Pathophysiology of HHV8+ Multicentric Castleman Disease

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CDCN
CASTLEMAN DISEASE COLLABORATIVE NETWORK

Inserm
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anRS
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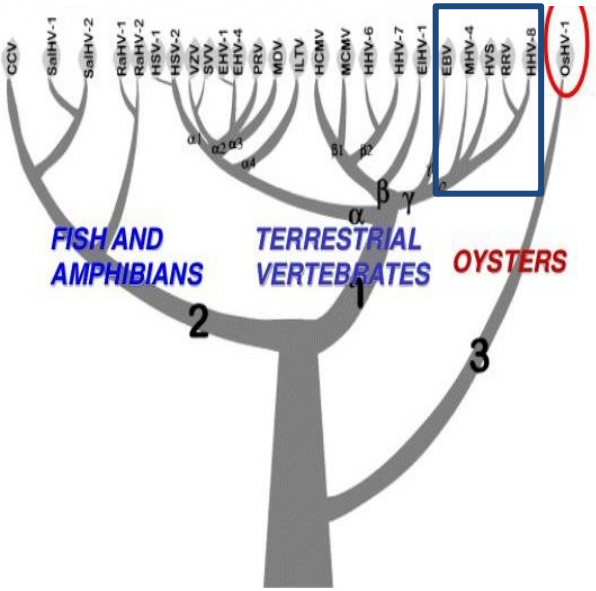
sur le sida et les hépatites virales

HHV-8 basics (1)



γ2 herpesvirus

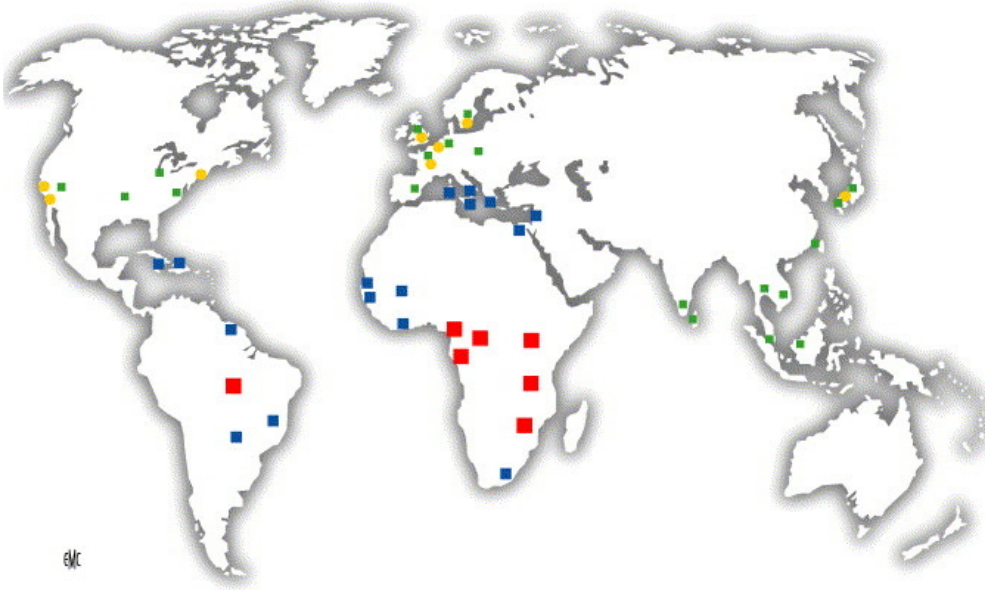
Double-stranded DNA virus
160 Kbp genome
100 ORF, 17 vmiRNA
Conserved genes within Herpes family + 20 unique genes (K genes)
Cellular homologs in both categories

Shares **similarities with Epstein-Barr virus** (cell cycle, B-cell lymphotropism)

HHV8 basics (2)

Transmission through saliva in young siblings
(high prevalence countries ■ = subsaharan Africa, West Indies)

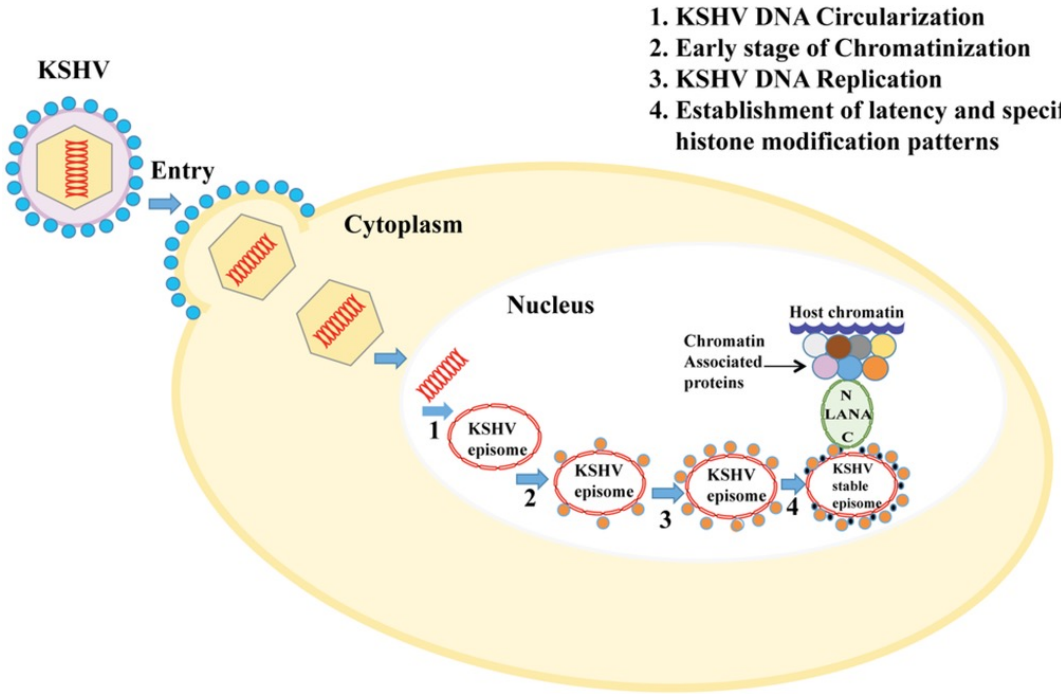
Sexual transmission, especially in MSM ■
(high and low prevalence countries ■)



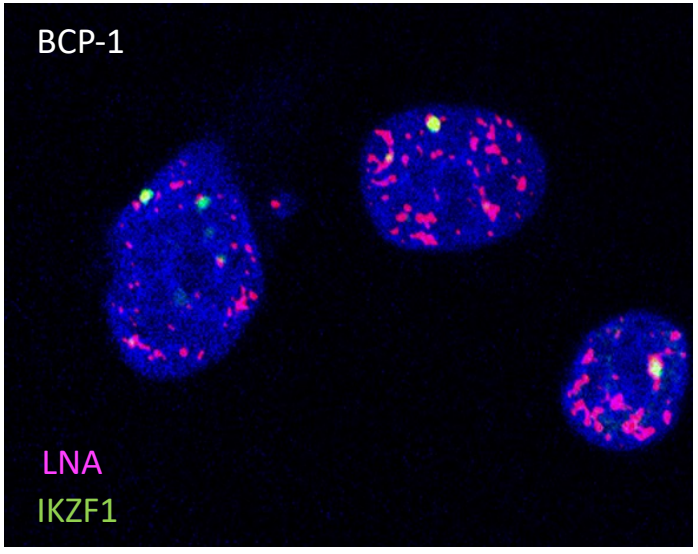
HHV8 target cells

Endothelial cells, epithelial cells and keratinocytes
Heparan sulfate, integrins, **Epha2R**

B cells and monocytes
DC-SIGN

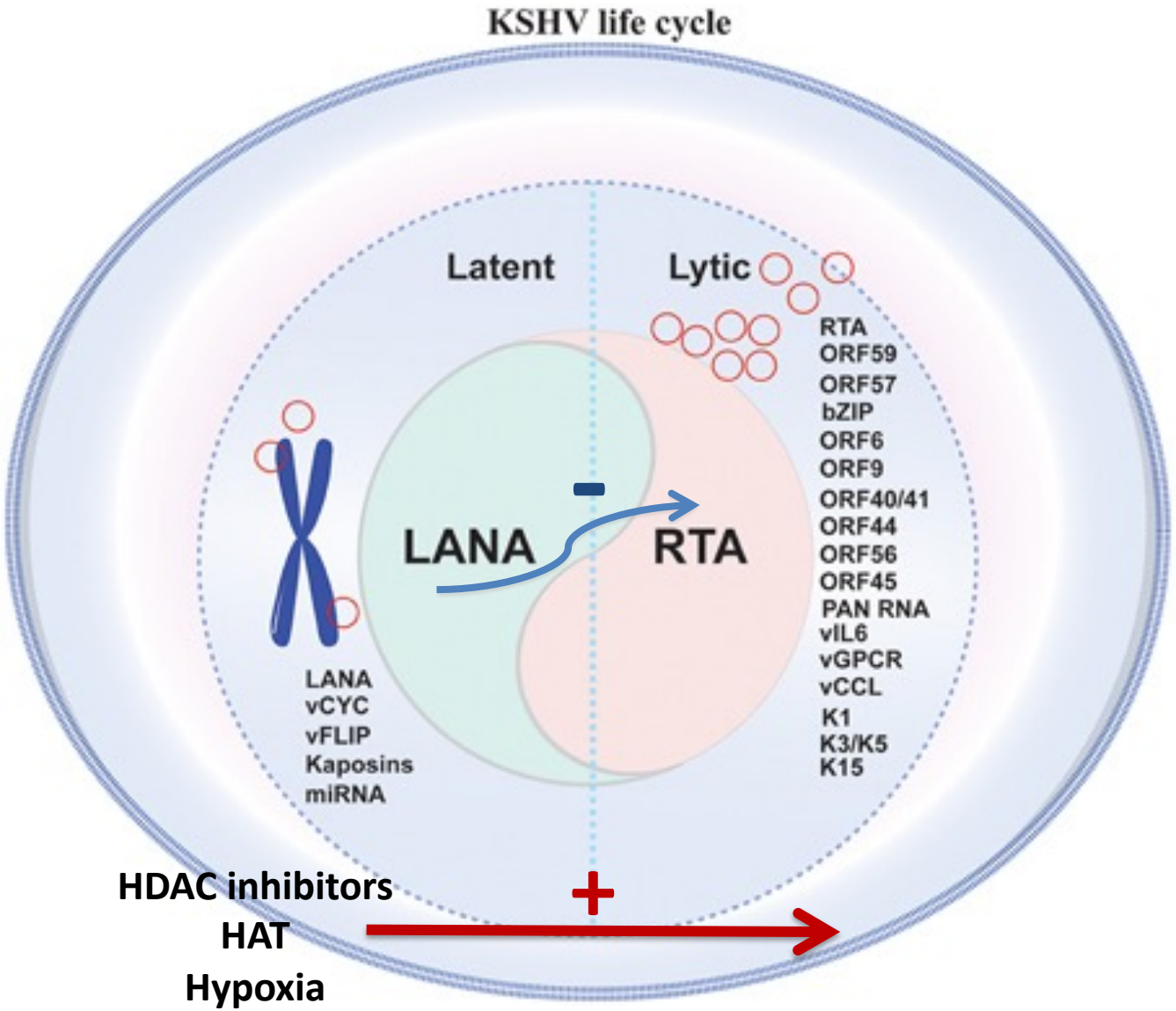


Various endocytic pathways
Latency establishment
Episomal maintenance
(LNA-mediated histone interaction)



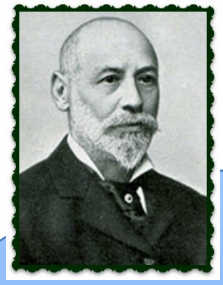
G. Fremont

Latent vs lytic viral cycles



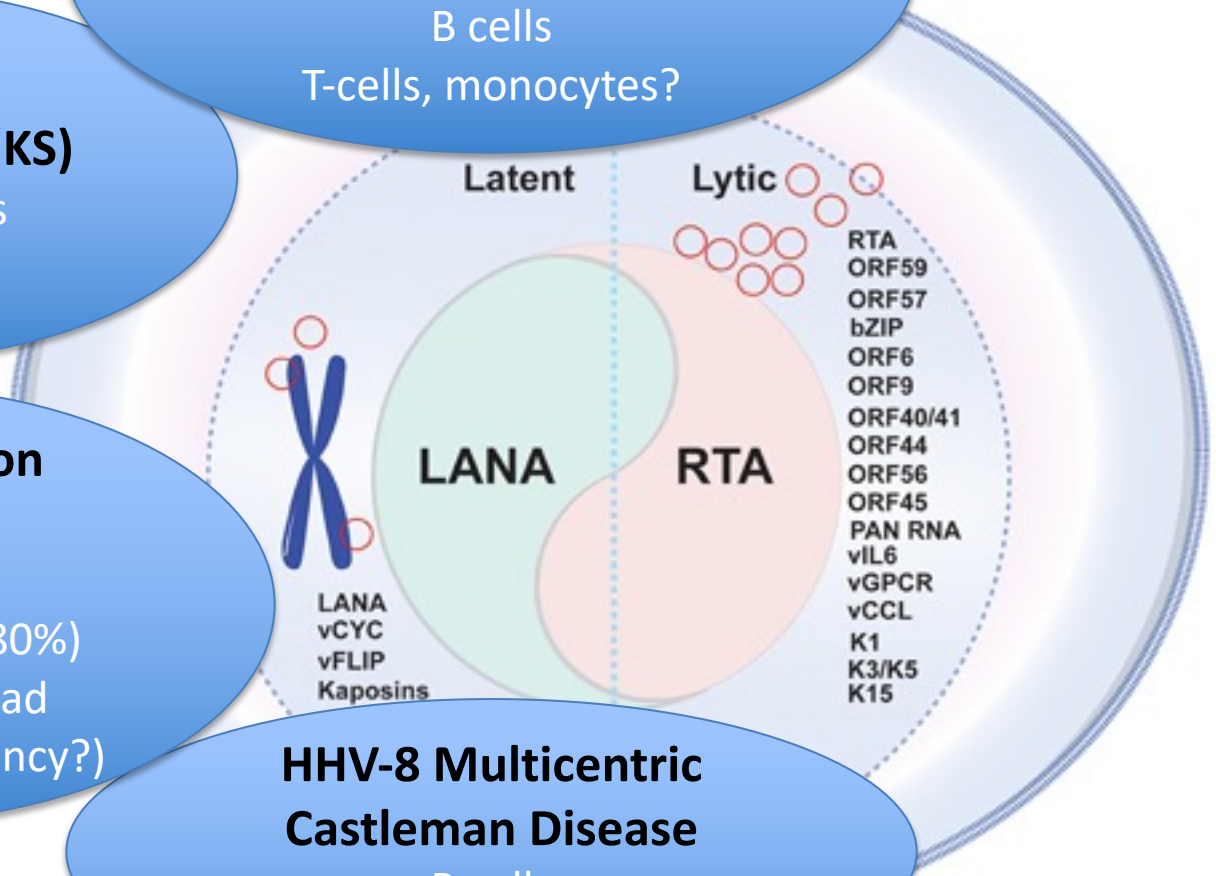
HHV8-associated diseases

Secondary (HIV, post-transplant) > primary ID setting



Kaposi Sarcoma (KS)
Endothelial cells

HHV-8 primary infection
Eptihelial cells
Endothelial cells
B cells
T-cells, monocytes?



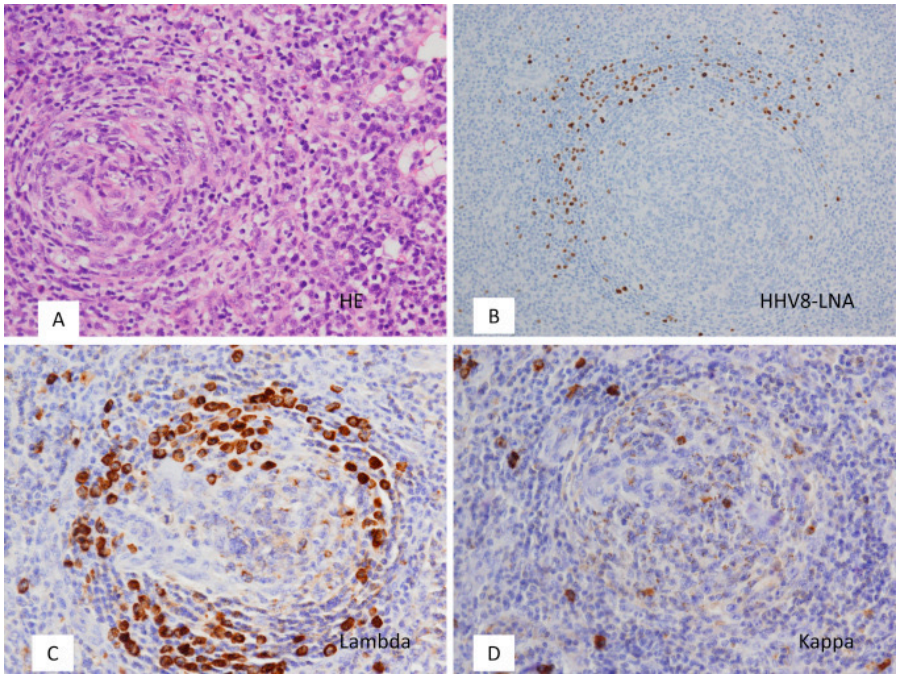
Primary Effusion Lymphoma
B cells
EBV co-infection (80%)
High mutation load
(DNA-repair deficiency?)

HHV-8 Multicentric Castleman Disease
B cells
T-cells, monocytes?

What is HHV8+ MCD?

Plasma-cell type MCD

Evidence of **HHV8 *in situ* infection** (LNA staining/qPCR)



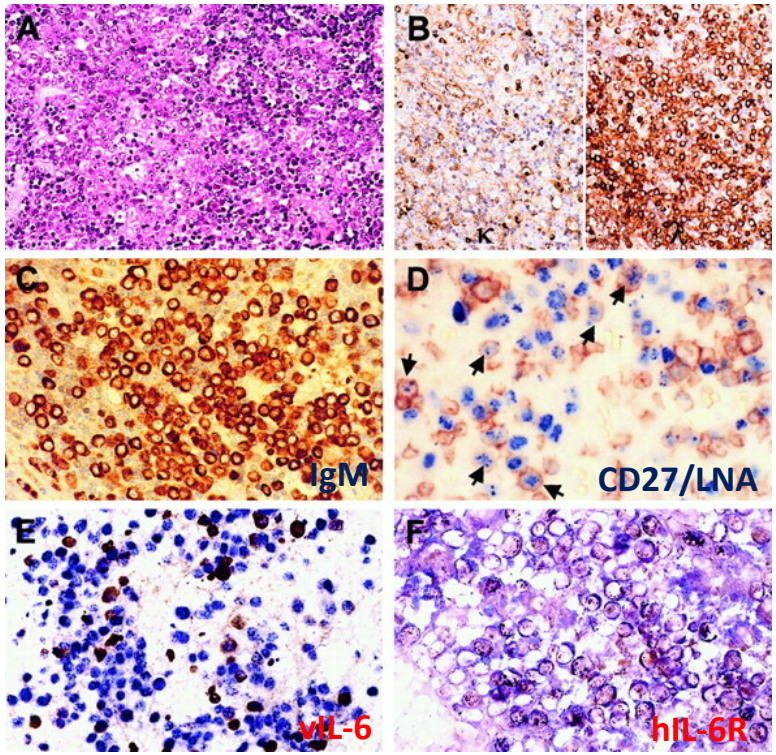
B-cell side

What drives HHV8+ plasmablast emergence?

T-cell side

Which T-cell defects allow HHV8+ MCD?

The B-cell side of HHV8+ MCD



Expansion of **monotypic (IgMλ) LNA+ plasmablast-like cells (CD19lowCD38++CD138-)**

CD27 and v-IL6 partial

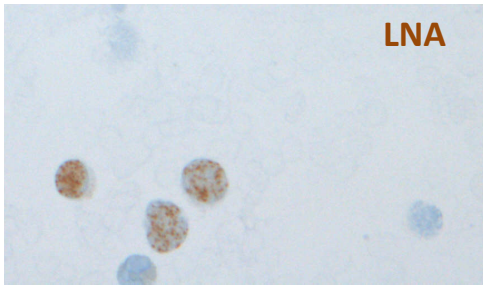
Most cells express human IL6-Receptor

Absent EBV integration (negative EBER HIS)

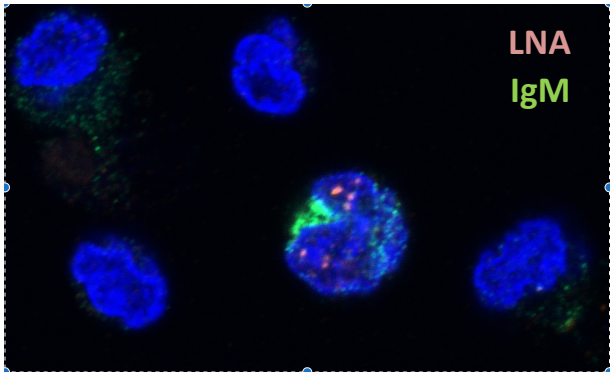
Du et al, Blood 2001

Flow cytometric phenotyping of LNA+ cells

LNA+ λ+ plasmablast-like cells are detectable in the blood of patients with active MCD and HHV-8 PCR>3 log (0.02-6% of PBMC)



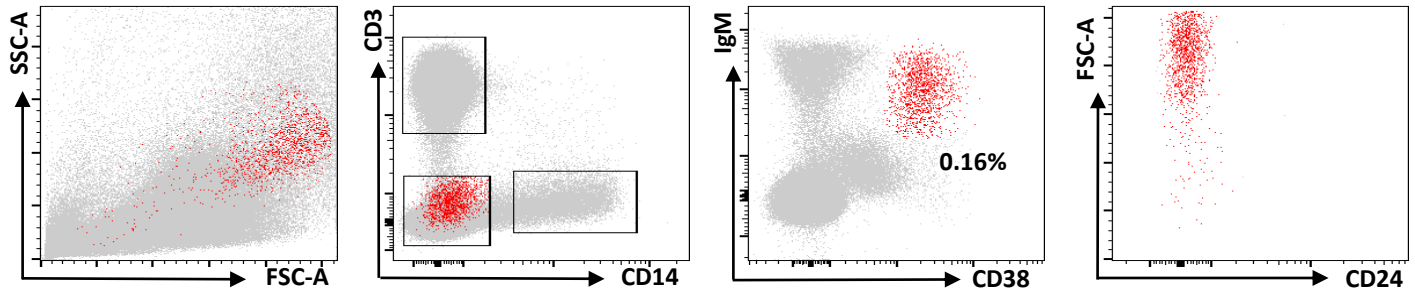
LNA staining, blood smear, V. Meignin



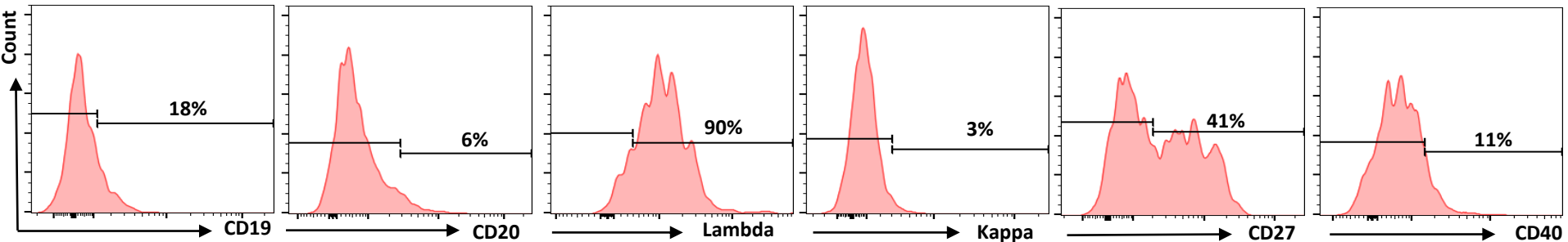
LNA/IgM staining, blood smear, G. Fremont

Their phenotype is similar to lymph node and splenic LNA+ cells and distinct from conventional plasmablasts found in reactive conditions

This could be a quick diagnostic tool for MCD and should be tested in KICS

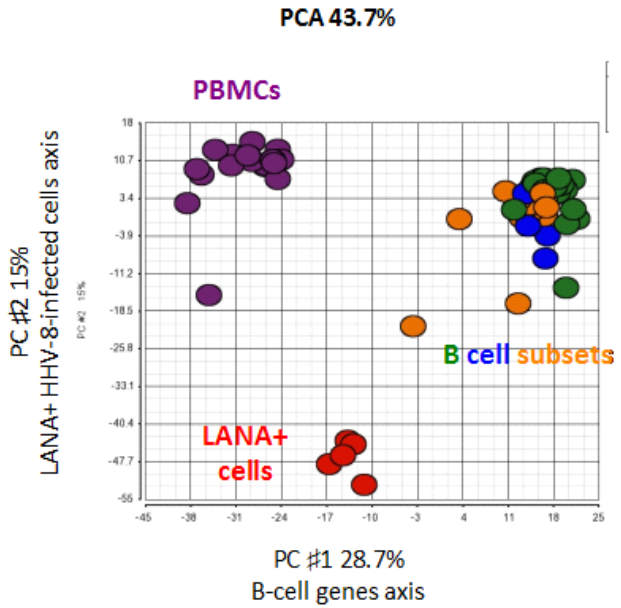


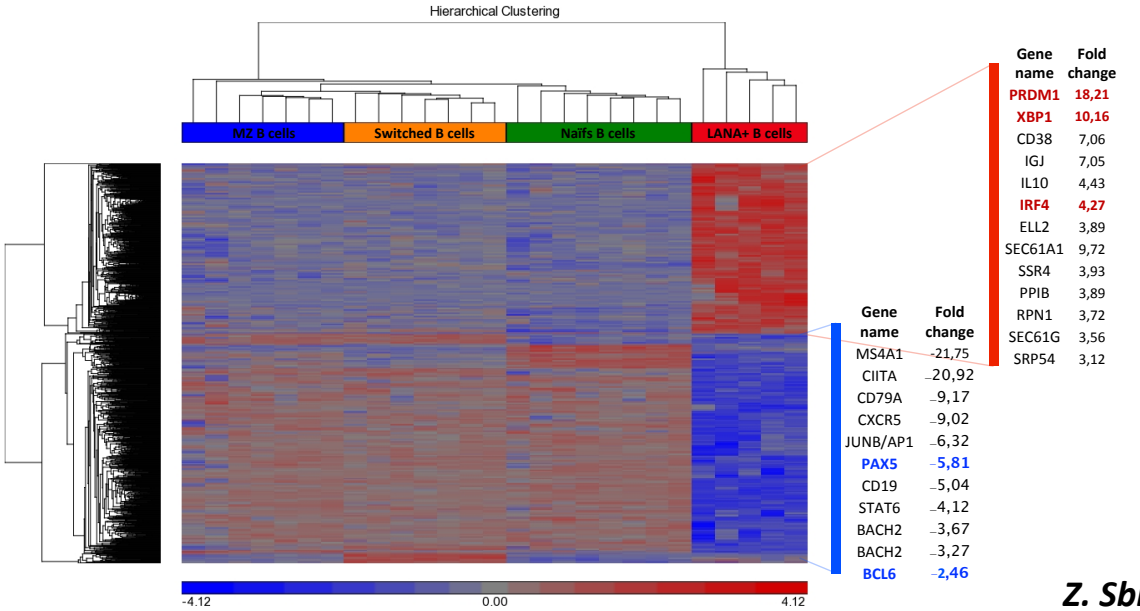
PBMcs from a patient with MCD flare
Frémont, Vanjak, in revision



LNA+ cells segregate from other B-cell subtypes and express a distinct plasma-cell signature

Microarray data from sorted populations





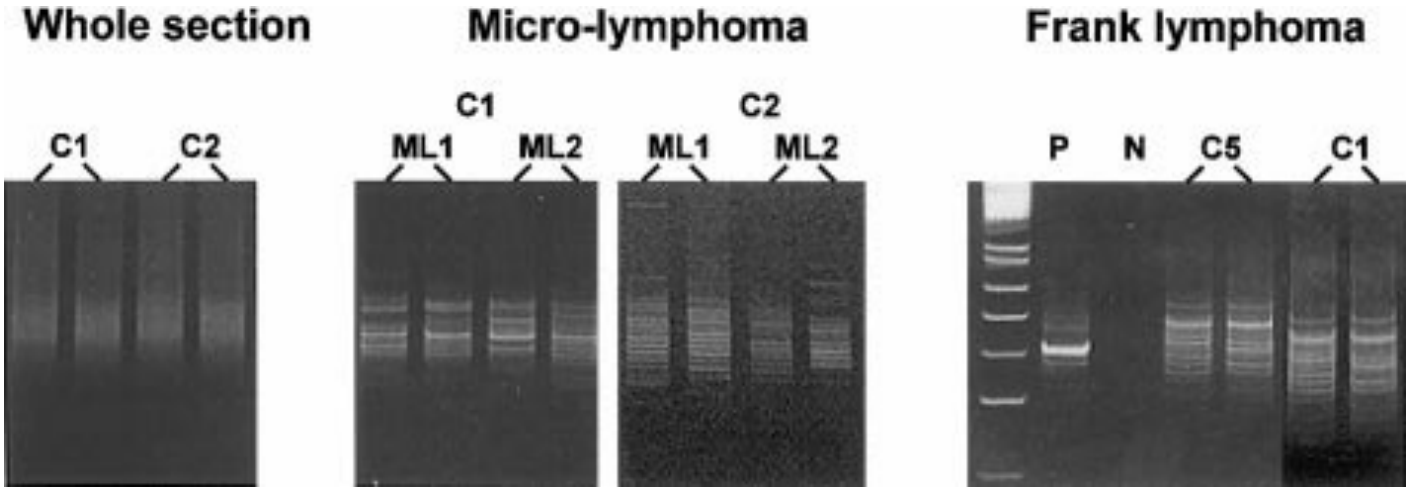
Gene name	Fold change
PRDM1	18,21
XBP1	10,16
CD38	7,06
IGJ	7,05
IL10	4,43
IRF4	4,27
ELL2	3,89
SEC61A1	9,72
SSR4	3,93
PPIB	3,89
RPN1	3,72
SEC61G	3,56
SRP54	3,12

Gene name	Fold change
MS4A1	-21,75
CIITA	-20,92
CD79A	-9,17
CXCR5	-9,02
JUNB/AP1	-6,32
PAX5	-5,81
CD19	-5,04
STAT6	-4,12
BACH2	-3,67
BACH2	-3,27
BCL6	-2,46

Z. Sbihi , unpublished data

The immune paradox of LNA+ cells

LNA+ cells are **polyclonal B cells** despite monotypic restriction



Fr3-JH PCR

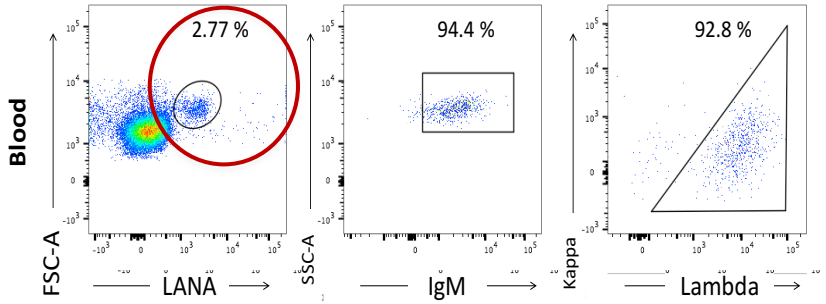
λ monotypic restriction may occur through **viral-induced and RAG-mediated DNA editing of B cells** (***Tontonchy, Plos Pathogen 2018***)

LNA+ cells show **no somatic hypermutations** despite a PB-like phenotype
This indicates that **naive B cells are putative targets of HHV8 in MCD**

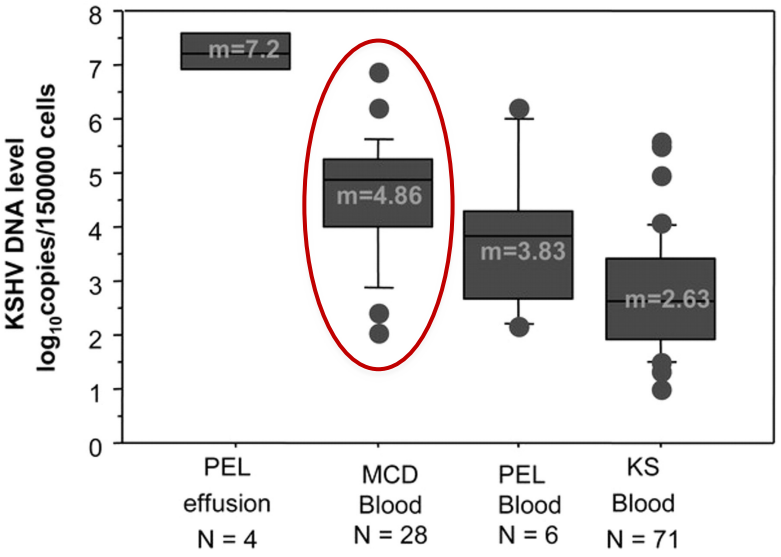
Du et al, Blood 2001

Further B-cell perspectives…

Is HHV8-MCD a pure latent disease?



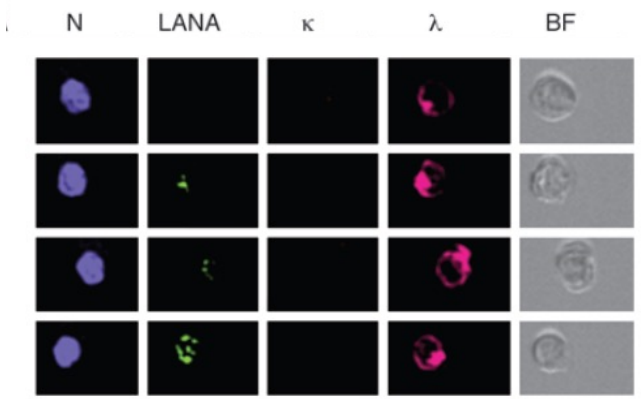
?

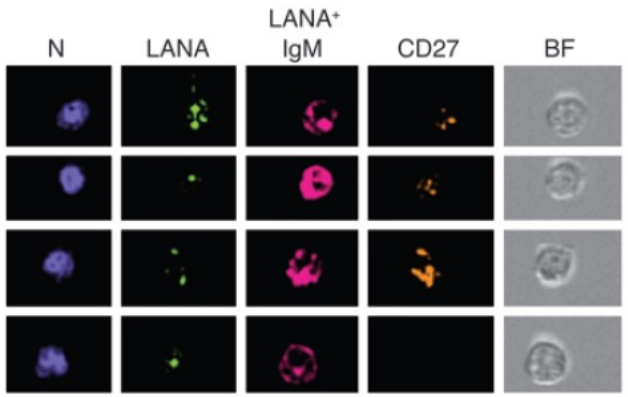


What are host and viral respective roles in MCD pathogenesis?

The tonsil model

HOST
hIL6,…

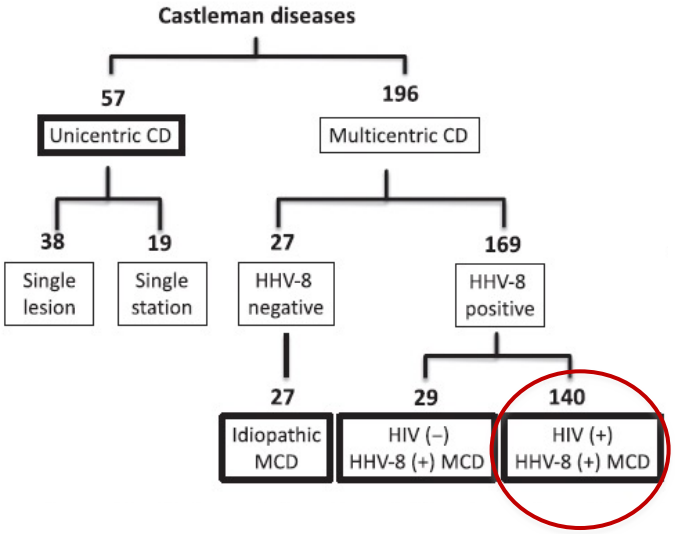




HHV8
vFLIP,…

Hassman et al, JCI 2011

Rationale for a T-cell side in MCD



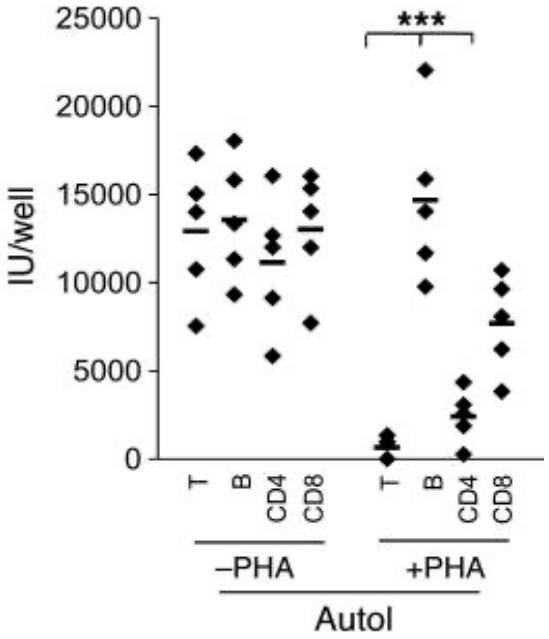
140/169 (82%) HHV8+ MCD patients are HIV co-infected

Induction of B Cell Hyperplasia in Simian Immunodeficiency Virus–infected Rhesus Macaques with the Simian Homologue of Kaposi's Sarcoma–associated Herpesvirus

By Scott W. Wong,*§ Eric P. Bergquam,* Ryan M. Swanson,* Felix W. Lee,* Stanley M. Shiigi,* Nancy A. Avery,* John W. Fanton,‡ and Michael K. Axthelm*

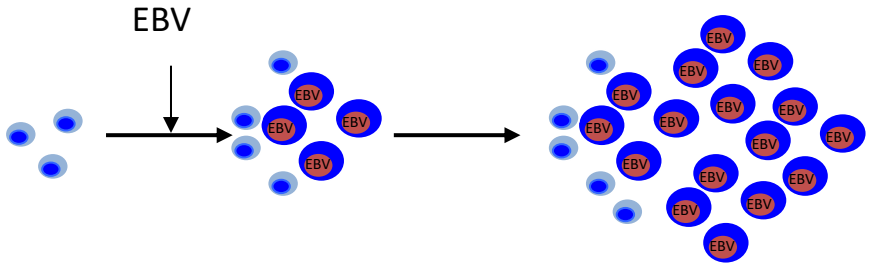
J Exp Med 1999

Activated CD4+ T-cell control of viral replication in B cells (Myoung, JCI 2011)

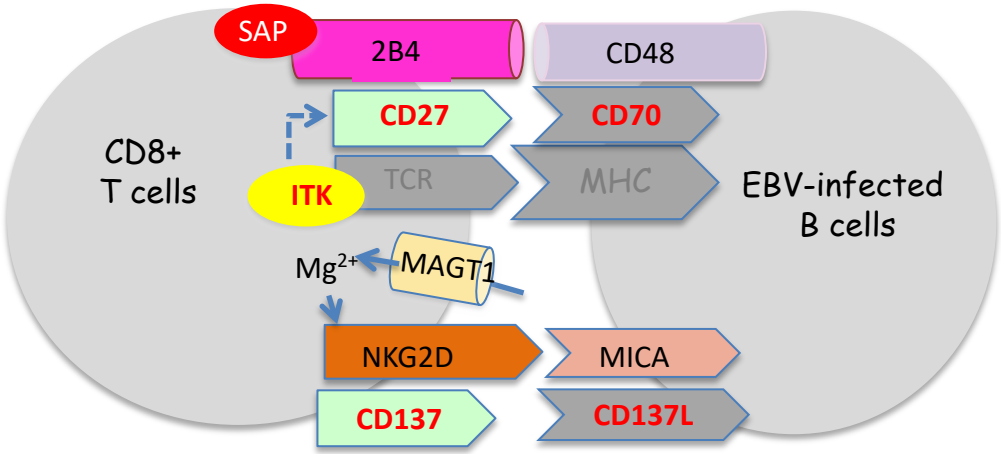


What could be learned from EBV-associated PIDs?

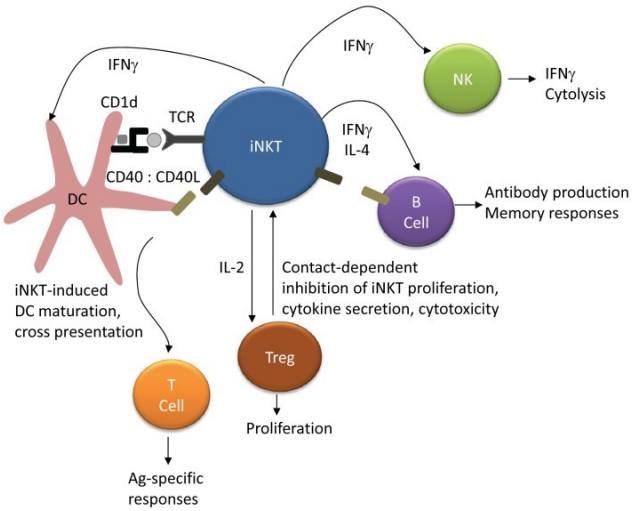
B-cell lymphoproliferation



Defective expansion and function of CD8+ T cells
Accumulation of proliferating infected B cells



Monogenic disorders associated with EBV PIDs
= T cell proliferation defects or **costimulation defects**

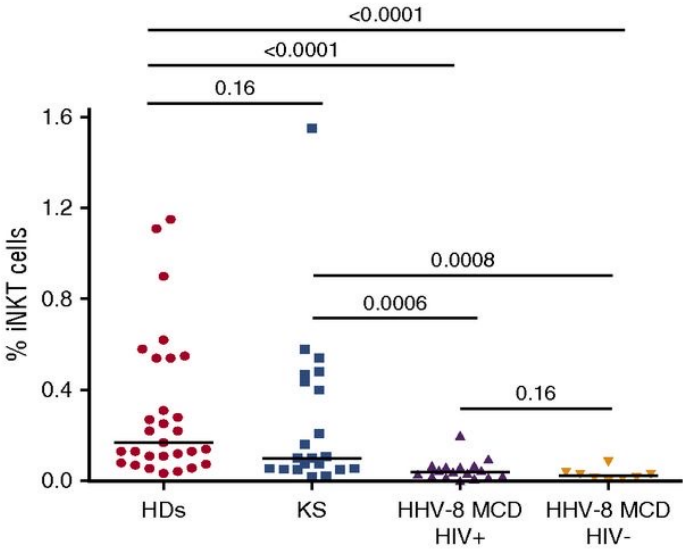


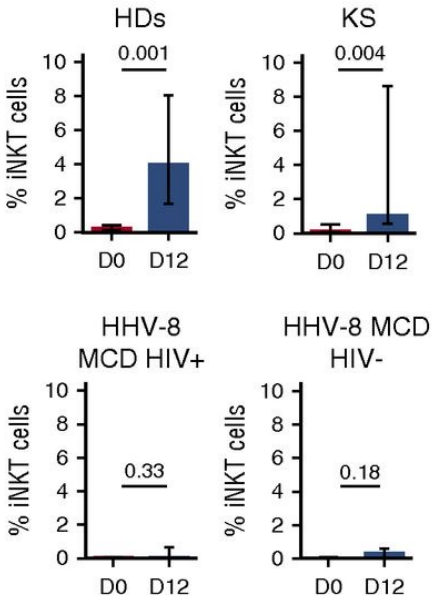
Invariant NKT cells are decreased in EBV-associated PIDs (extrinsical bystander effect on T-cell proliferation?)

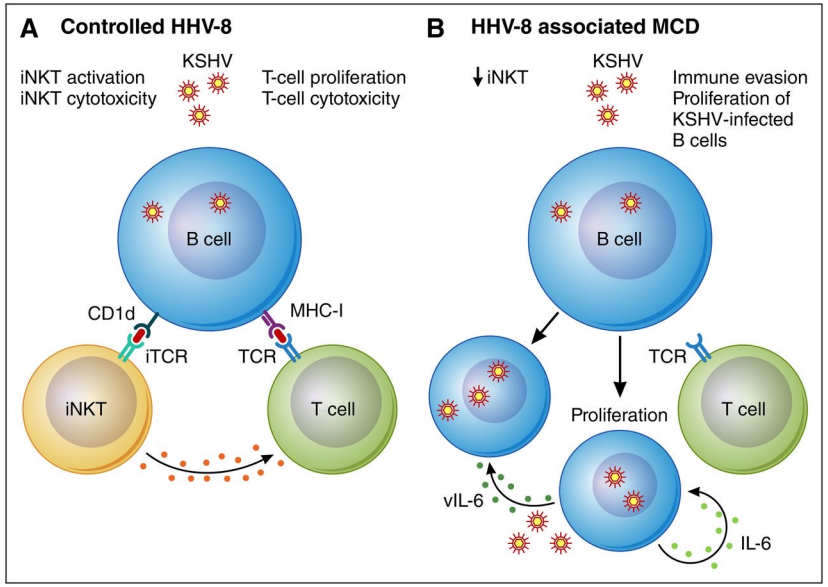
S. Latour, Nature (2006)
COACI (2013)
Nature (2014)
J Exp Med (2016, 2019)
EMBO Mol Med (2018)

The iNKT side of MCD

IL-2 + αGalCer stimulation







Sbihi et al, Blood 2017

What could be learned from HHV8 related PIDs?

HHV8-related diseases are **weakly penetrant** in children, even in children with primary or secondary (HIV+) immune deficiencies in endemic areas (role of chronic infection?, high redundancy of immune clearance mechanisms towards HHV8?)

No known monogenic causes of HHV8+ MCD to date

Few genetically-defined PIDs have been associated with Kaposi Sarcoma

TABLE II. Genetic Predisposition to Pediatric Kaposi Sarcoma

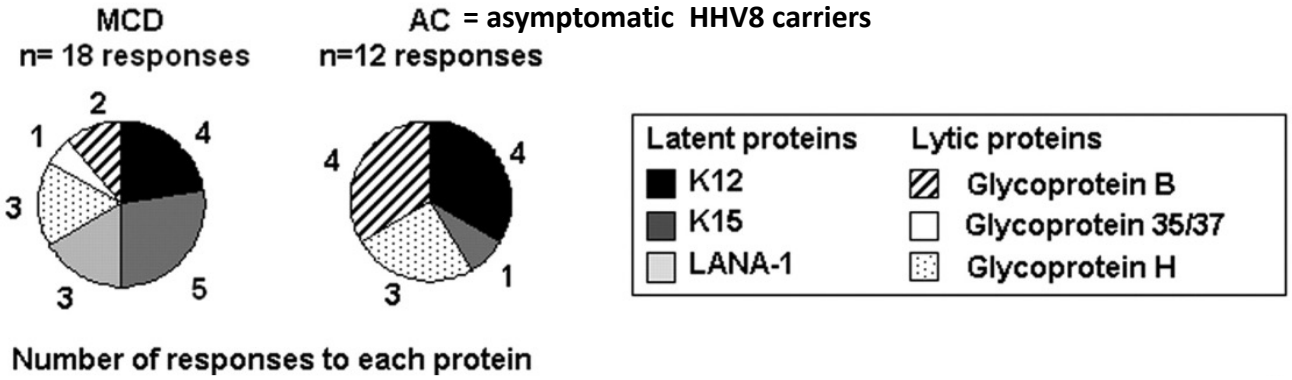
Gene	Type	Inheritance	PID	Onset age	Presentation	Clinical outcome
WAS	Classic	XR	Wiskott-Aldrich syndrome	14 months	Aggressive disseminated cutaneous and systemic KS	Complete remission
IFNGR1	Classic	AR	IFN-γR1 deficiency	10 years	Aggressive disseminated cutaneous and systemic KS	KS progression and death
STIM1	Classic	AR	STIM1 deficiency	2 years	Aggressive disseminated cutaneous and systemic KS	KS progression and death
TNFRSF4	Classic	AR	OX40 deficiency	14 years	Aggressive disseminated cutaneous and systemic KS	Complete remission

Abbreviations: KS, Kaposi sarcoma; XR, X-linked recessive; AR, autosomal recessive; PID, pediatric immunodeficiency.

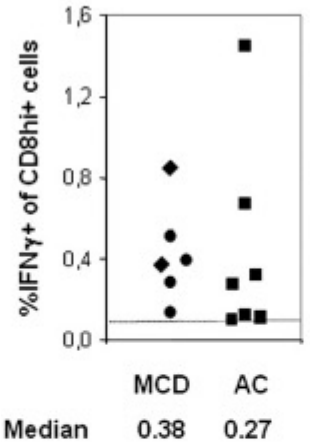
Jackson et al, Ped Blood Cancer 2016

Further T-cell perspectives…

Multicentric Castleman disease is associated with polyfunctional effector memory HHV-8–specific CD8⁺ T cells (Guihot et al, Blood 2008)

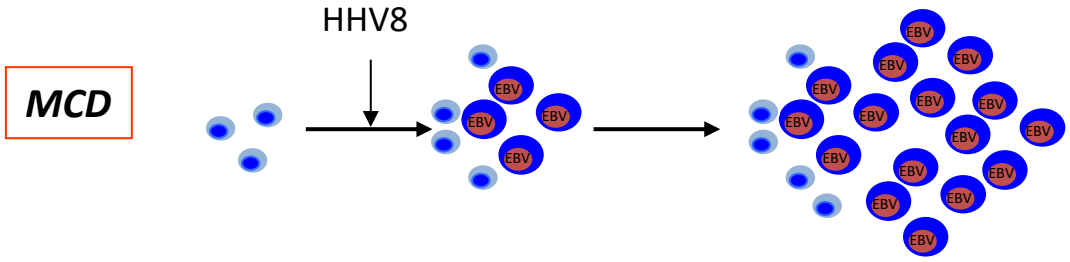


ELISPOT and IC



Same results for
IL-2
TNFα
CD107
MIP1

Are these data reproducible in a cell-to-cell setting?
What are the role of costimulatory molecules in MCD pathogenesis?



Defective expansion and function of CD8+ T cells?
Accumulation of proliferating infected PB like cells

Summary

HHV8+ MCD is a complex B- and T-cell disorder, sharing similarities with EBV-driven lymphoproliferative disorders

B-cell side

- Infection of naive B cells leads to the emergence of λ monotypic and naive plasmasblast-like cells
- These cells are detectable in the blood of MCD patients
- Host and viral factors are implicated in B-cell transformation
- Viral-induced and RAG-mediated DNA editing may explain monotypic restriction despite a polyclonal B-cell repertoire

T-cell side

- HHV8+ MCM mainly occurs in a T-cell deficiency setting
- These defects involve CD4+, CD8+ but also iNKT cells
- Despite polyfunctional HHV8 specific CD8+ T cells, subtle defects including costimulatory defects (CD40, Ox40 pathways) may lead to T-cell proliferation defects towards HHV8 infected cells

Thank you!

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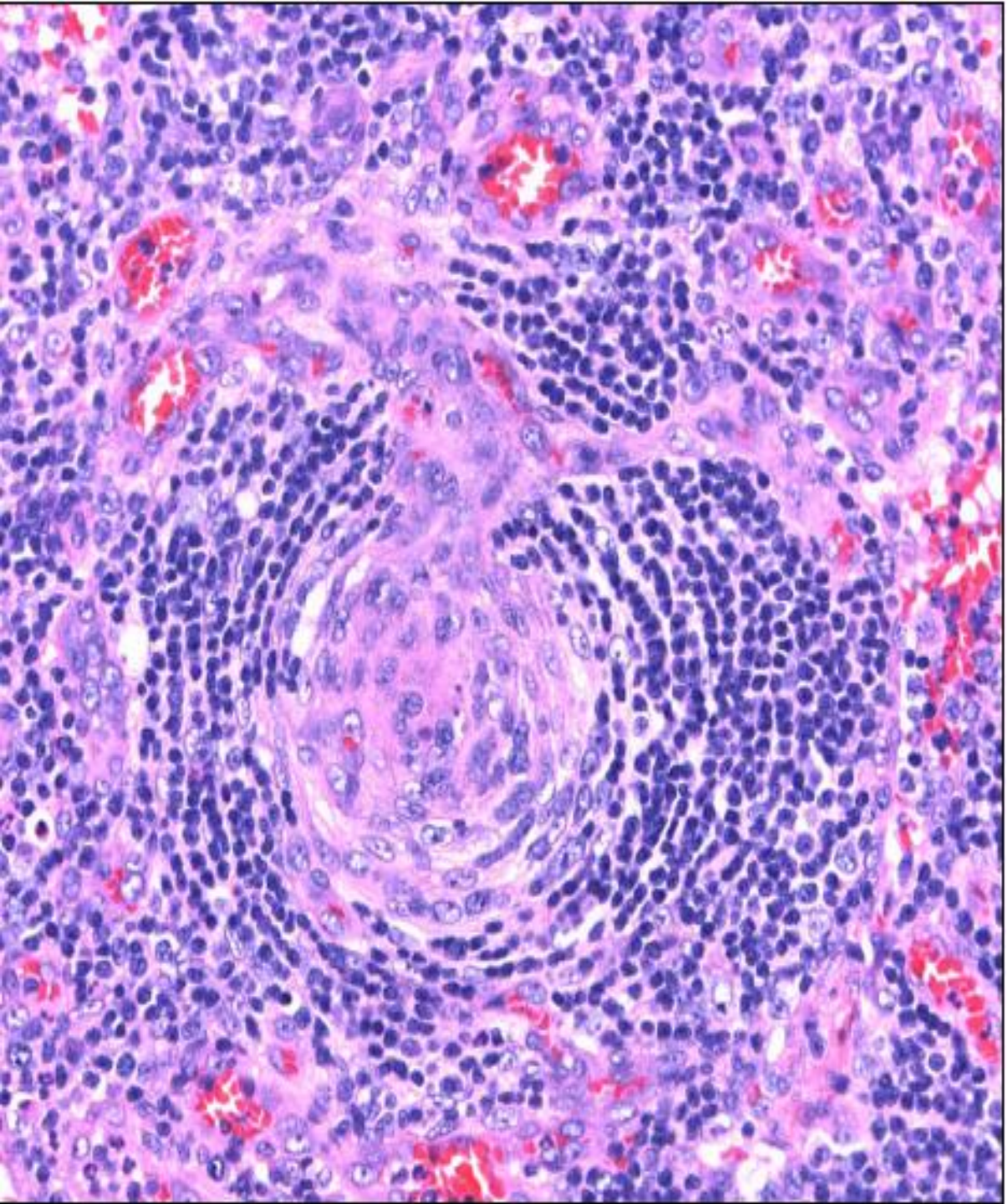
UMR1163, INSTITUT IMAGINE

S. Latour, A. Fischer, JP. de Villartay E. Martin, S. Winter, B. Fournier

CASTLEMAN DISEASE COLLABORATIVE NETWORK



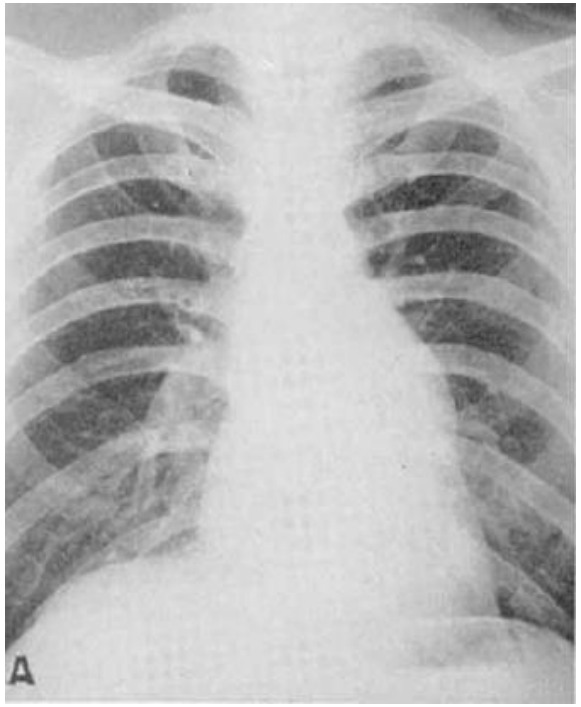
KSHV/HHV8 related Castleman's disease

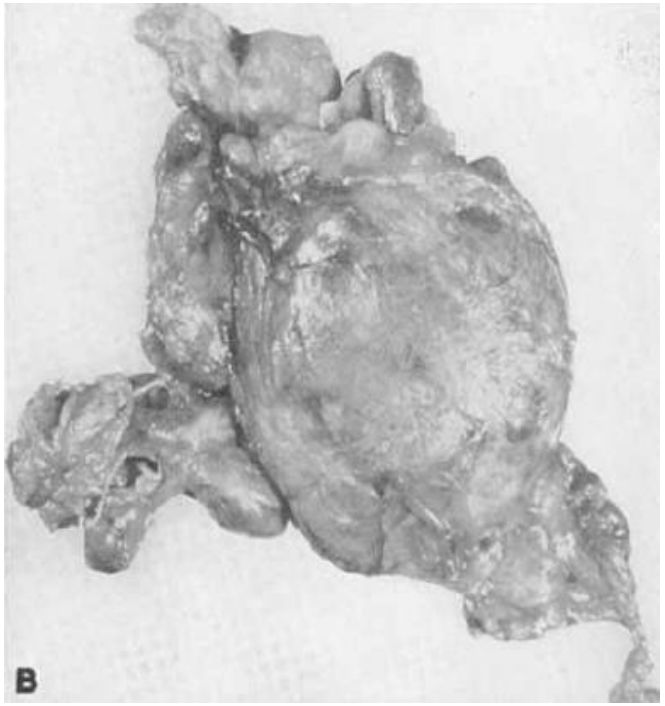
Benjamin Castleman

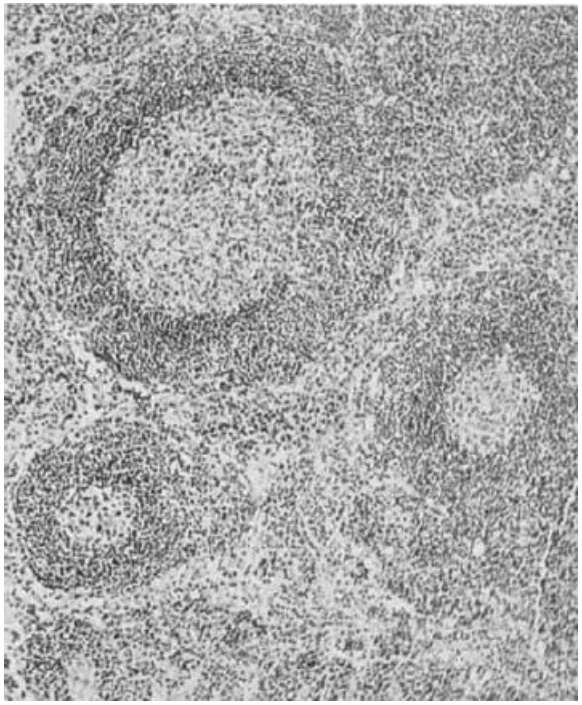
Castleman B and Towne VW: *Case Records of Massachusetts General Hospital, Case 40001.* NEJM,1954; 26: 250

Castleman B, Iverson L, Menendez VP: *Localized mediastinal lymph-node hyperplasia resembling thymoma.* Cancer, 1956; 9: 822-830

The first ever case….

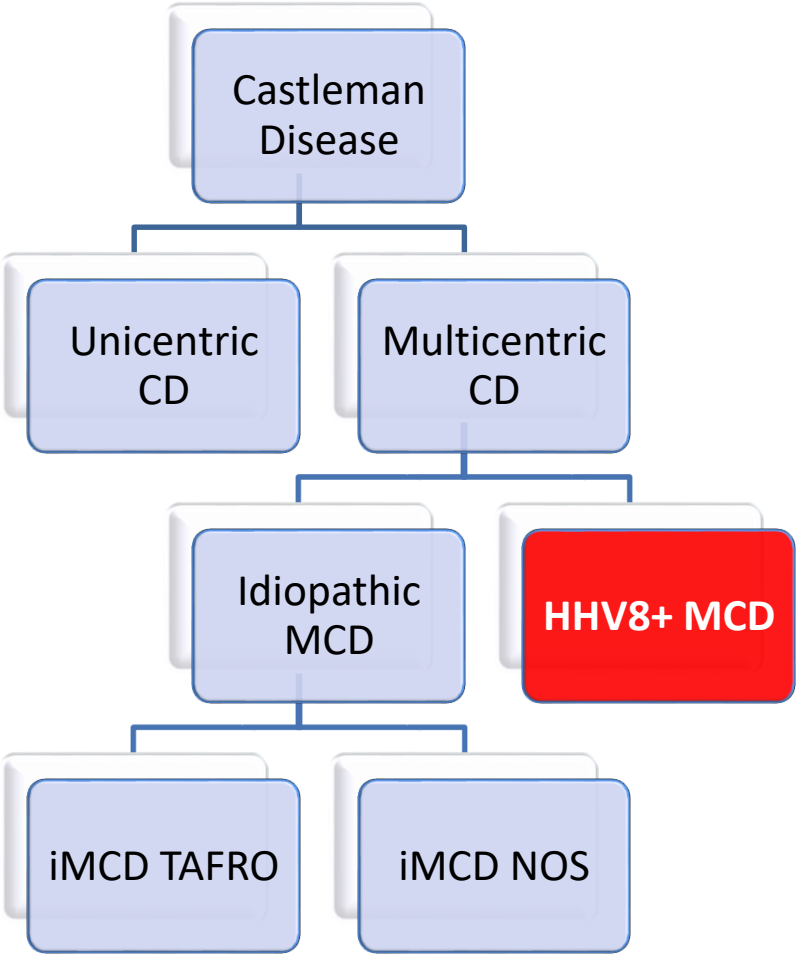


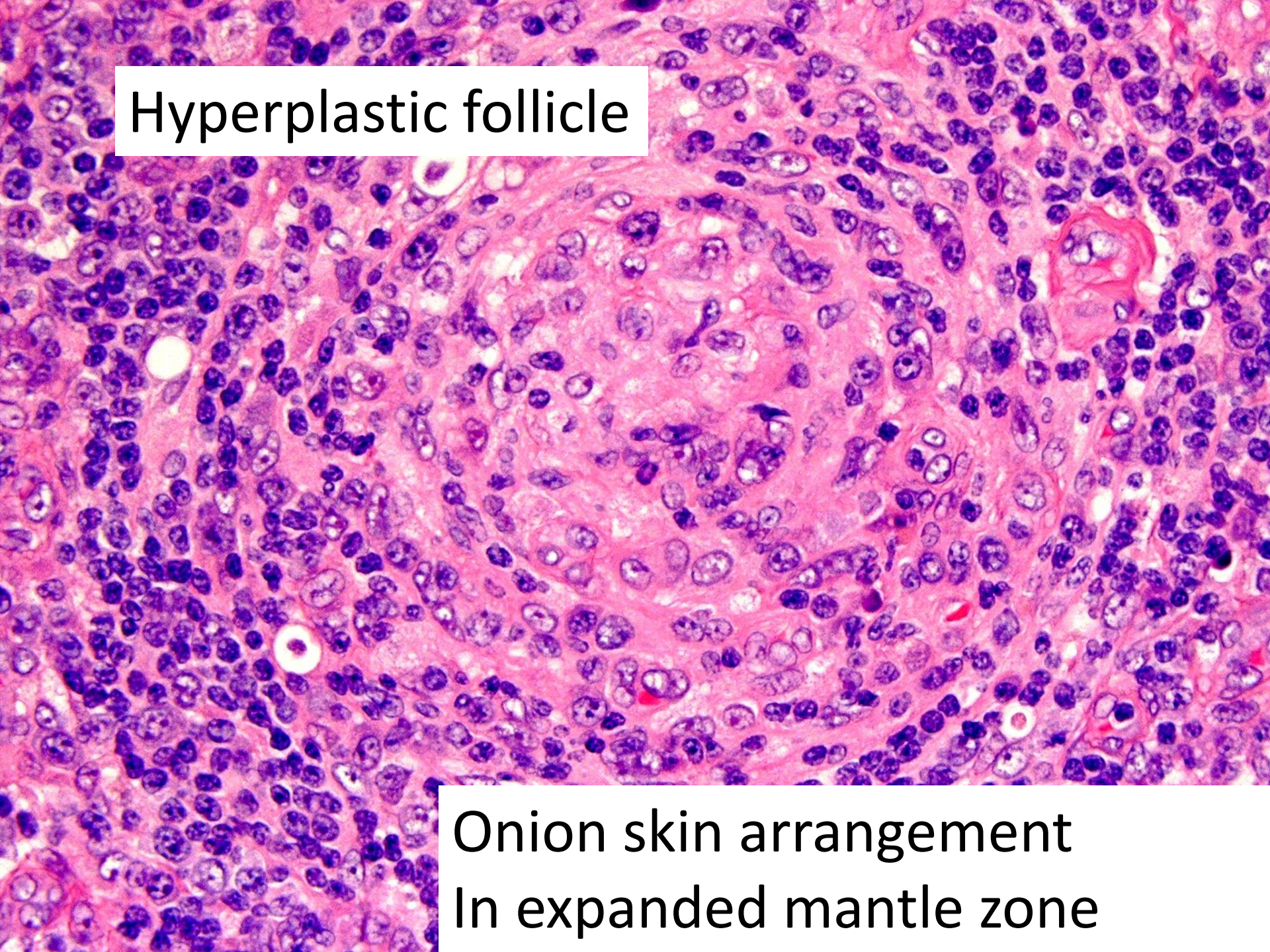




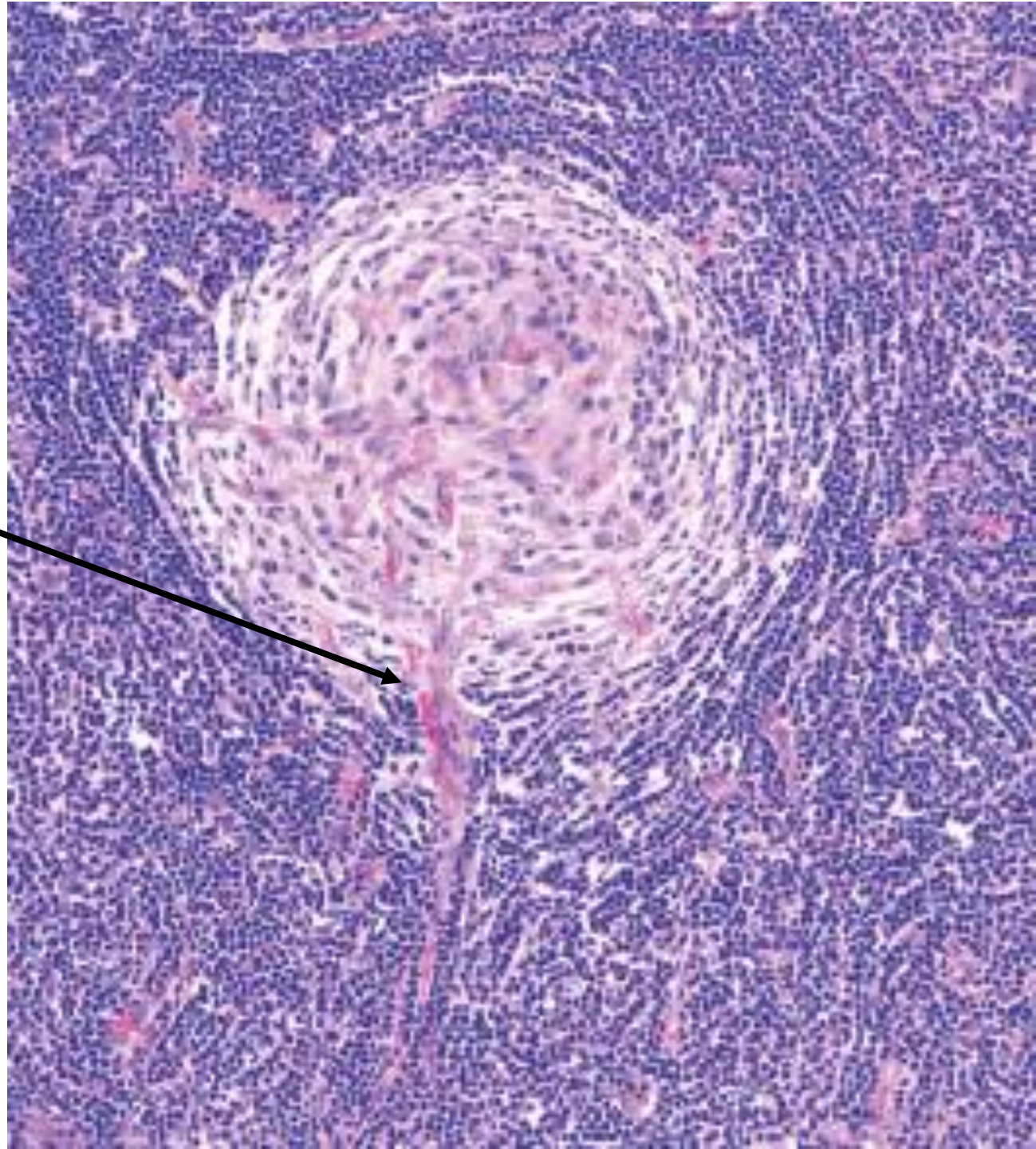
NEJM 1954; 250: 26-30

A classification of Castleman disease

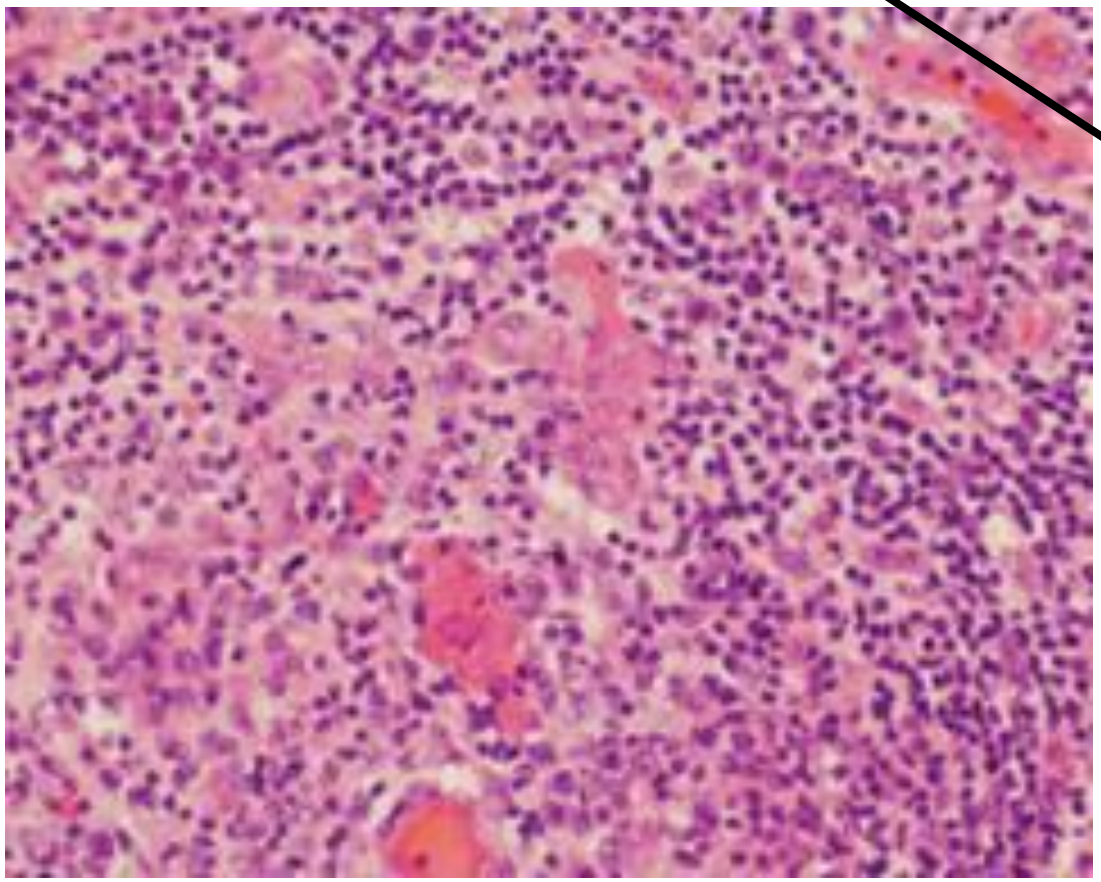


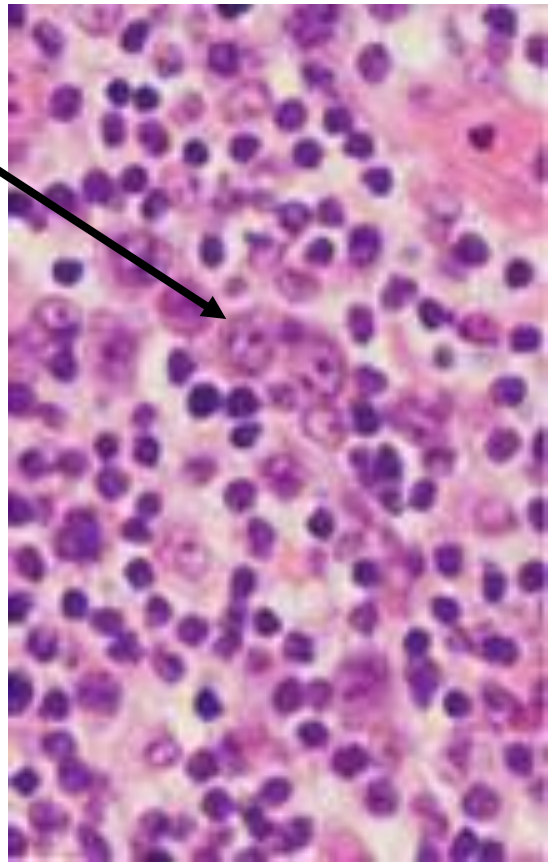

Hyperplastic follicle
Onion skin arrangement
In expanded mantle zone

Germinal centre pierced by blood vessel

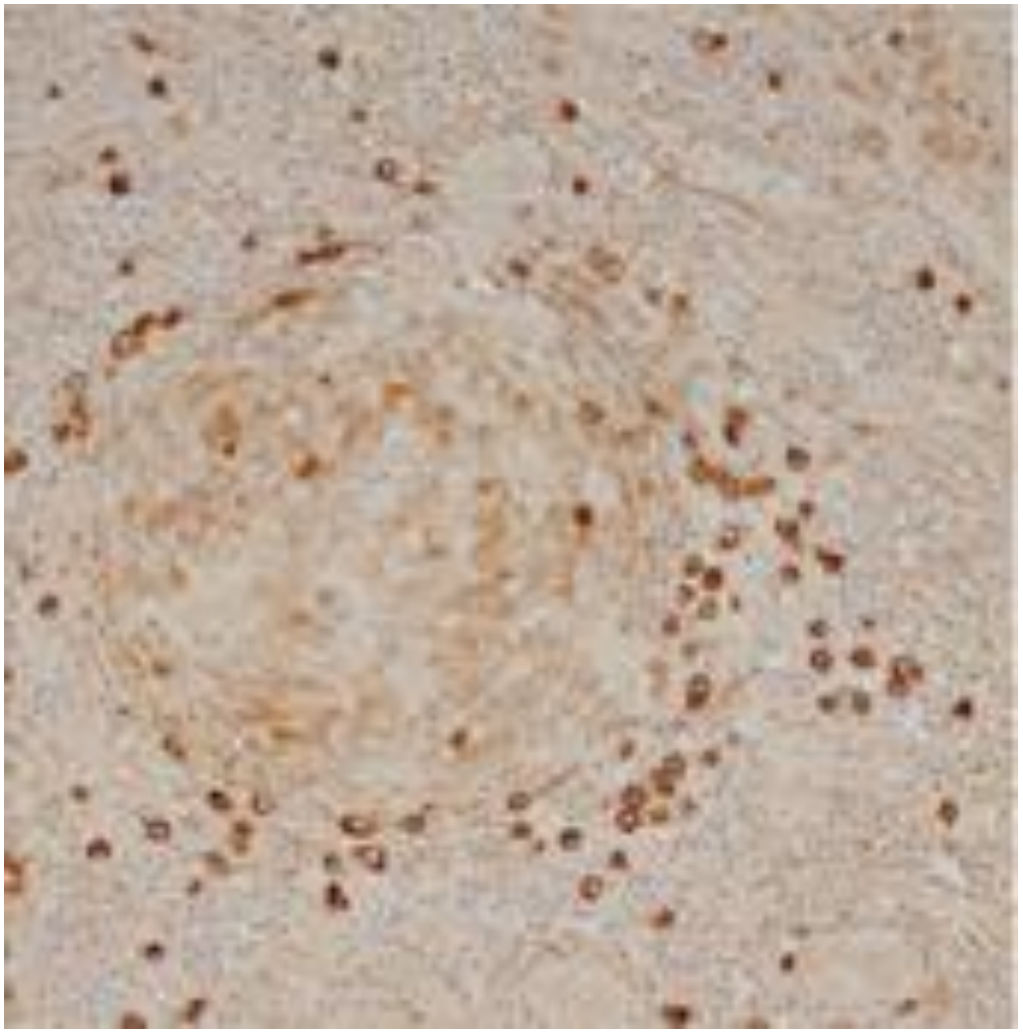


Mantle zone with large lymphoid cells with prominent nucleoli (plasmablasts)

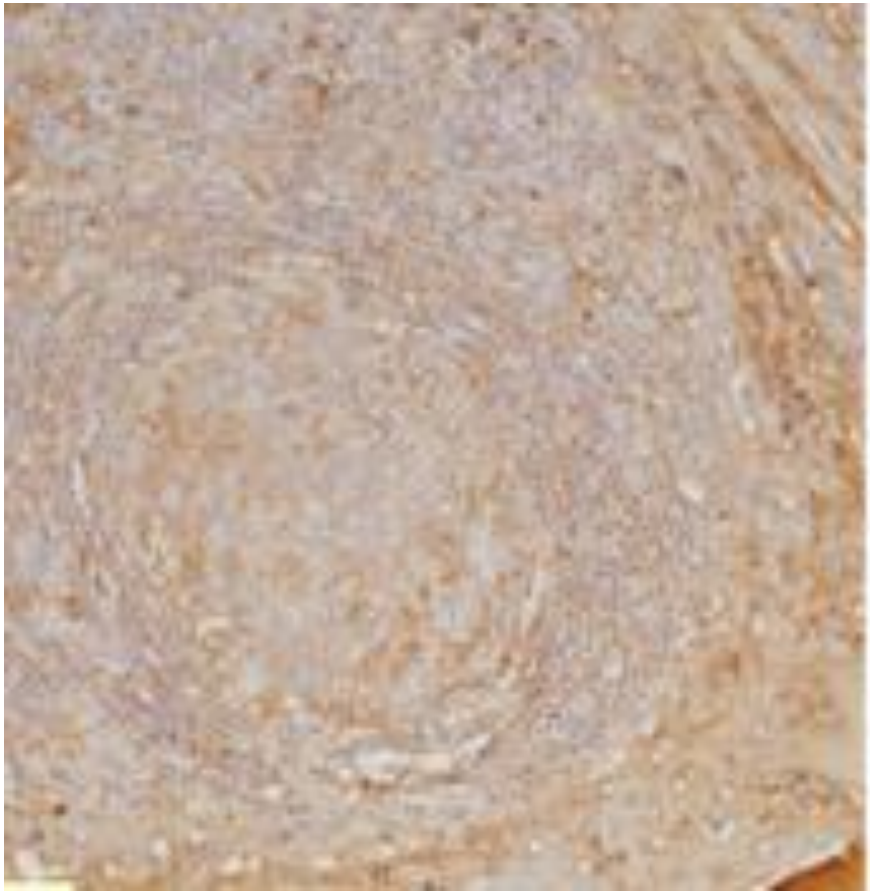




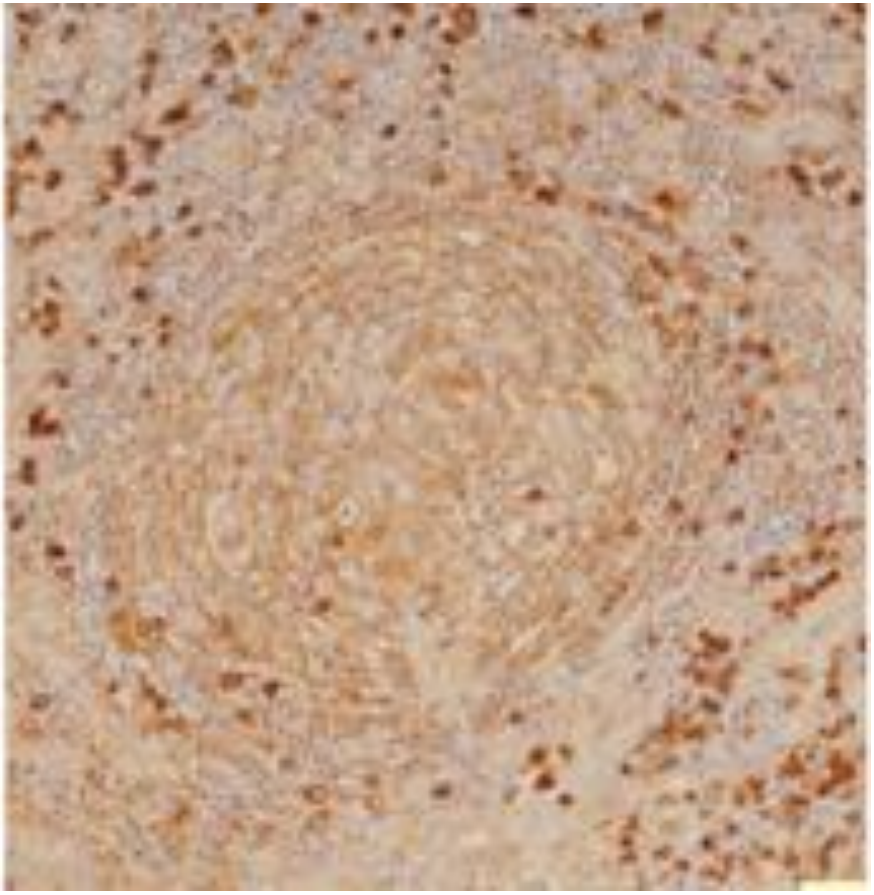
Plamablasts IgM+



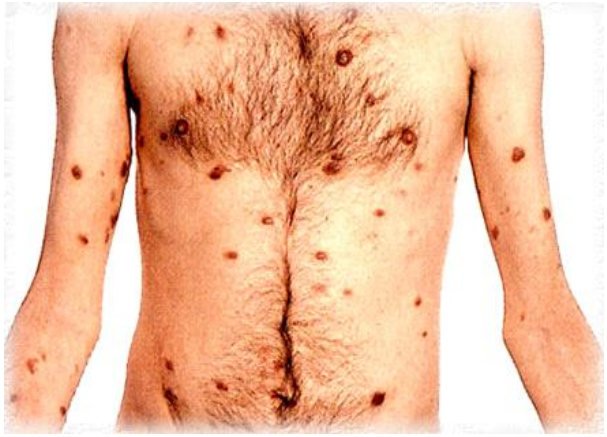
Plasmablasts λ light chain restricted



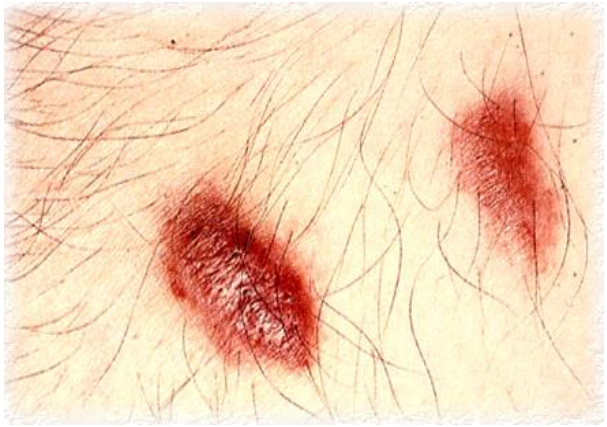
κ Light chain



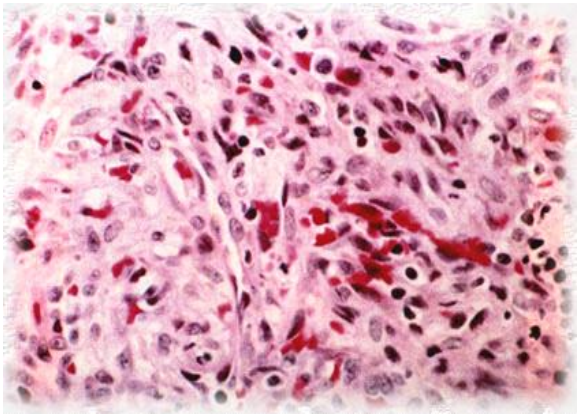
λ Light chain



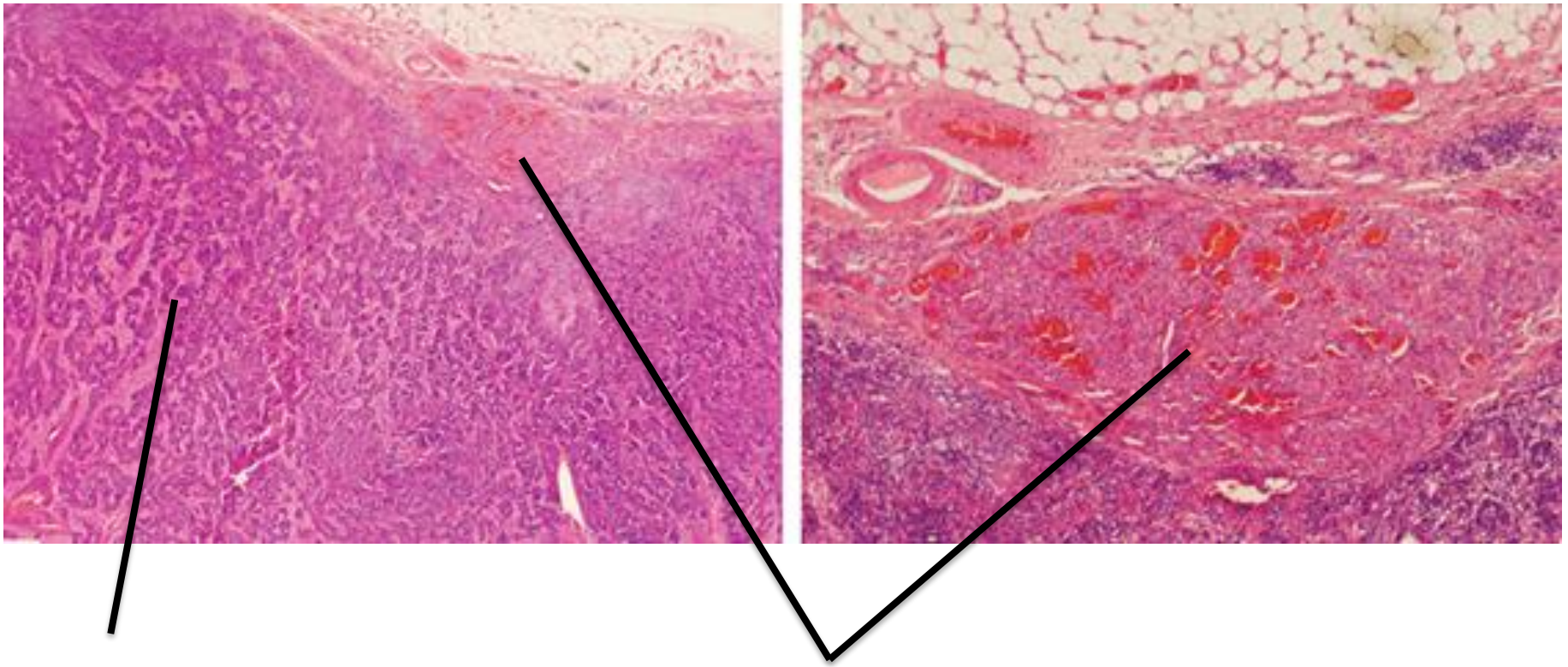
HIV-MCD associated with KS



60% of 72 MCD patients had KS at MCD diagnosis

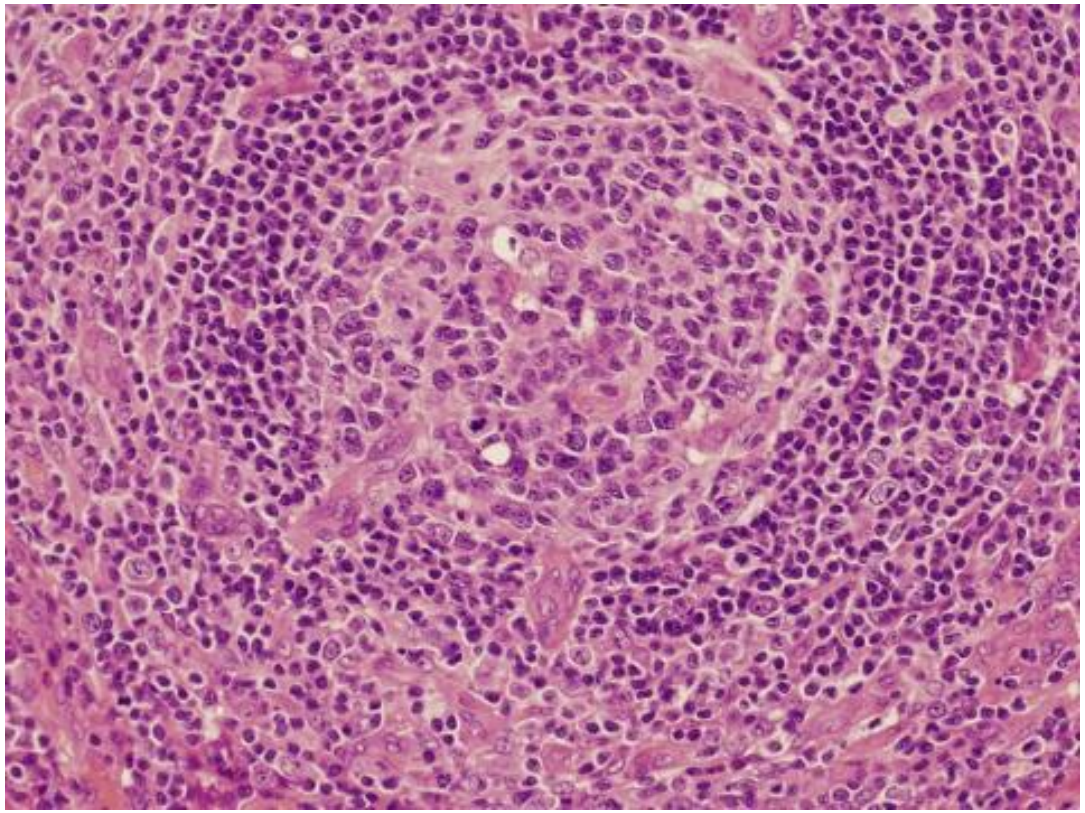


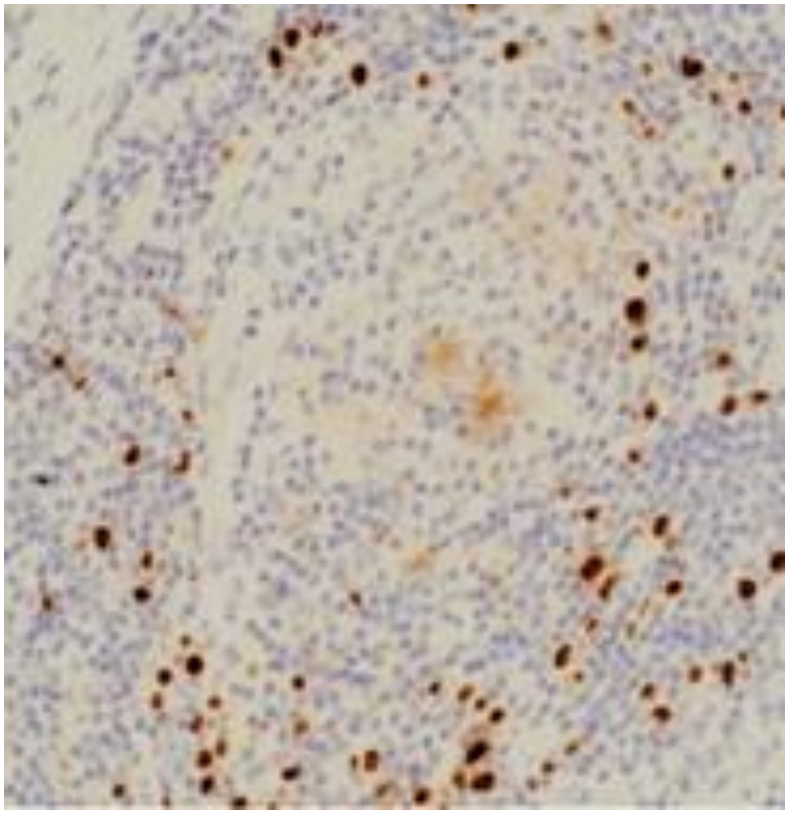
MCD & microscopic KS in LN



Am J Surg Pathol. 2008; 32:1006-12

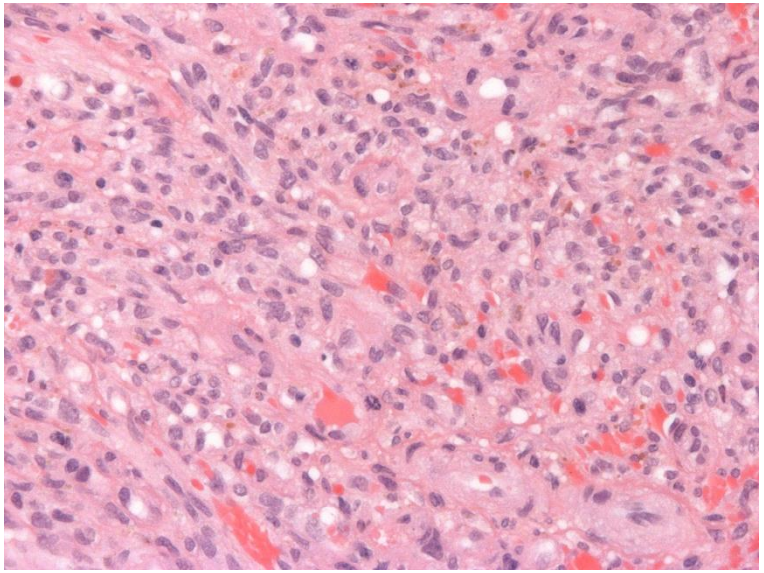
Plasmablasts infected by HHV8



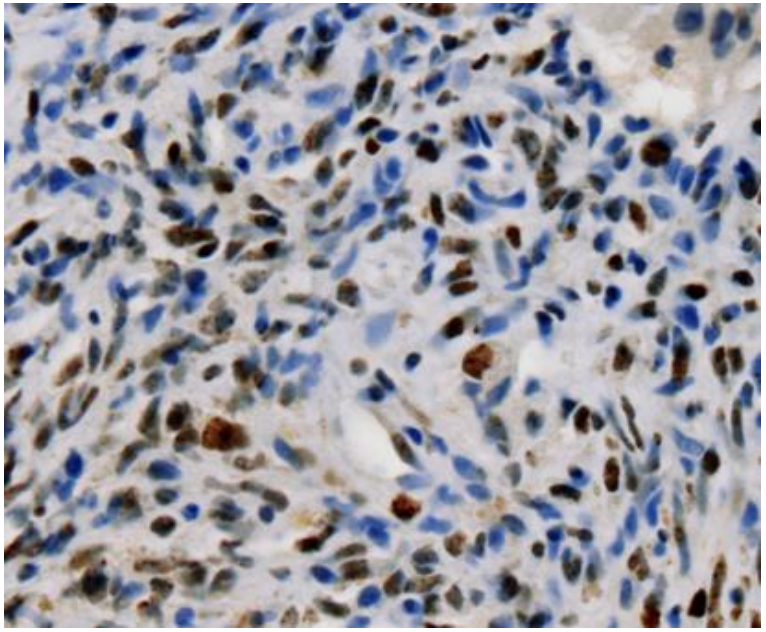


HHV8 LANA staining

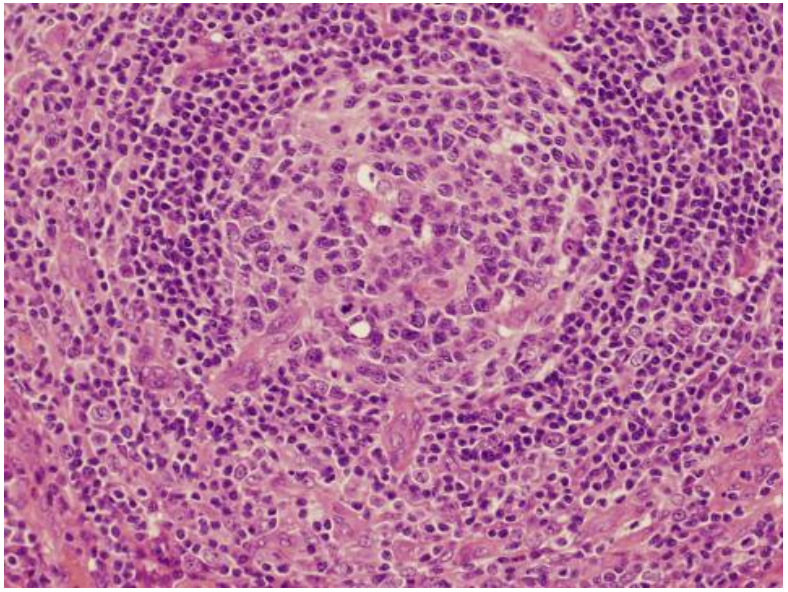
Kaposi sarcoma



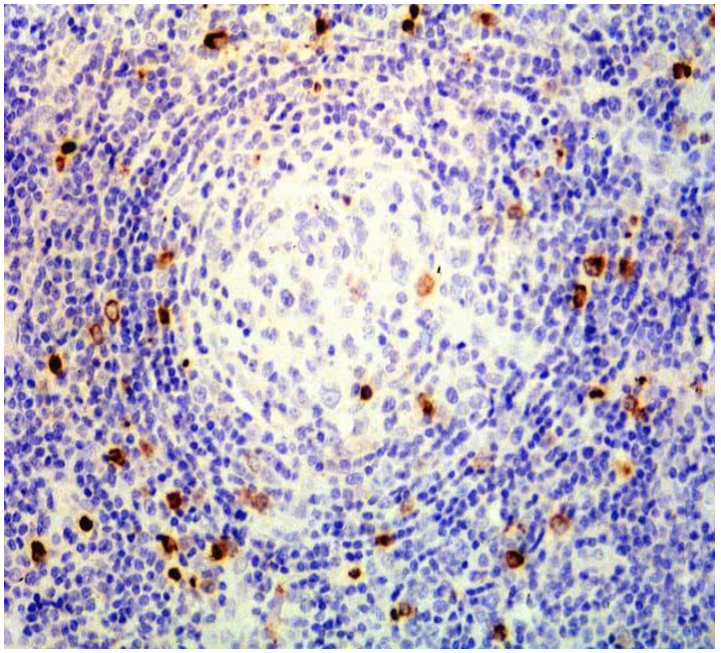
KSHV is
latent (LANA)



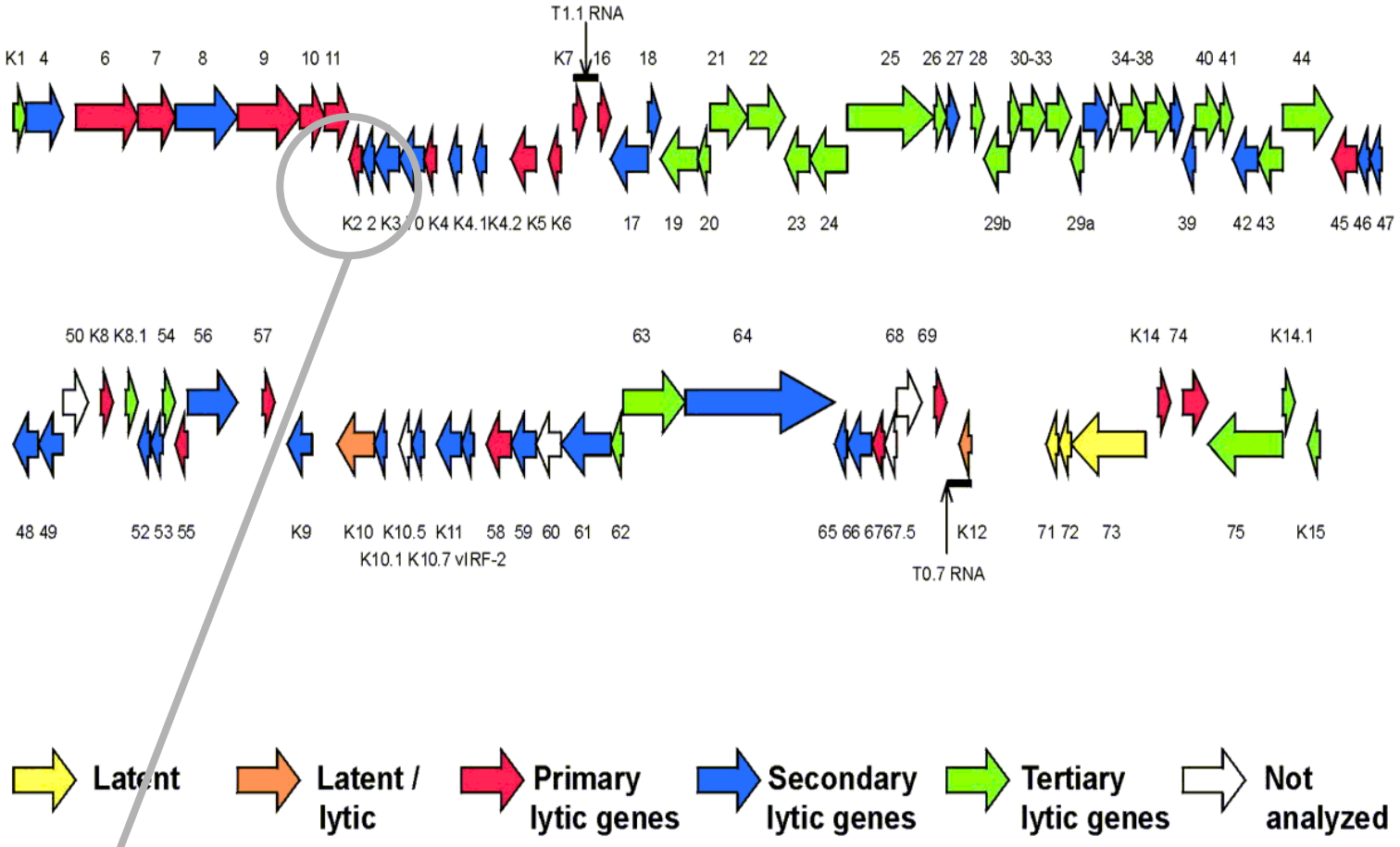
Castleman's disease



KSHV is *lytic* (vIL6 staining in MCD)



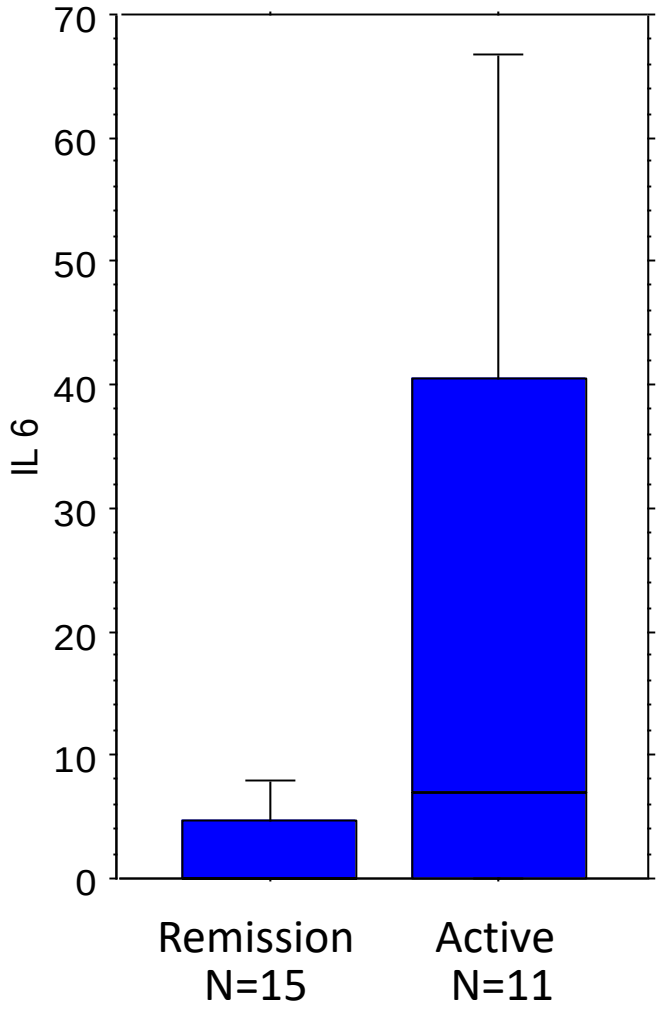
HHV8 genome expression (lytic/latent)



ORF K2 (vIL6) a primary lytic gene

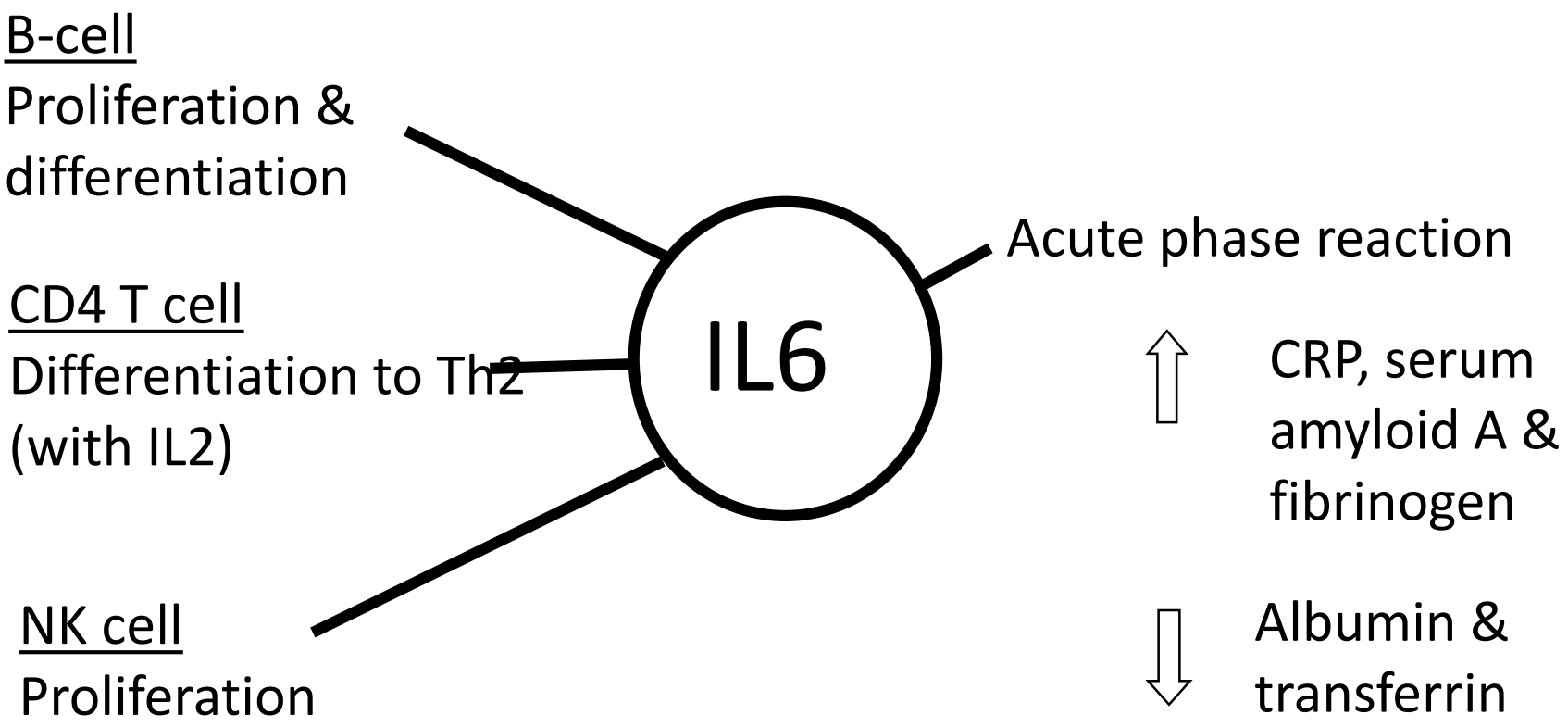
Serum IL6 in MCD

Serum IL6



MW p=0.06

Functions of IL6



What's an attack of MCD?

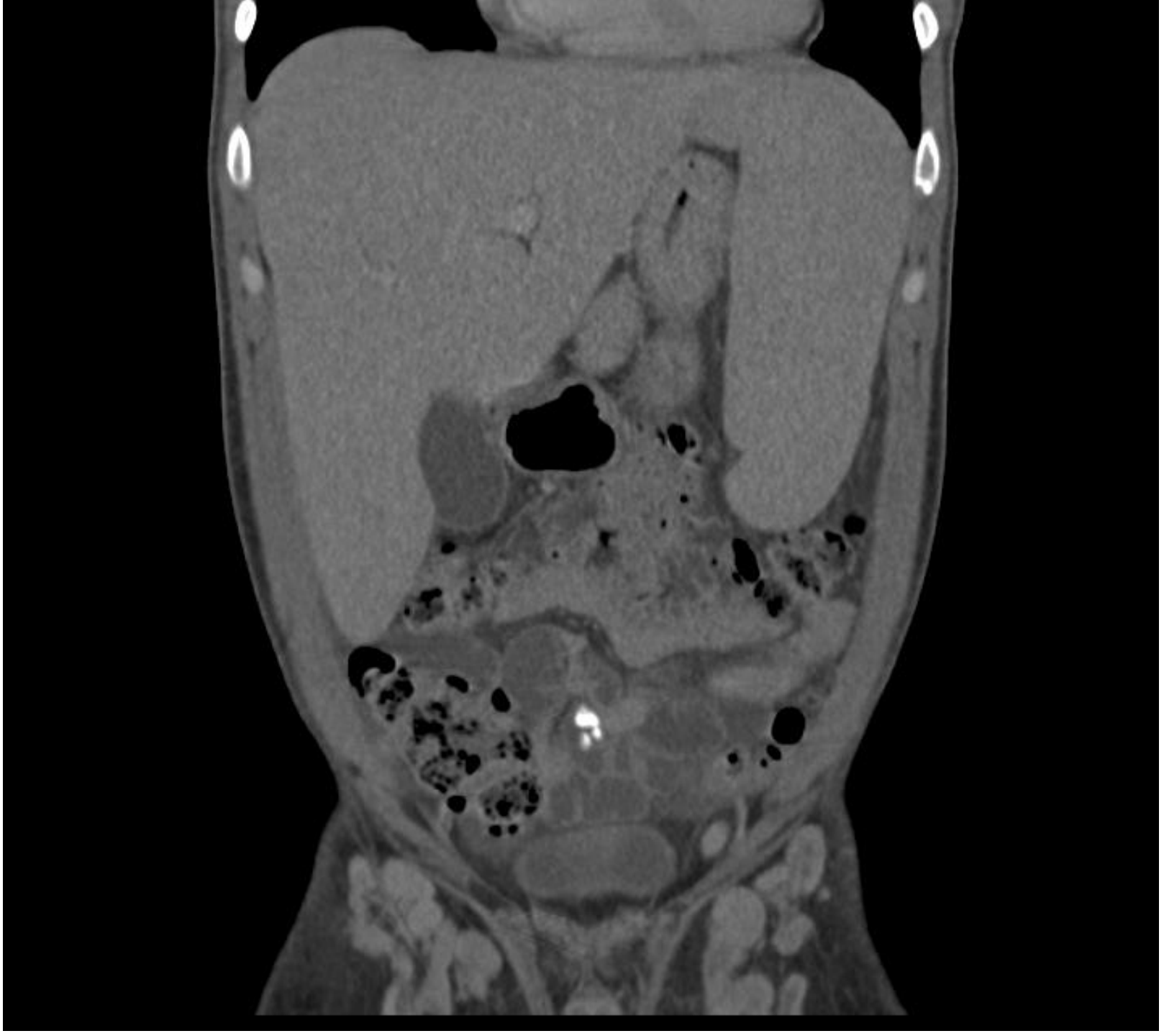
1. Fever
2. At least 3 of the following:
    1. Lymphadenopathy
    2. Splenomegaly
    3. Oedema
    4. Pleural effusion
    5. Ascites
    6. Cough
    7. Nasal obstruction
    8. Xerostomia
    9. Rash
    10. Central neurologic symptoms
    11. Jaundice
    12. Autoimmune haemolytic anaemia
3. Serum C-reactive protein level > 20 mg/L

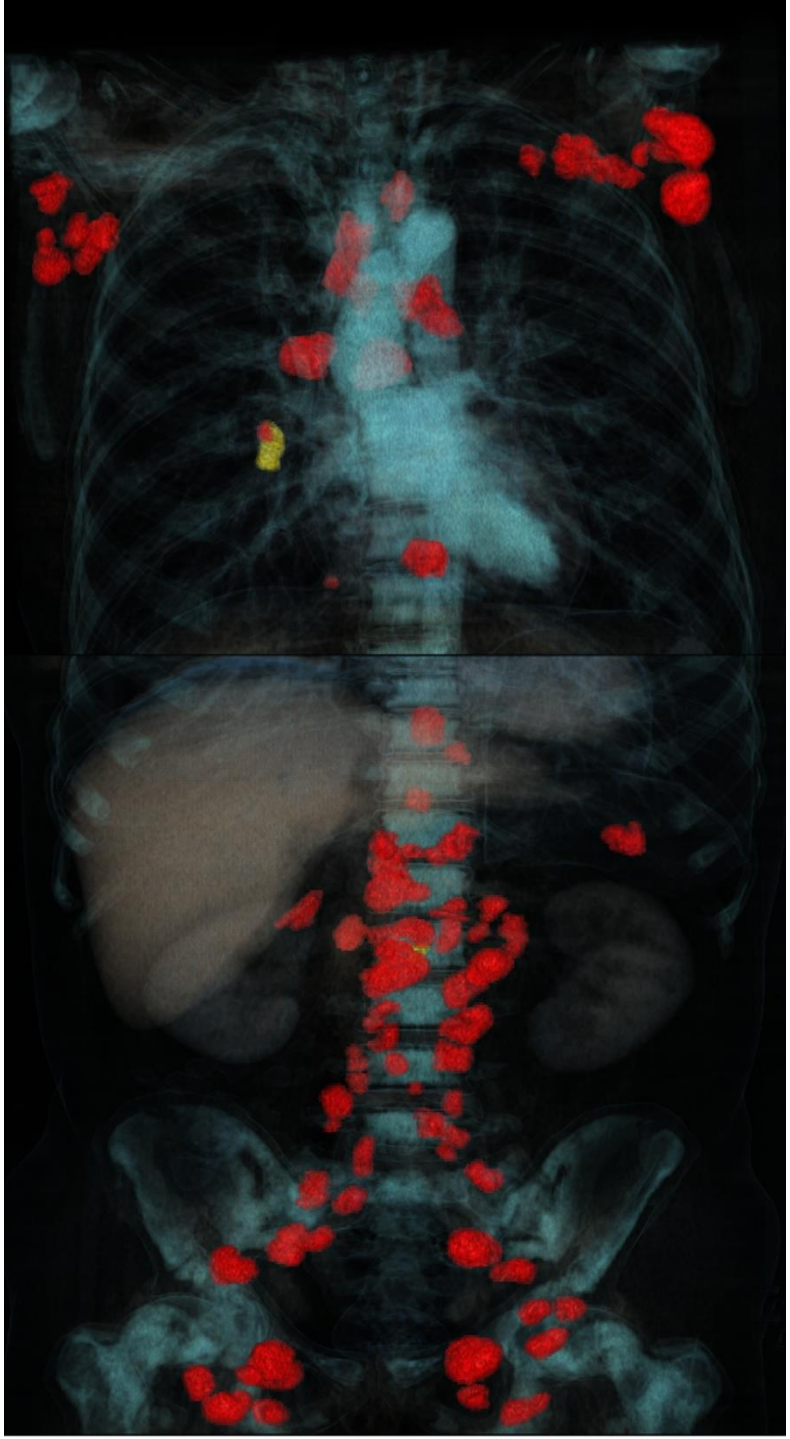
(in the absence of any other cause)

J Clin Oncol. 2007;25:3350-6.

Features at diagnosis of HIV+ KSHV/HHV8-MCD at CWH (n=84)

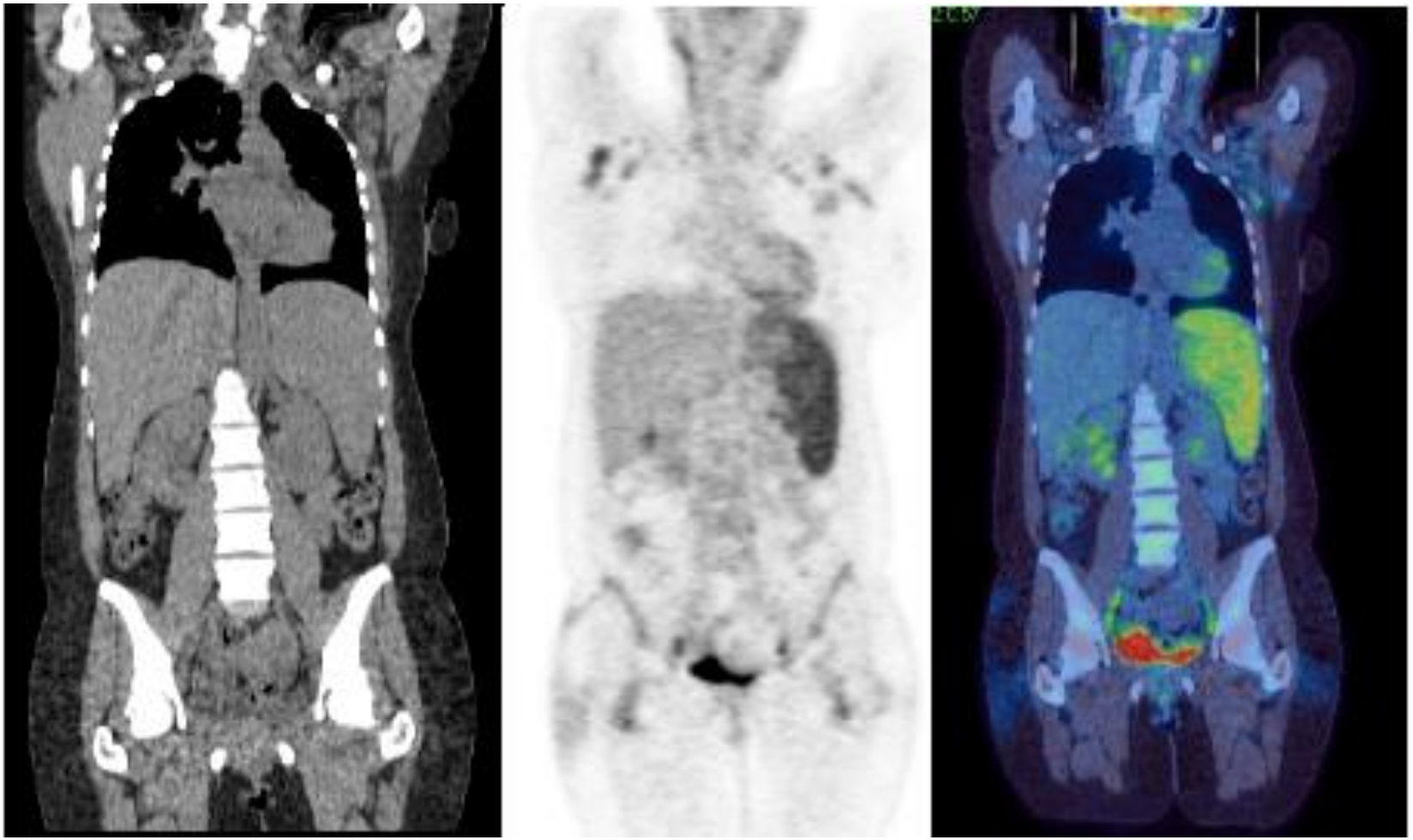
Mean Age	42 years (21-69)
Male	86 %
Prior AIDS	39%
Median CD4 count (range)	283 cells/mL (24-834)
On HAART >3m	41 (49%)
On HAART & VL<50 copies	27 (33%)
Median duration symptoms (range)	3 months (0.5-48)



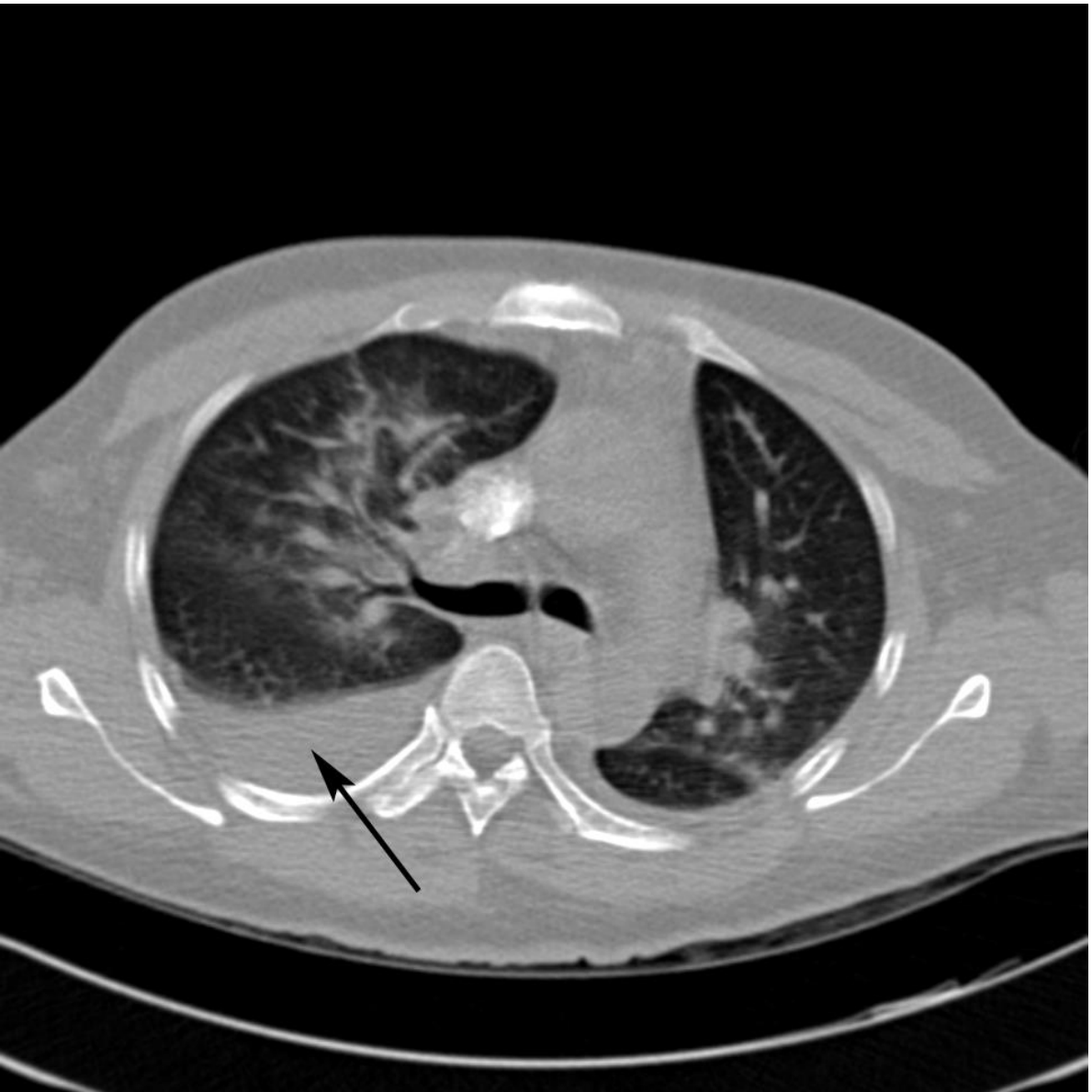


Fused volume rendered CT images of relapsed multicentric Castleman's disease (previous splenectomy)

Newly diagnosed MCD



Pulmonary involvement (20%)

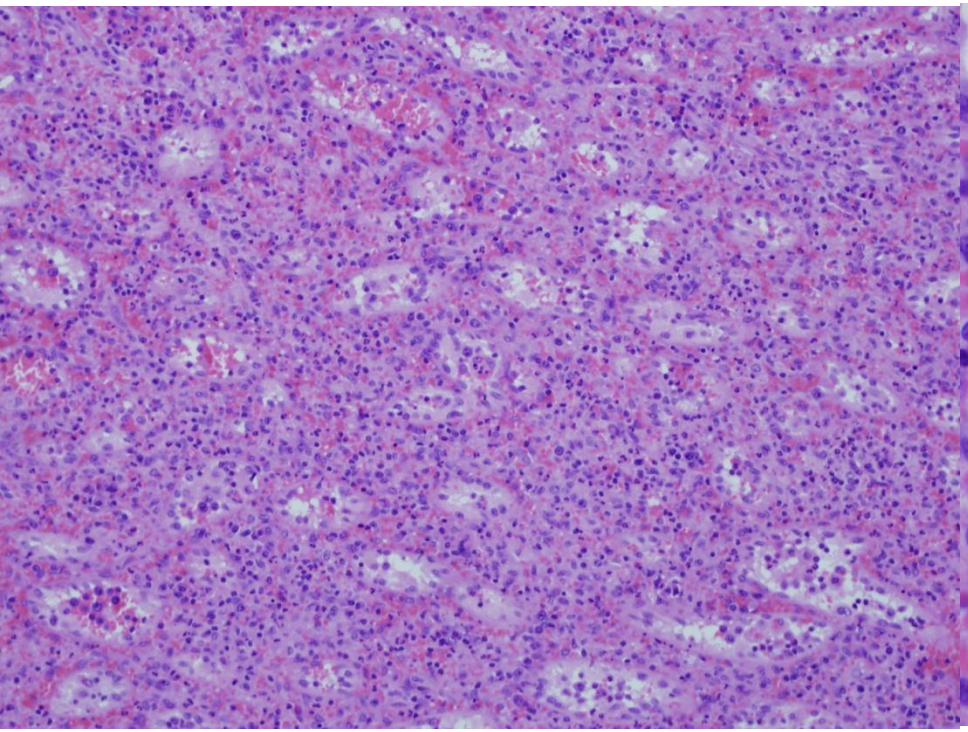


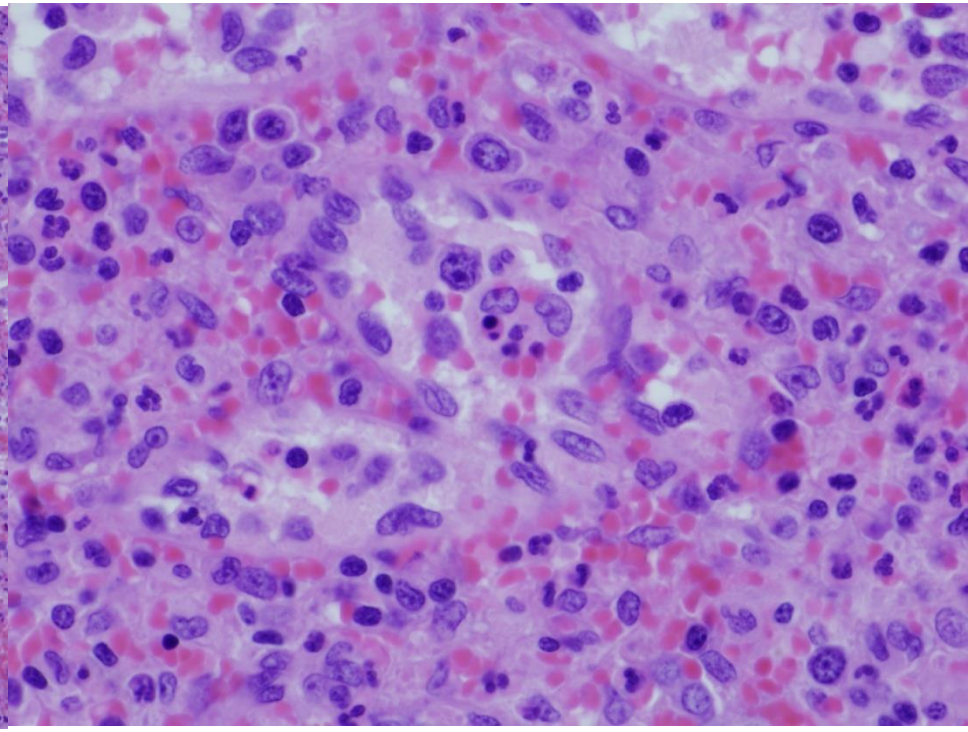
Pleural effusion

Septal thickening (peribronchovascular and interlobular)

Ground glass opacification (acute pneumonitis)

Haemophagocytic Syndrome in MCD (5-10%)

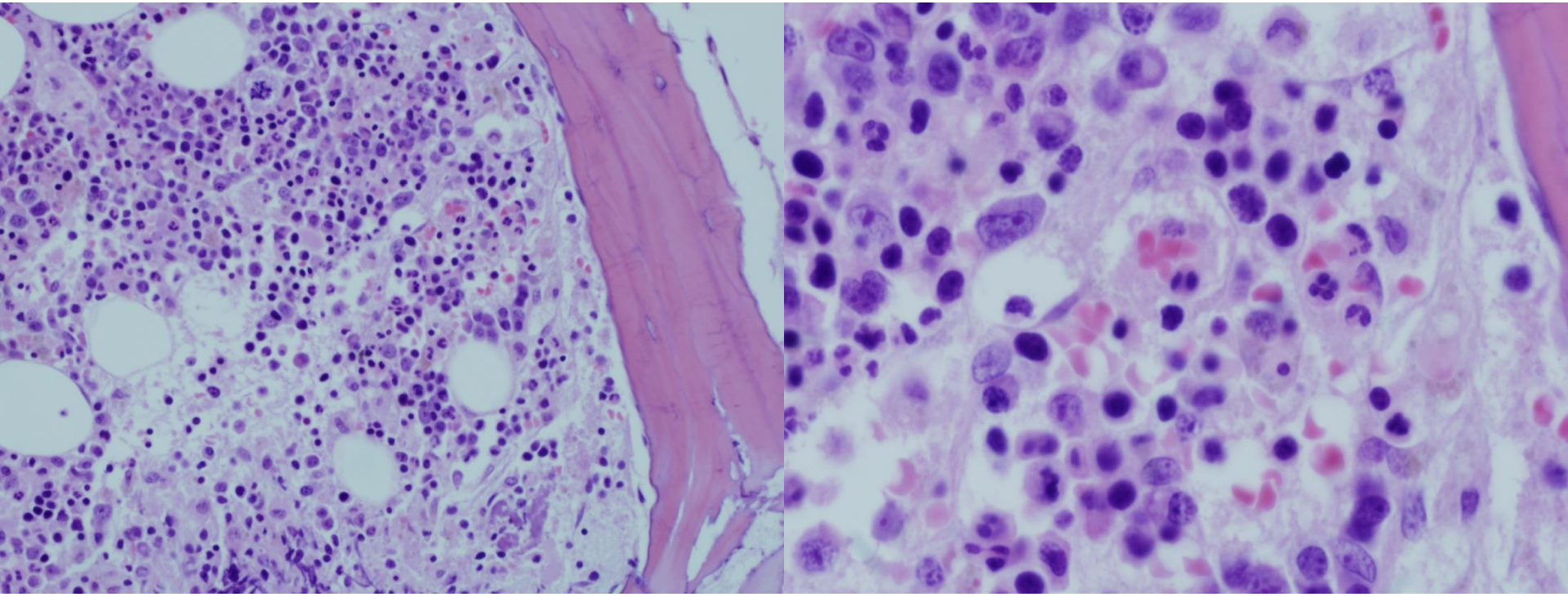




Histiocytes in splenic sinusoids phagocytosing erythrocytes

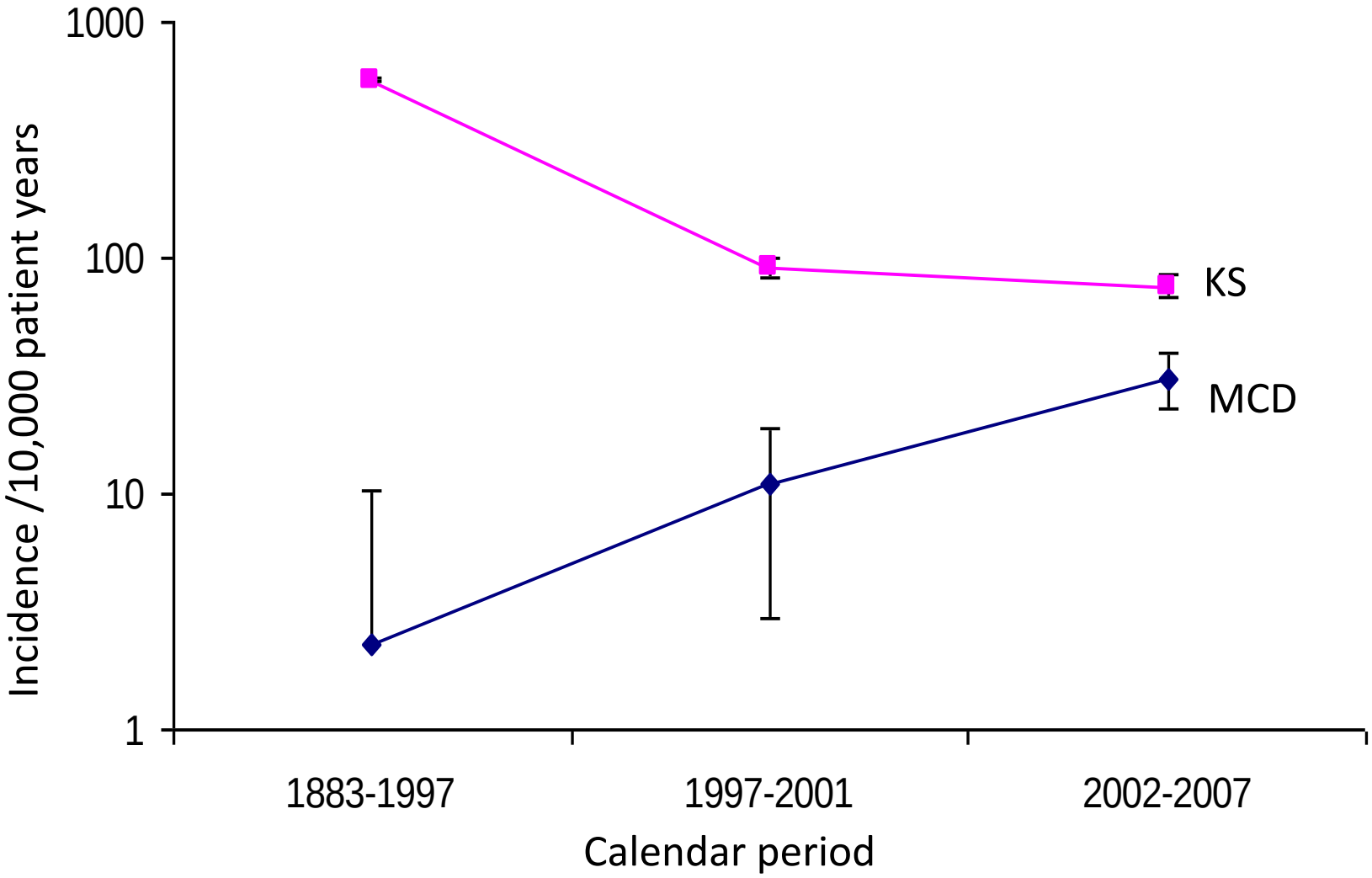
Clin Exp Immunol. 2008, 154: 399-405

Haemophagocytic Syndrome in MCD

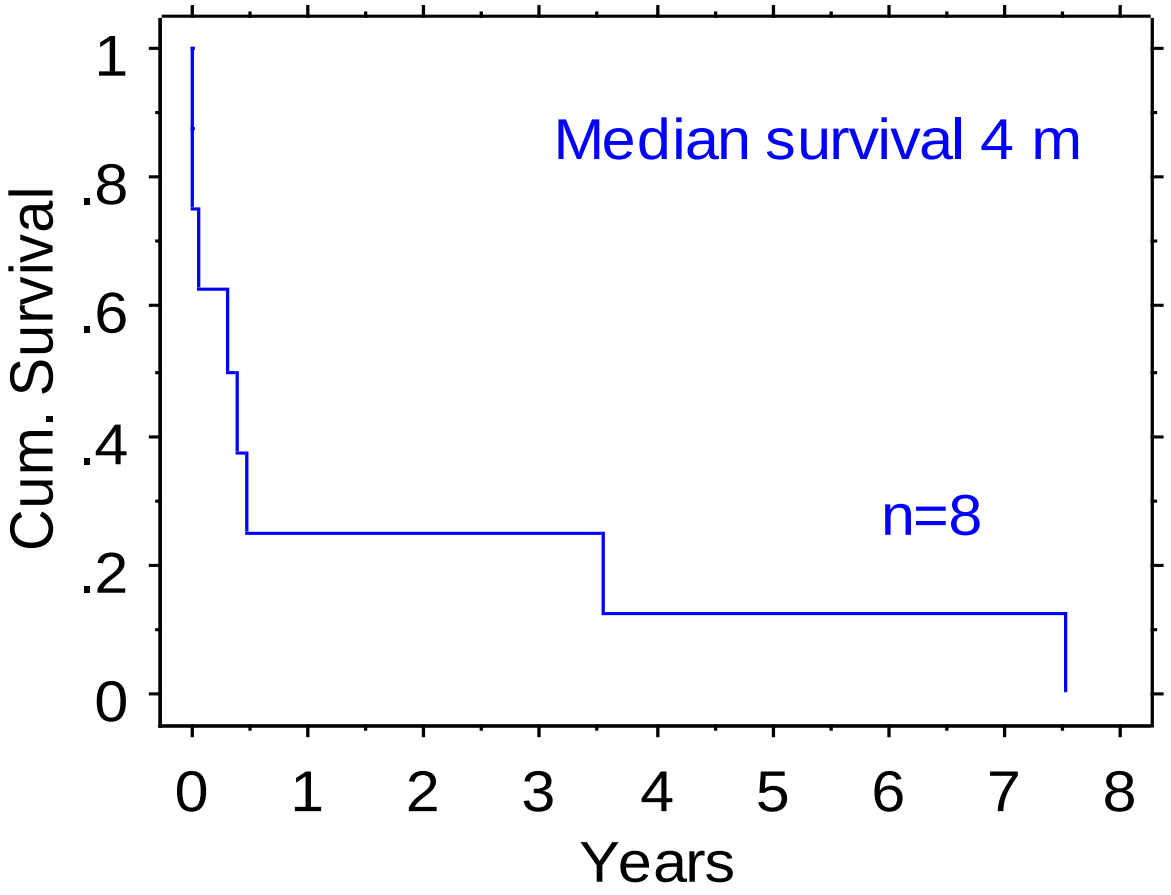


Histiocytes in bone marrow phagocytosing erythrocytes

Changing incidence of MCD & KS



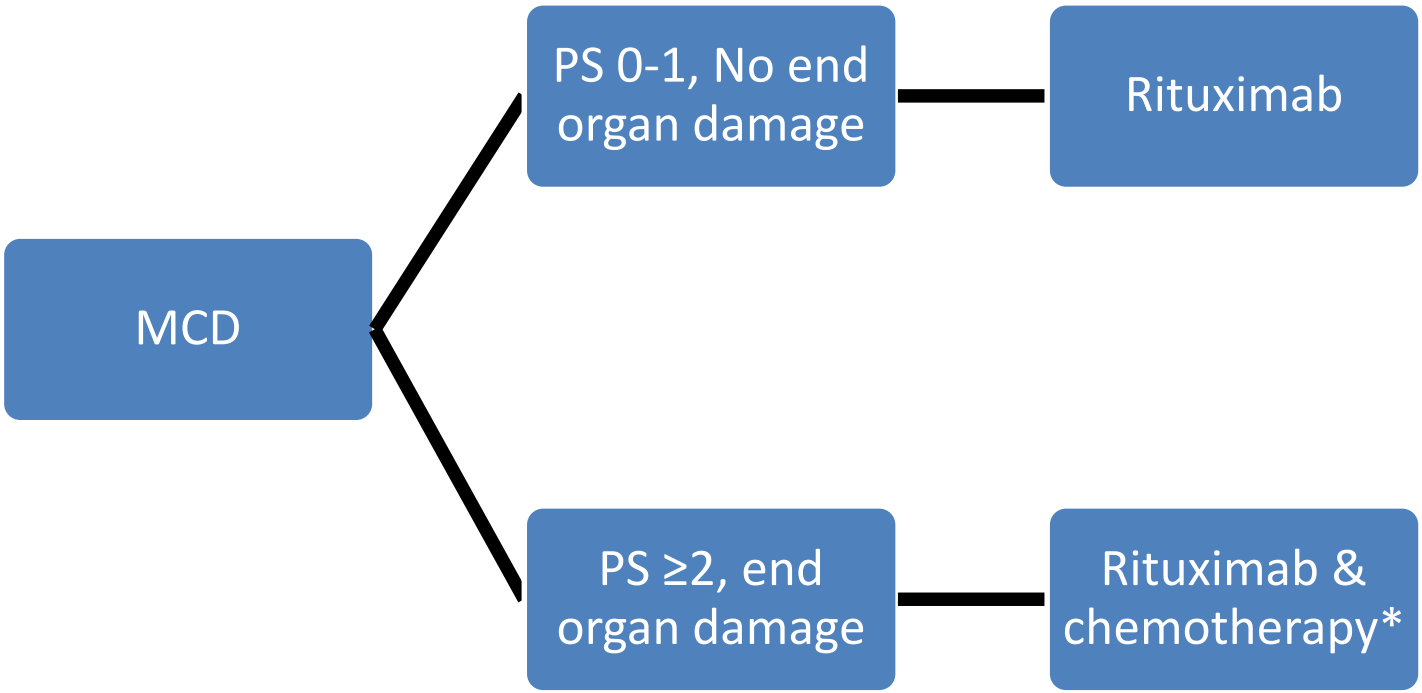
Survival pre 2002



Largest published series
n=20
Median survival 14m

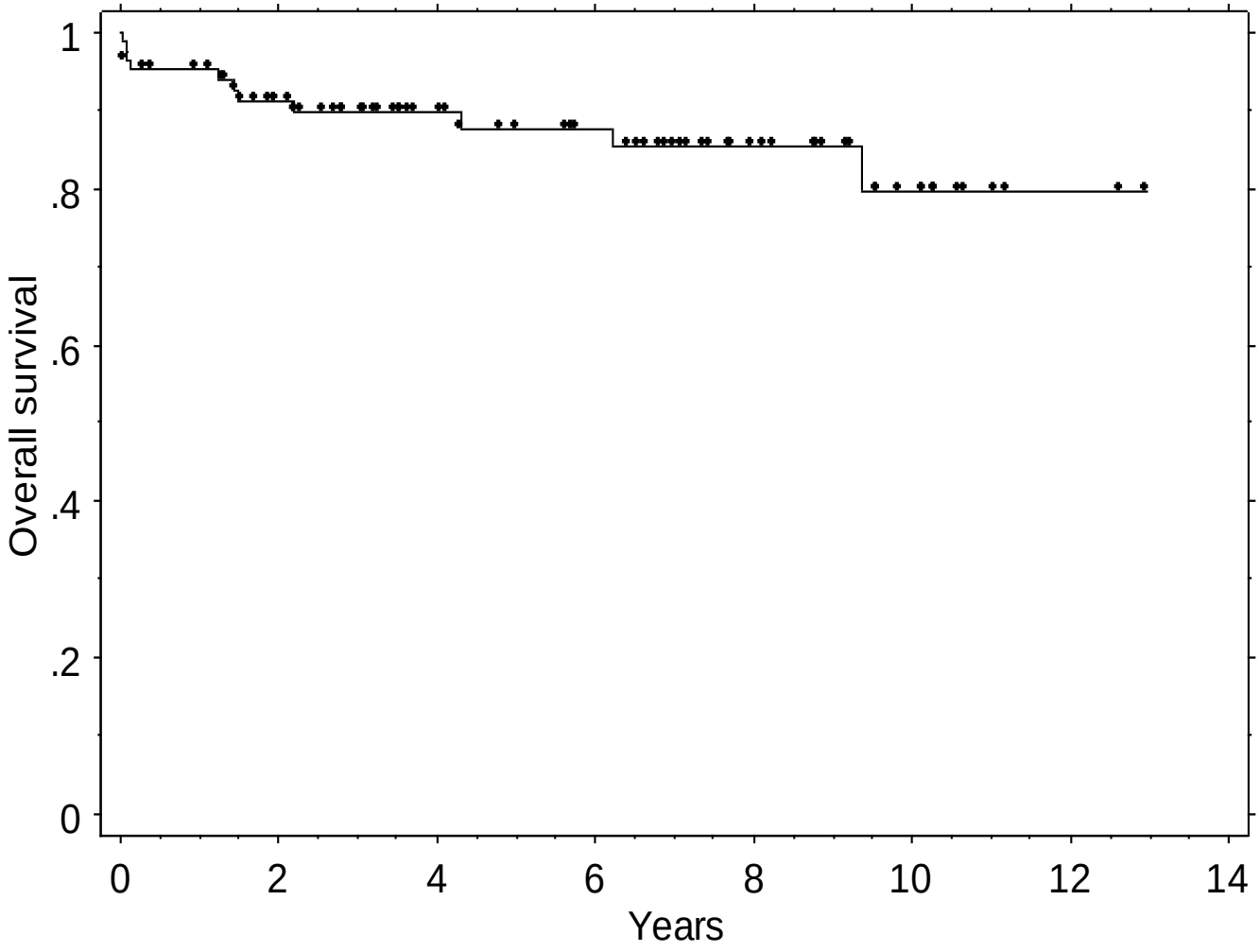
AIDS 1996, 10: 61

C&W risk-stratified algorithm

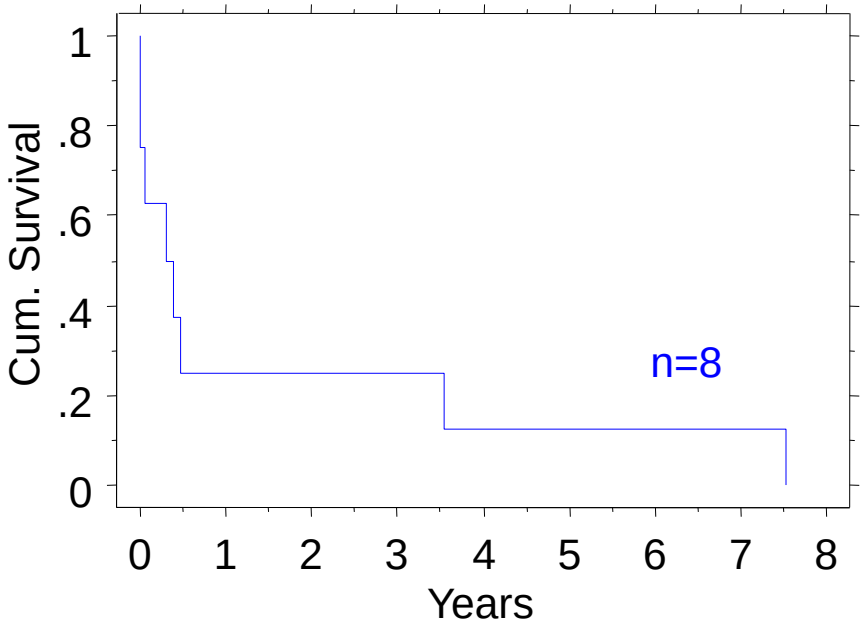


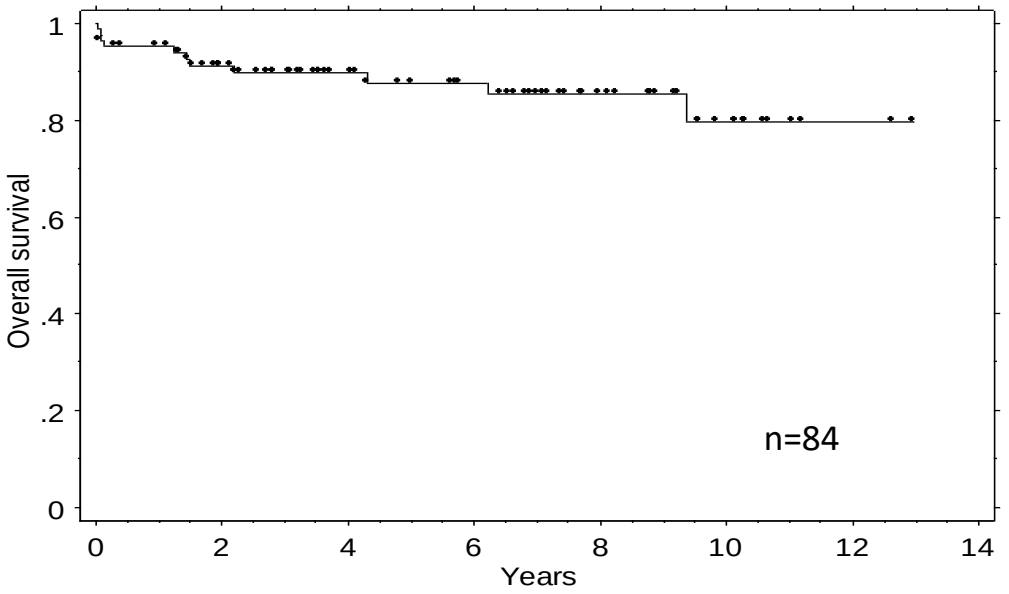
*Liposomal doxorubicin if concurrent active Kaposi sarcoma, otherwise Etoposide

Overall survival of 84 patients treated with rituximab-based therapy



Overall survival pre/post Rituximab

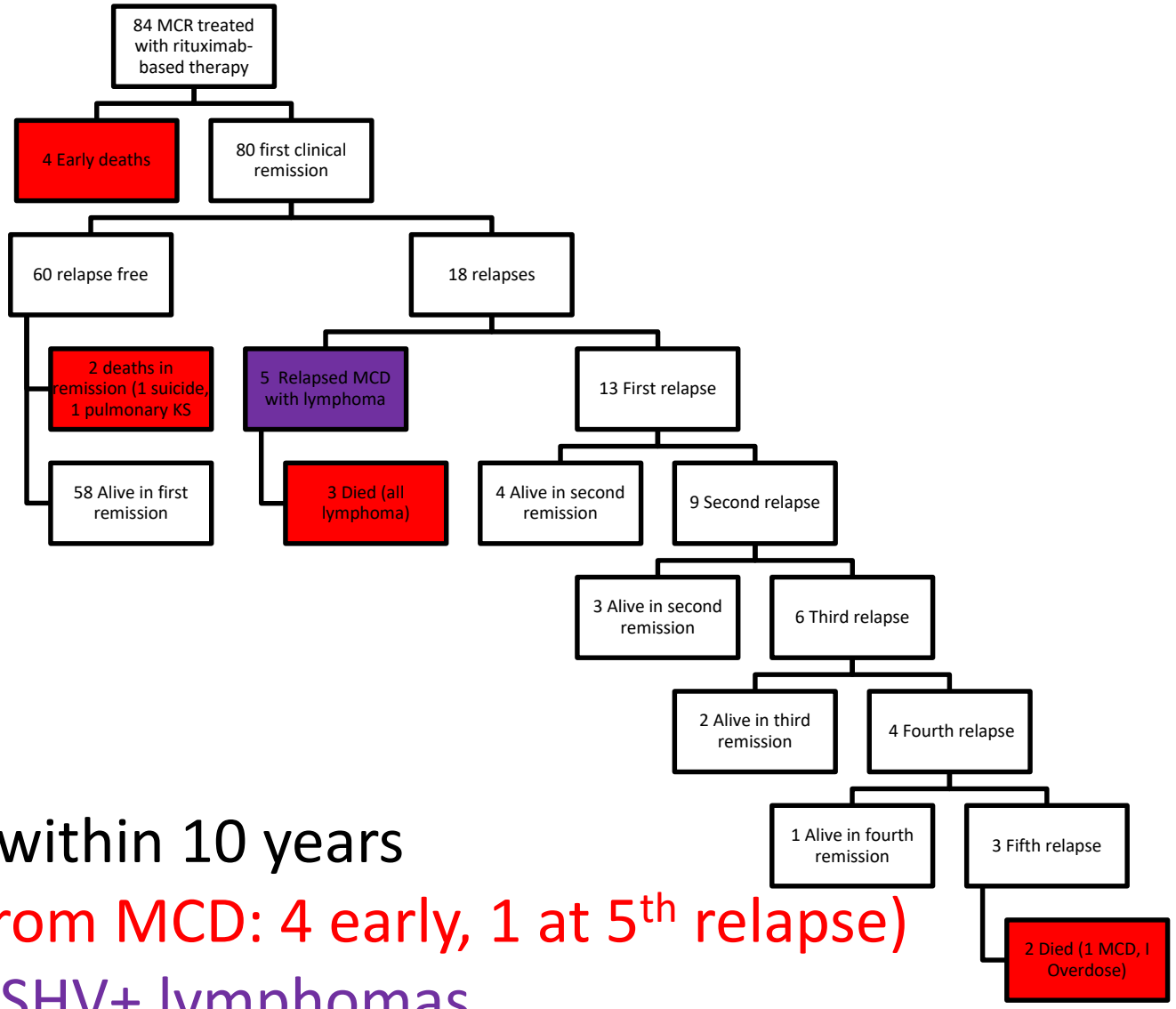




5 year survival 18%

5 year survival 88%

Outcomes in 84 HIV+ MCD patients



25% relapsed within 10 years

11 deaths (5 from MCD: 4 early, 1 at 5th relapse)

5 developed KSHV+ lymphomas

KSHV/HHV8 MCD

- Relatively easy to diagnose (HHV8, IgM λ restriction)
- Rituximab based treatment
- Relapse common
- High rates of KSHV/HHV8 related lymphoma