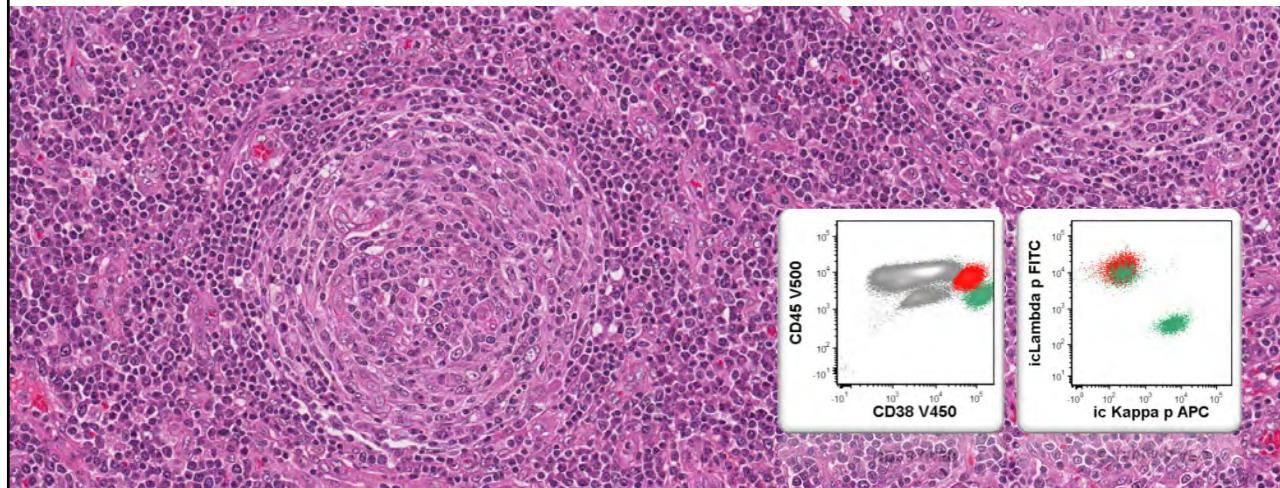


On KSHV/HHV8-associated Lymphoproliferation



Hao-Wei Wang M.D., Ph.D.

Head, Clinical Flow Cytometry Laboratory
Laboratory of Pathology, NCI, NIH, USA



1

Disclosure

I have no conflict of interest in relation to this presentation.

2

About the NIH Clinical Center



Opened in July 1953

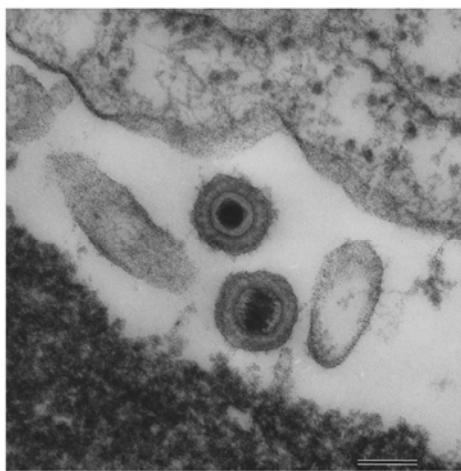


NIH Clinical Center, Bethesda, Maryland

"The nation's largest hospital devoted entirely to clinical research."

3

Kaposi Sarcoma-associated Herpesvirus (KSHV)



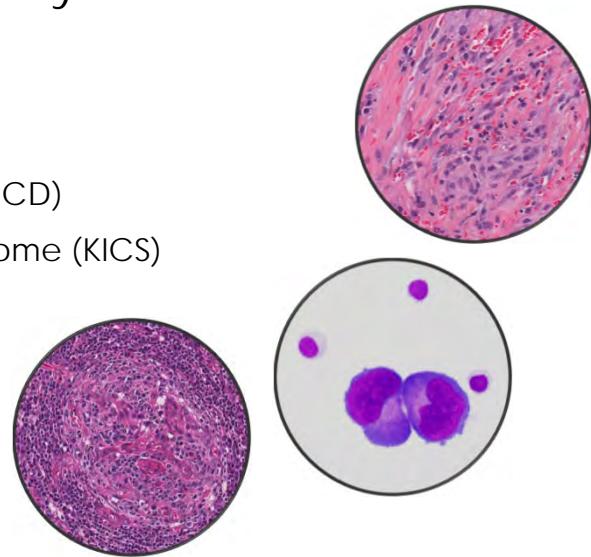
Schulz TF. J Gen Virol. 1998

- Also known as Human Herpesvirus-8 (HHV8)
- Double-stranded DNA virus
- Latent vs. lytic cycles
- “Molecular piracy” – homologs of human cytokines → systemic inflammation
 - e.g. vIL-6

4

Diseases Caused by KSHV/HHV8

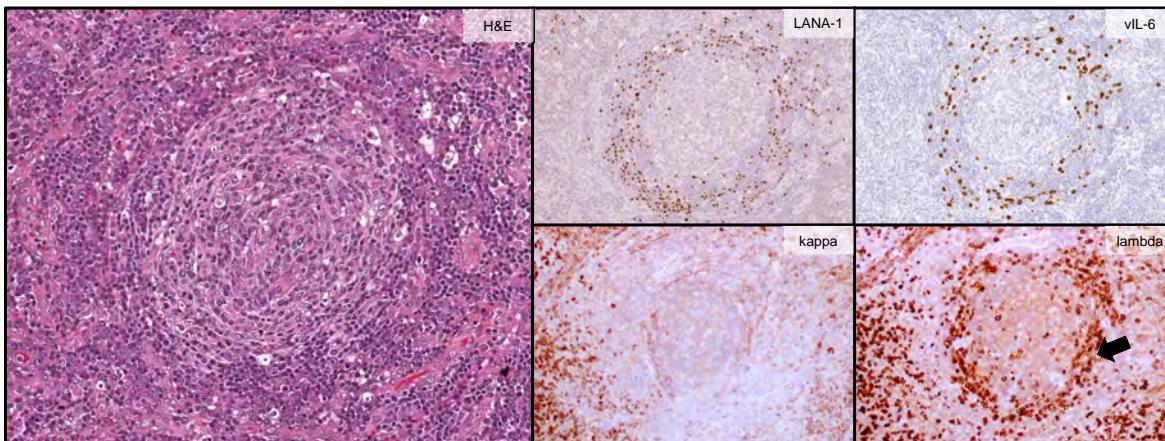
- Kaposi sarcoma
- Primary effusion lymphoma (PEL)
- Multicentric Castleman disease (MCD)
- KSHV inflammatory cytokine syndrome (KICS)



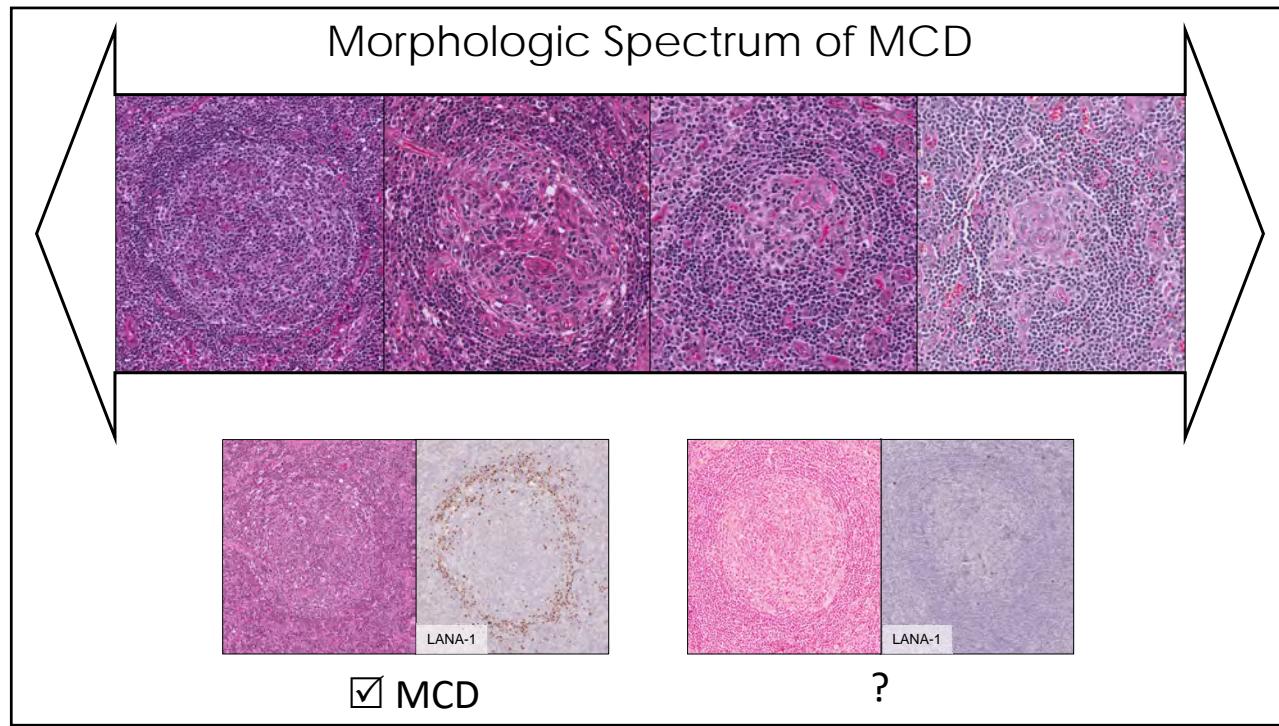
5

Multicentric Castleman Disease (MCD)

- Waxing and waning inflammatory symptoms
 - Fever, edema, ascites, effusion, lymphadenopathy, splenomegaly
- Lambda-restricted KSHV/HHV8-positive plasmablasts

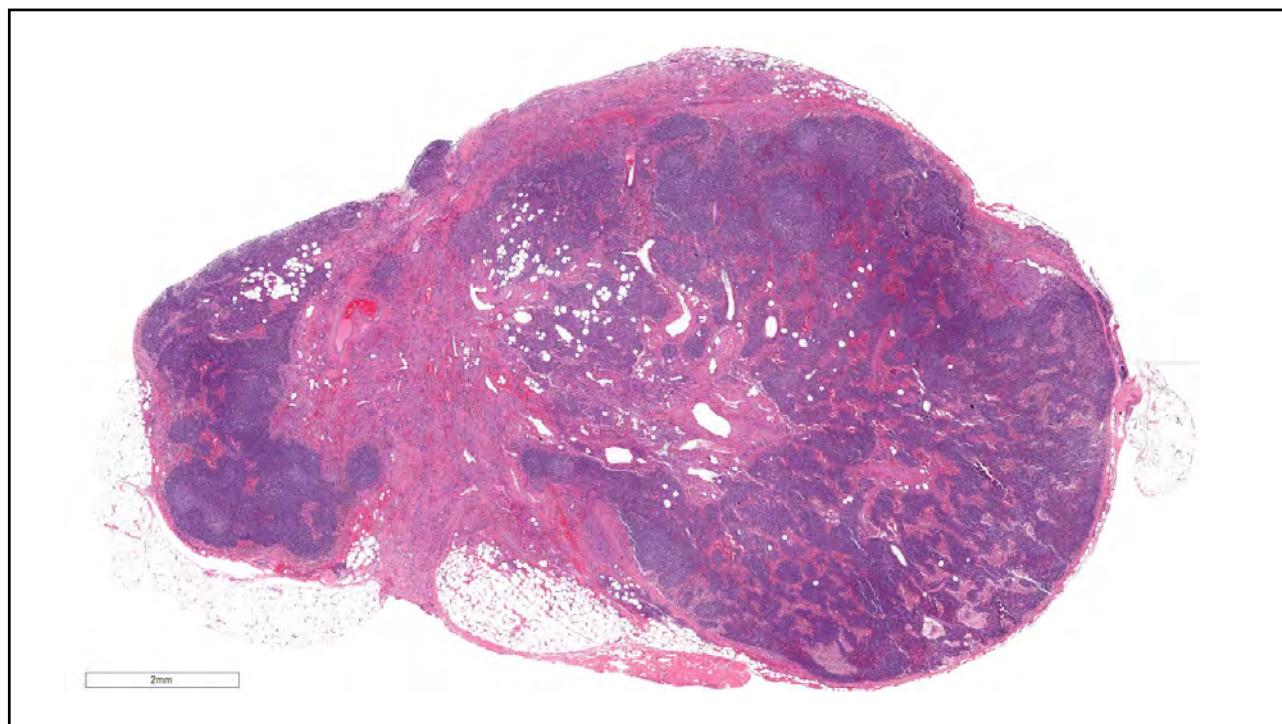


6

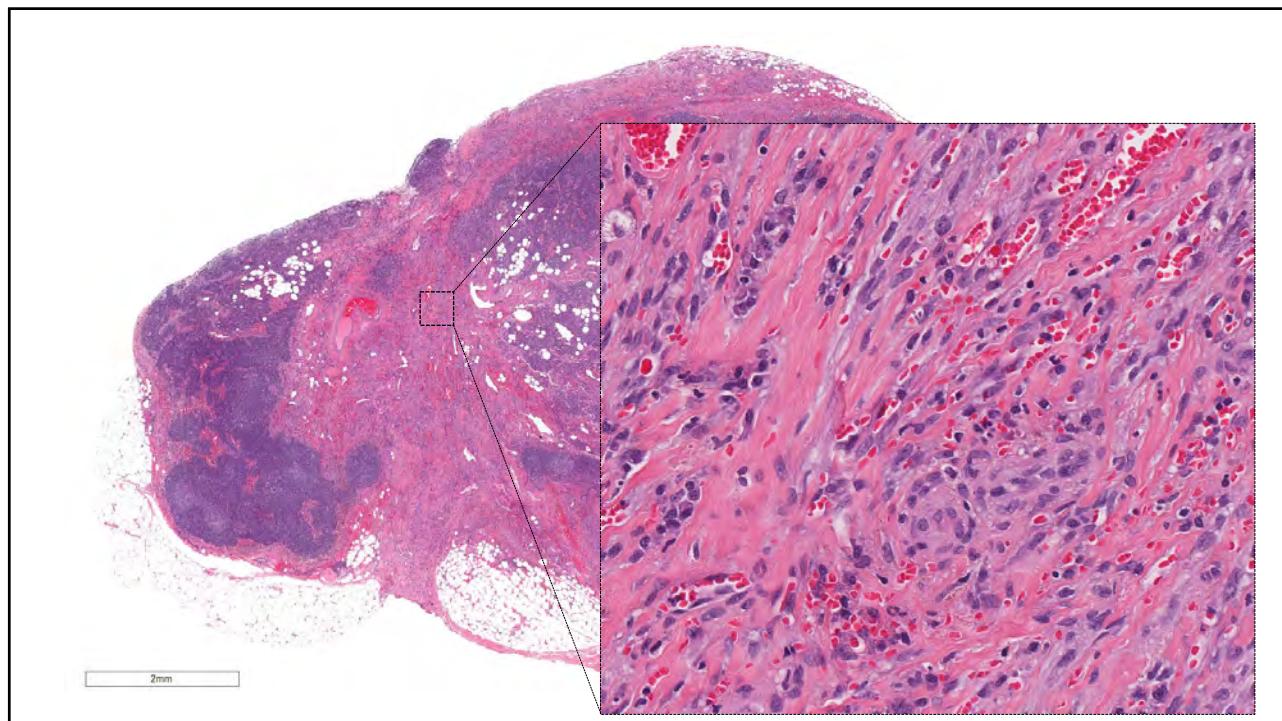


Case #1

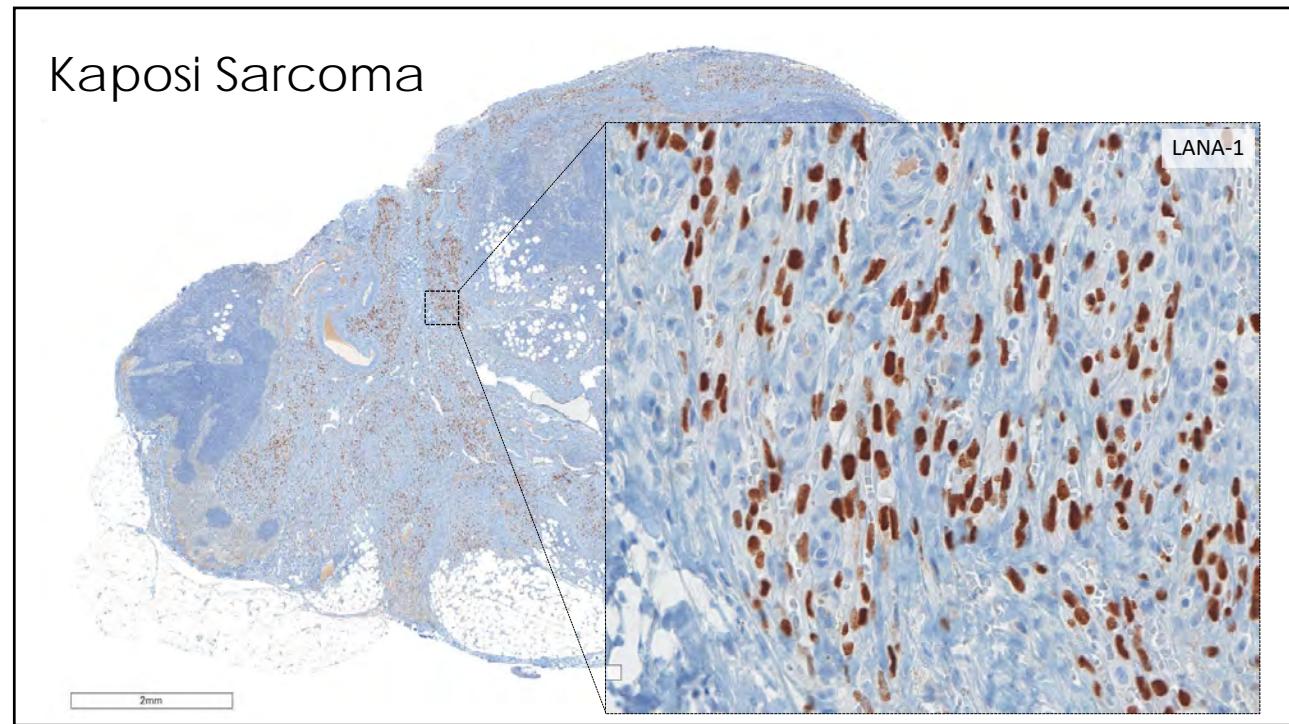
- 60 y/o HIV+ male patient
- History of Kaposi sarcoma
- Fever, diffuse lymphadenopathy, trace pleural effusion
- Left axillary lymph node excisional biopsy



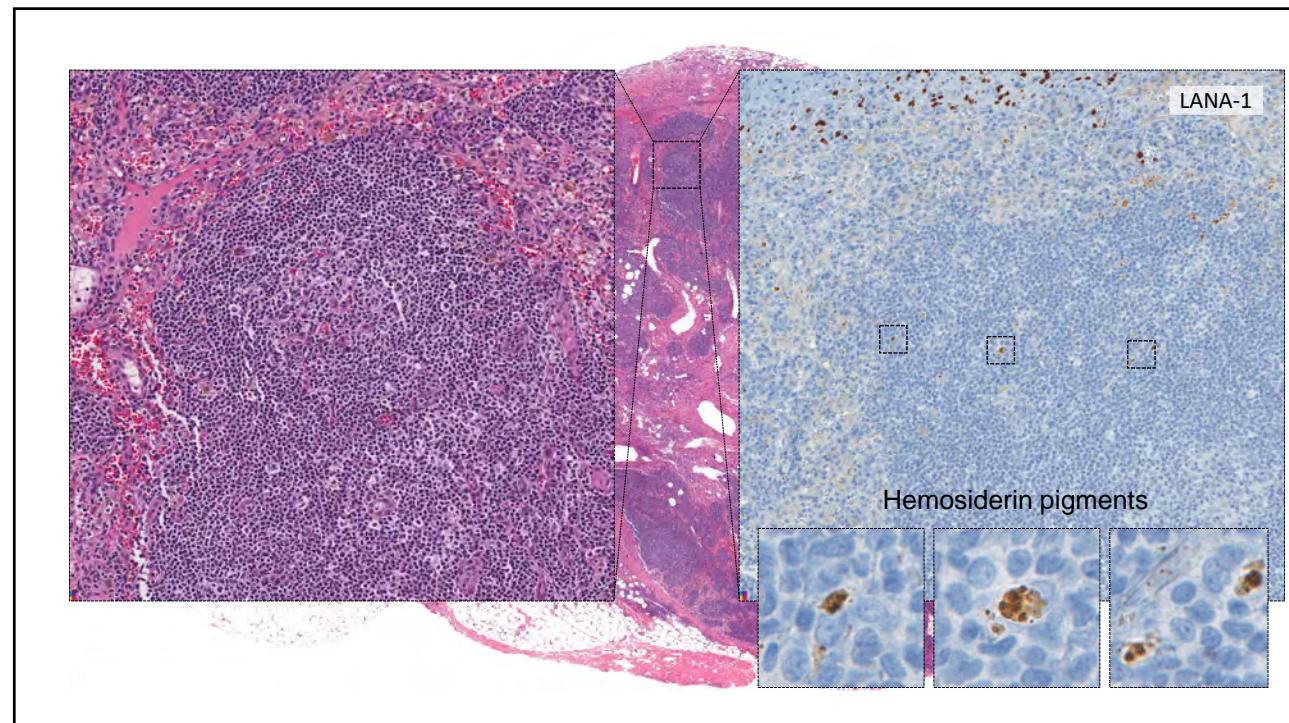
9



10

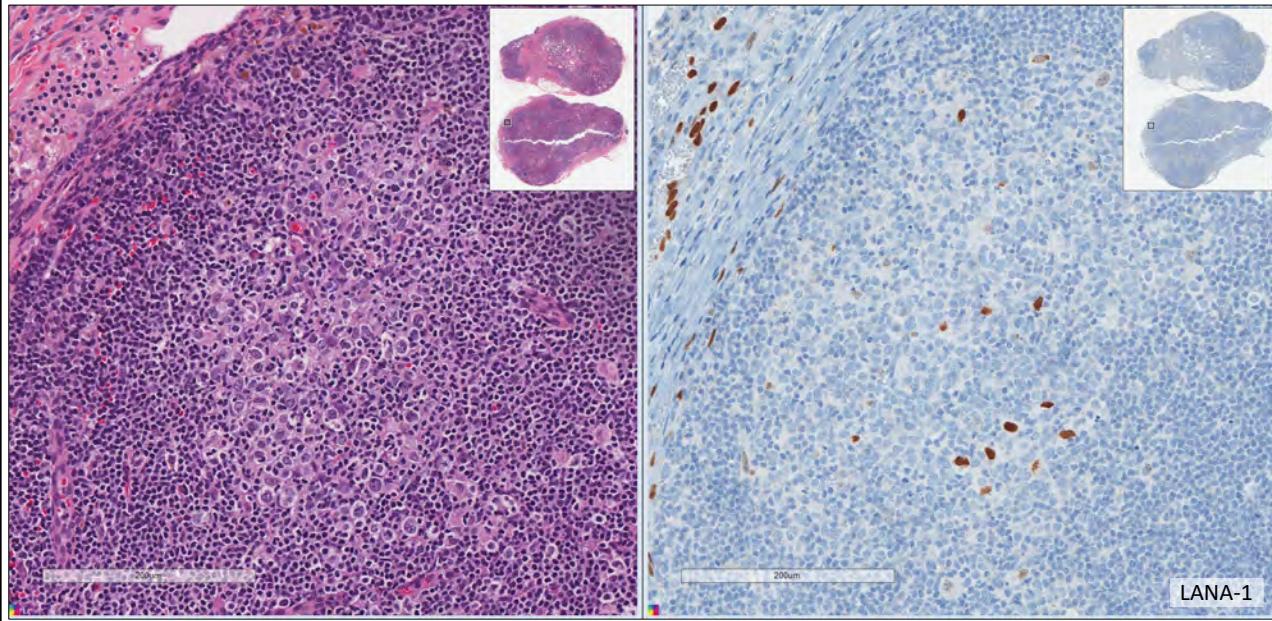


11



12

Is this sufficient for the diagnosis of MCD?



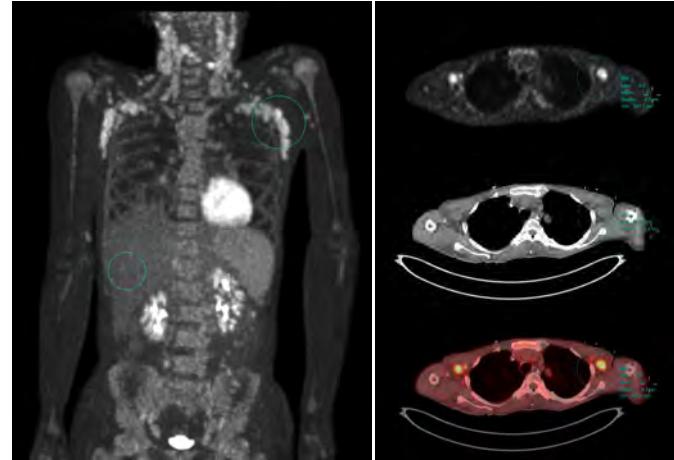
13

Can Flow Cytometry aid the diagnosis of MCD?

14

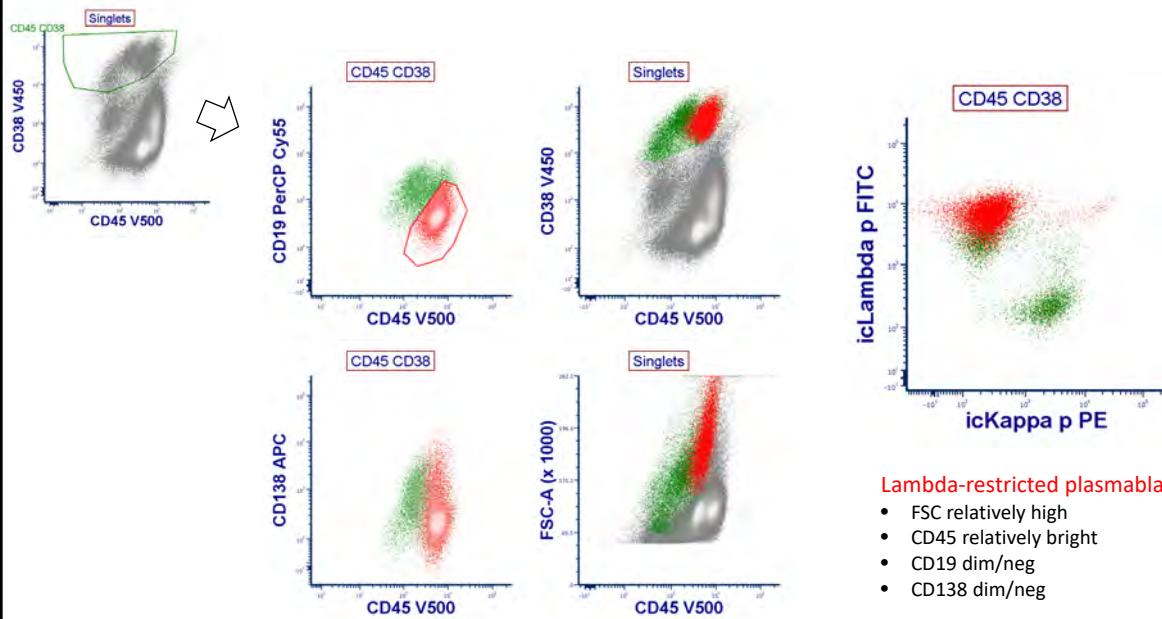
Case #2

- 38 y/o HIV+ male on HAART
- Shortness of breath, mental status change
- Lymph node swelling
- Left axillary lymph node excisional biopsy:
 - KSHV/HHV8+ MCD



15

Lymph Node Suspension



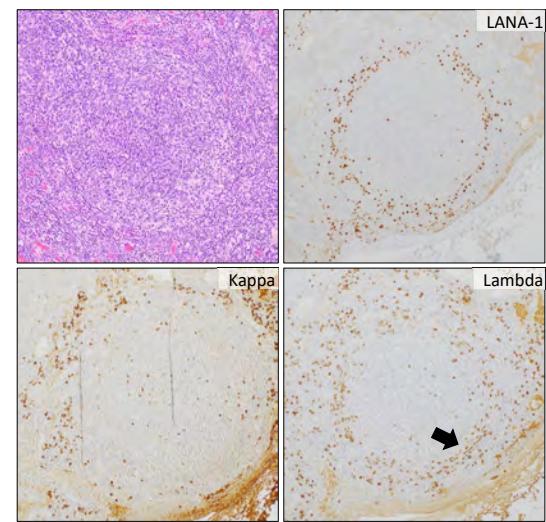
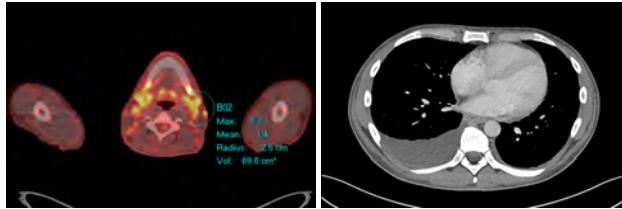
16

Can Flow Cytometry aid the diagnosis of MCD on effusion specimen?

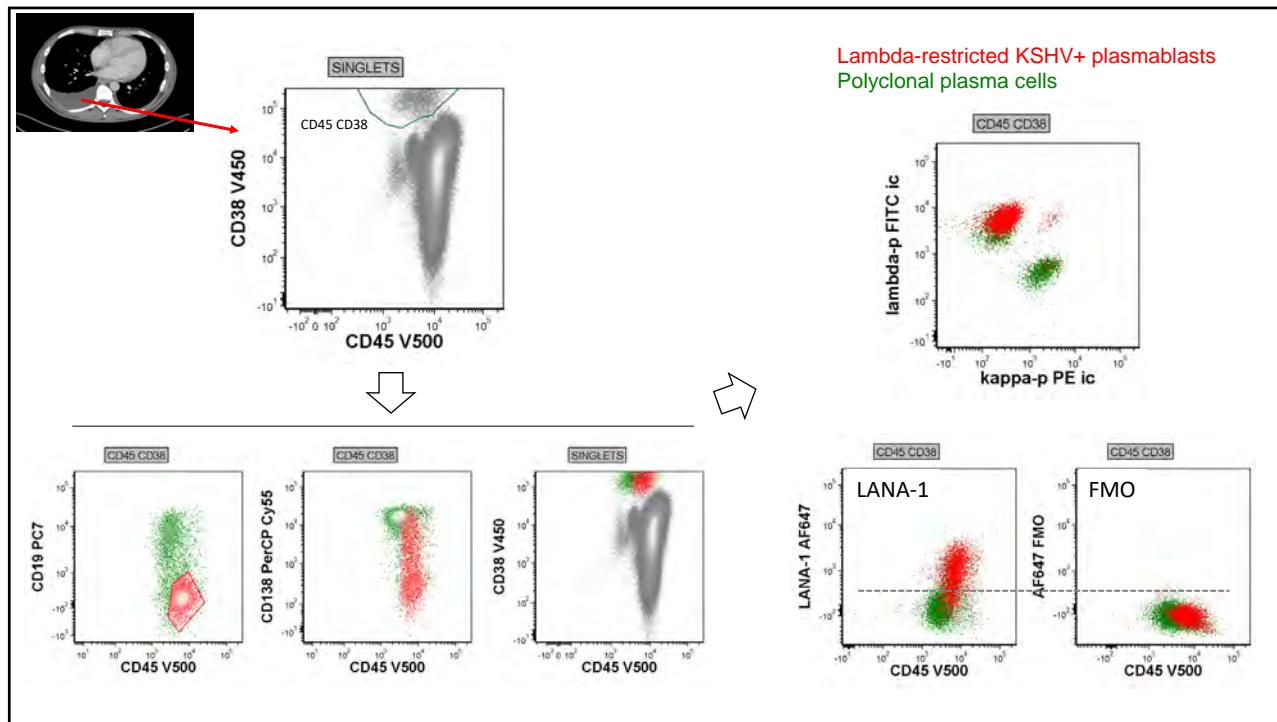
17

Case #3

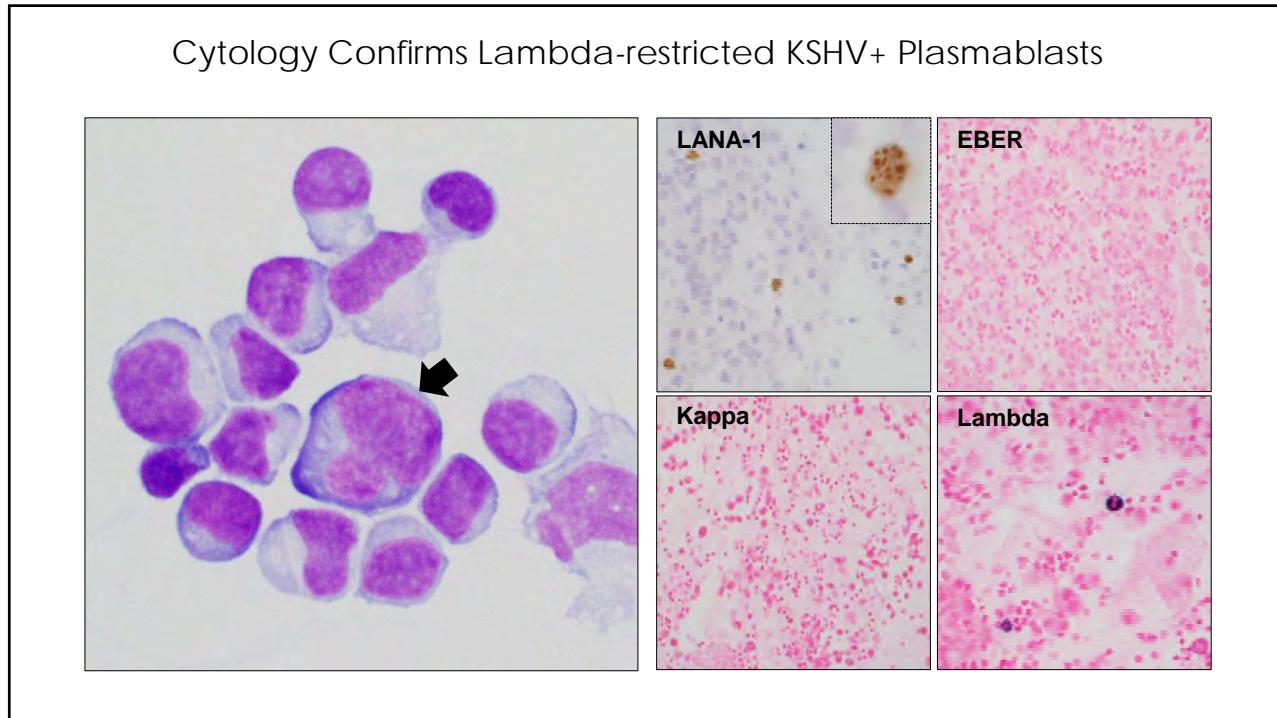
- 30 y/o HIV+ male
- Fatigue, lymphadenopathy
- Left neck lymph node:
 - KSHV/HHV8+ MCD



18



19



20

What are the implications ?

- Flow may be a useful diagnostic tool for MCD
- KSHV-infected lambda-restricted plasmablasts are not restricted to the lymph nodes

21

What about KICS?

22

Diagnostic Criteria of KICS

1. Clinical manifestations

a. Symptoms	b. Laboratory abnormalities	
Fever	Anemia	
Fatigue	Thrombocytopenia	
Edema	Hypoalbuminemia	
Cachexia	Hyponatremia	
Respiratory symptoms	c. Radiographic abnormalities	
Gastrointestinal disturbance	Lymphadenopathy	
Arthralgia and myalgia	Splenomegaly	
Altered mental state	Hepatomegaly	
Neuropathy with or without pain	Body cavity effusions	

2. Evidence of systemic inflammation

Elevated C-reactive protein (≥ 3 g/dL)

3. Evidence of KSHV viral activity

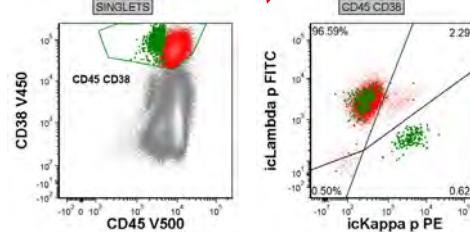
Elevated KSHV viral load in plasma (≥ 1000 copies/mL) or peripheral blood mononuclear cells (≥ 100 copies/ 10^6 cells)

4. No evidence of KSHV-associated multicentric Castleman disease or primary effusion lymphoma

23

Case #4

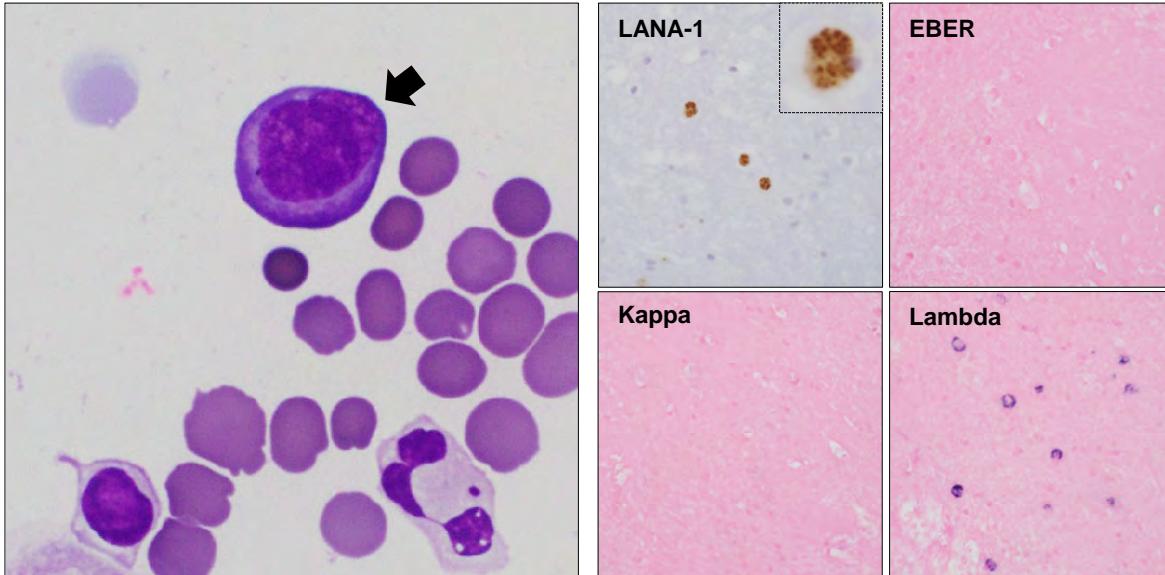
- 67 y/o HIV+ female with cutaneous and gastrointestinal KS
- Shortness of breath, fatigue, weight loss, pleural effusions
- Multiple lymph node biopsies:
 - Kaposi sarcoma only
- Clinical diagnosis of KICS



Lambda-restricted KSHV+ plasmablasts
Polyclonal plasma cells

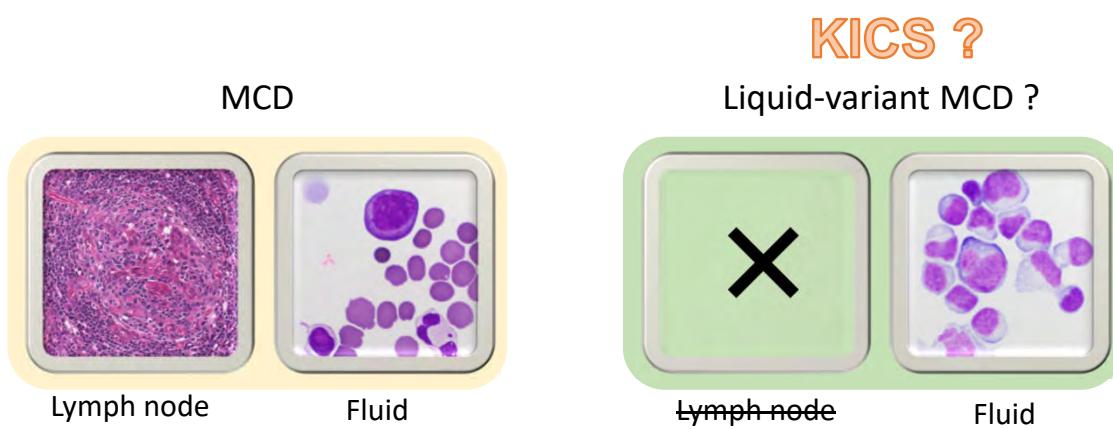
24

Cytology Confirms Lambda-restricted KSHV+ Plasmablasts



25

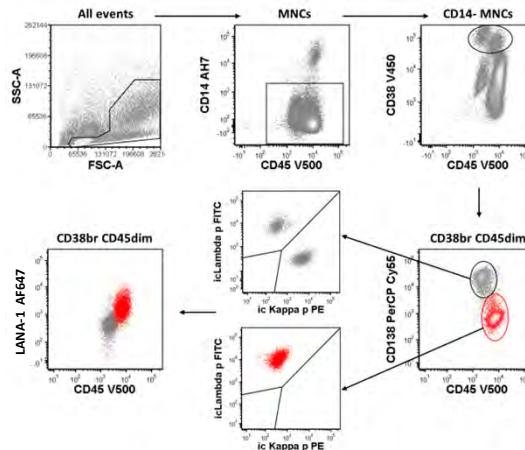
Is there a “liquid-phase” or “liquid-variant” of MCD ?



26

Flow Cytometric Assessment of MCD/KICS Effusion Samples

- Retrospective study 2012-2022
- 62 body fluid specimens
- 30 patients (11 MCD and 19 KICS) at NCI



Dr. Ting Zhou

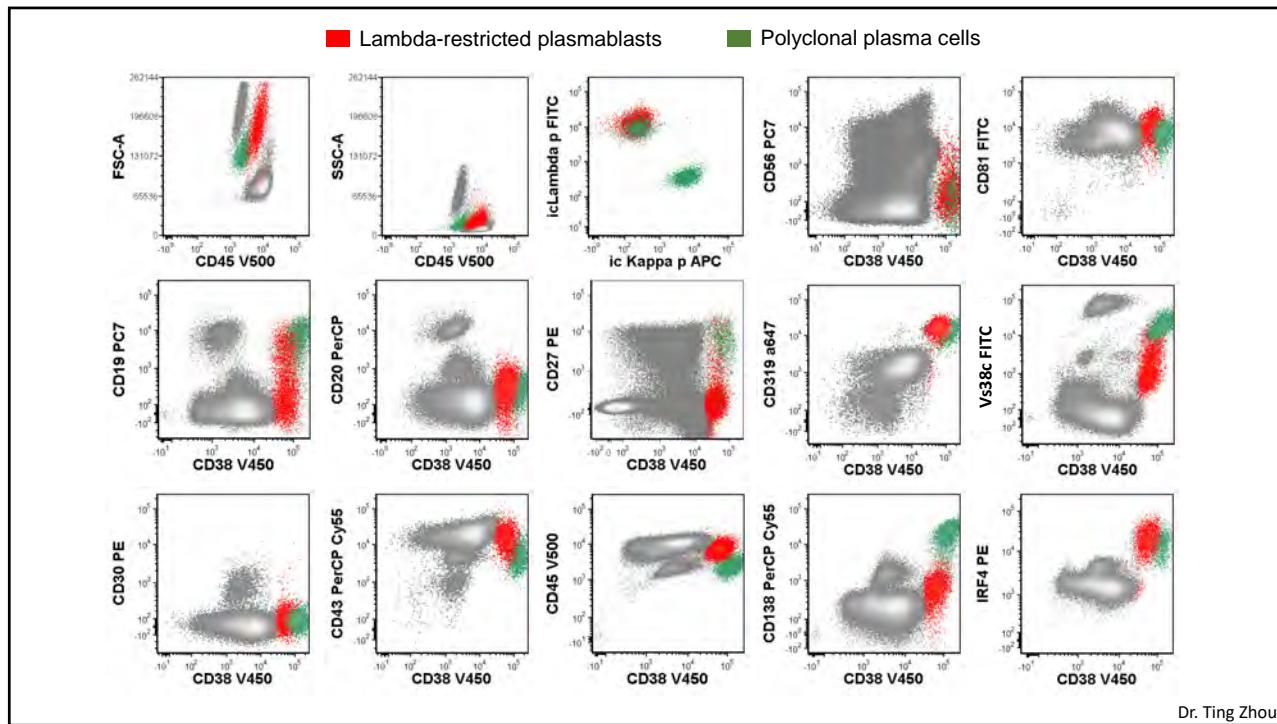
27

Flow Cytometric Assessment of MCD Effusion Samples

- 8 of 11 MCD patients had **lambda-restricted plasmablasts** detected in the effusions with distinctive immunophenotype
 - CD45 relatively bright
 - CD19 dim/negative
 - CD138 dim/negative
 - CD38 dimmer than PC
 - VS38c dimmer than PC
 - CD319 brighter than PC
 - FSC higher than PC
 - Lambda-restricted
 - LANA-1 positive by flow or IHC

Dr. Ting Zhou

28

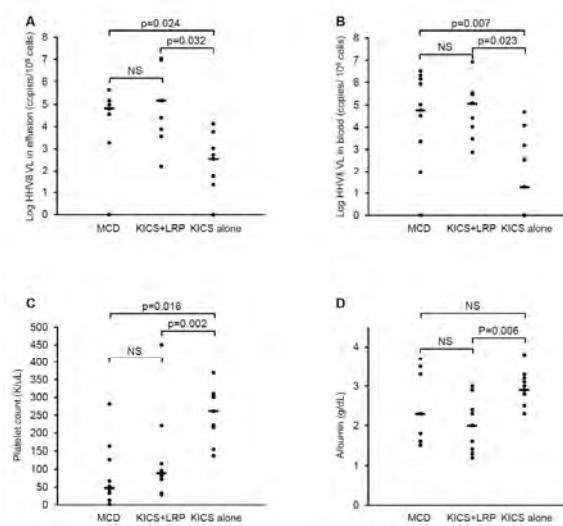


29

Flow Cytometric Assessment of KICS Effusion Samples

- 9 of 19 KICS patients also had "LRP" detected in effusions, with the same immunophenotype as in MCD
- LRP-positive KICS cases closely resemble MCD clinically
 - Higher KSHV/HHV8 viral load
 - Lower platelet and albumin
 - More frequent hepatosplenomegaly

	MCD	KICS with LRP	KICS w/o LRP
HHV8 VL in effusion	Higher	Lower	
HHV8 VL in blood	Higher	Lower	
Platelet	Lower	Higher	
Albumin	Lower	Higher	
Hepatosplenomegaly	More frequent	Less	



Dr. Ting Zhou

30

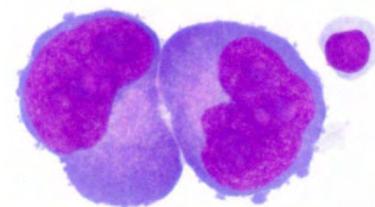
Conclusions and Implications

- ✓ Flow is a useful diagnostic tool for MCD and KICS
- ✓ Flow examination on effusions can be used in MCD diagnosis
- ✓ KSHV-infected plasmablasts present in effusions may play a role in pathogenesis
- ✓ KICS with detectable lambda-restricted plasmablasts may represent "liquid-variant" of MCD

31

Primary Effusion Lymphoma (PEL)

- Variable morphology
 - Immunoblastic / plasmablastic / anaplastic / Hodgkin or Reed-Sternberg-like
- Lack B-cell markers
- Usually undetectable immunoglobulin
- Often express CD30, CD38, VS38c, CD138 (?)
- LANA-1 positive
- EBER positive

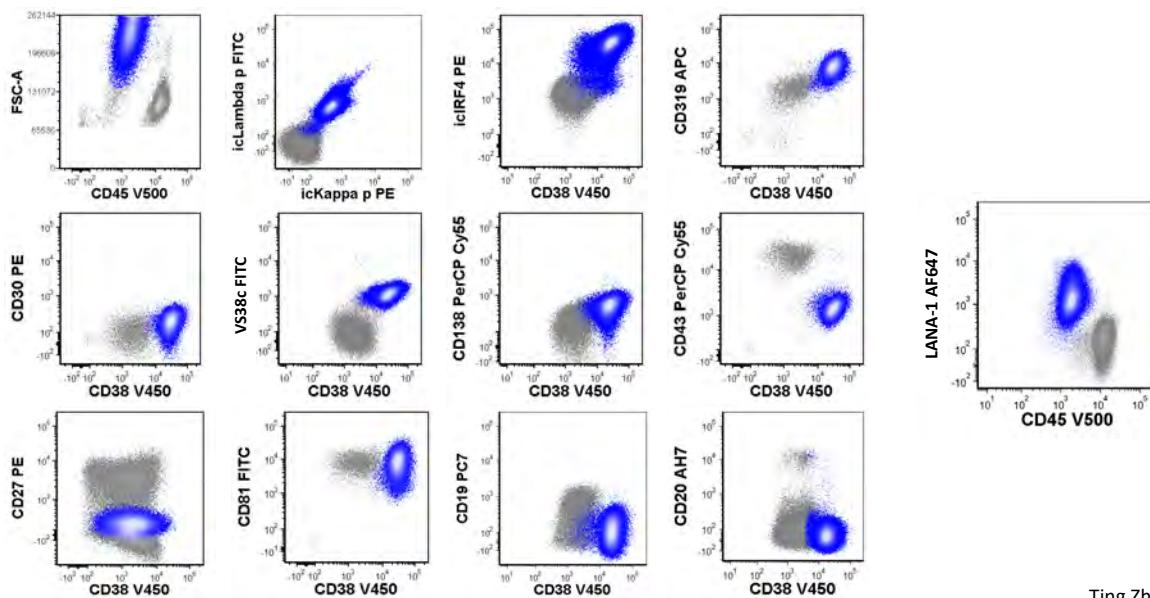


32

How to distinguish PEL from MCD?

33

Typical Flow Cytometric Finding of PEL



Ting Zhou

34

17

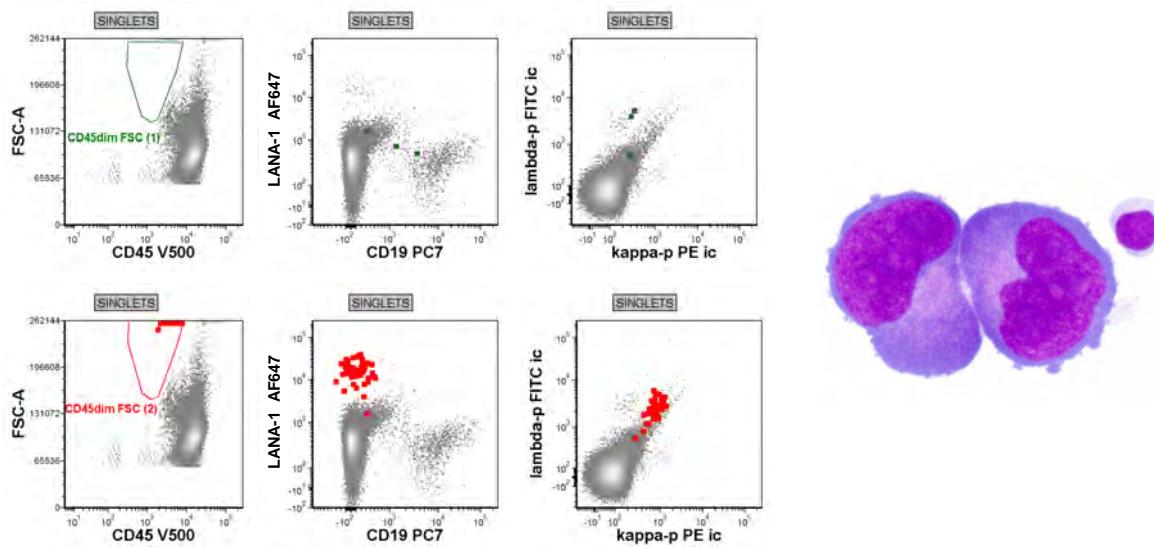
Summary - Phenotypic Features of PEL

- Plasma cell markers
 - Positive: CD38, VS38c, IRF4, CD319, (CD138)
- Negative B-cell markers
 - Negative CD19, CD20
- CD45 usually dim
- Light chain negative in most cases
 - Occasionally positive; when positive, usually with a spectrum and not tight as the lambda-restricted plasmablasts in MCD
- LANA-1 positive

35

PEL – Diagnostic Pitfalls

CSF from a HIV+ patient with history of CNS PEL s/p intrathecal methotrexate/cytarabine/hydrocortisone



36

Acknowledgements



Flow Cytometry Unit

Heart **Marylalice Stetler-Stevenson**

Constance Yuan

Ting Zhou

Cathy McCoy

Linda Weaver

Rob Honec

Truc Ho

Ryan Sochacki

Alyssa Doverte

Leandra Moukoudi Ndoko

Jake Wellek

Dan Moyer

Aaron Nelson

Tyler Lowe

Naomi Hniang

Hematopathology

Elaine Jaffe

Stefania Pittaluga

NCI Investigators

Robert Yarchoan

Ramya Ramaswami

Kathryn Lurain

Thomas Uldrick



hao-wei.wang@nih.gov