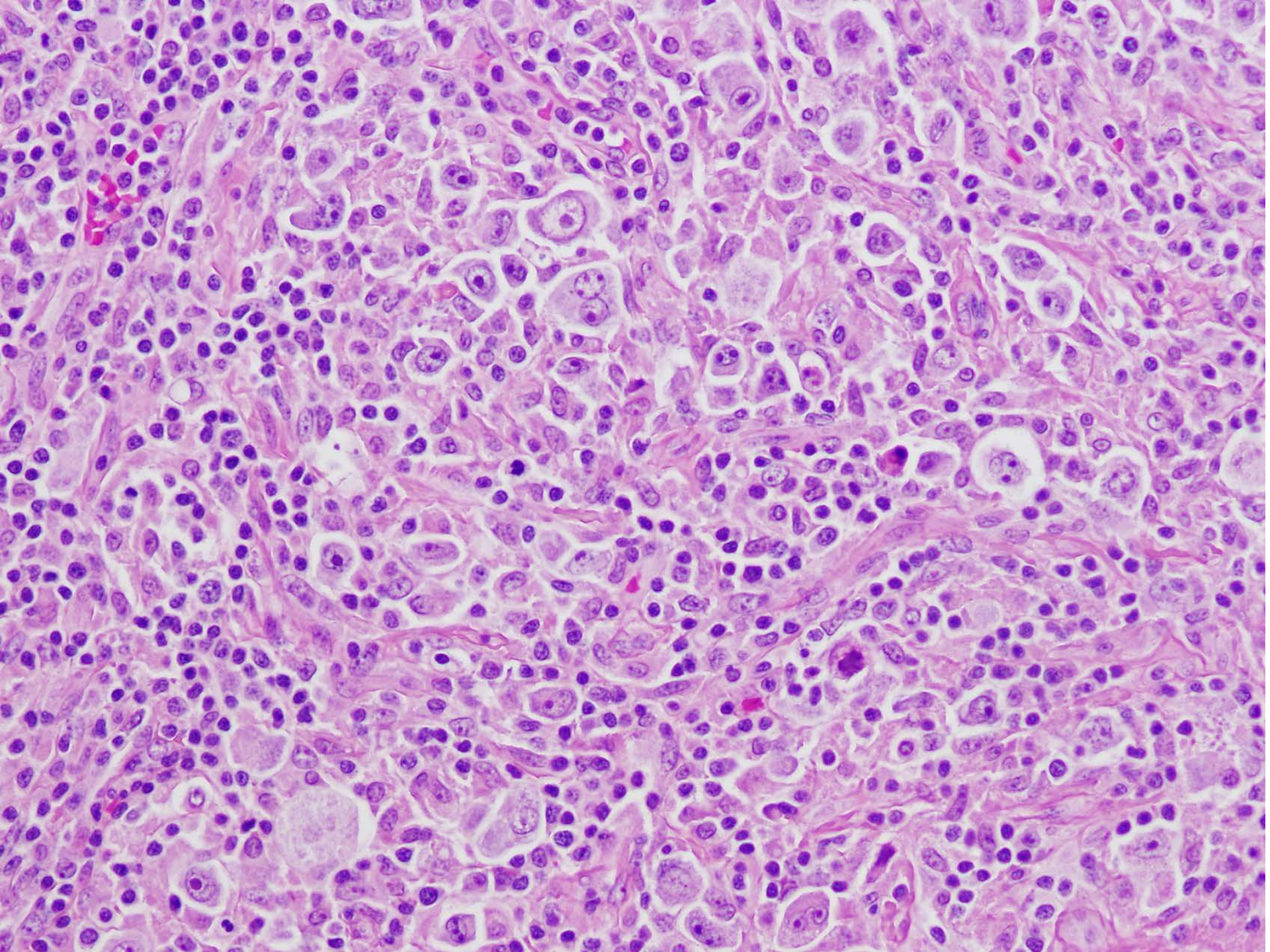
20 million specificities in young age

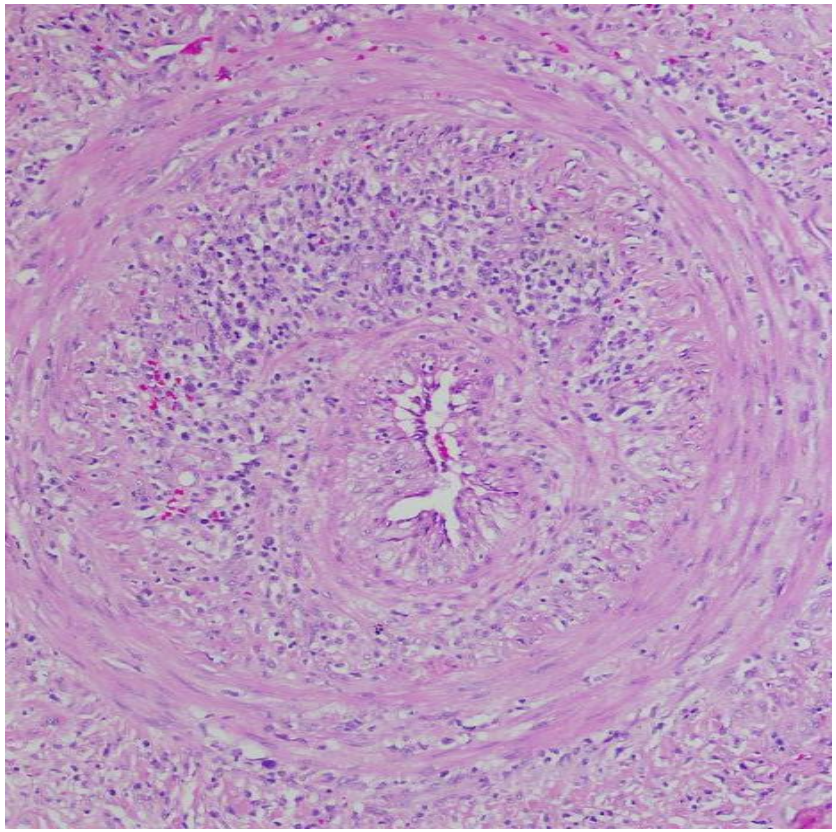
100 fold reduction in old age

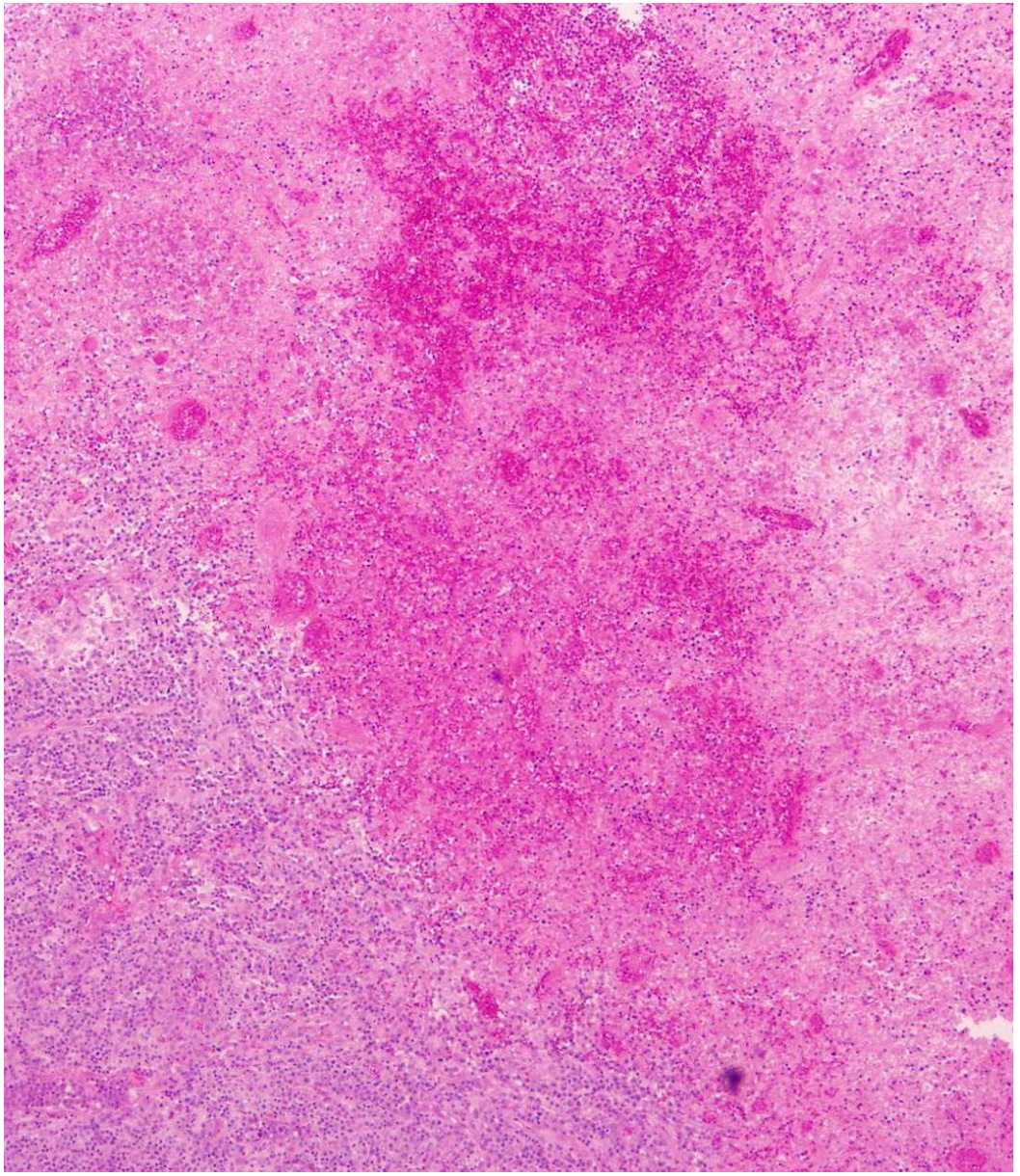
Cell numbers remain the same

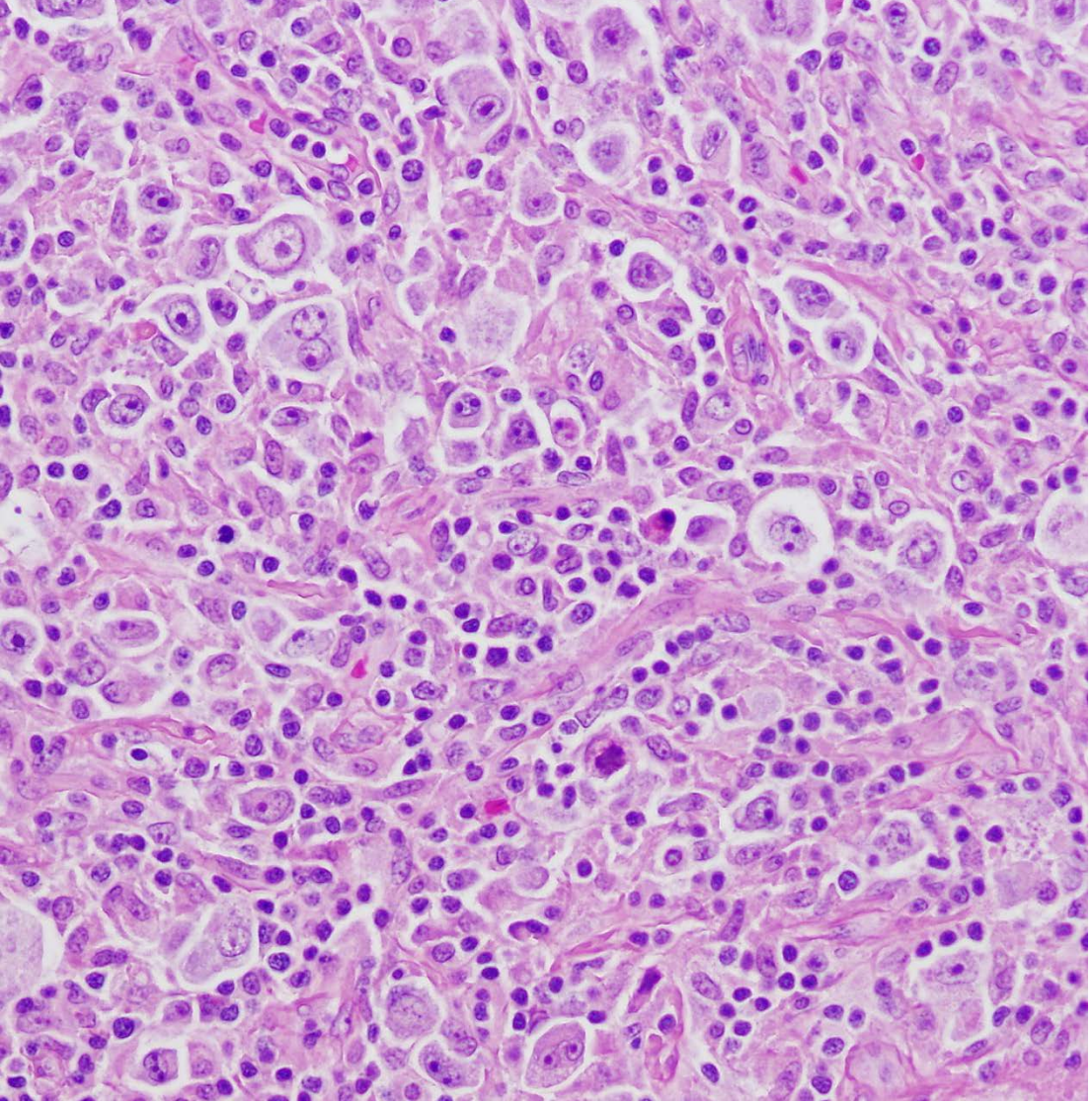
¾ reactivity to persistent viruses (CMV & EBV)

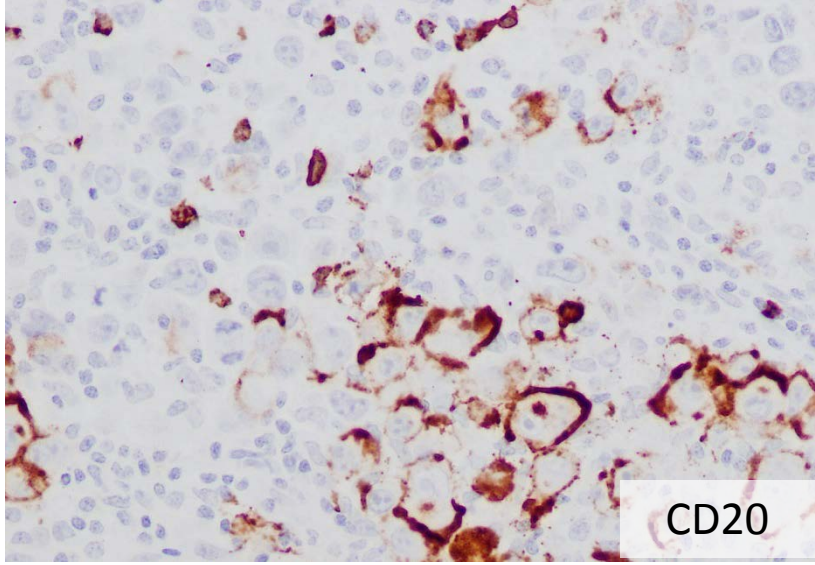
Olsson J, Wikby A, Johansson B, et al. *Mech Ageing Dev*. 2000;121:187-201.
Ouyang Q, Wagner WM, Zheng W, et al. *Exp Gerontol*. 2004;39:607-13.
Vescovini R, Telera A, Fagnoni FF, et al. *Exp Gerontol*. 2004;39:1233-43.
Wikby A, Johansson B, Olsson J, et al. *Exp Gerontol*. 2002;37:445-53.

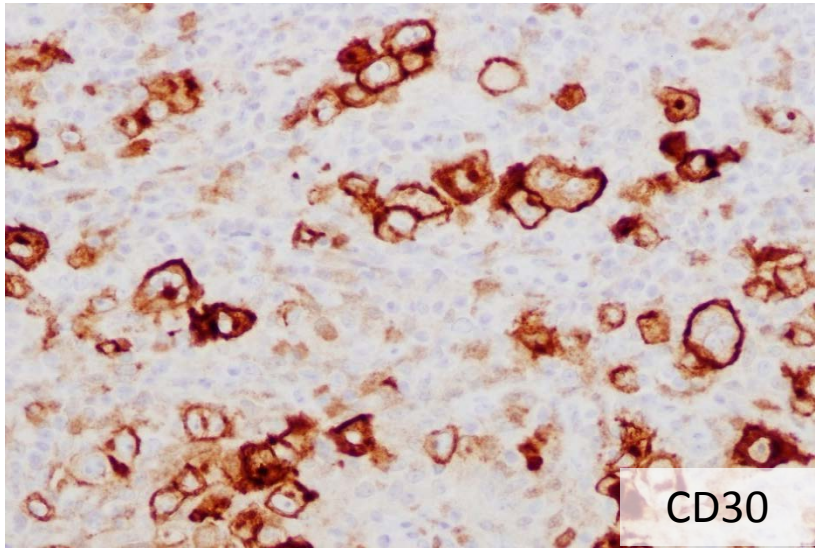


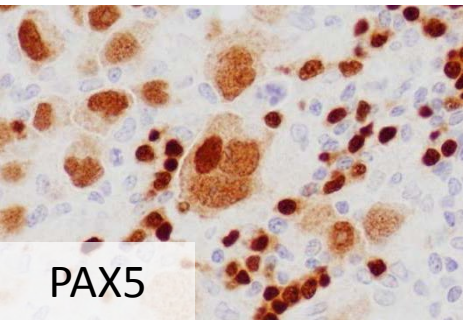


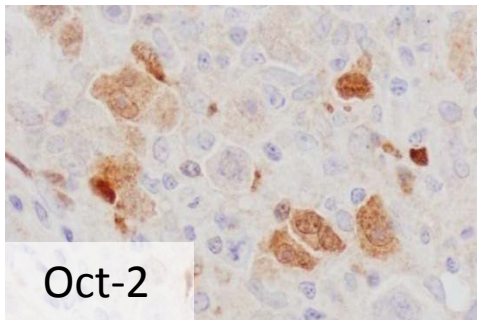


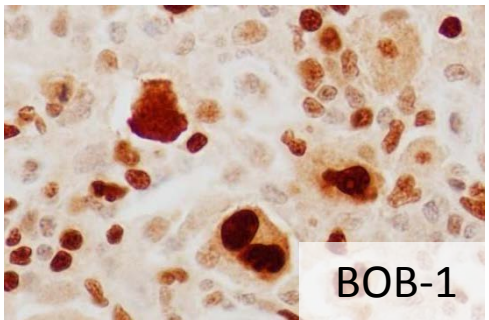


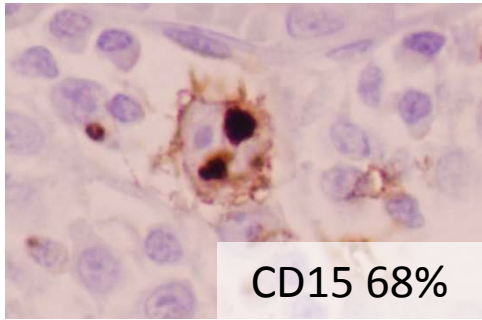

CD20


CD30

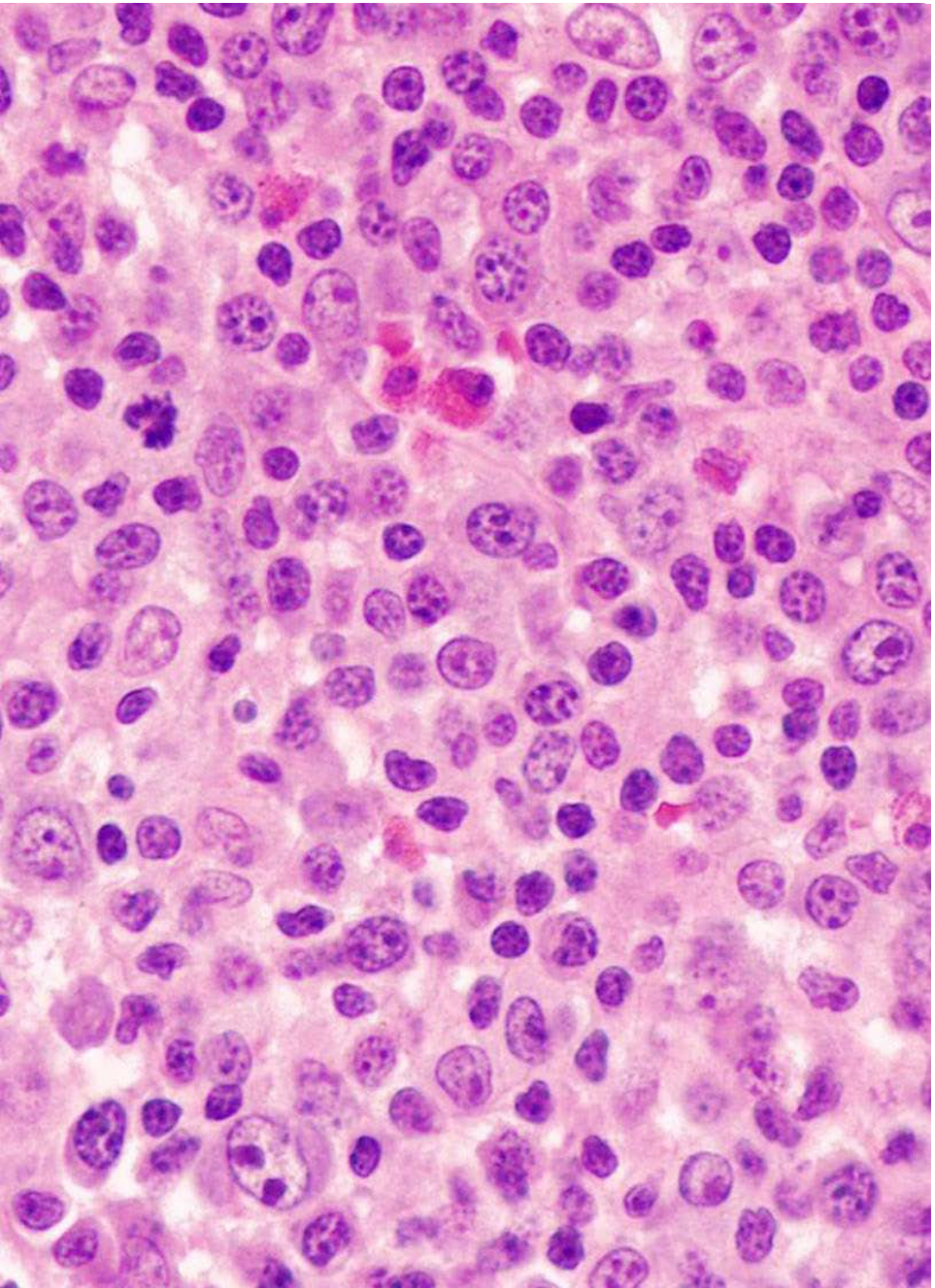

PAX5


Oct-2

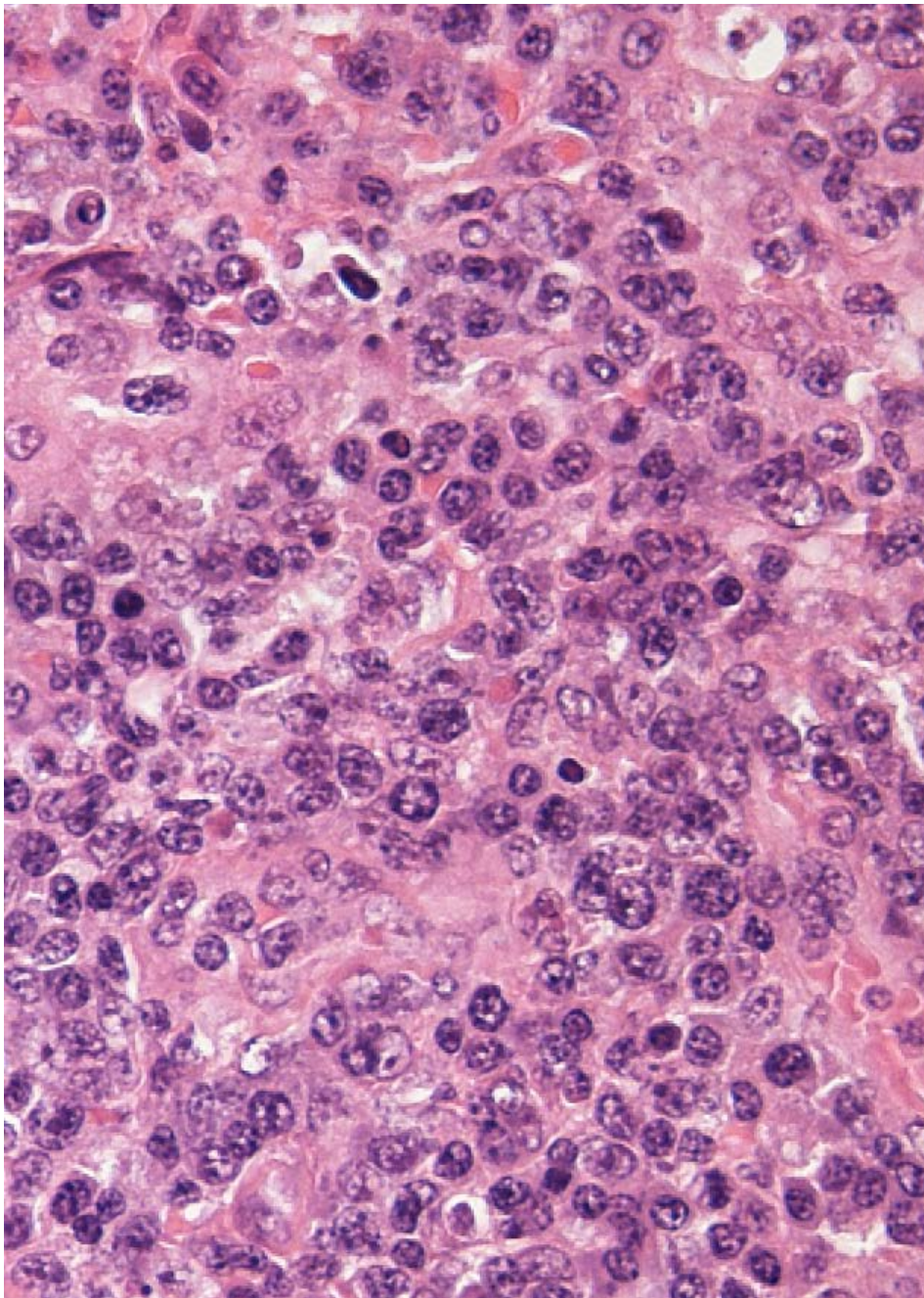

BOB-1


CD15 68%

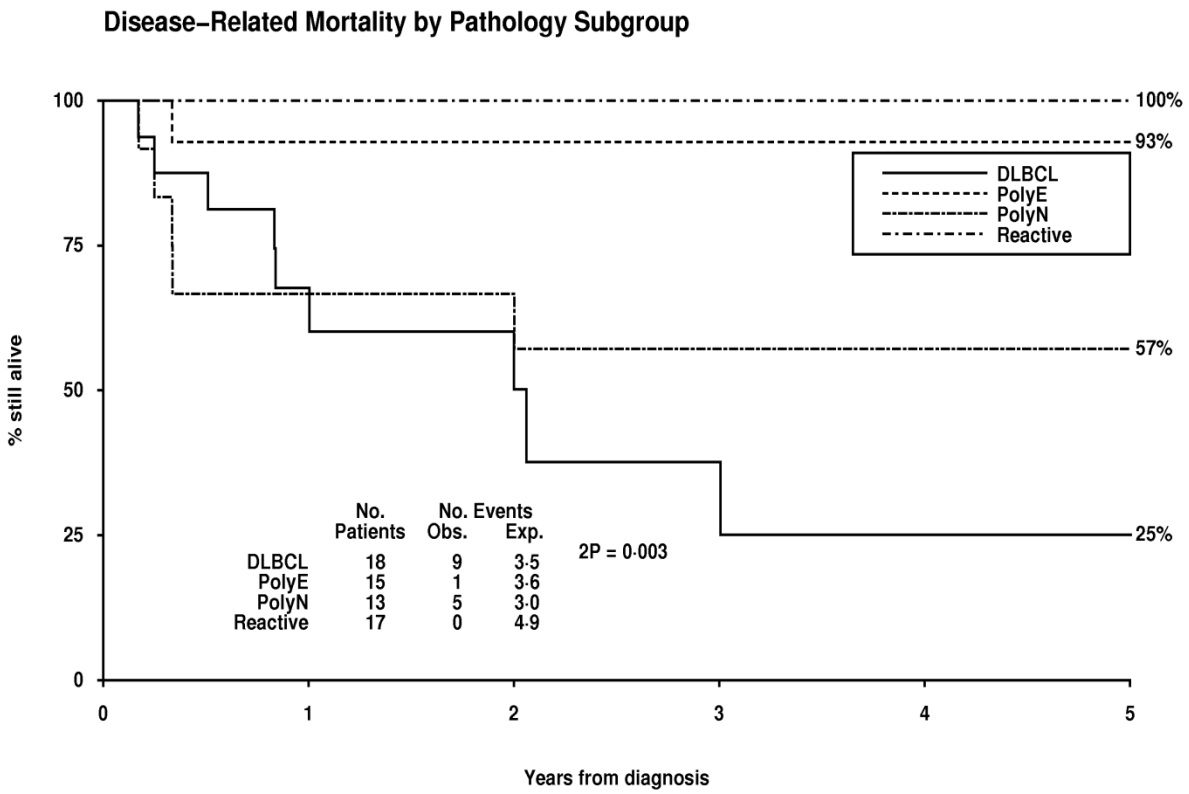
Polymorphous PTLD



Polymorphous Age Related Lymphoma

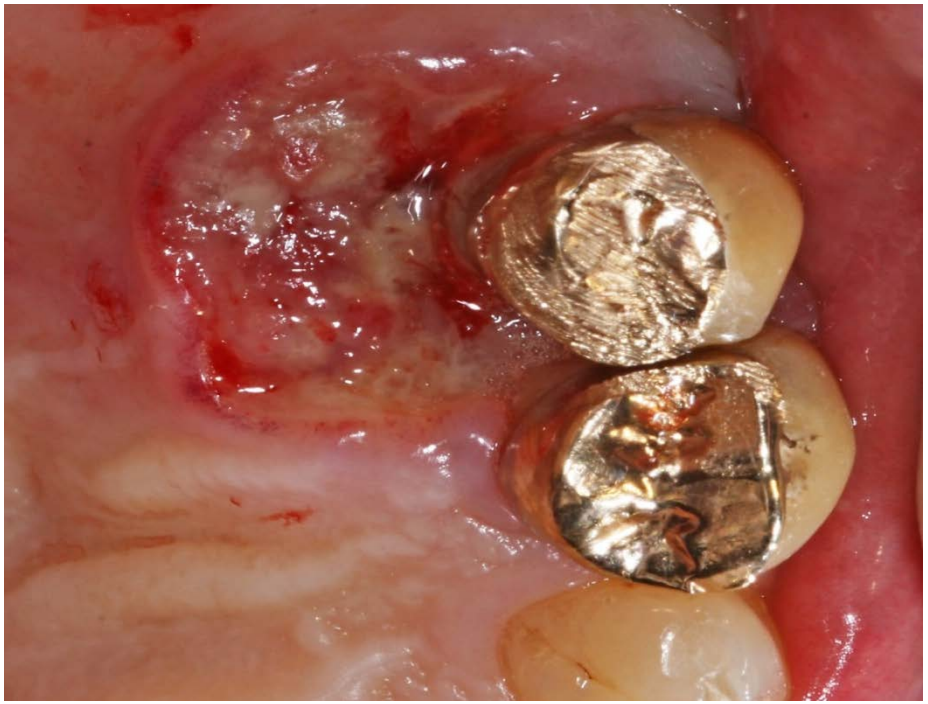


Age Related EBV+ B-cell LPD



Median survival
Poly N 27 m
DLBCL 24 m

Dojcinov et al. *Blood. 2011;117(18):4726-4735*



EBV+ Mucocutaneous Ulcer

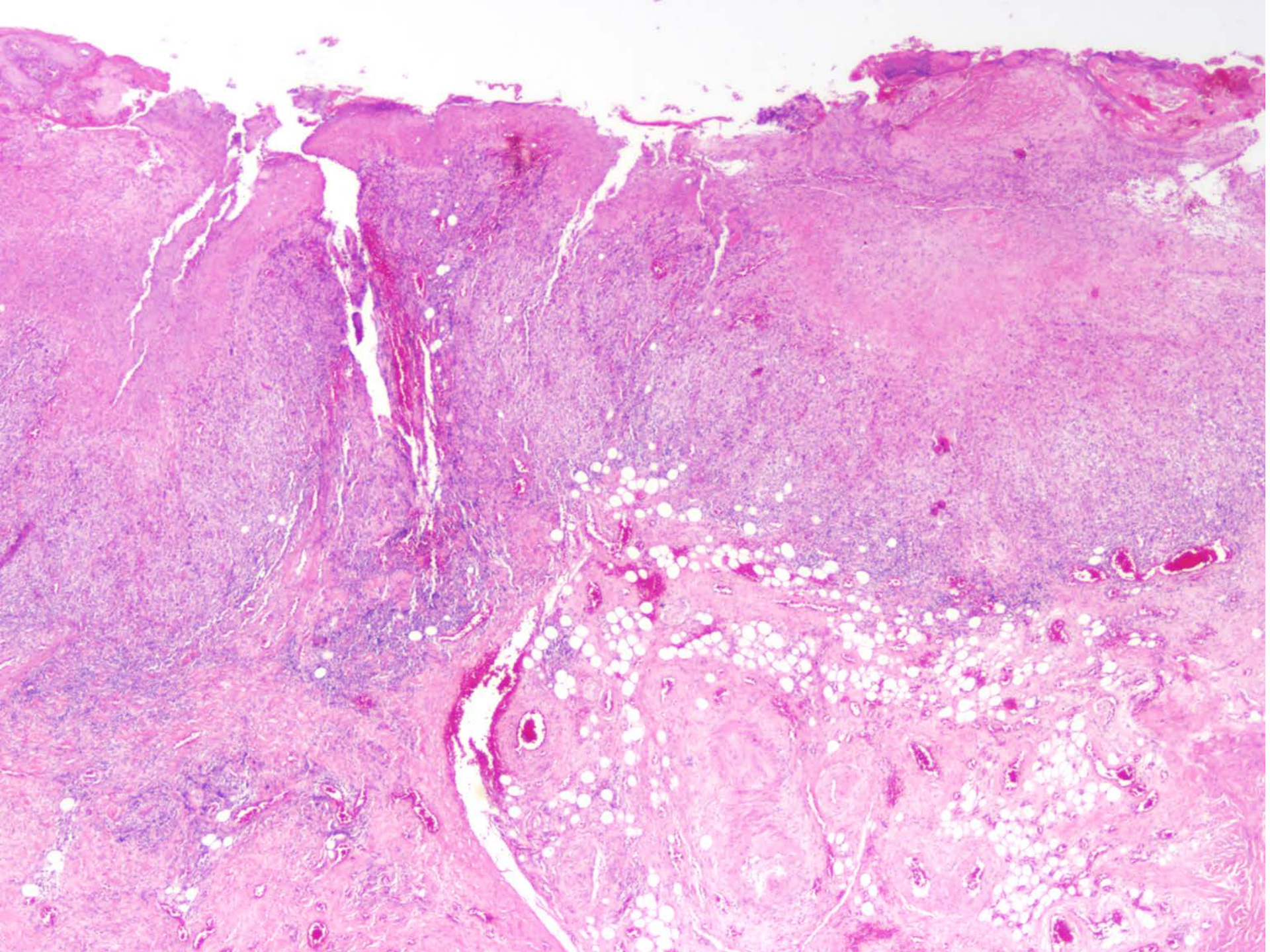
Localised Ulcerating Mucosal and Cutaneous Lesions

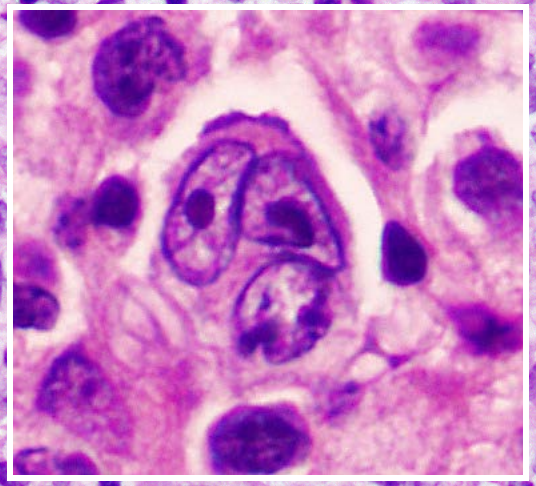
Dojcinov et al. Am J Surg Pathol 2010; 34: 405-417

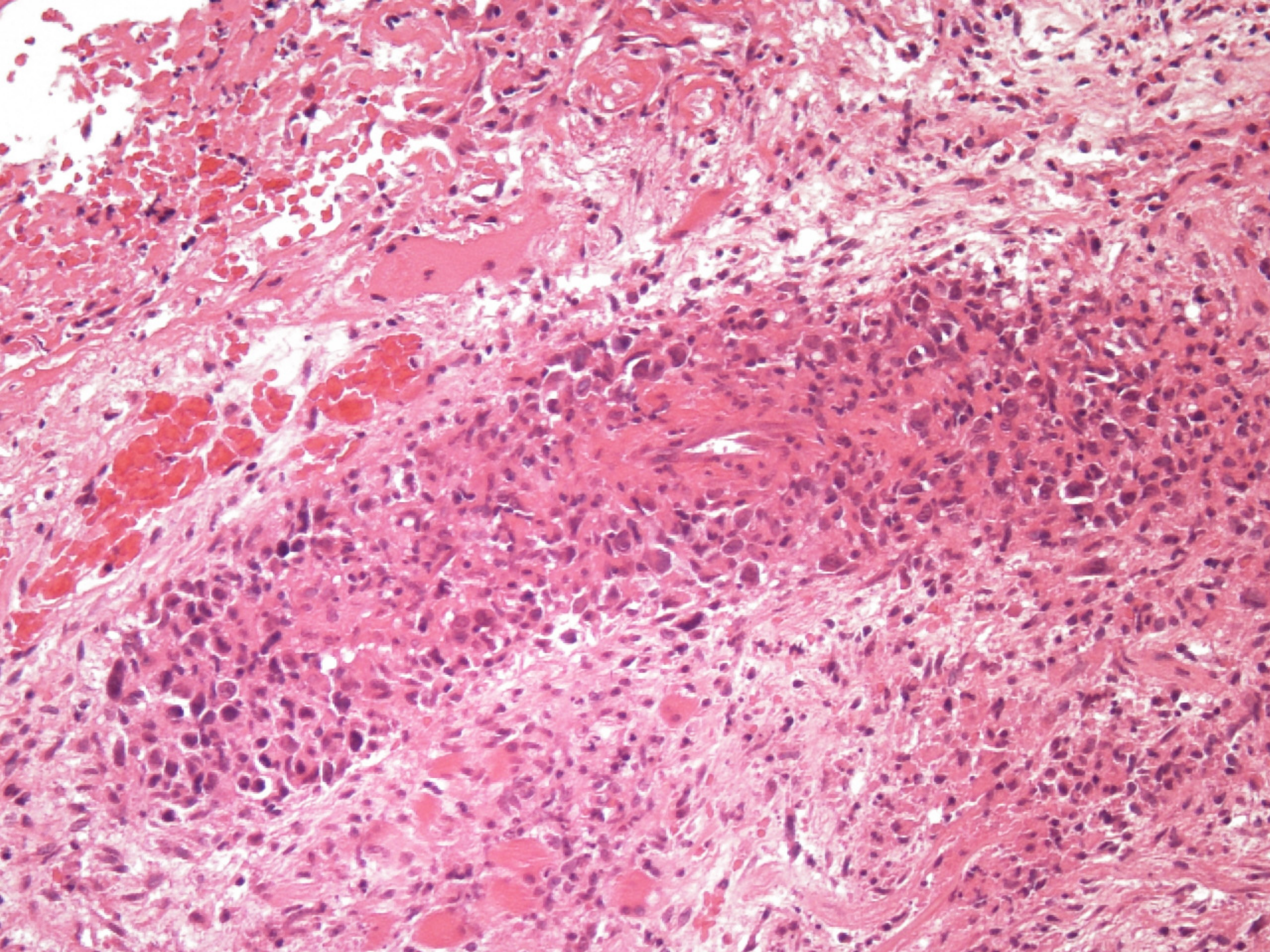
Immunosenescence
Iatrogenic systemic IS
(SLE, Sarcoidosis, RA, IBD, Transplant)

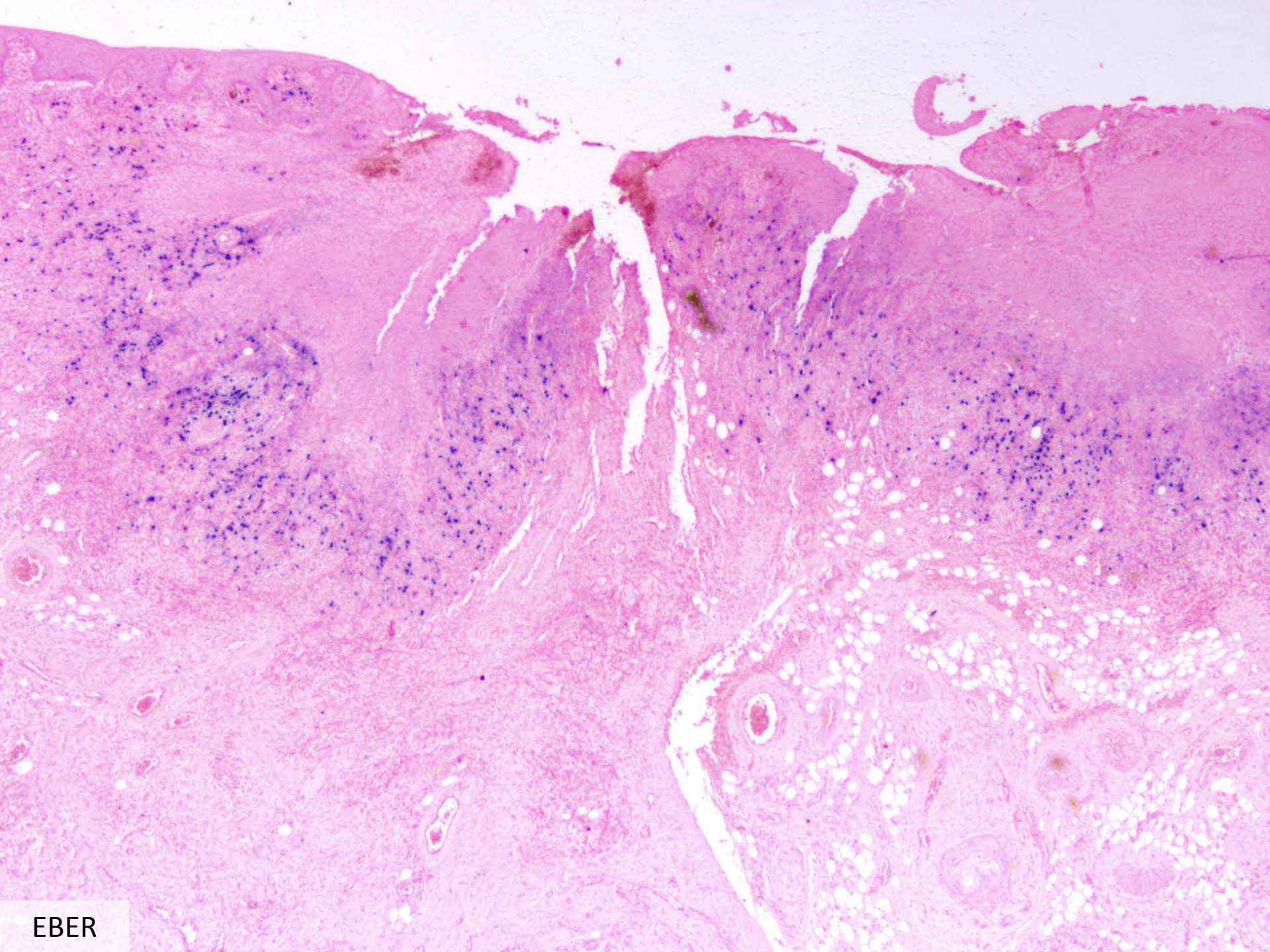
- Methotrexate
- Cyclosporin A
- Azathioprine
- MMF
- Topical steroid treatment

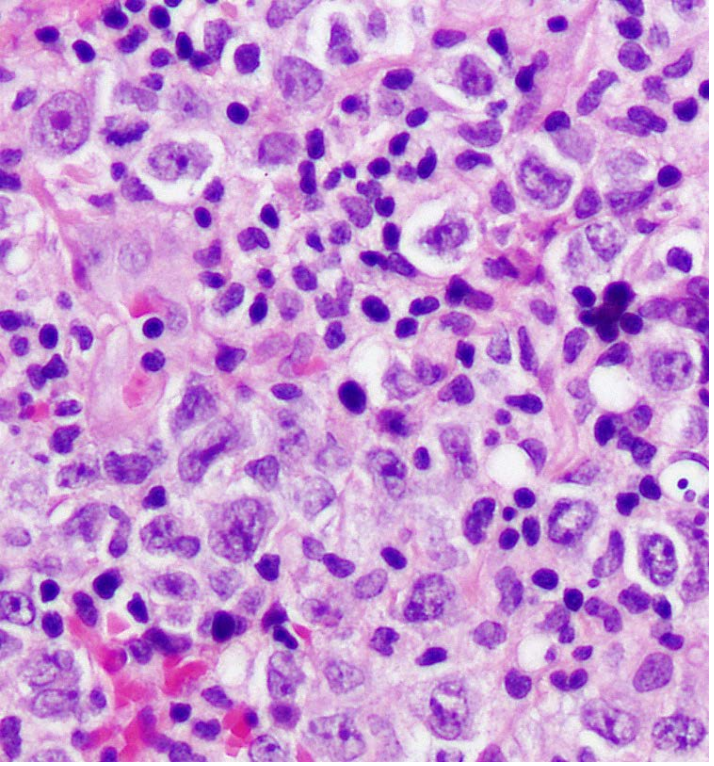
HIV

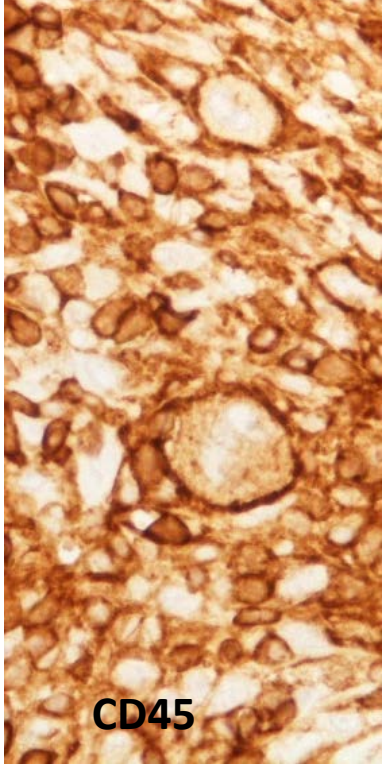


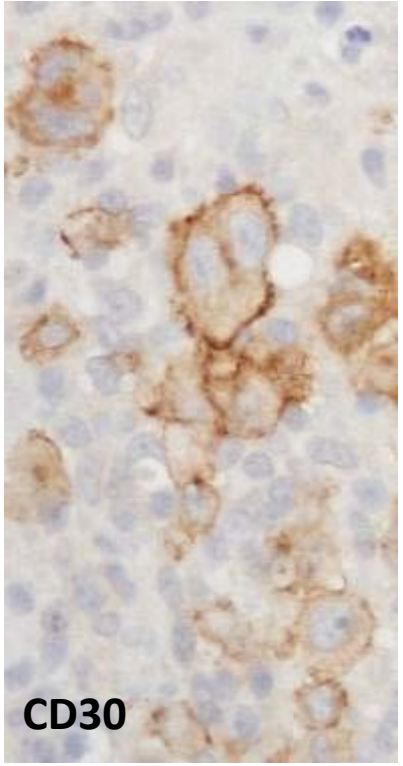


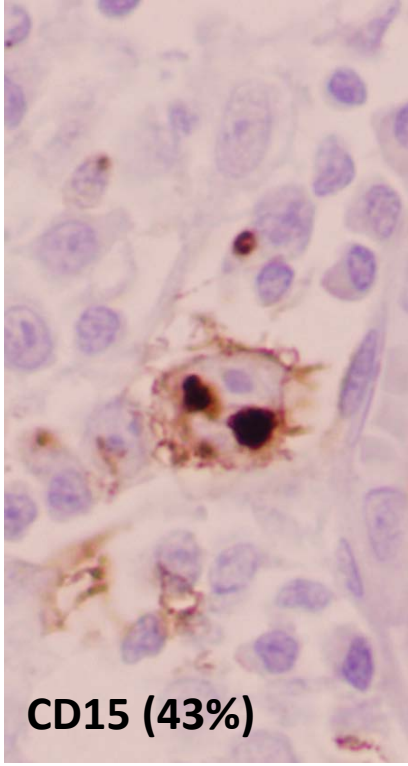


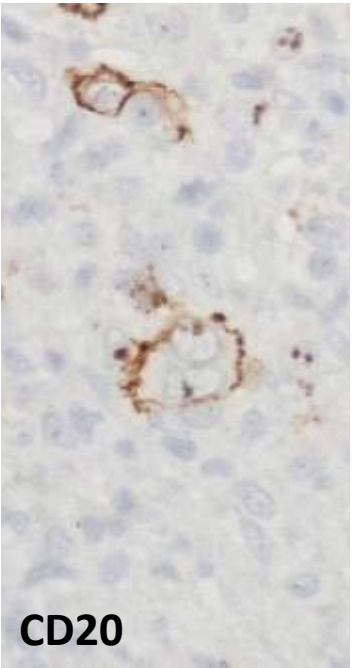

EBER

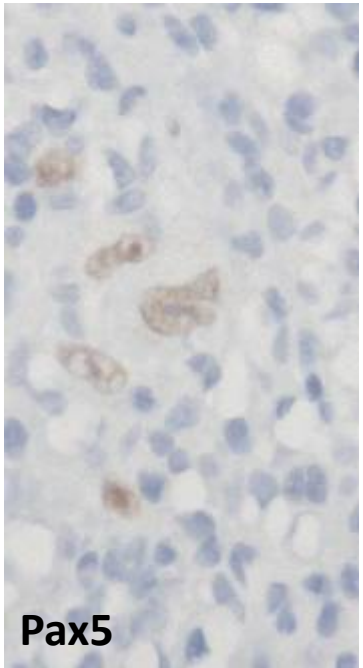


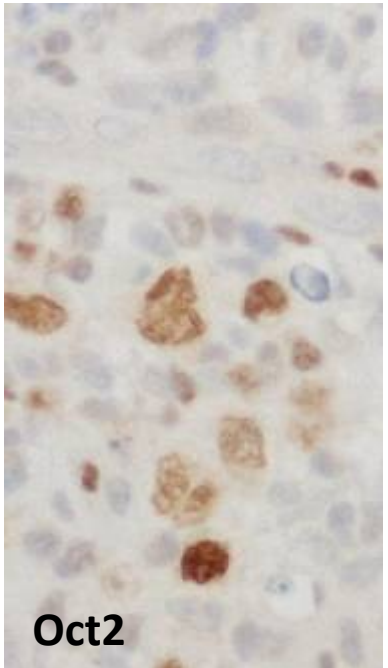

CD45

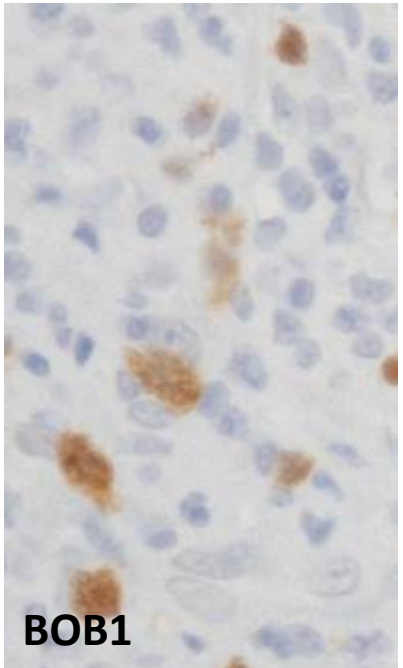

CD30

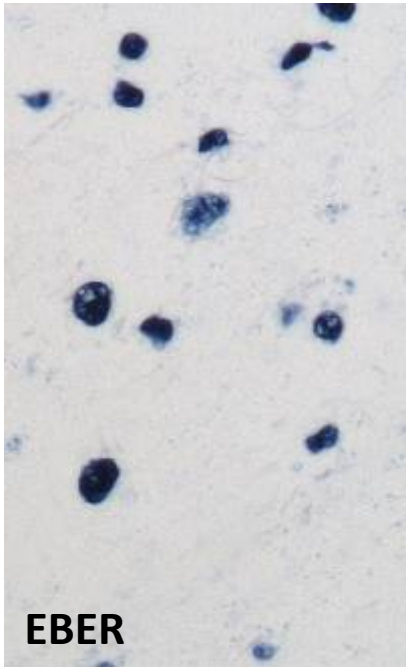

CD15 (43%)


CD20


Pax5


Oct2


BOB1


EBER

Pathology of IS Related LPD

- Pathological overlap between different aetiologies
  - Most common “conventional” lymphomas
    - DLBCL, BL, cHL, Hodgkin-like features
    - NK, gamma/delta, LGL
    - Small BCLs relatively uncommon
  - Polymorphous lymphoma
  - Plasma cell / Plasmablastic differentiation

Age Related EBV+ LP (AREBVLP) or cHL?

	AREBVLP	cHL
Presentation	Nodal and extranodal	Nodal (primarily)
Morphology	Range of cell sizes Histiocytes, lymphocytes and PCs Angioinvasion Necrosis common Granulomas could be seen Numerous EBER+ cells (variable size)	**HRS cells only** **Mixed infiltrate (NP, EO)** **No angioinvasion** **Necrosis not so common** Granulomas could be seen **Fewer EBER+ cells (uniform size)**
Phenotype	CD45+/- CD20 +/- PAX5+ OCT2+ BOB1+ MUM1+ CD30+ CD15+/- (68%)	CD45- CD20- PAX5+ (weak) OCT2- BOB1- MUM1+ CD30+ CD15+

Lymphomatoid Granulomatosis

Extranodal EBV associated B-cell LPD

Angiocentricity and angiodestruction

Immunological deficit

- CD8 dysfunction
- Association with ID (WA, HIV, HTLV1, Transplant)

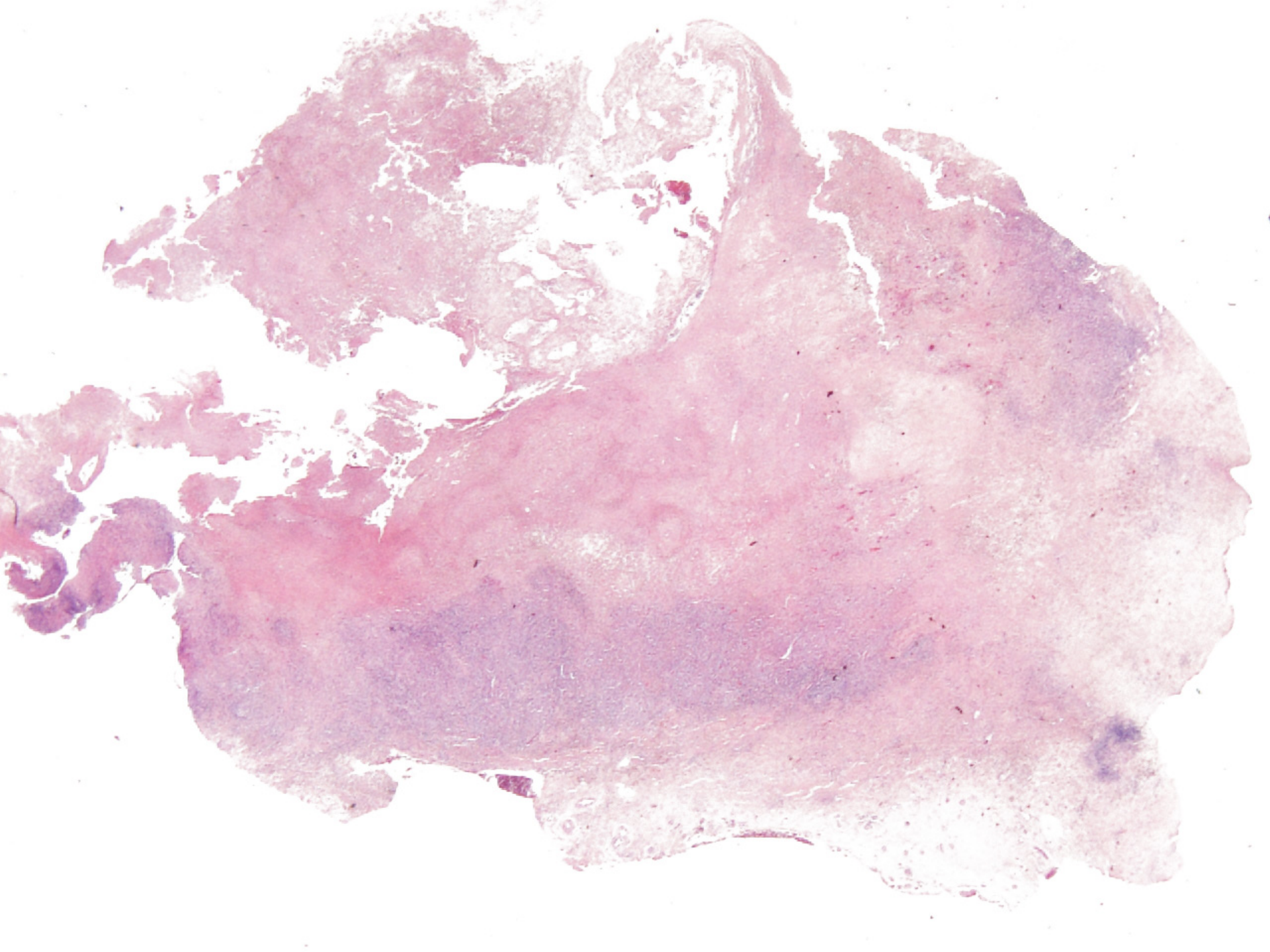
LyG – Epidemiology & Presentation

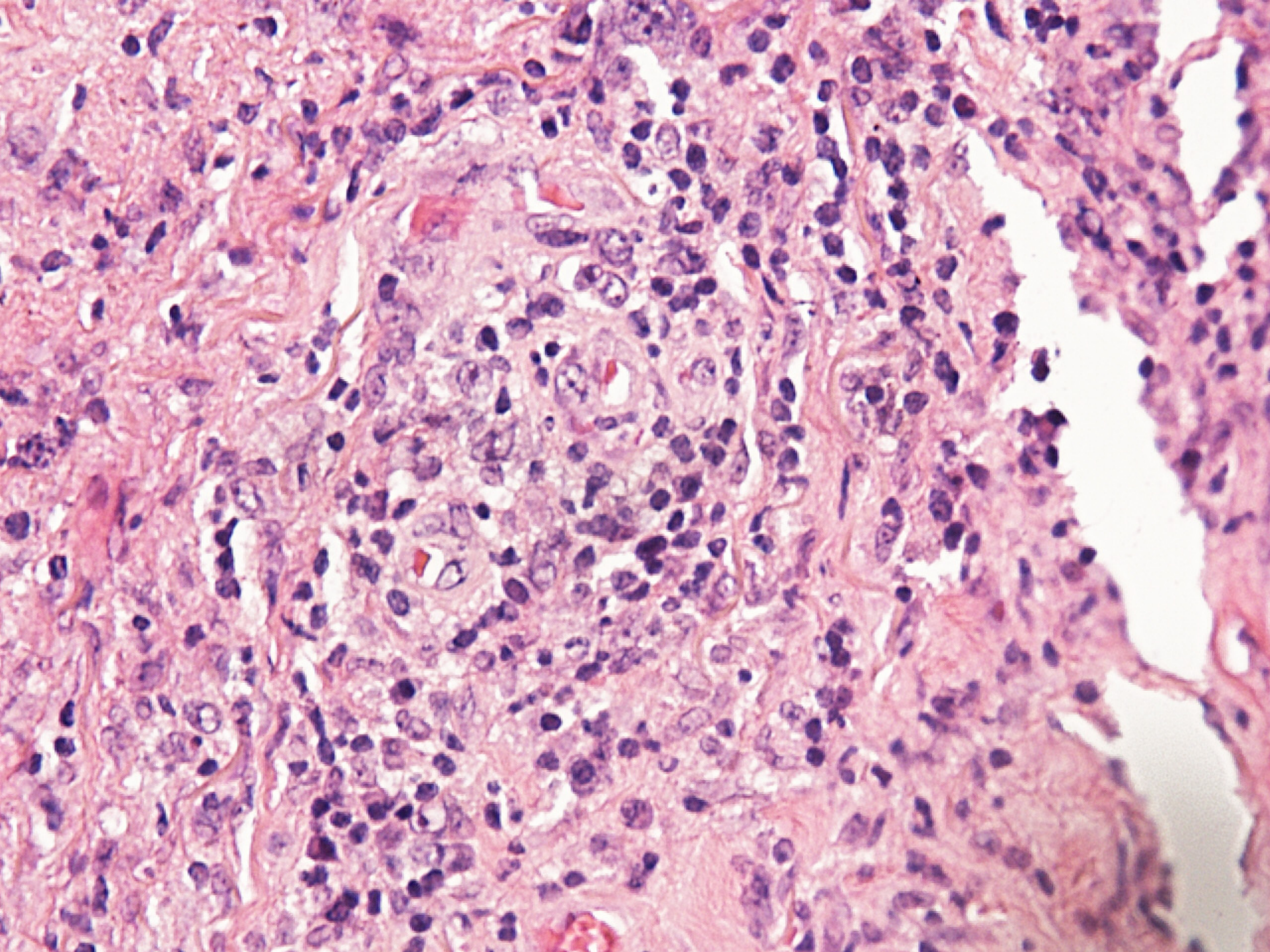
Age 30s-40s M:F=2:1
Western population

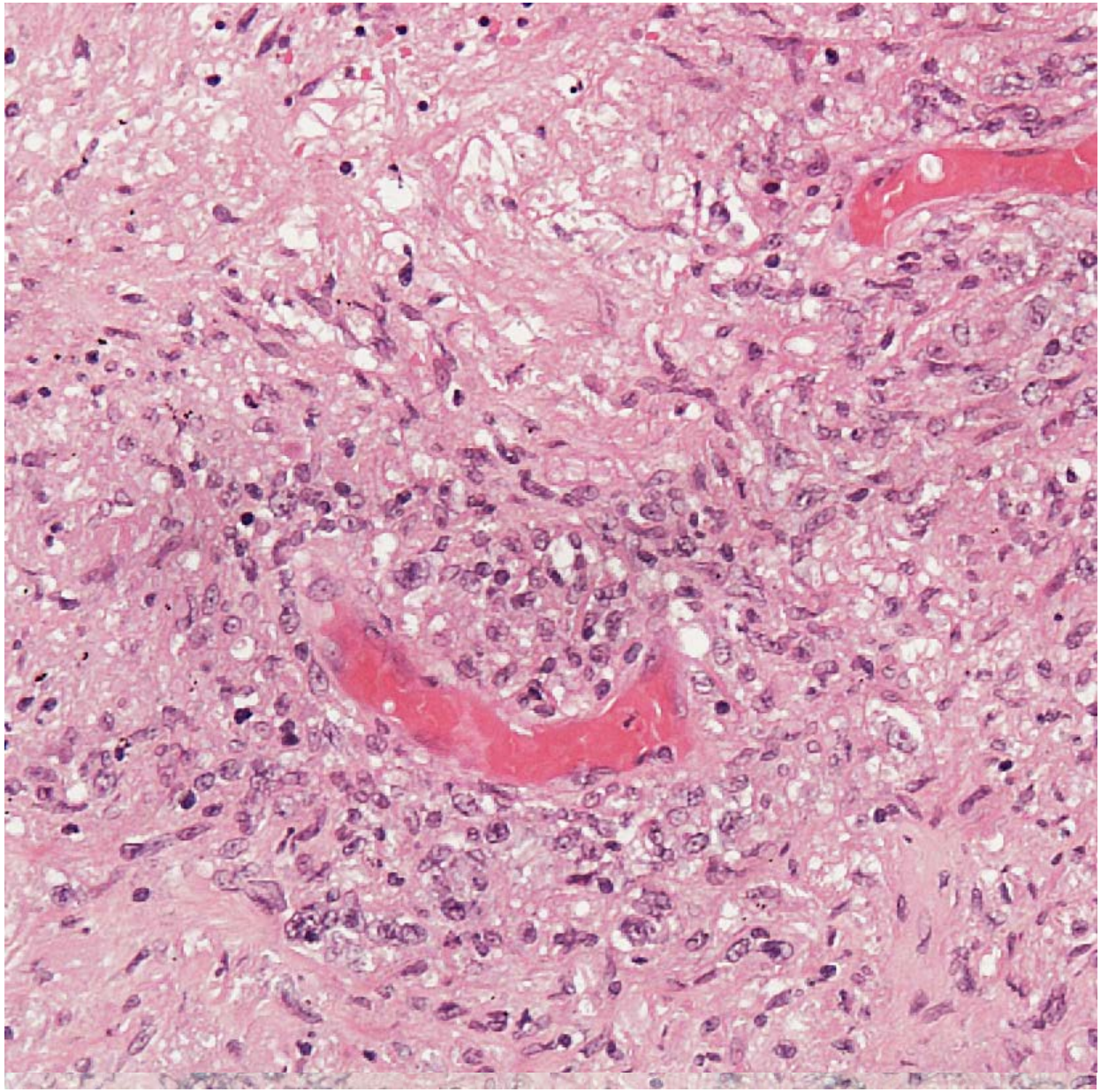
Lung

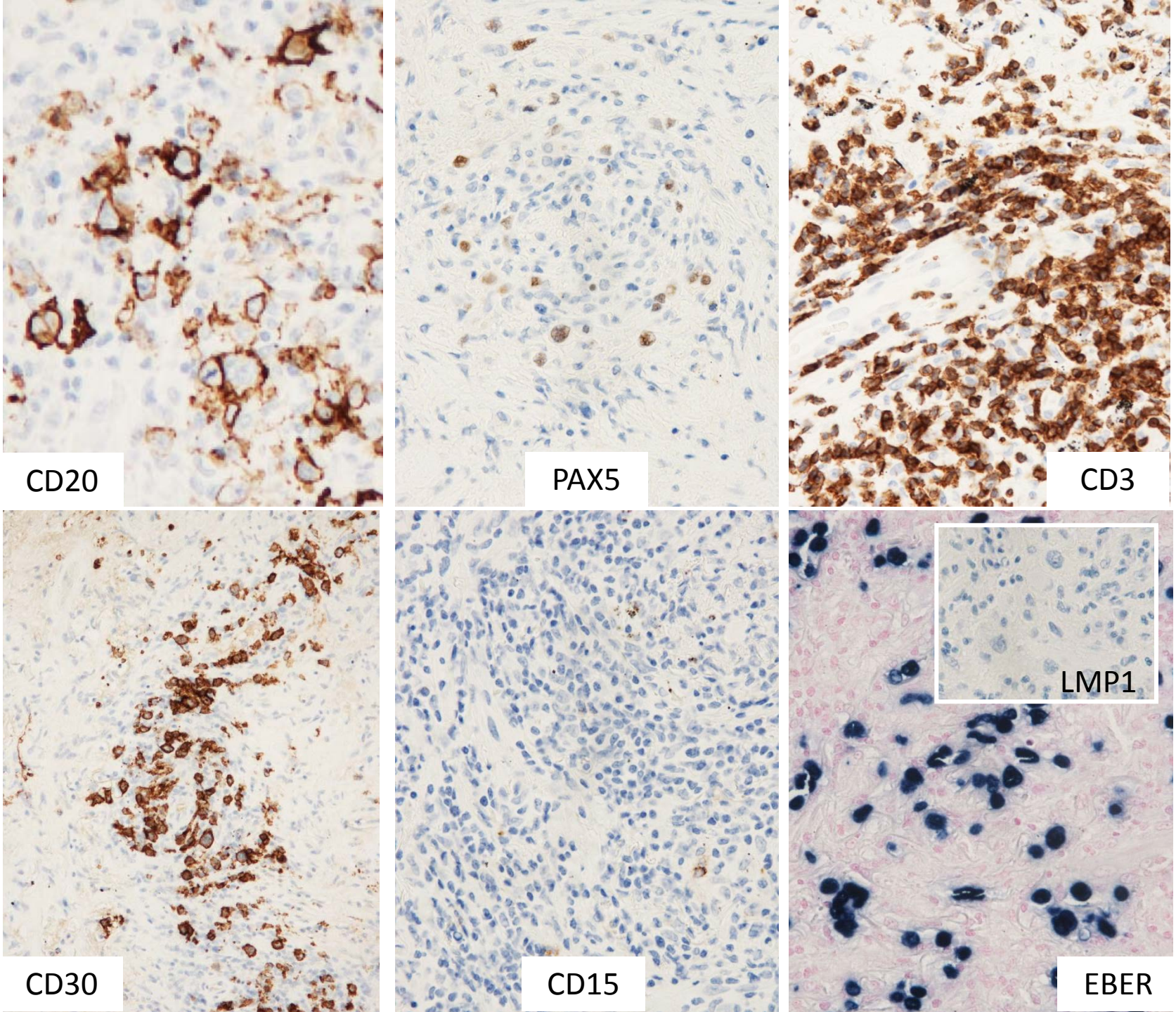
Skin
Liver
Kidney
Brain

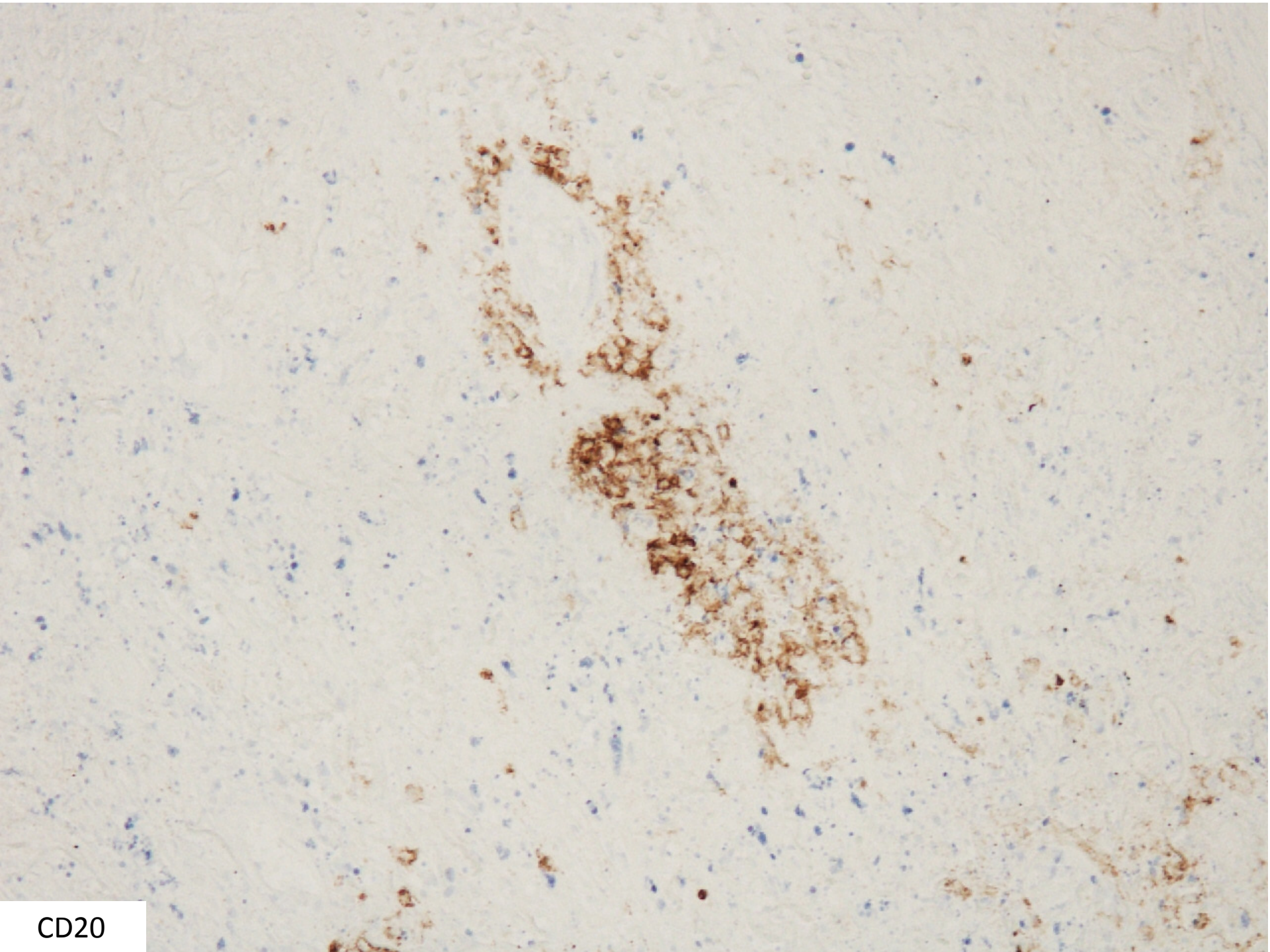
No lymphadenopathy
No bone marrow involvement








CD20
PAX5
CD3
CD30
CD15
LMP1
EBER


CD20

LyP or cHL?

	LyG	cHL
Presentation	Extranodal **No diagnosis without lung involvement**	Nodal (primarily)
Morphology	**Range of cell sizes** **Mainly lymphocytes** **Angioinvasion** **No granulomas** **Necrosis** Numerous EBER+ cells (variable size)	**HRS cells only** **Mixed infiltrate (NP, EO)** No angioinvasion Granulomas could be seen Necrosis not so common **Fewer EBER+ cells (uniform size)**
Phenotype	CD45+ CD20+ PAX5+ OCT2+ BOB1+ CD30+ CD15- **LMP1-**	CD45- CD20- PAX5+ (weak) OCT2- BOB1- MUM1+ CD30+ CD15+

PTLD

- Type of Tx
  - <1% BMTR
  - 1 % renal transplant
  - 1-2% liver transplant
  - 5% heart and lung
  - 20% small bowel transplant
  - 30-50% multiorgan transplantation
- Children
  - 4-10% renal transplant
  - 4-15% liver transplant
- Greatest in first year after transplant
- Post transplant status (D+/R-)
- Associated with ATG, OKT3

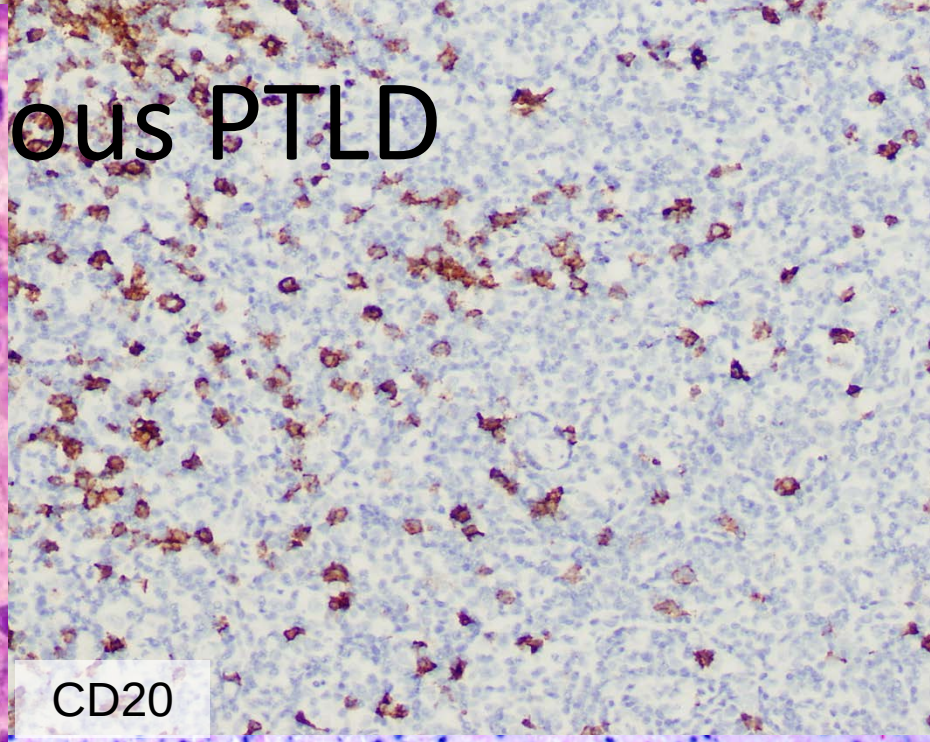
PTLD - presentation

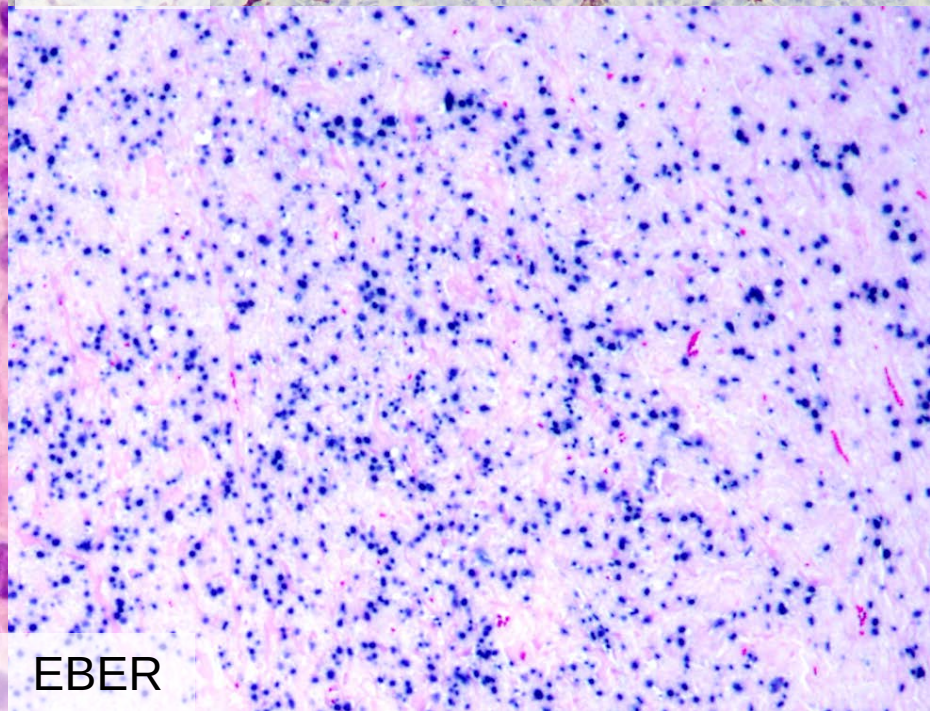
- Localised or diffuse and generalised
  - LN, tonsil: IM – like, early lesions
  - GI, lung, liver
  - BM, brain
  - Graft involvement 20%

PTLD CLASSIFICATION (WHO)

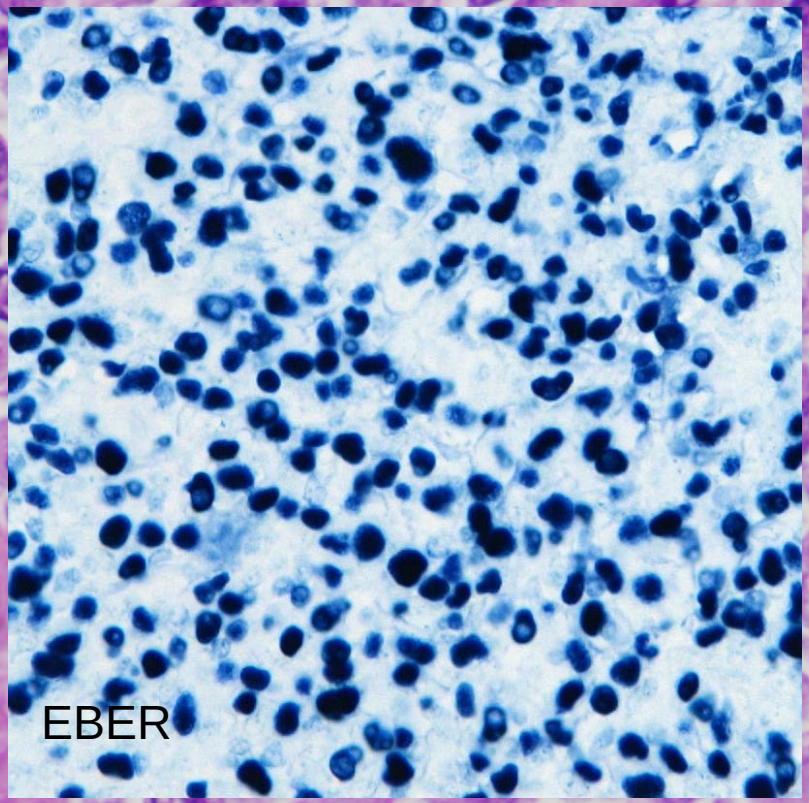
- **EARLY LESIONS**
    - Infectious mononucleosis-like
    - Reactive plasma cell hyperplasia
- **POLYMORPHIC PTLD**
- **MONOMORPHIC PTLD**
  - B-cell:
    - DLBCL
    - Burkitt
    - Plasma cell myeloma / Plasmacytoma-like lesions
  - T/NK-cell:
    - Peripheral T-cell lymphoma NOS
    - Hepatosplenic T-cell lymphoma
    - Other
- **HL AND HL-LIKE PTLD**

Polymorphous PTLD





PTLD DLBCL



EBV NEGATIVE PTLD

- Up to 20% cases
- Occur later (median 50 months)
- Monomorphic
- Aggressive
- Origin:
    - Sporadic tumours
    - Constant stimulation of lymphocytes related to chimeric post-transplant state
    - ‘hit & run’ theory. EBV induces preneoplastic injury and then is disposed of by cell when full neoplasia ensues.

PTLD - Prognosis

Favourable		Unfavourable
Graft based Localised		BMTR Generalised
Early		Late
IM-like PCH Plasmacytoma-like	Polymorphic	Monomorphic Myeloma-like
EBV+		EBV-
Polyclonal		Monoclonal

PTLD: Diagnosis Formulation

Lineage (B/T)

EBV+ vs EBV –

Polyclonal vs monoclonal

Polymorphous vs monomorphic

"B-cell PTLD: Polymorphous, EBV+, monoclonal"

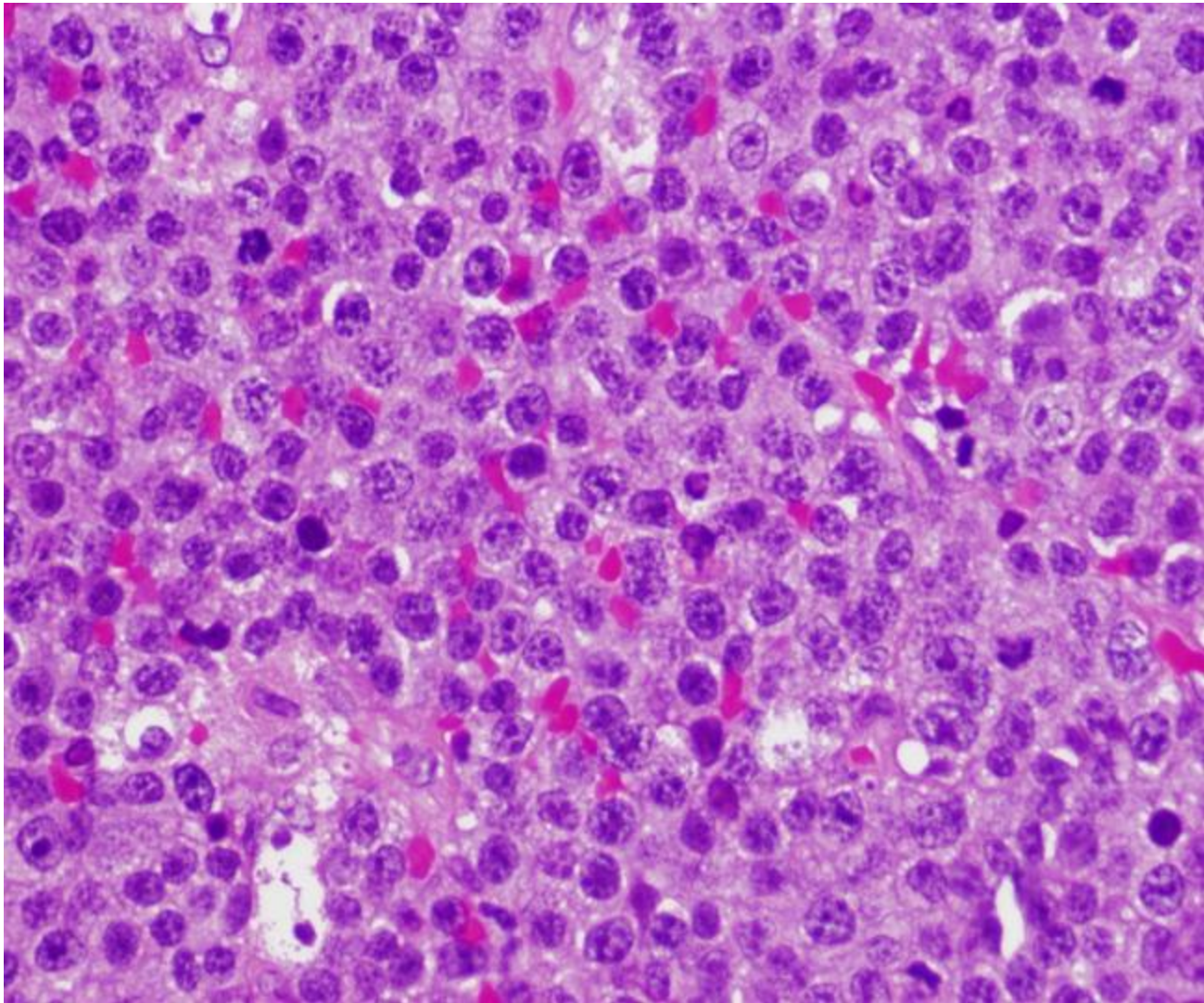
PTLD: Management

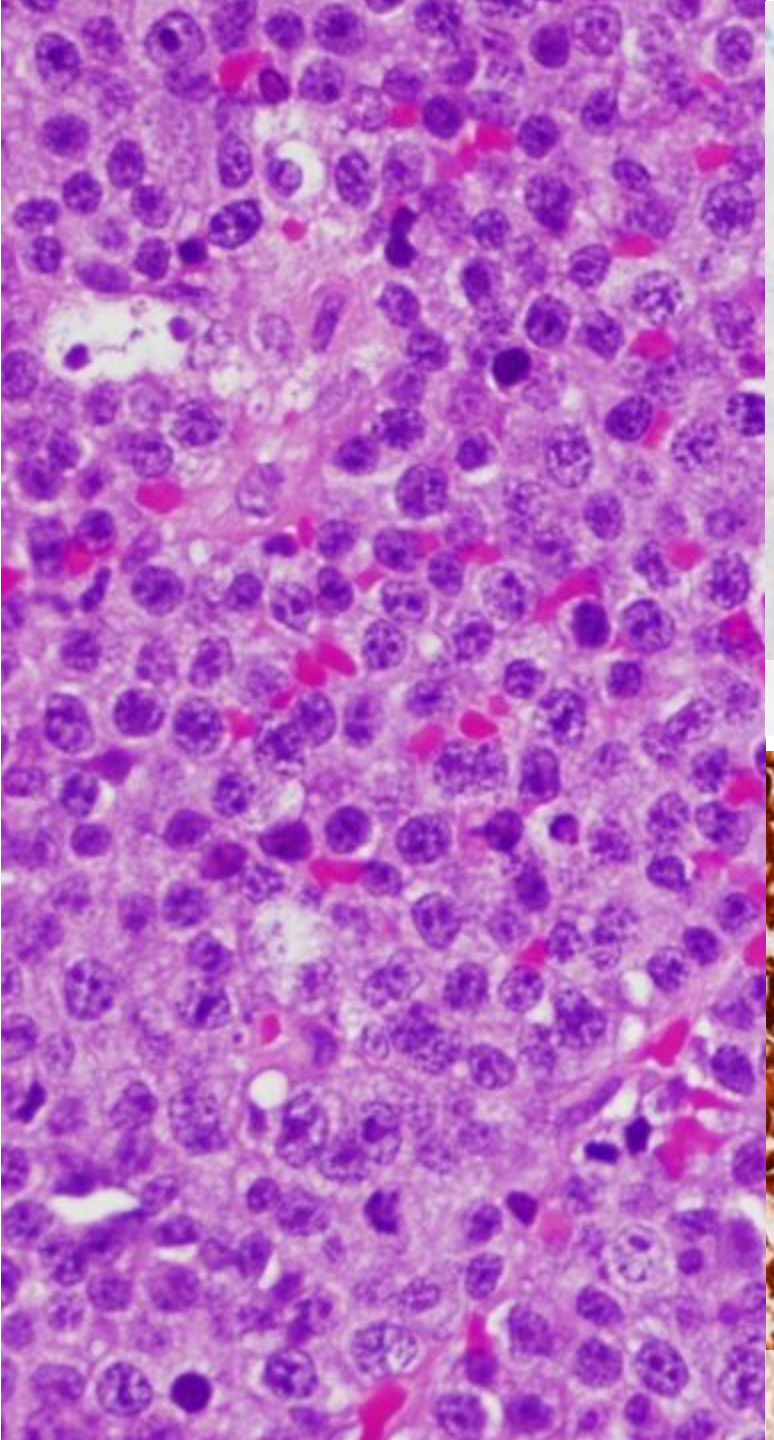
- No standardised and regulated approach
- Early lesions:
  - Spont. regression / Conservative
- Polymorphous:
  - Reduction of IS
  - Local control / surgical excision
  - Rituximab
  - Chemotherapy
- Late monomorphic:
  - Lymphoma treatment
  - Adoptive Immunotherapy: Allogeneic EBV specific CD8+ cytotoxic T-cells

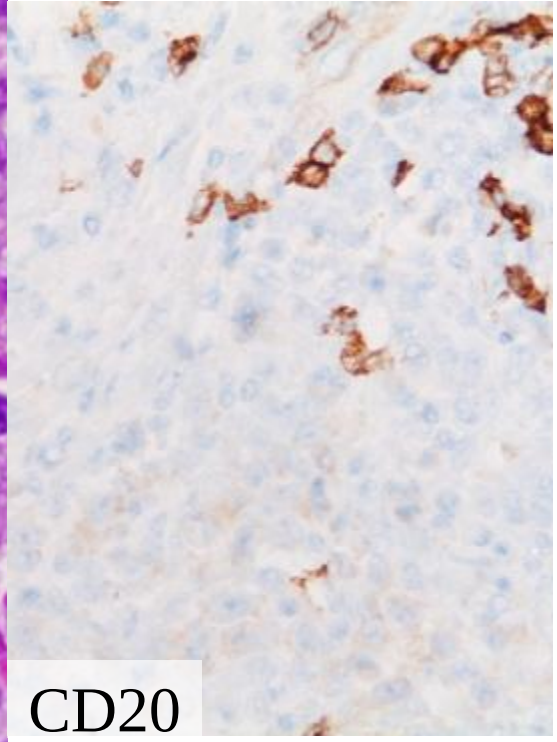
Plasmablastic Lymphoma

- Mouth involvement
- HIV+, Immunosuppressed, Elderly
- EBV positive

- Lymph nodes



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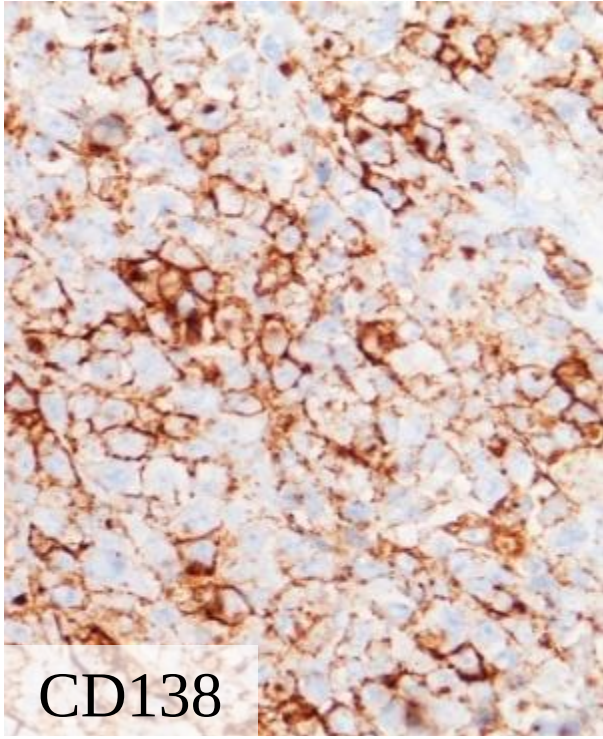
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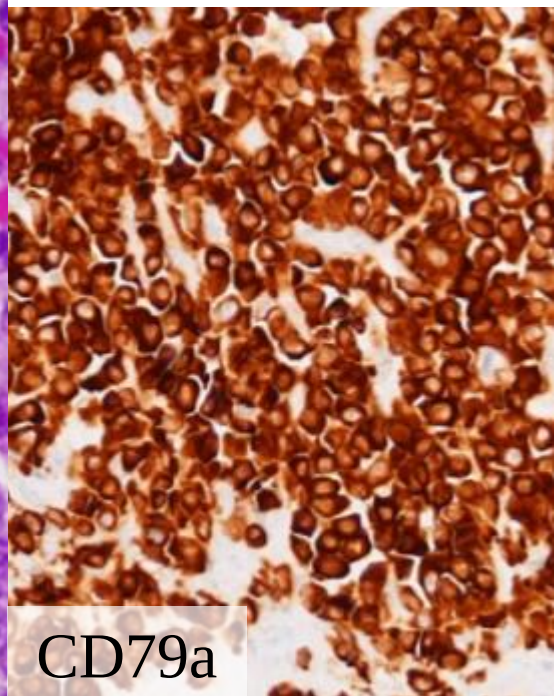
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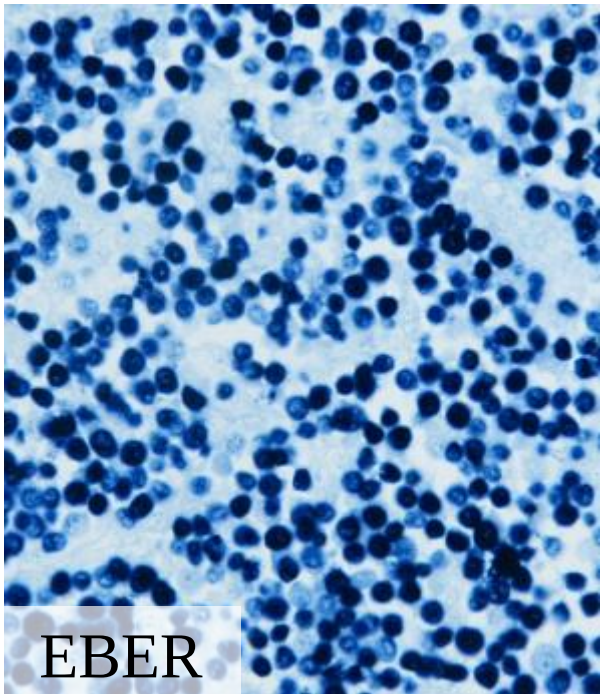
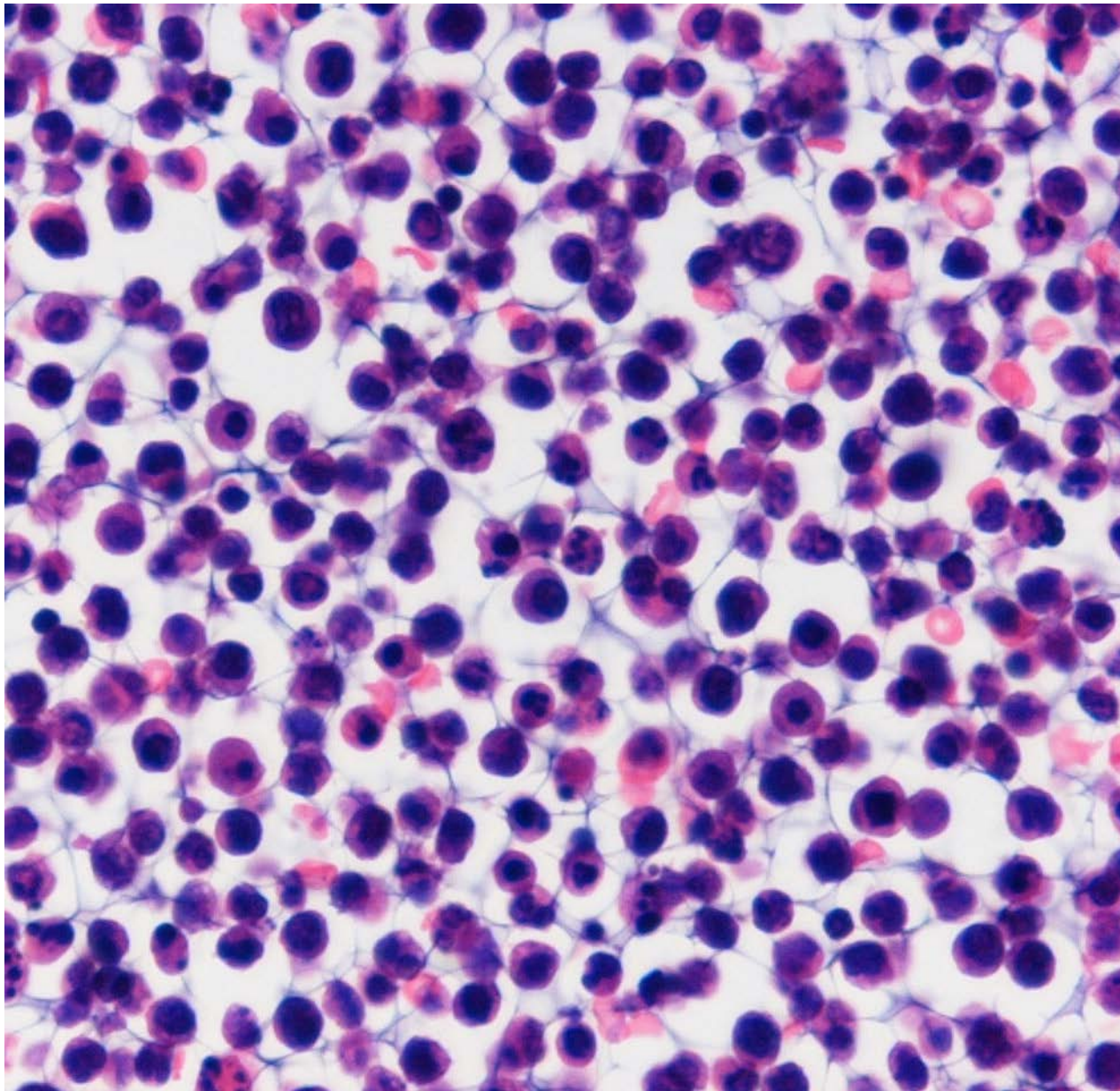
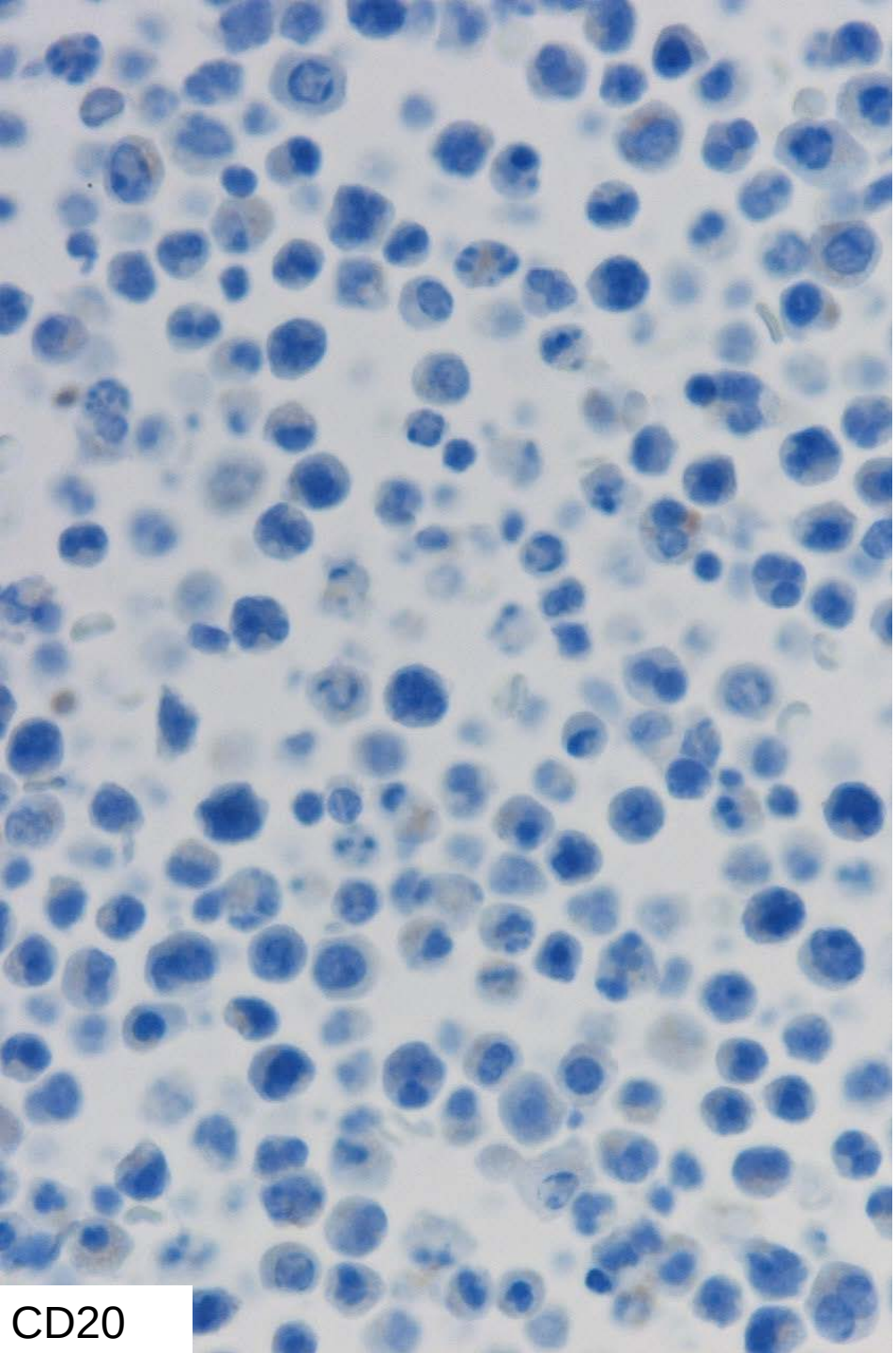

EBER

Primary effusion lymphoma

- Ascites
- ”Solid” variant
- HIV, elderly, PTLD, alcoholic cirrhosis
- Poor prognosis

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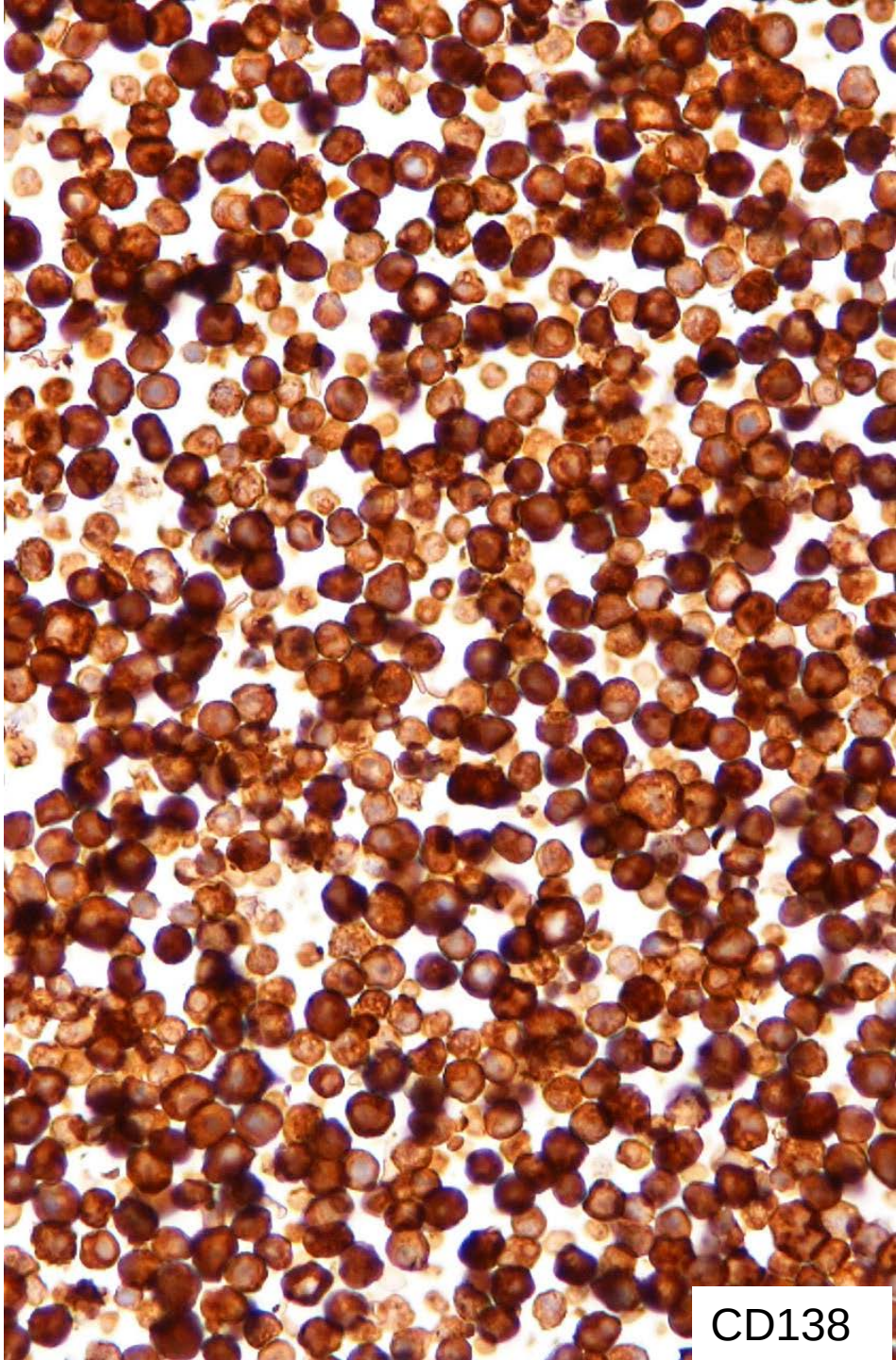
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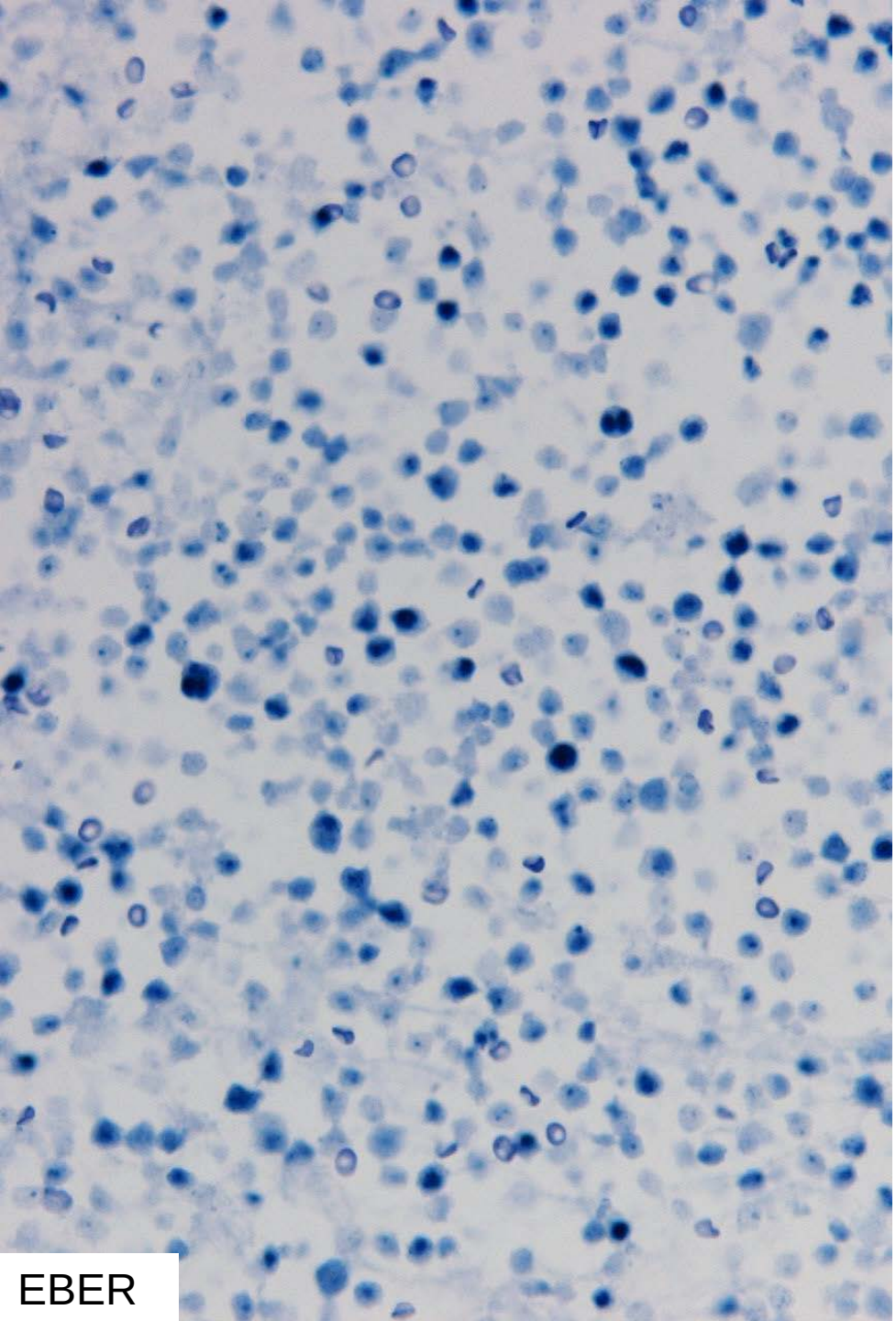
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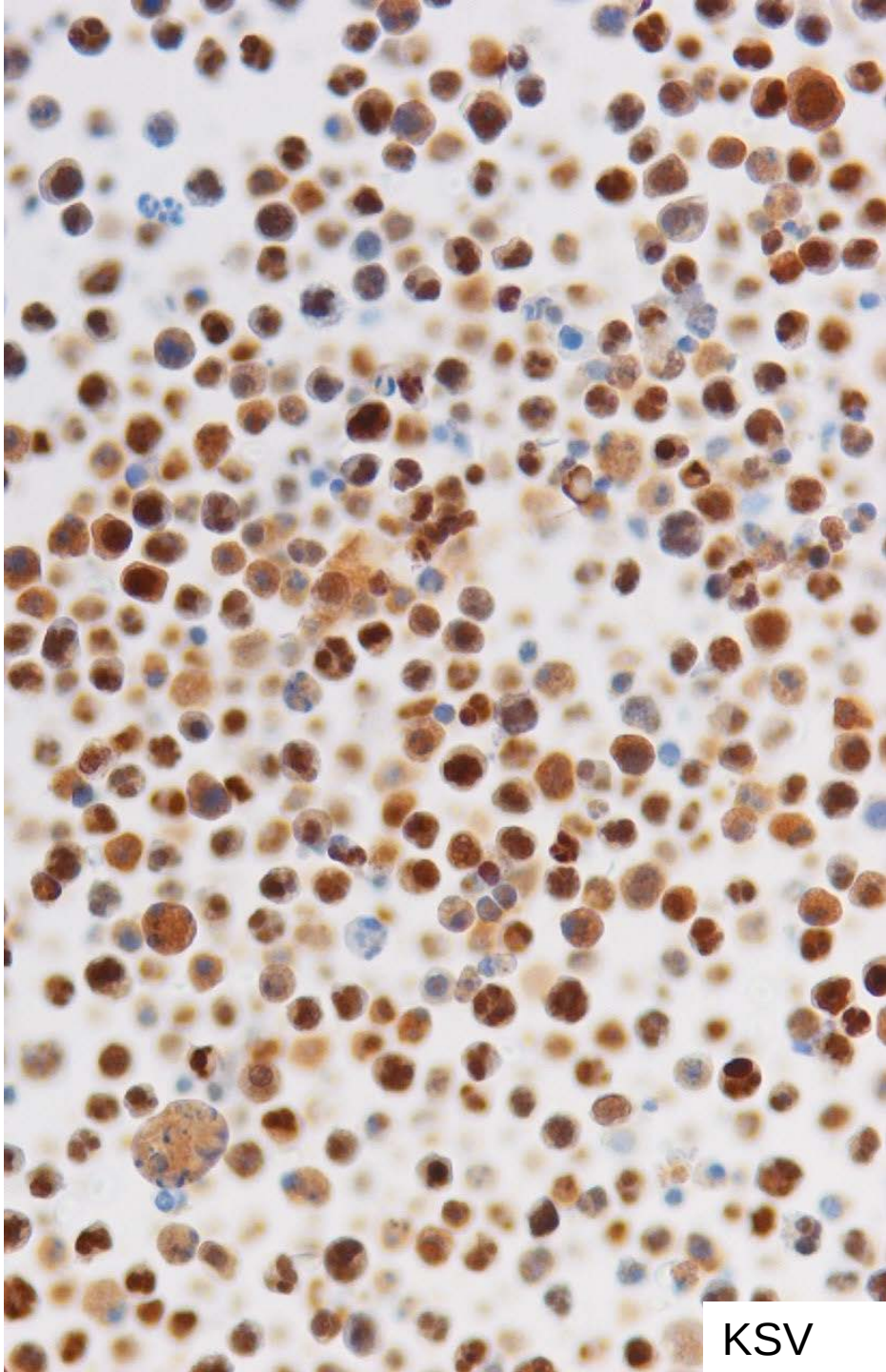

KSV

Extranodal NK/T-cell lymphoma, Nasal Type

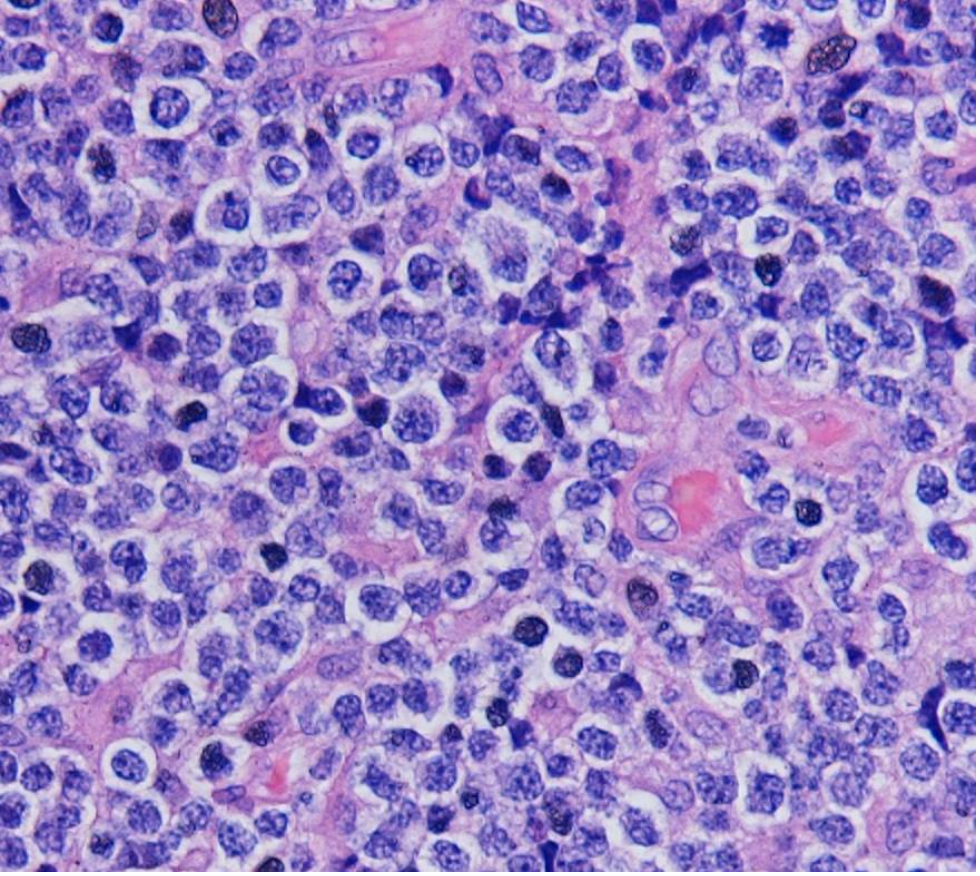
Adult patients

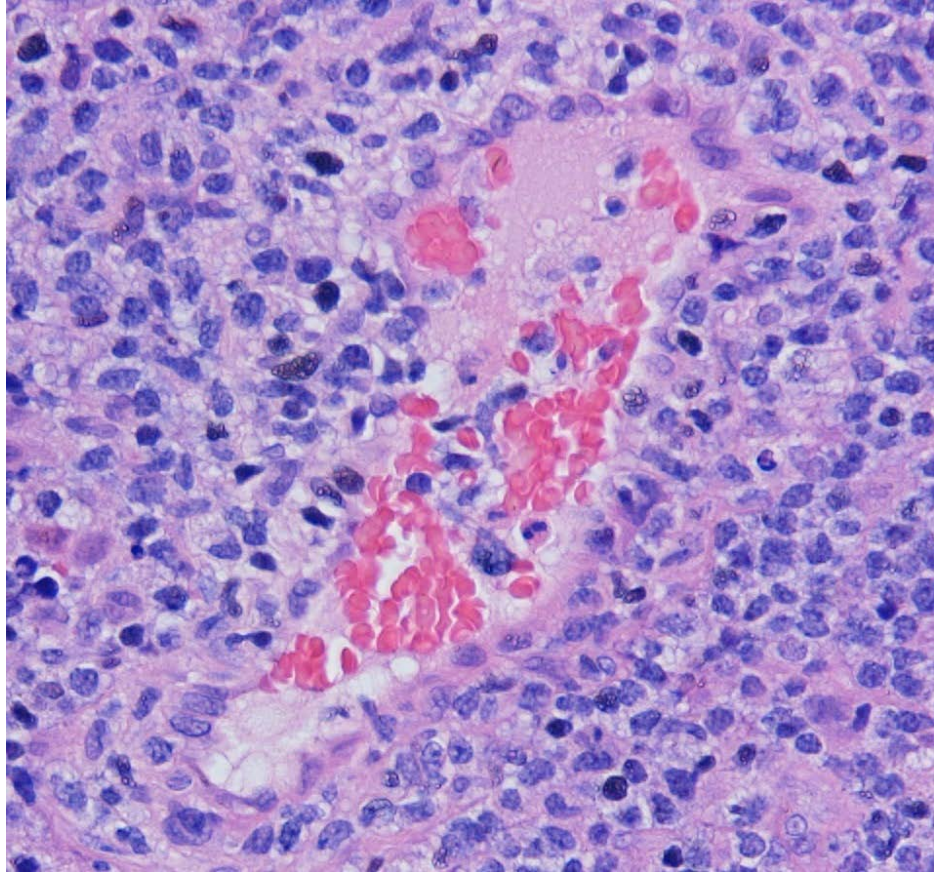
Asia, Mexico, Central & South America

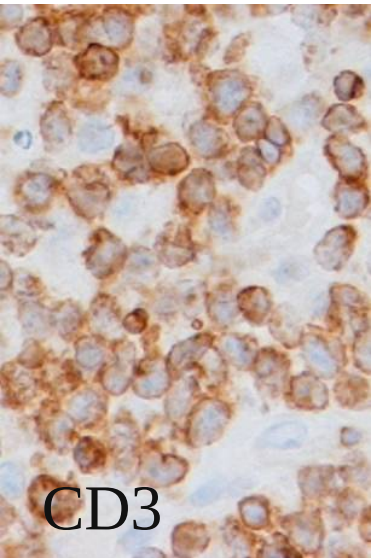
Nasopharyngeal region, GI tract, other extranodal sites

Cytogenetics: germline TCR

Extremely aggressive clinical course



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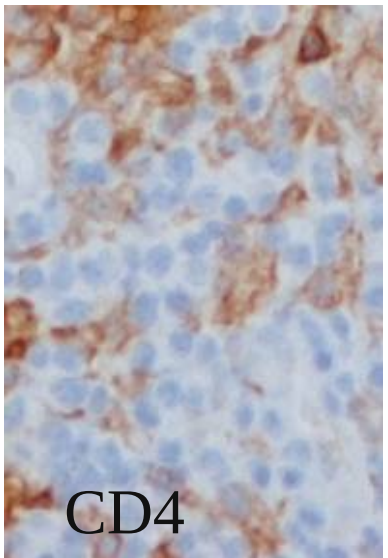
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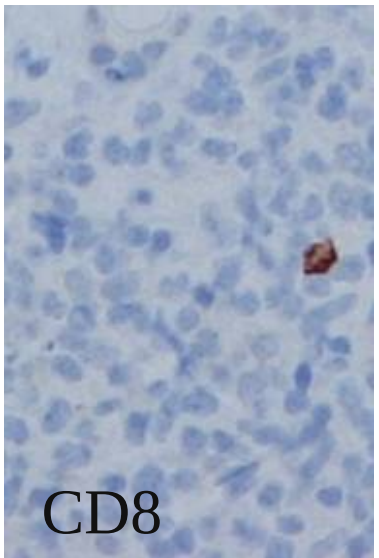
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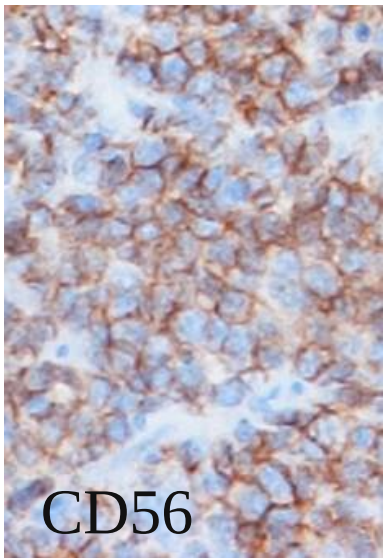
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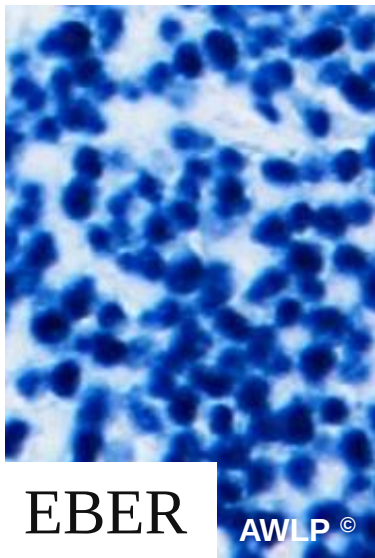
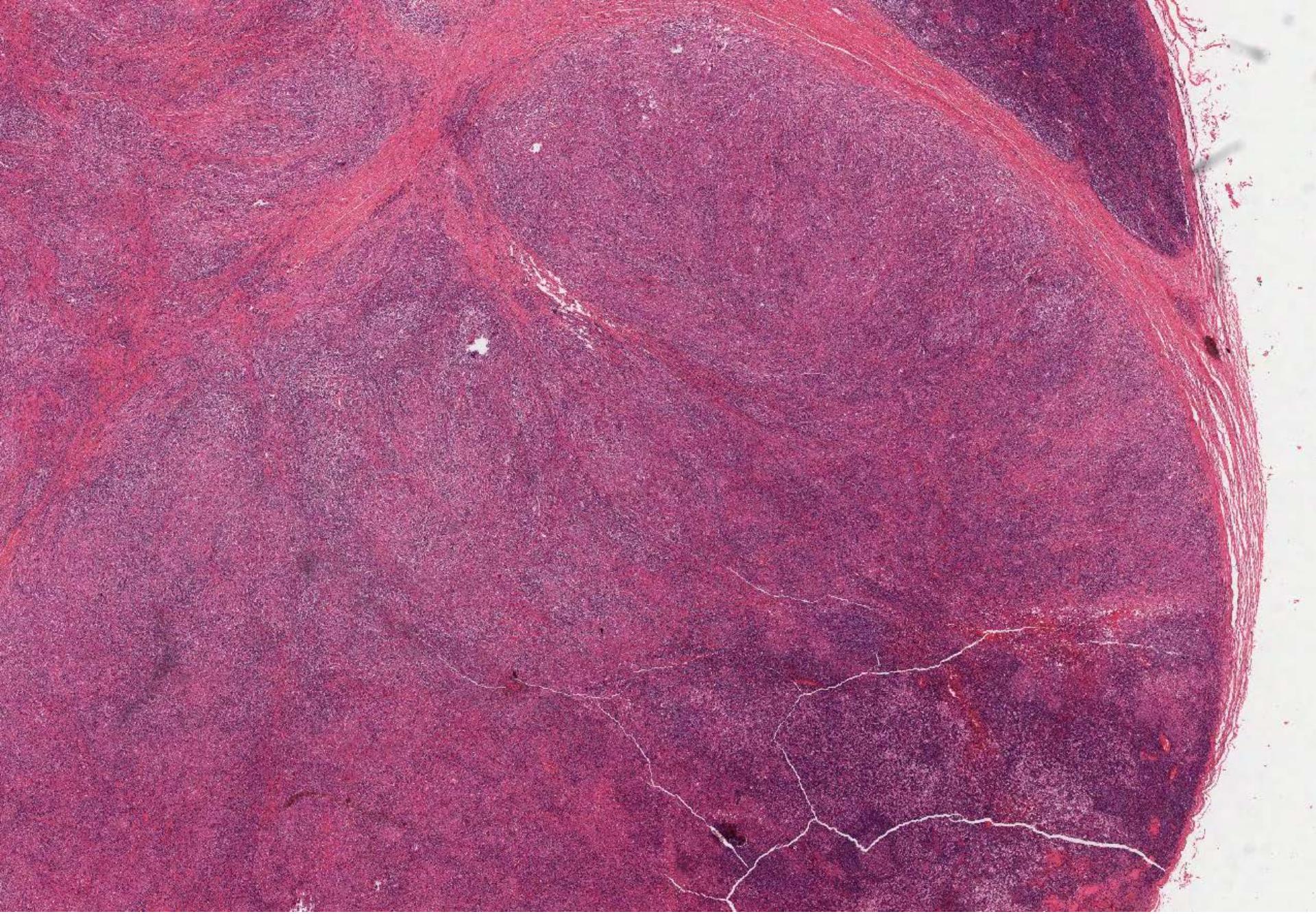

EBER
AWLP ©

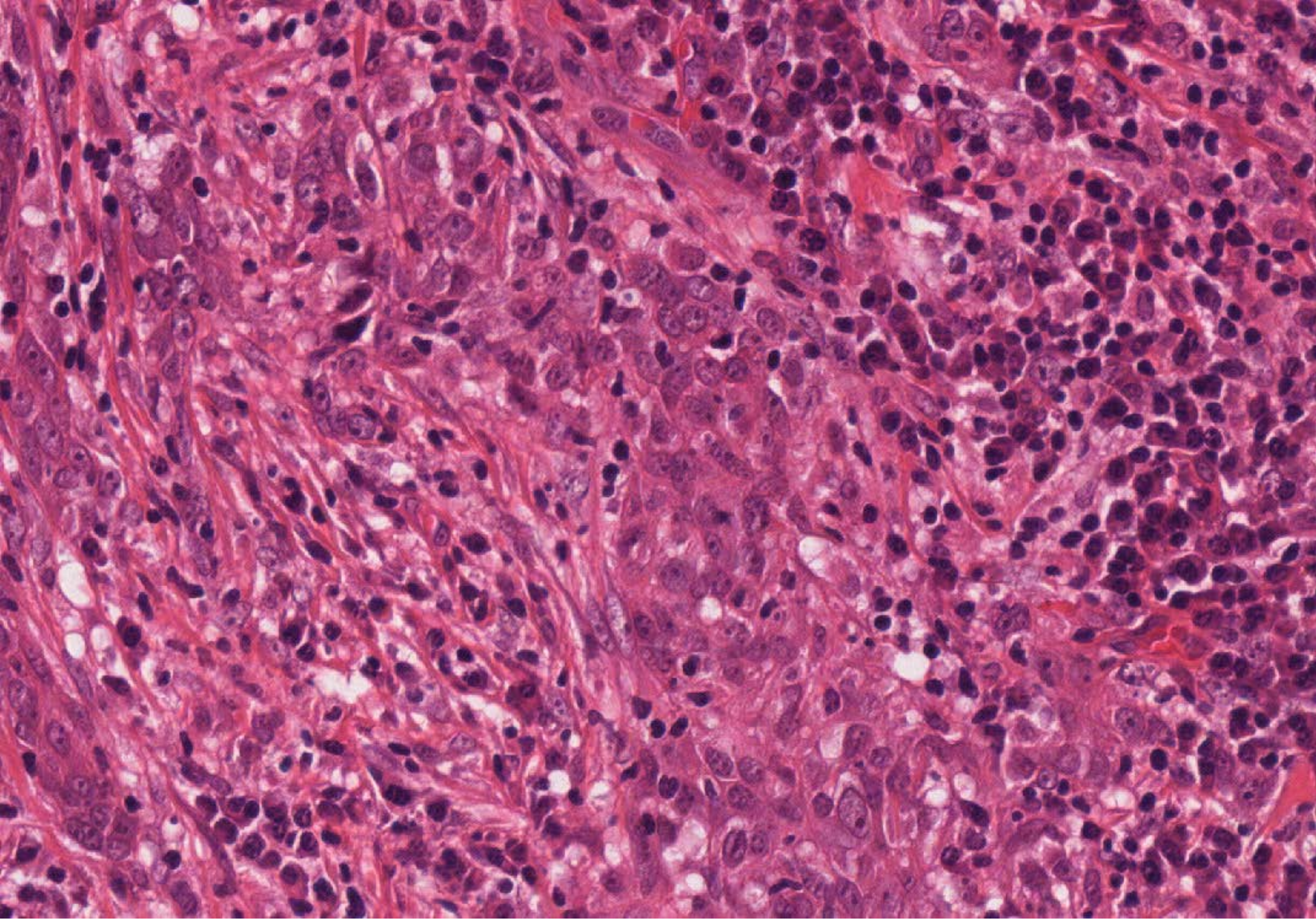
Systemic EBV positive T-cell Lymphoproliferative Disease of Childhood

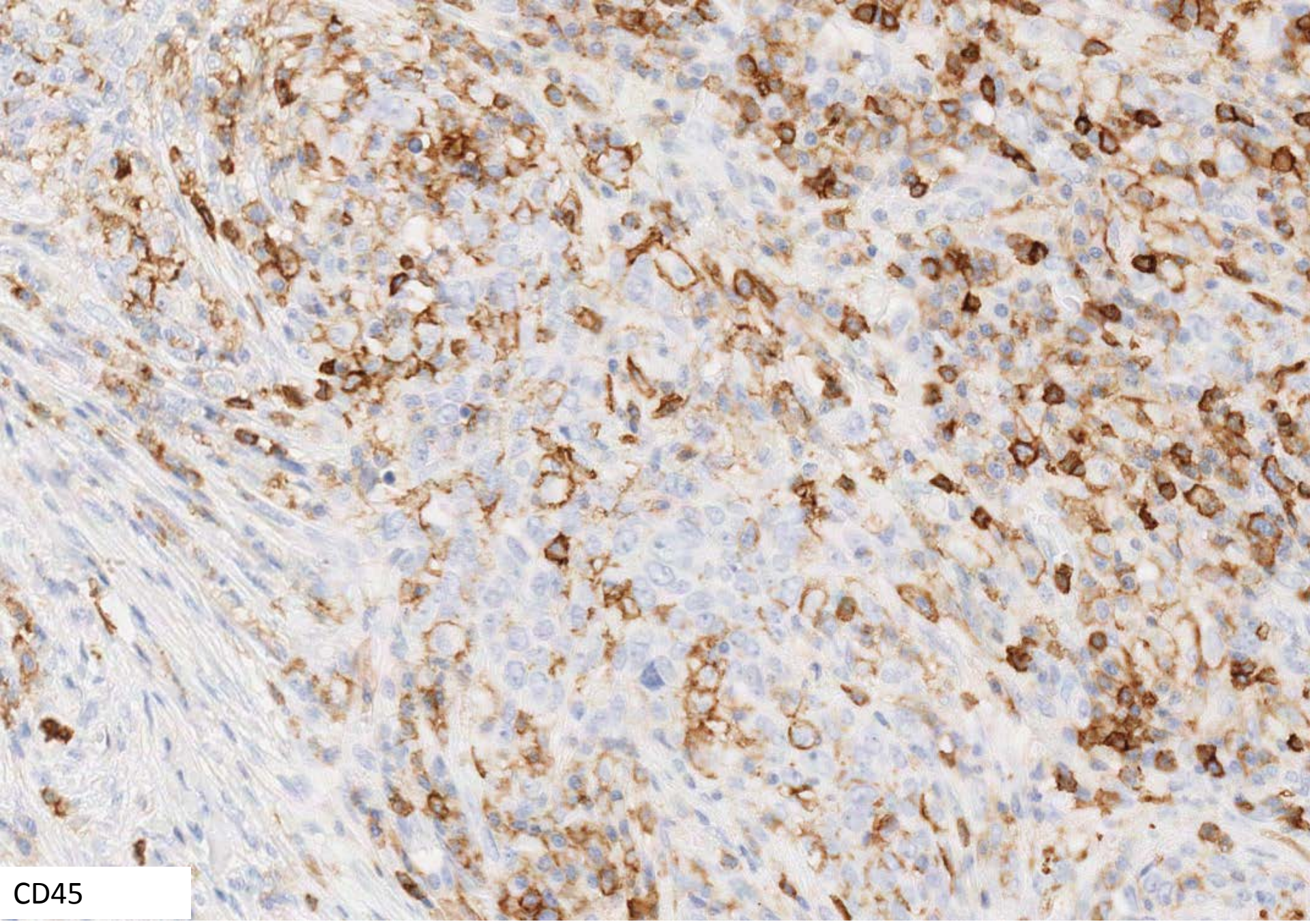
- Hispanic and Asian children
- Acute systemic illness
- Haemophagocytic syndrome
- High viral load
- Clonal EBV positive T-cells

Hydroa Vacciniforme-like Lymphoma

- Hispanic and Asian Children
- Cutaneous lesions exacerbated by sun
- T/NK phenotype
- Protracted course, progression



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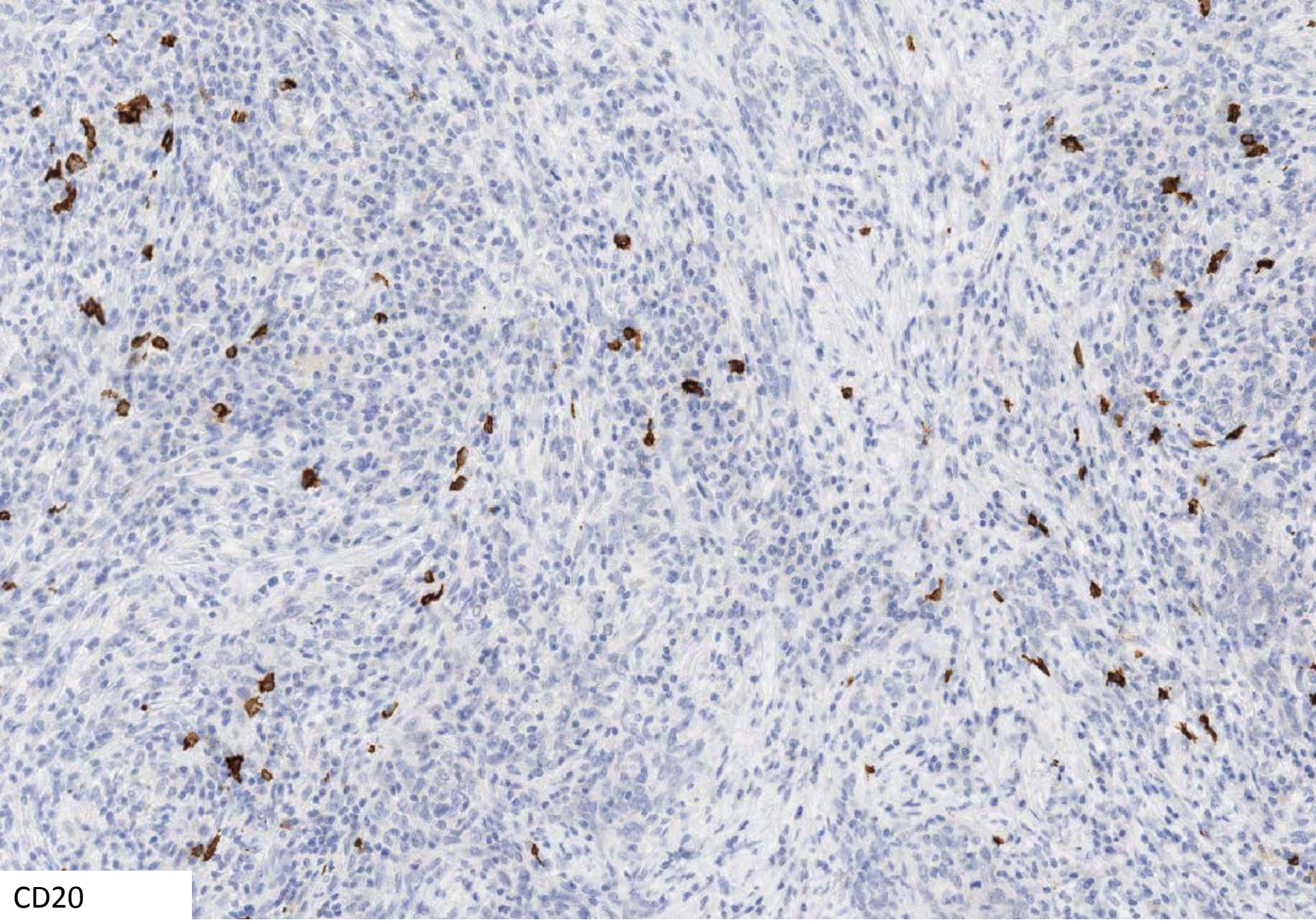

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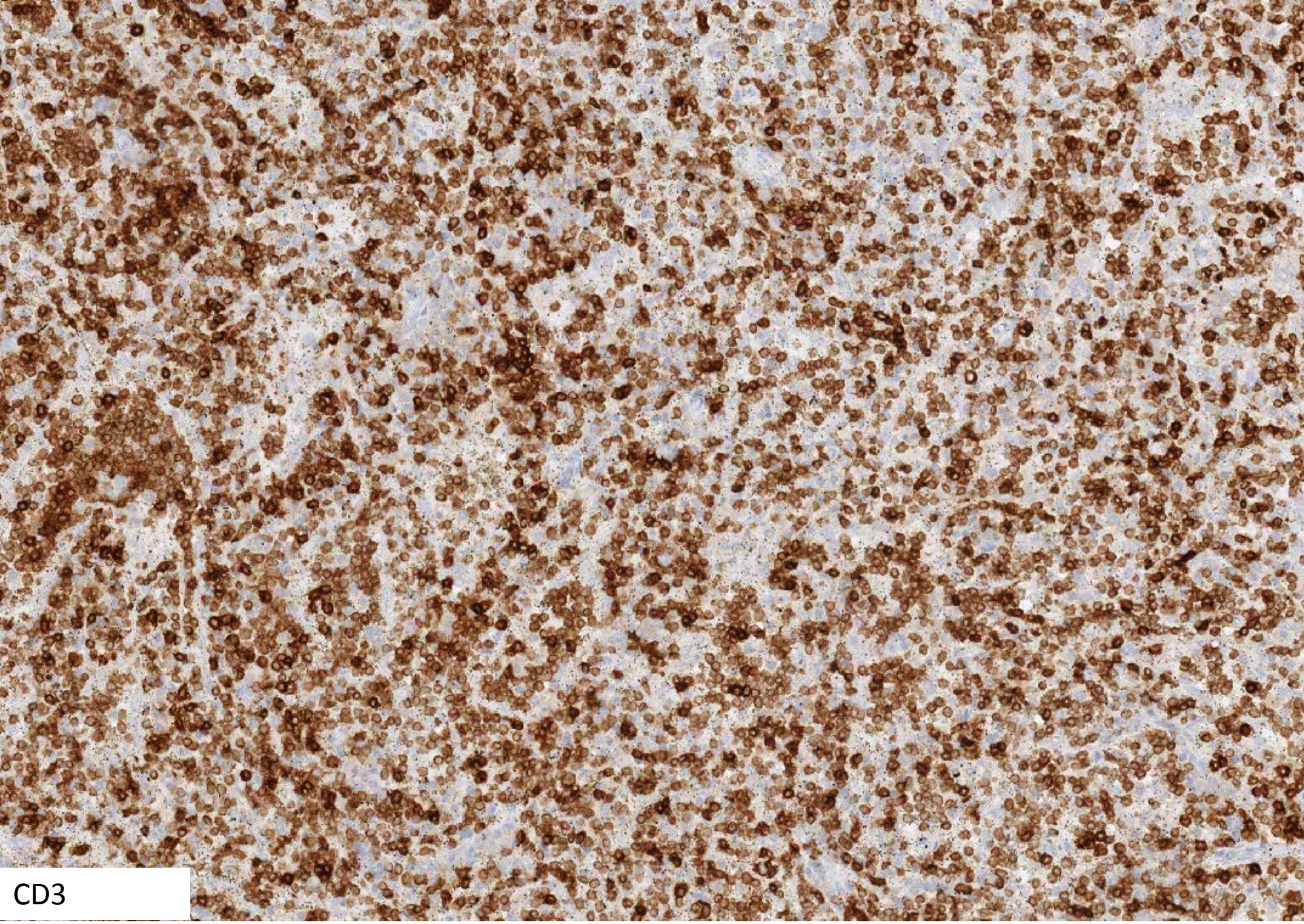
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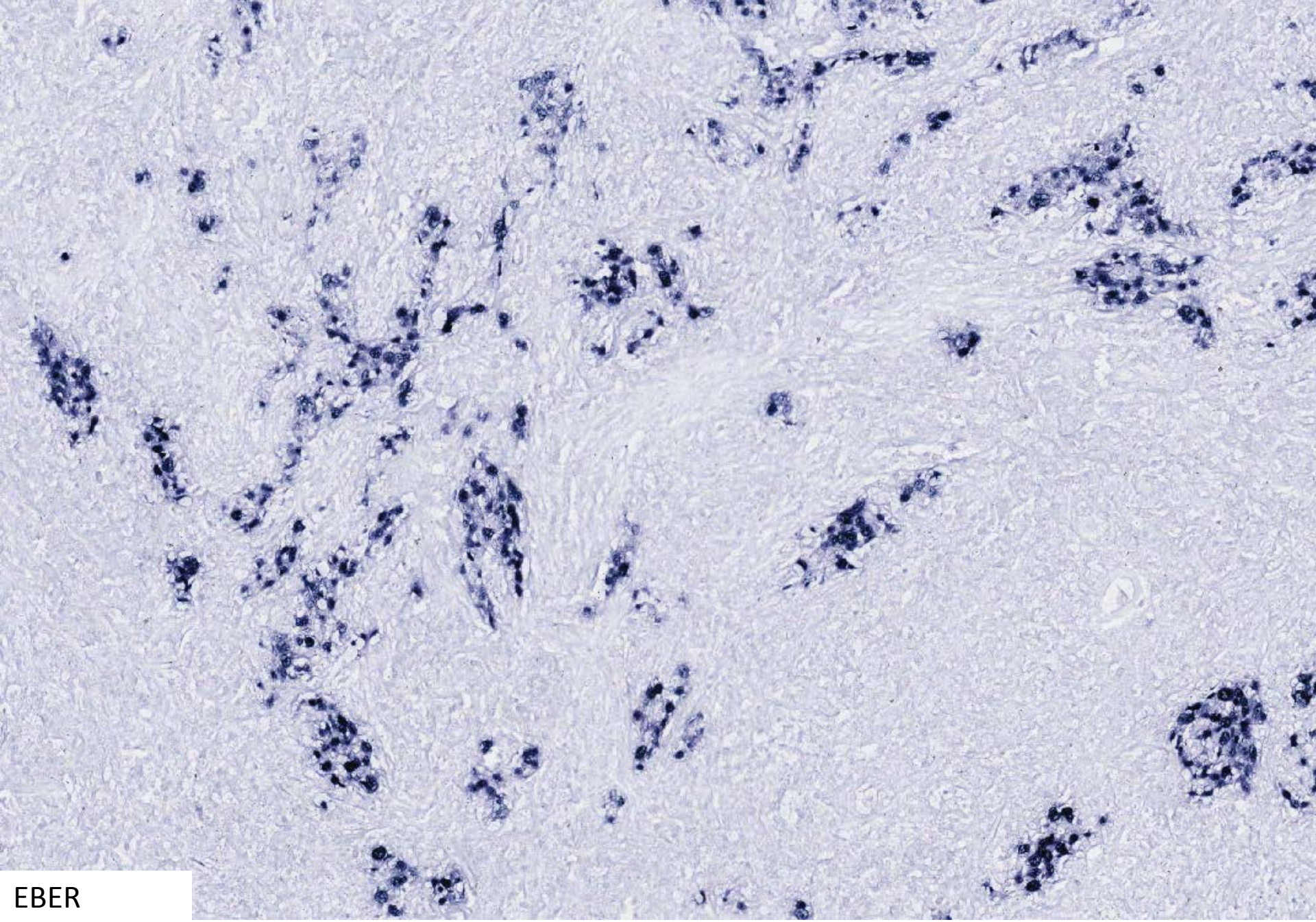

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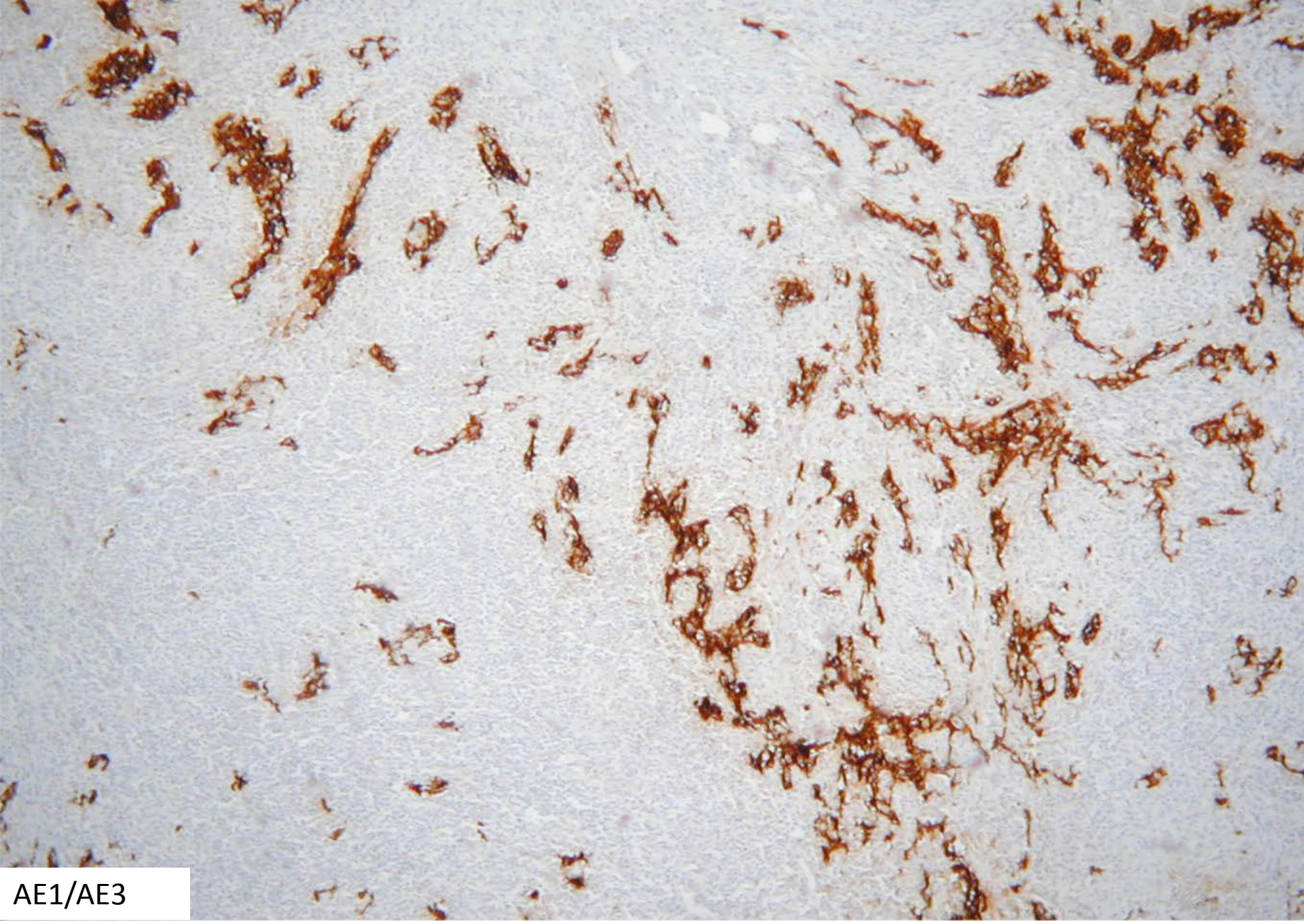
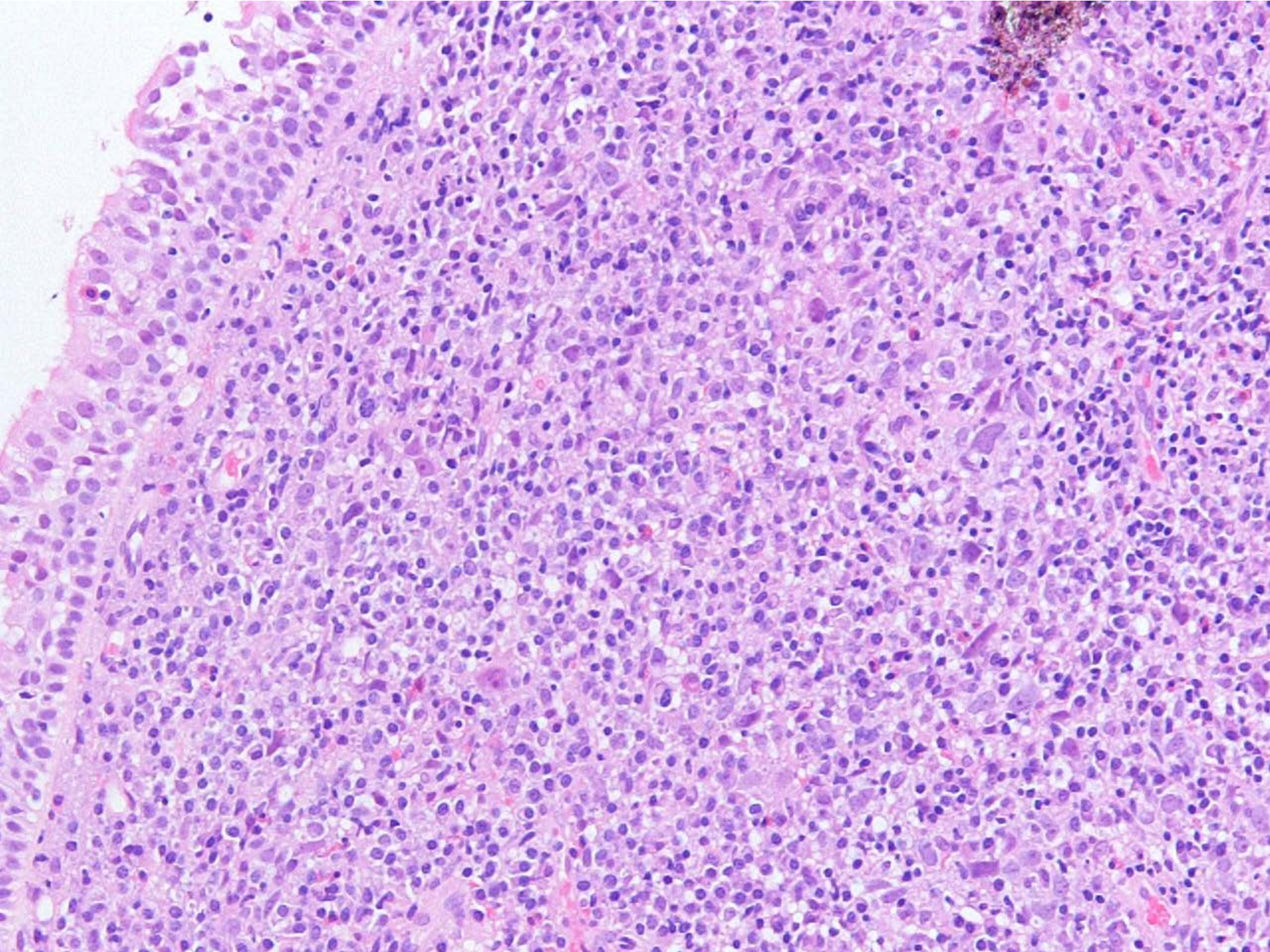
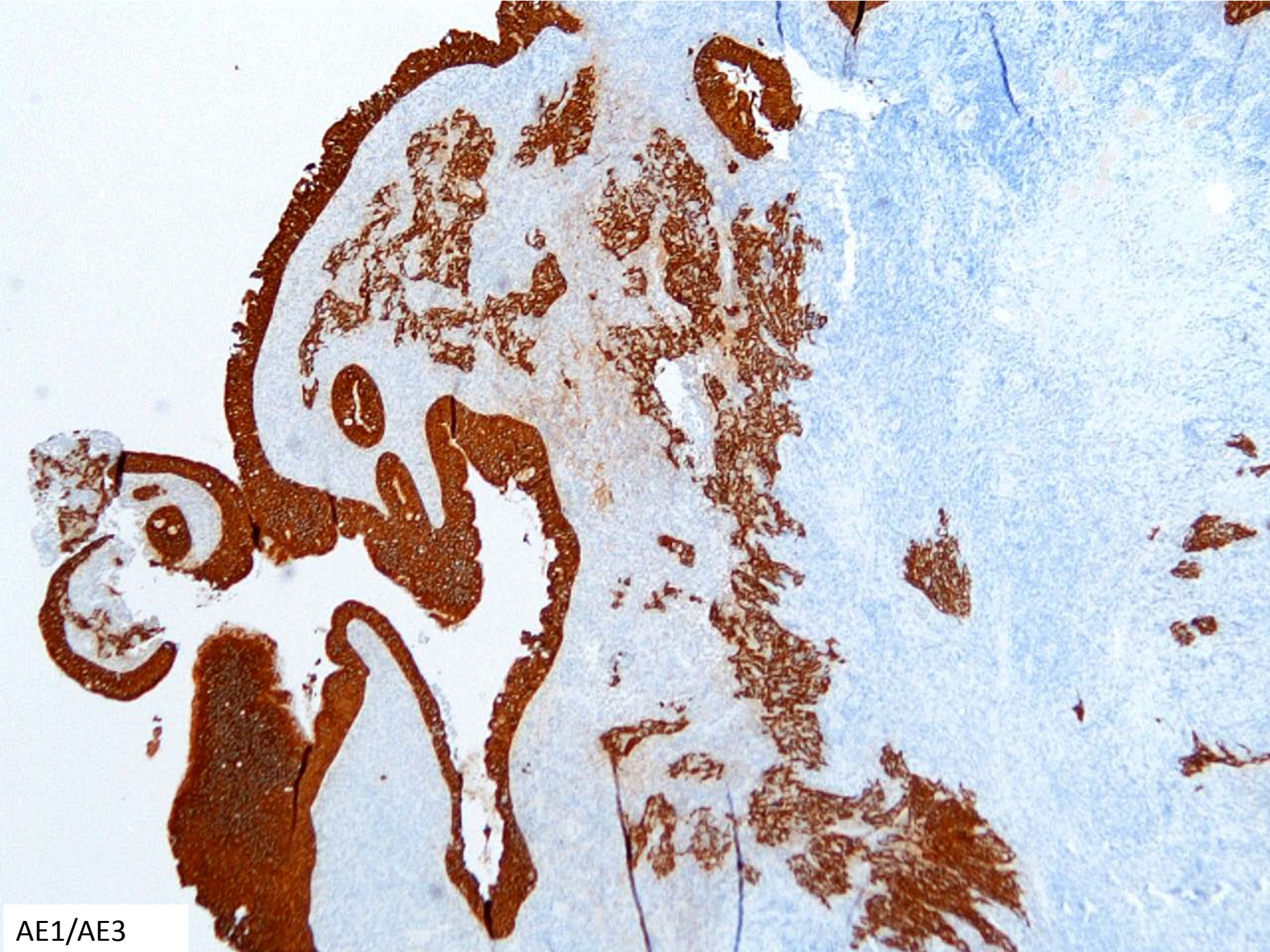


AE1/AE3

Undifferentiated Nasopharyngeal Carcinoma ("Lymphoepithelioma-like Carcinoma")

- 60% of NPC
- Common in children and young adults
- Neck mass - Lymph node metastasis without evident primary tumour
- Meticulous examination of the pharynx with random biopsies




AE1/AE3

Take Home…

- EBER-ISH is the right test for EBV
- Think of IM – Looks ugly – Retention, retention, retention of architecture
- EBV – Old virus, many new entities
  - Age related spectrum
  - Systemic T-cell LPD of childhood
- Overlapping morphology
  - Polymorphous
  - Hodgkin-like
- Differential diagnosis with HL
  - Complex of features
  - Clinical aspects of disease very important
  - Important clinical implications – MCU
- Not everything EBV+ is lymphoma
  - “Lymphoepithelioma-like” carcinoma, very frequently misdiagnosed as cHL