



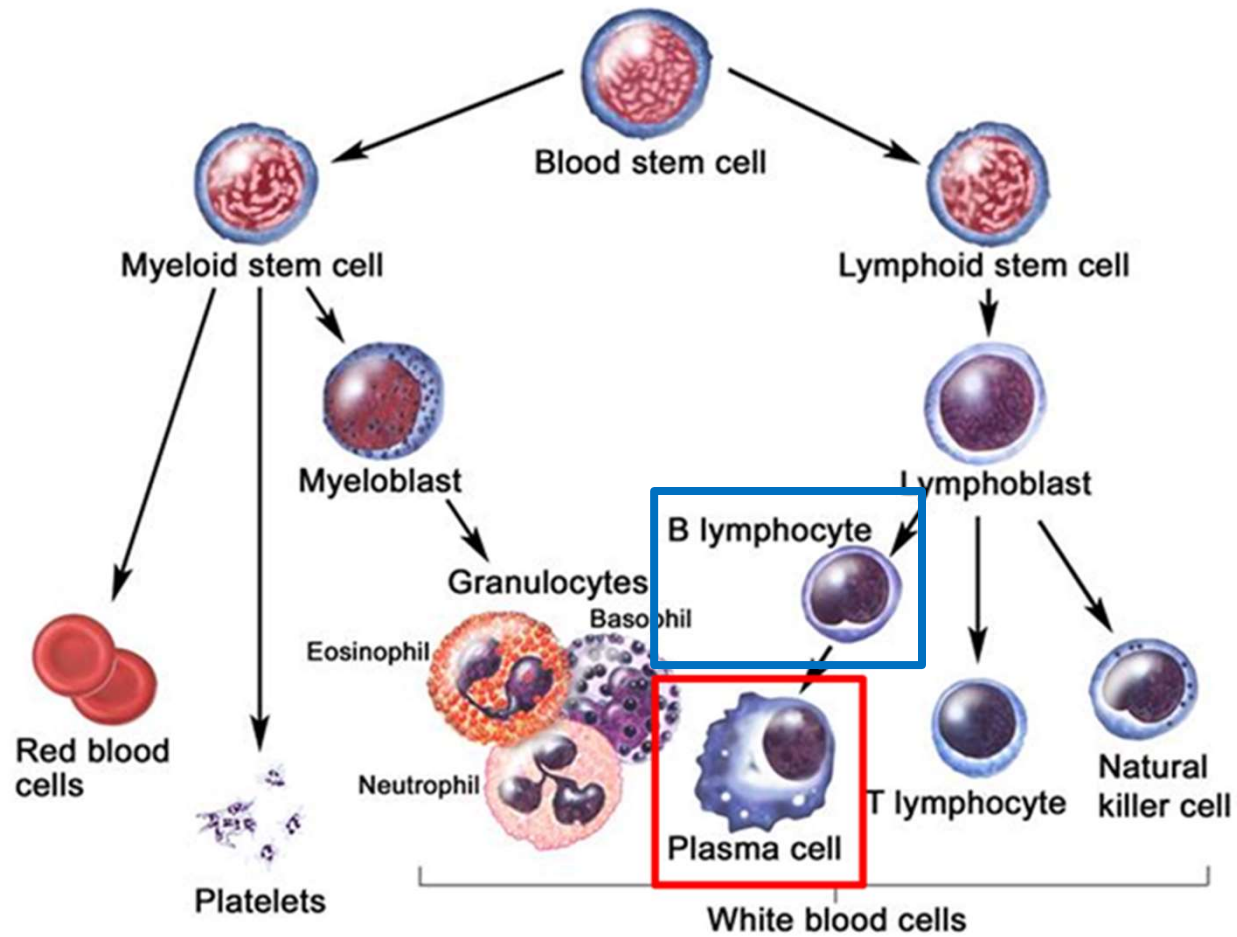
B-CELL LYMPHOMAS & PLASMA CELL DISORDERS

Adam Seegmiller, M.D., Ph.D.







Indonesian Cytometry Association Online Workshop

May 27, 2016







B Cells and Plasma Cells



B Cell / Plasma and Immunophenotype

	Mature Naïve B Cells	Activated B Cells/ Plasmablasts	Mature Plasma Cells
CD19	Positive		Positive
CD20	Positive		Negative
CD38	Variable		Bright
CD138	Negative		Positive
CD45	Bright		Dim
slg	Positive		Negative
clg	Positive		Positive

B Cell / Plasma and Immunophenotype

	Mature Naïve B Cells	Activated B Cells/ Plasmablasts	Mature Plasma Cells
CD19	Positive		Positive
CD20	Positive		Negative
CD38	Variable		Bright
CD138	Negative		Positive
CD45	Bright		Dim
sIg	Positive		Negative
clg	Positive		Positive

B Cell / Plasma and Immunophenotype

	Mature Naïve B Cells	Activated B Cells/ Plasmablasts	Mature Plasma Cells
CD19	Positive	Positive	
CD20	Positive	Negative	
CD38	Variable	Bright	
CD138	Negative	Positive	
CD45	Bright	Dim	
sIg	Positive	Negative	
clg	Positive	Positive	

Selected B-cell Lymphomas (WHO)

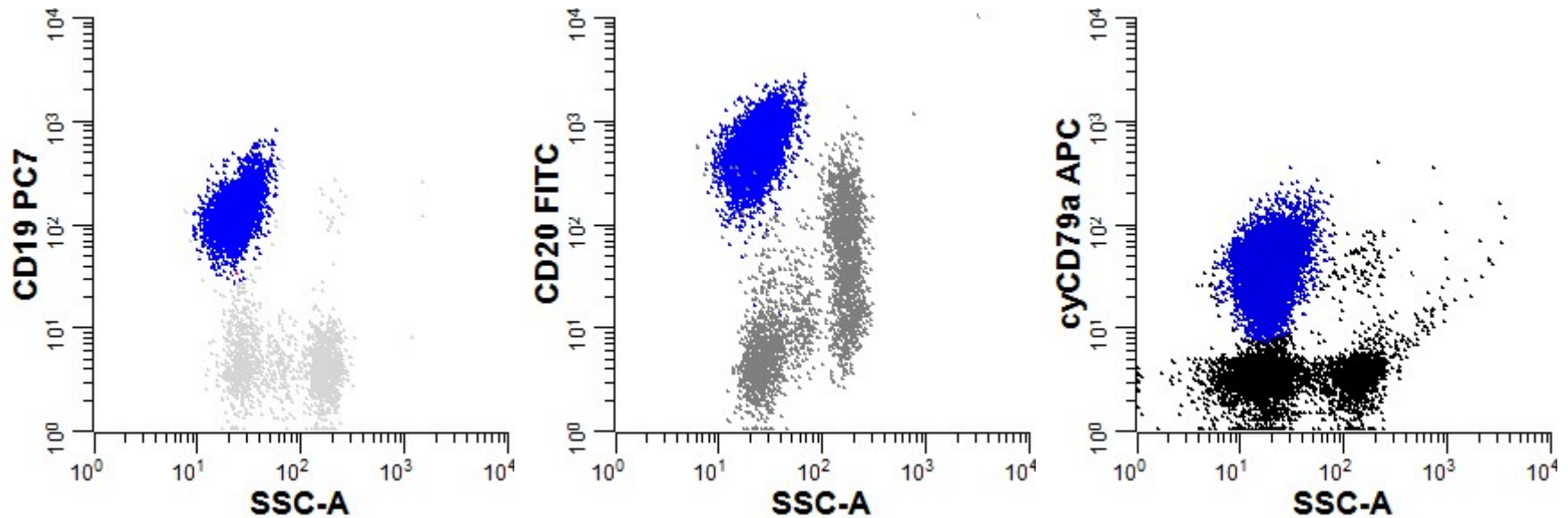
- Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- Lymphoplasmacytic lymphoma
- Extranodal marginal zone lymphoma (MALT)
- Nodal marginal zone lymphoma
- Follicular lymphoma
- Mantle cell lymphoma
- Burkitt Lymphoma
- Diffuse Large B-cell Lymphoma & Variants

A Standard Approach

- Five questions to ask for any population...
 1. What lineage are these cells?
 2. Are these cells mature or immature?
 3. Are these cells benign or neoplastic?
 4. What size are these cells?
 5. Subclassification

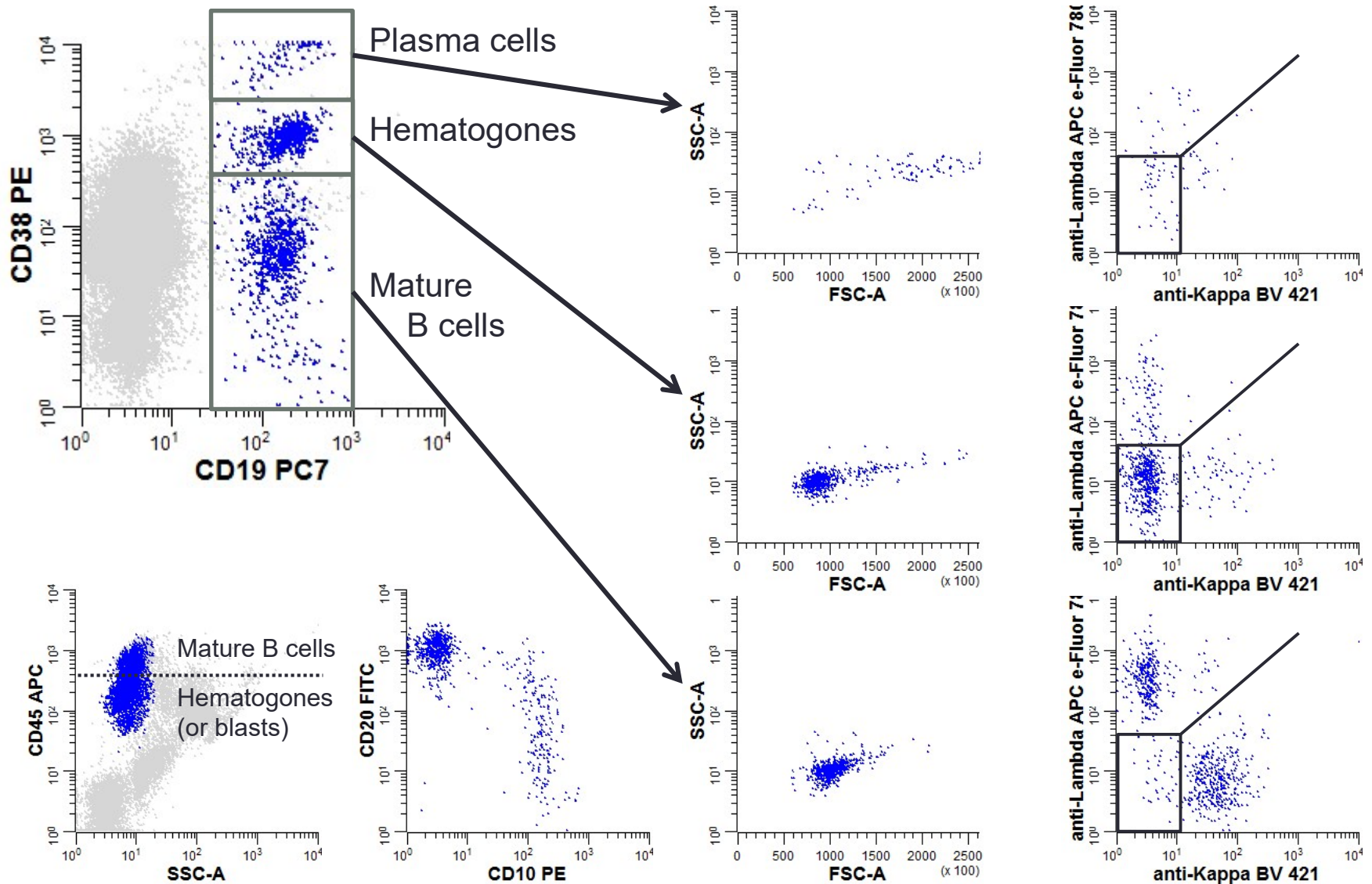
1. What lineage are these cells?

- Markers of B cells: CD19, CD20, CD22, iCD79a

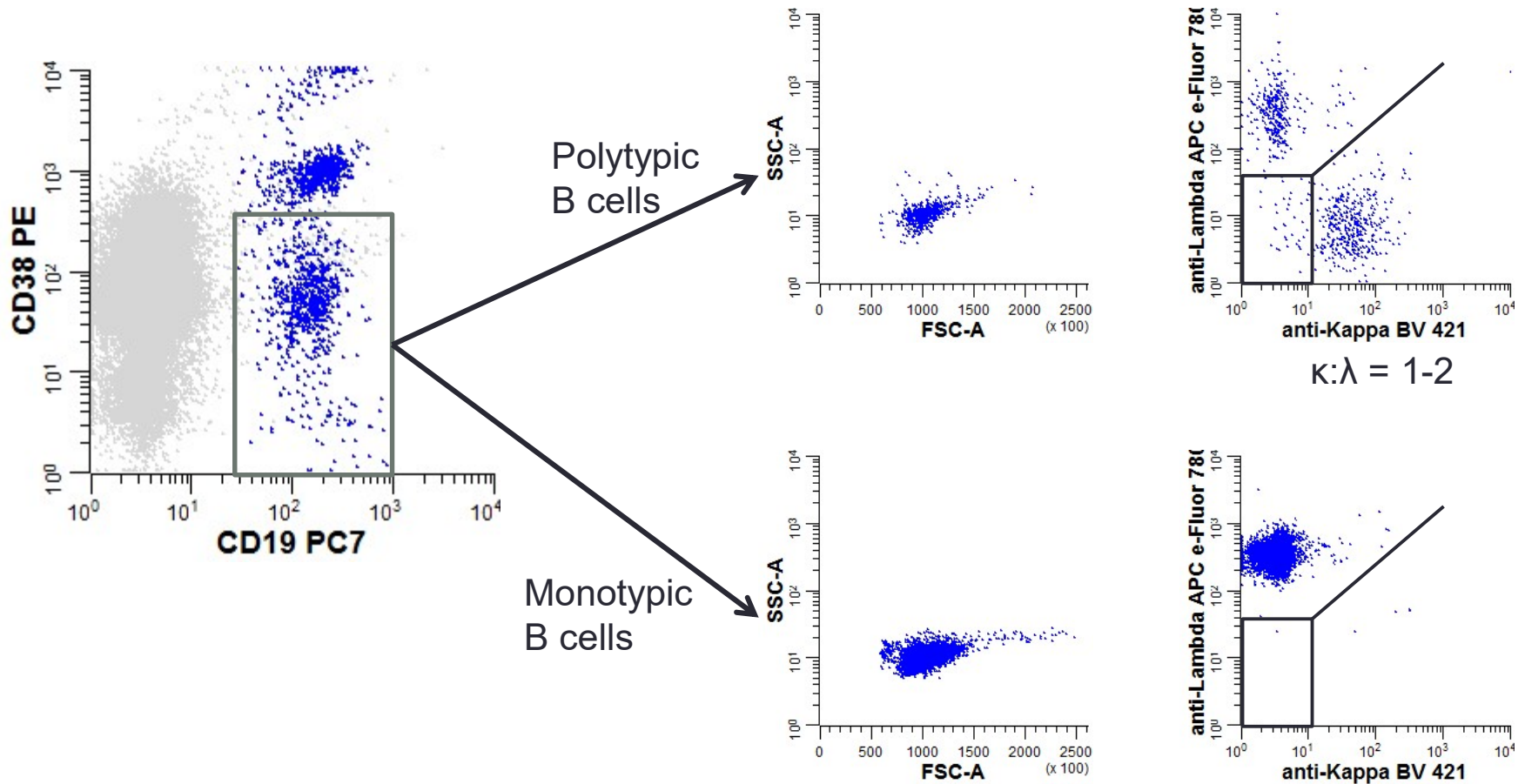


- Caveats:
 1. Anti-antigen therapies: rituximab, blinitumomab, etc.
 2. Some lymphomas down-regulate CD20 (CLL).

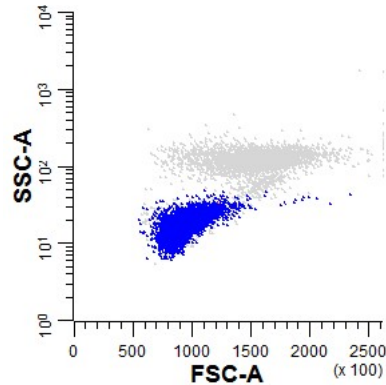
2. Are these cells mature or immature?



3. Are these cells benign or neoplastic?

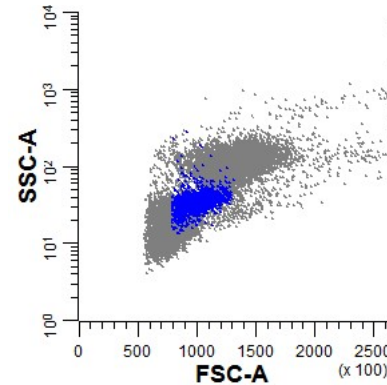


4. What size are these cells?



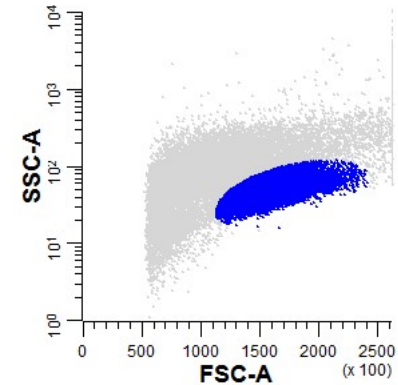
Small

CLL/SLL
Mantle cell lymphoma
Follicular lymphoma
Marginal zone
lymphoma
Lymphoplasmacytic
lymphoma



Medium

Hairy cell leukemia
Follicular lymphoma
(higher grade)
Burkitt lymphoma

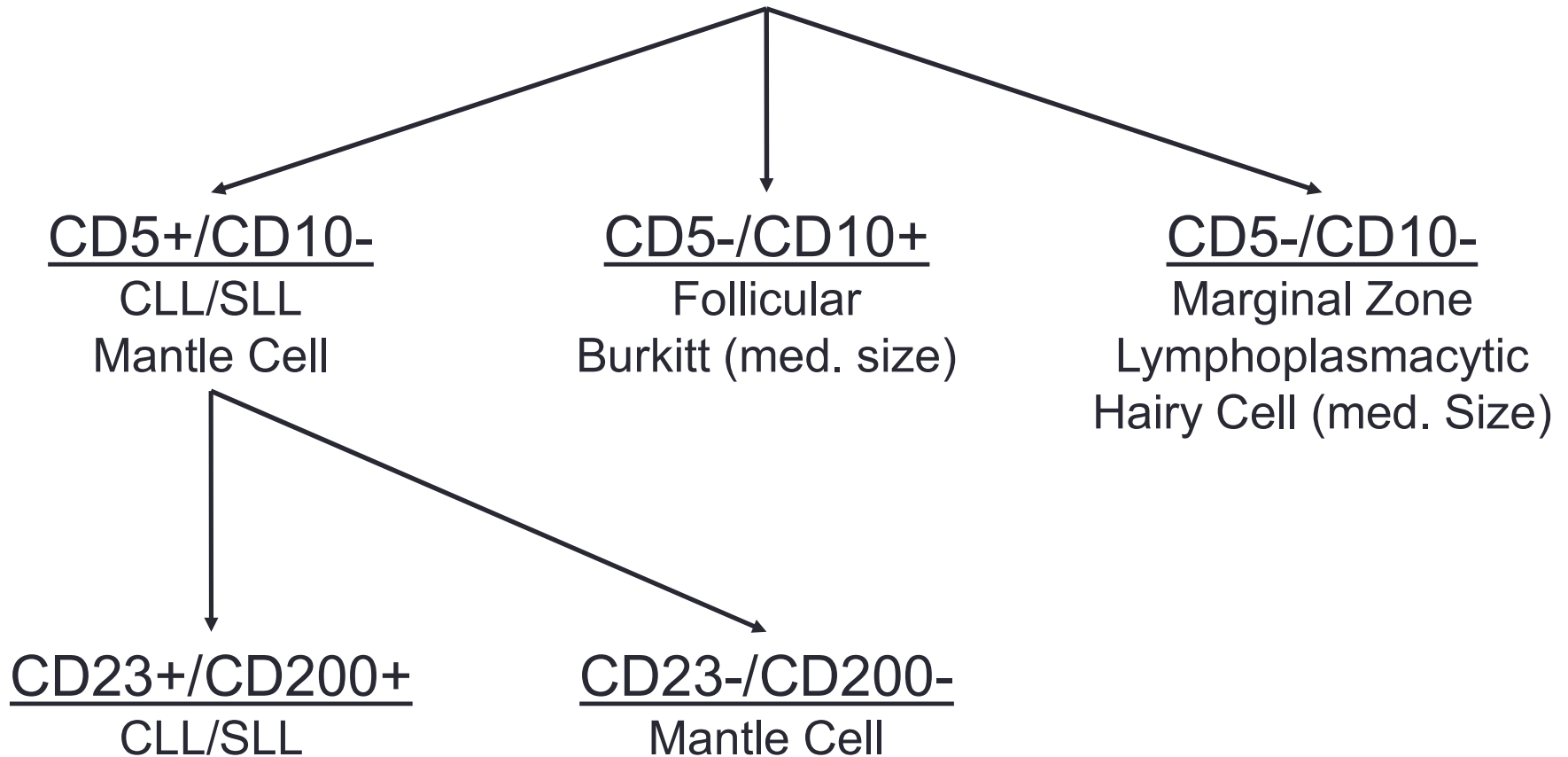


Large

Diffuse large B-cell
lymphoma

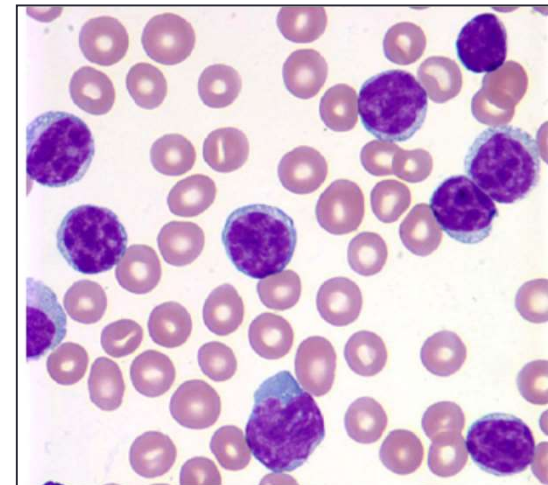
5. Subclassification

Small/Medium Mature B-lineage Leukemia/Lymphoma



Example #1:

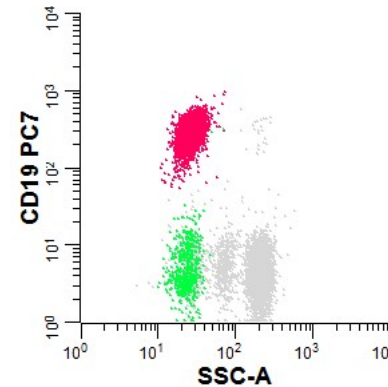
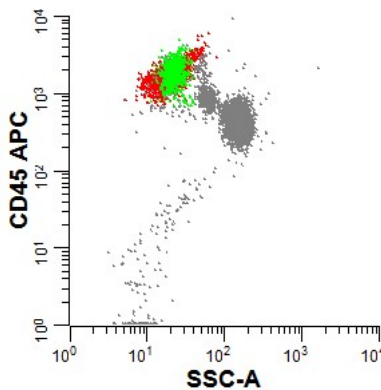
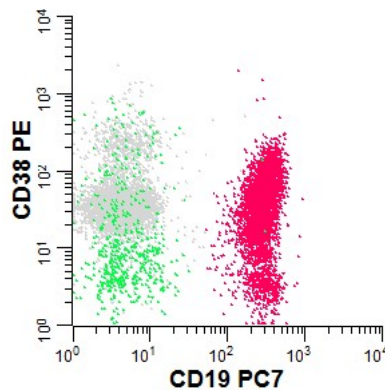
- 62 year-old male presenting with dysphonia (vocal changes) and found to have cervical lymphadenopathy.
- CBC
 - WBC: $16.9 \times 10^3/\mu\text{L}$ [3.9-10.7] – 58% lymphocytes
 - Hbg: 13.5 g/dL [14.0-18.1]
 - Plt: $207 \times 10^3/\mu\text{L}$ [135-371]
- Peripheral blood smear:
- Flow cytometry performed on blood.



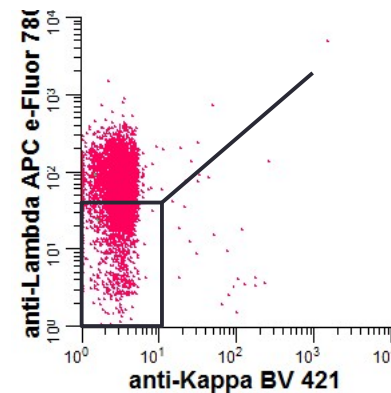
Example #1:

1. Lineage: 46% CD19(+) B cells

2. Maturity: CD38 (mod/dim)
CD45 (bright)

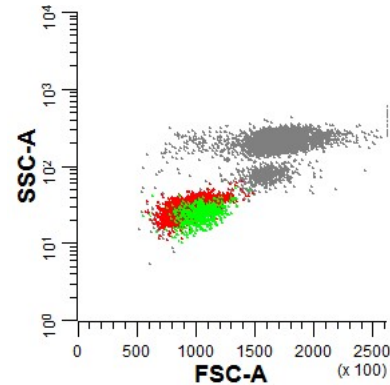


3. Light chain restricted (lambda dim)

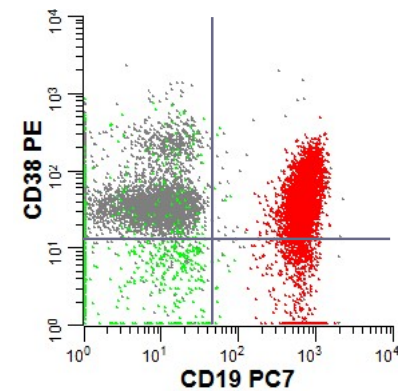
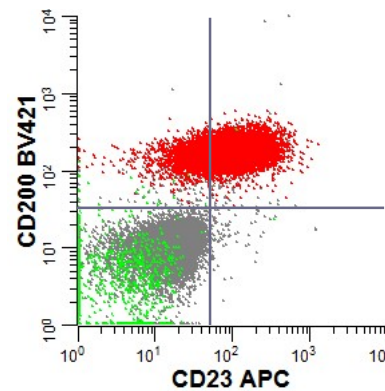
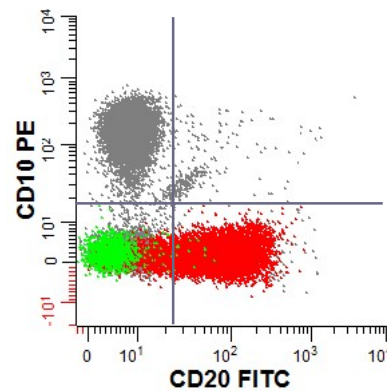
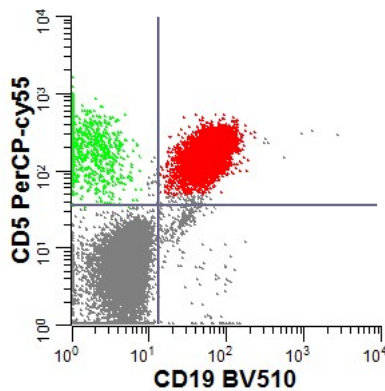


Example #1:

4. Small size:



5. Positive: CD5, CD23, CD38 (mod/dim), CD200 (bright)
Negative: CD10



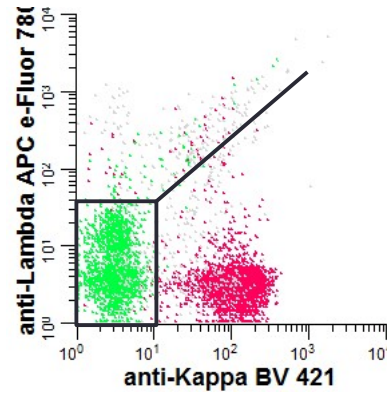
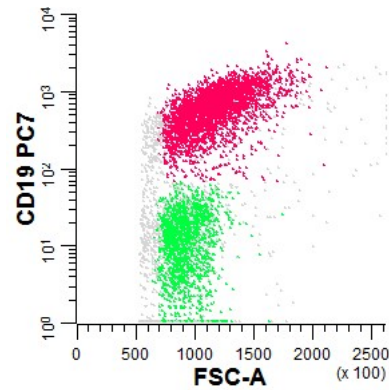
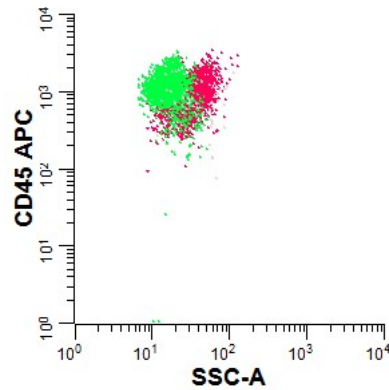
CLL/SLL

- Monotypic mature CD19(+) B cells, often with dim light chain expression.
- Usually small; larger size may indicate increased prolymphocytes.
- Aberrantly expresses CD5.
- Also expresses CD23 and CD200 (bright); differentiates it from mantle cell lymphoma.
- CD20 is often decreased (except with trisomy 12).

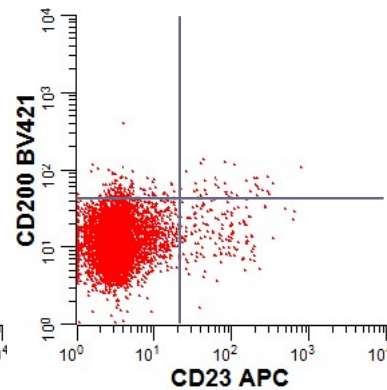
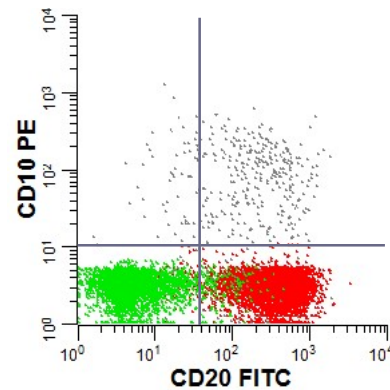
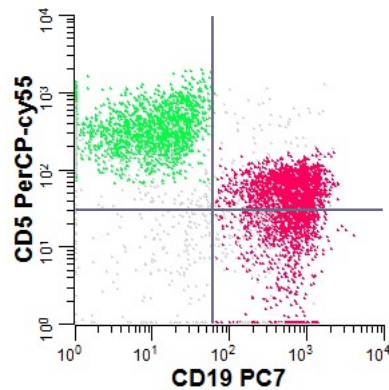
Example #2:

- 61 year-old female presented with chest pain and shortness of breath.
- Imaging studies showed widespread lymphadenopathy and mild splenomegaly.
- Flow cytometry was performed on a cervical lymph node.

Example #2:



1. B-cell process
2. Mature
3. Monotypic
4. Small-medium sized



5. Positive for CD5 (dim), CD20

Negative for CD10, CD23, CD200

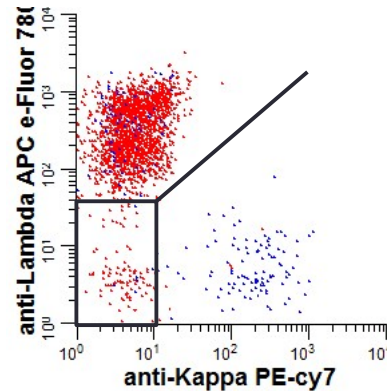
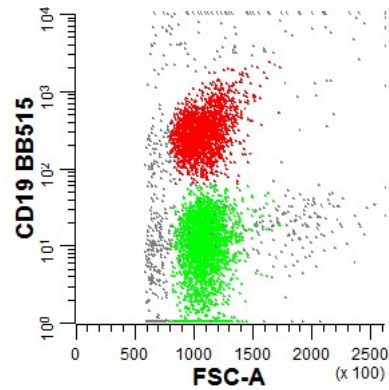
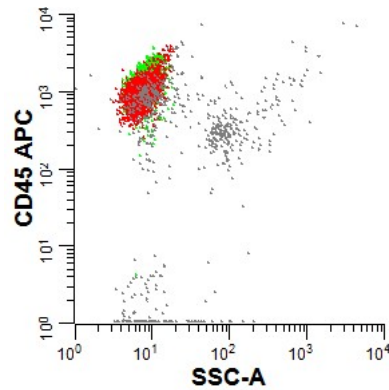
Mantle Cell Lymphoma

- Monotypic mature CD19(+) B cells, with bright light-chain expression
- Usually small; larger size may indicate more aggressive variant (pleomorphic or blastic).
- Aberrantly expresses CD5, but is generally negative for CD23 and CD200, differentiating it from CLL/SLL.
- Rare variants: CD5(-) or CD10(+) or CD23(+).
- Confirmation is by cyclin D1 expression or t(11;14) rearrangement.

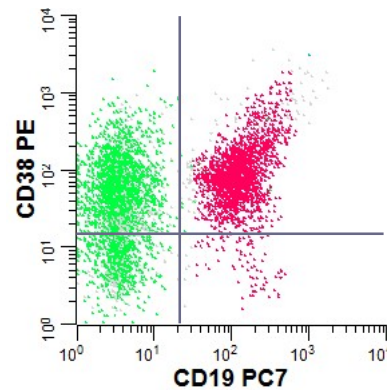
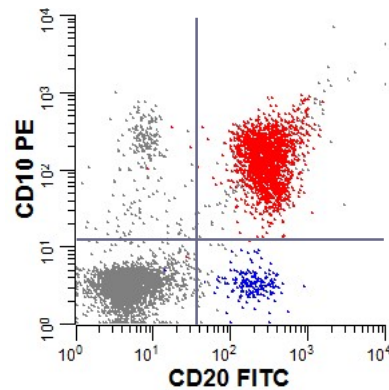
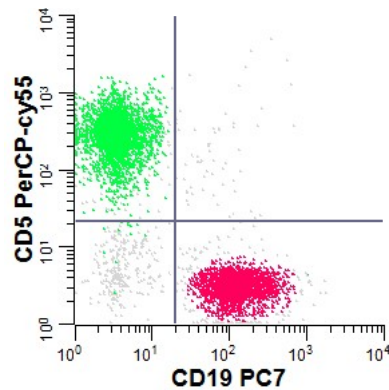
Example #3:

- 53 year-old male presented with abdominal pain.
- Imaging studies showed enlarged mediastinal, mesenteric, and retroperitoneal lymph nodes.
- Flow cytometry was performed on a retroperitoneal lymph node.

Example #3:



1. B-cell process
2. Mature
3. Monotypic
4. Small



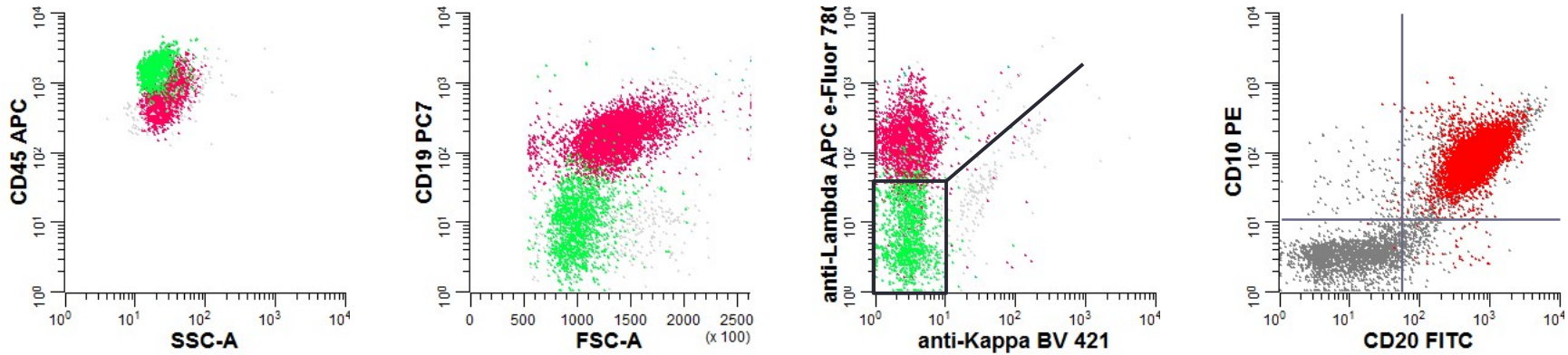
5. Positive for CD10, CD20, CD38 (mod)

Negative for CD5, CD23, CD200 (not shown)

Follicular Lymphoma

- Monotypic mature CD19(+) B cells
- Expresses CD10, consistent with germinal center origin.
- Usually small or variably-sized.
- Higher-grade follicular lymphoma may be negative for CD10. Morphology is essential for the diagnosis.

What about this?

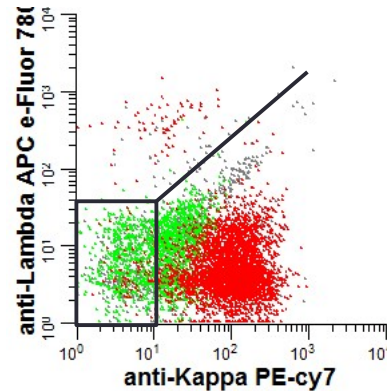
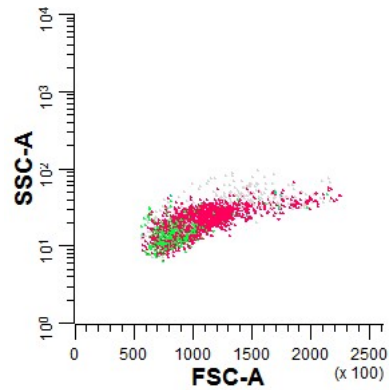
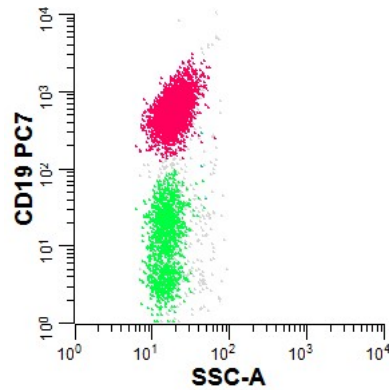


- If CD10(+) monotypic B cells are larger, then consider the following differential diagnosis:
 1. Higher grade follicular lymphoma
 2. Burkitt lymphoma (typically CD38 bright)
 3. Diffuse large B-cell lymphoma

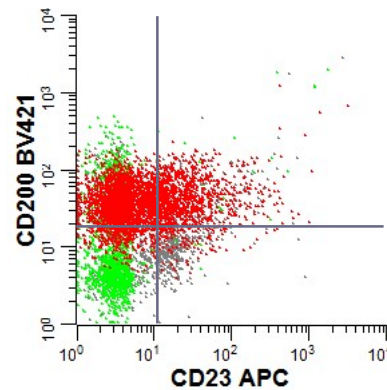
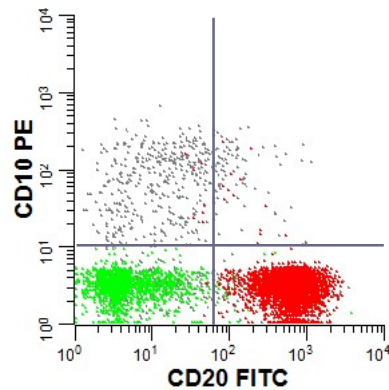
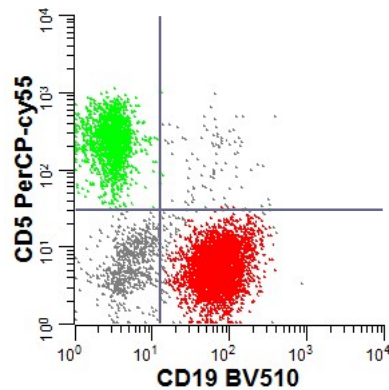
Example #4:

- 44 year-old female that presented complaining of abdominal fullness and fatigue.
- Review of systems also revealed frequent drenching night sweats and a recent 30-pound weight loss.
- Abdominal ultrasound showed splenomegaly.
- CBC:
 - WBC: $4.2 \times 10^3/\mu\text{L}$ [3.9-10.7]
 - Hbg: 11.9 g/dL [11.8-16.0]
 - Plt: $115 \times 10^3/\mu\text{L}$ [135-371]
- Flow cytometry was performed on a splenectomy specimen.

Example #4:



1. B-cell process
2. Mature
3. Monotypic
4. Small



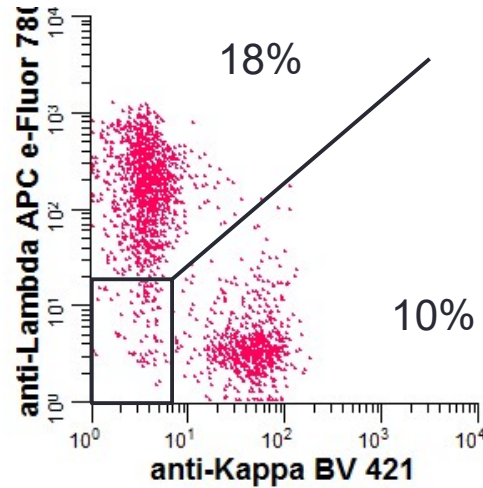
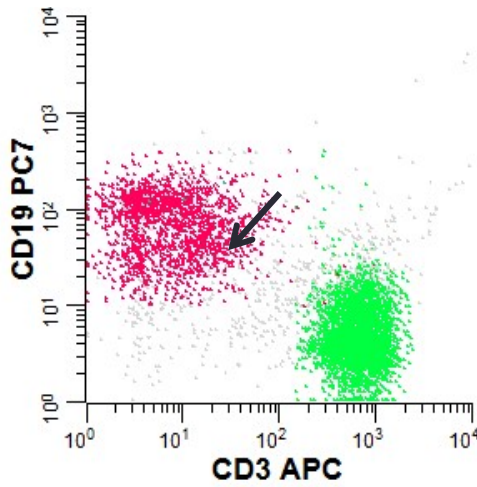
5. Positive for CD20, CD23 (dim), CD200 (mod)

Negative for CD5, CD10

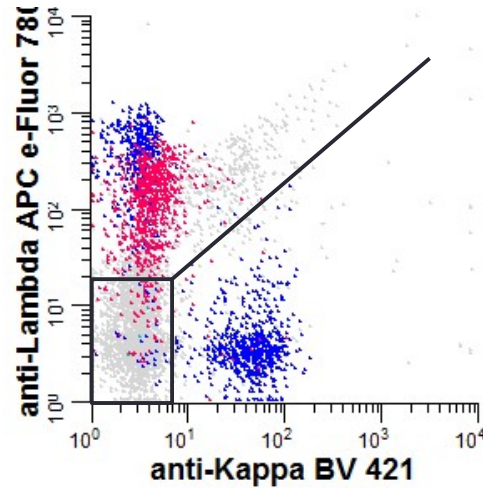
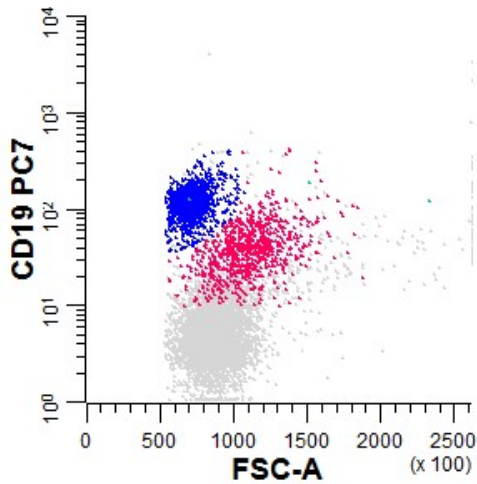
Marginal Zone Lymphoma

- Three different presentations with similar phenotype:
 - Splenic – marrow, peripheral blood, spleen
 - Nodal – lymph nodes
 - Extranodal (MALT) – mucosal tissues: GI tract, salivary gland, lung, ocular adnexa, skin, thyroid, etc.
- Phenotype is non-specific: negative for CD5 and CD10
- May occur in a background of polytypic B cells – can be difficult to separate.
- Frequent plasmacytic differentiation.
- Difficult to distinguish from lymphoplasmacytic lymphoma; requires correlation with morphologic, clinical, and molecular findings.

Separating MZL from Background



Kappa:Lambda = 0.56:1
(normal = 1-2:1)



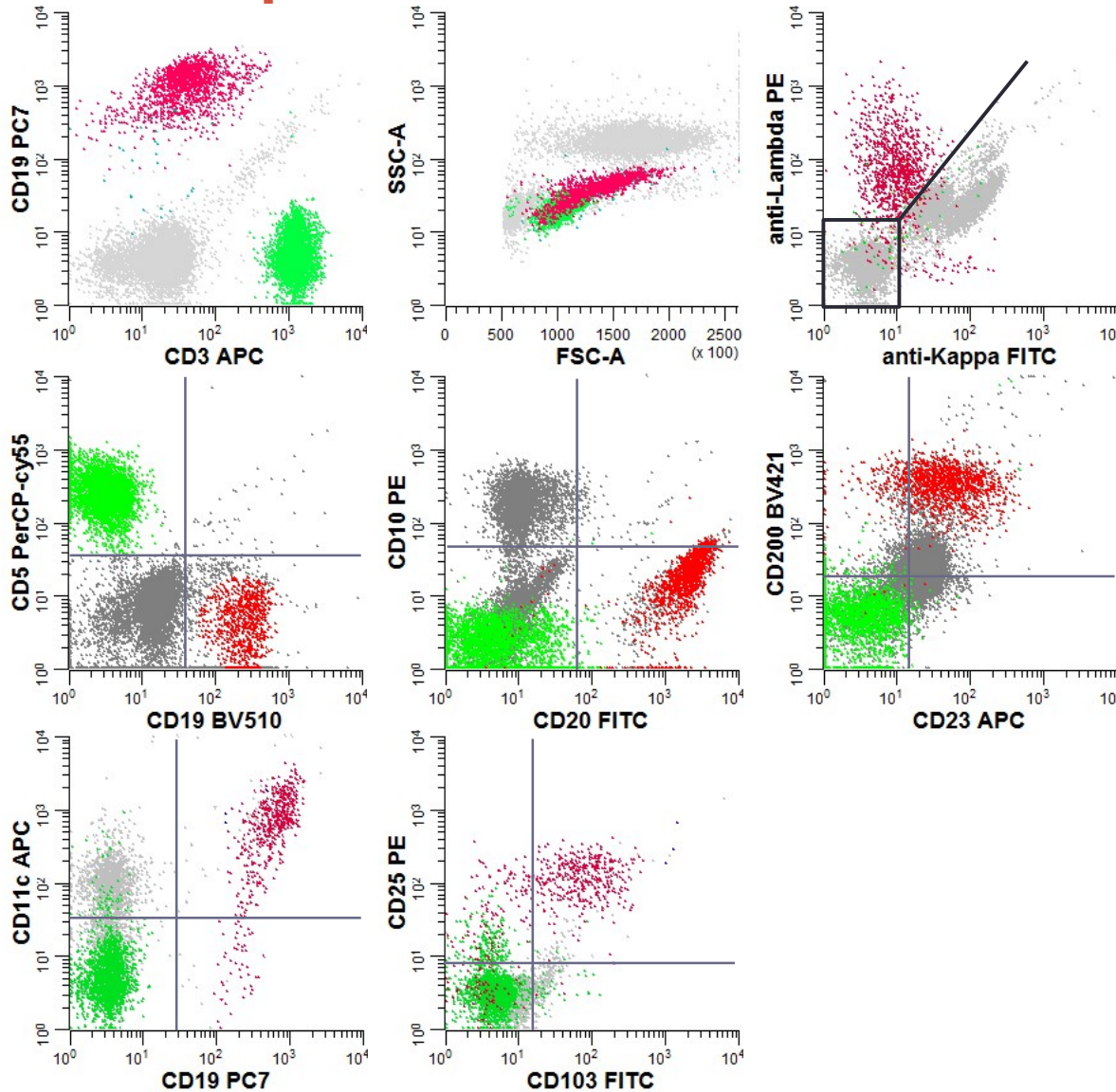
Marginal Zone Lymphoma

- Three different presentations with similar phenotype:
 - Splenic – marrow, peripheral blood, spleen
 - Nodal – lymph nodes
 - Extranodal (MALT) – mucosal tissues: GI tract, salivary gland, lung, ocular adnexa, skin, thyroid, etc.
- Phenotype is non-specific: negative for CD5 and CD10
- May occur in a background of polytypic B cells – can be difficult to separate.
- Frequent plasmacytic differentiation.
- Difficult to distinguish from lymphoplasmacytic lymphoma; requires correlation with morphologic, clinical, and molecular findings.

Example #5:

- 56 year-old male presented complaining of fatigue, early satiety, and weight loss.
- CBC:
 - WBC: $7.9 \times 10^3/\mu\text{L}$ [3.9-10.7]
 - 81% lymphocytes: $6.4 \times 10^3/\mu\text{L}$ [1.1-3.5]
 - 16% neutrophils: $1.3 \times 10^3/\mu\text{L}$ [1.6-8.1]
 - 3% monocytes: $0.2 \times 10^3/\mu\text{L}$ [0.3-1.1]
 - Hbg: 12.4g/dL [14.0-18.1]
 - Plt: $61 \times 10^3/\mu\text{L}$ [135-371]
- Imaging shows massive splenomegaly.
- Flow cytometry was performed on bone marrow.

Example #5:



1. B-cell process
2. Mature
3. Monotypic
4. Medium-sized with increased side scatter.
5. Positive for CD20, CD23, CD200 (bright)

Negative for CD5, CD10

Positive for CD11c (bright), CD25, and CD103

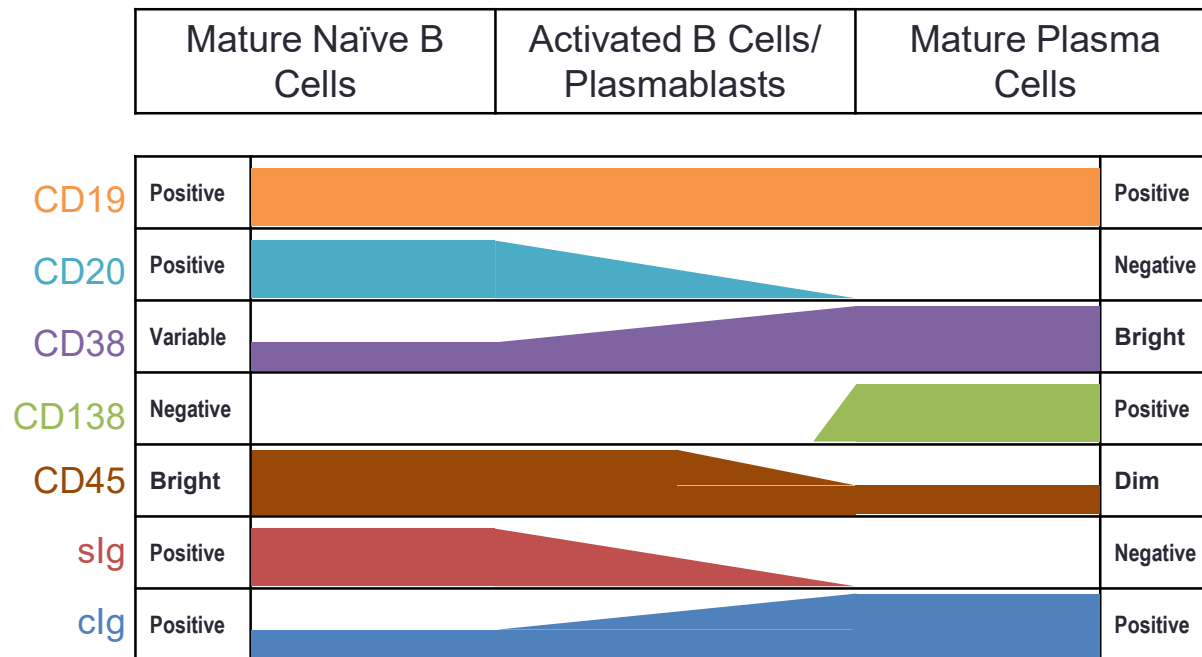
Hairy Cell Leukemia

- Same phenotype as marginal zone and lymphoplasmacytic lymphomas: CD5(-)/CD10(-)
- Clues to consider diagnosis:
 - Older male
 - Leukopenia, especially monocytopenia
 - Large spleen
 - Medium-sized monotypic B cells with increased side scatter
- Other phenotypic features:
 - Bright expression of CD11c, CD22, and CD200.
 - Double-positive for CD25 and CD103 (SMZL may express CD25, but not CD103)



PLASMA CELL DISORDERS

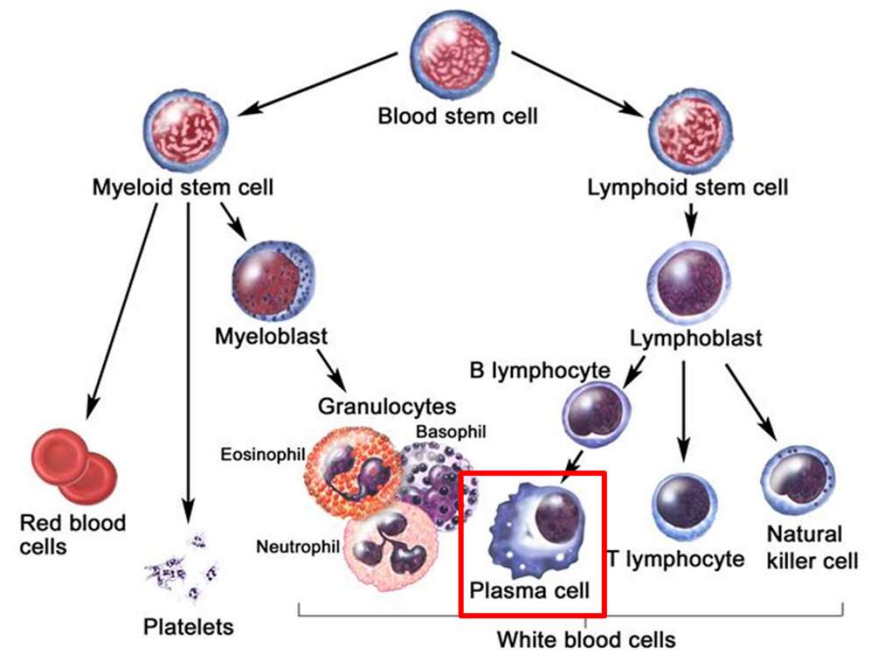
Plasma Cell Maturation and Immunophenotype



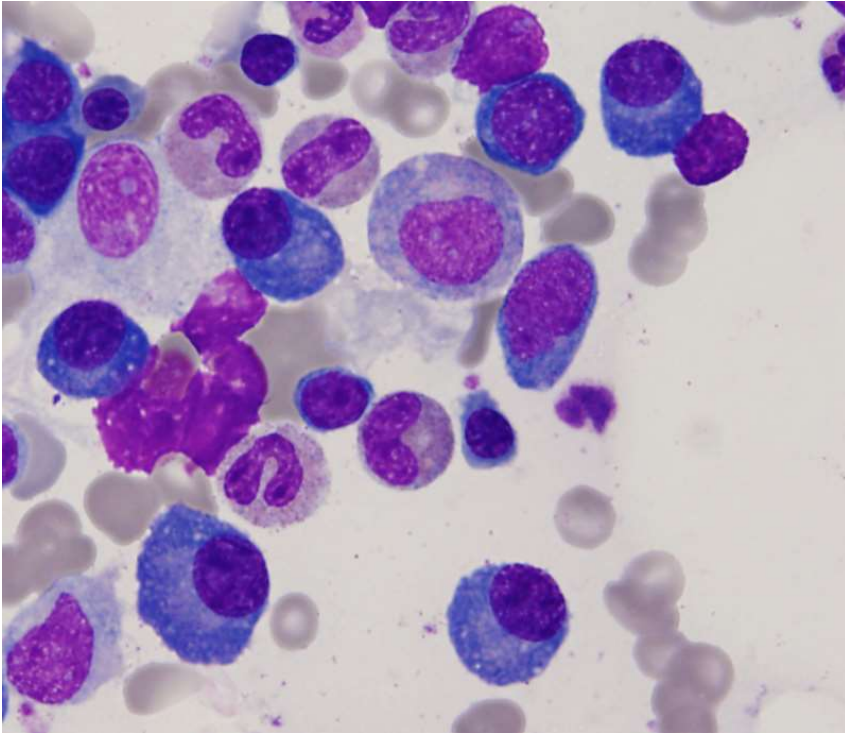
Plasma Cells

What is a plasma cell?

- Terminally differentiated B cells.
- Develops from naïve B cells in lymph nodes after exposure to antigen and stimulation by helper T cells.
- Produces large quantities of soluble immunoglobulin

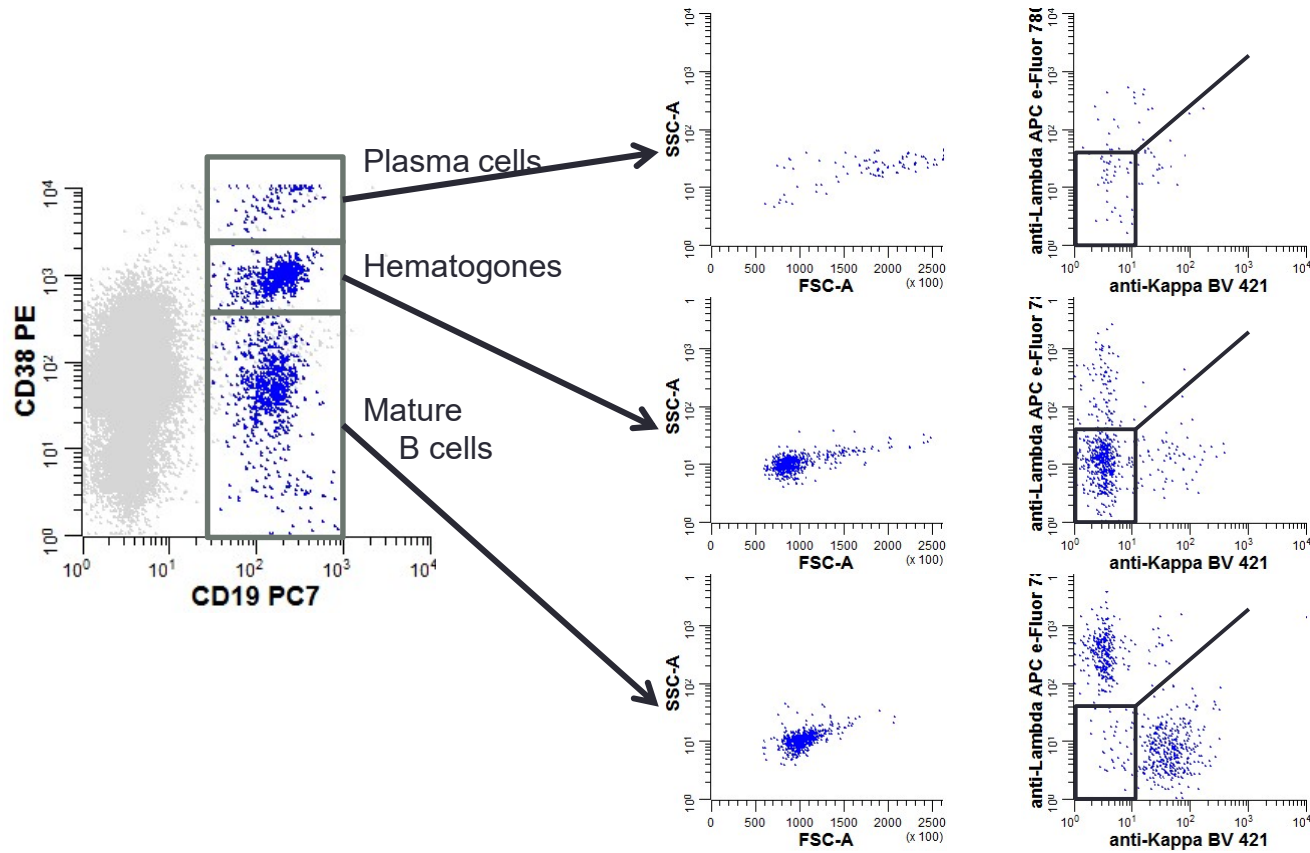


Plasma Cells



- Medium-sized
- Eccentrically-placed nucleus
- Condensed chromatin without visible nucleoli
- Basophilic cytoplasm
- Peri-nuclear hof

Identification of Plasma Cells



Single Tube Plasma Cell Evaluation

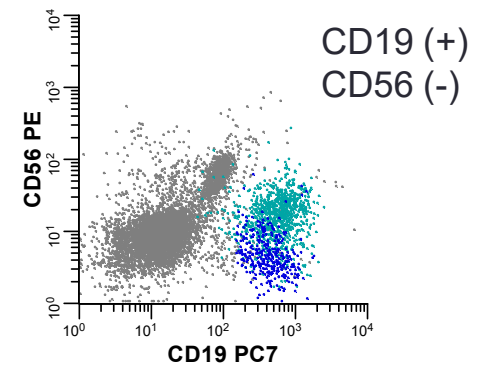
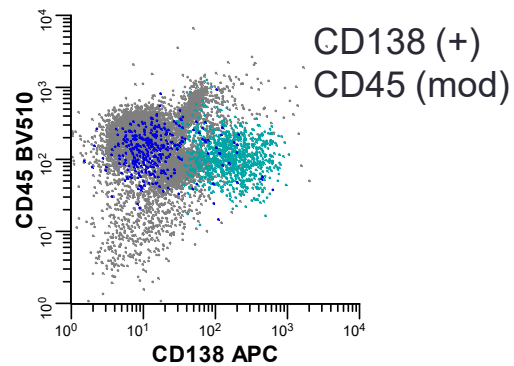
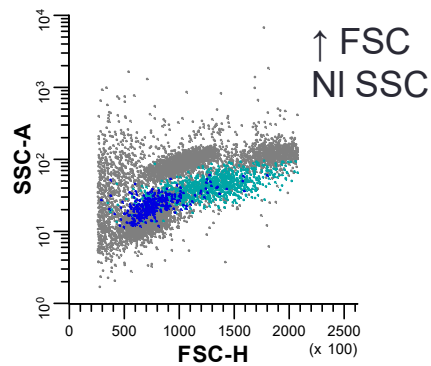
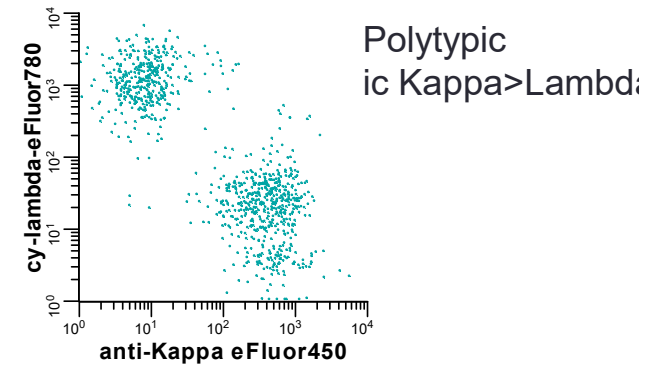
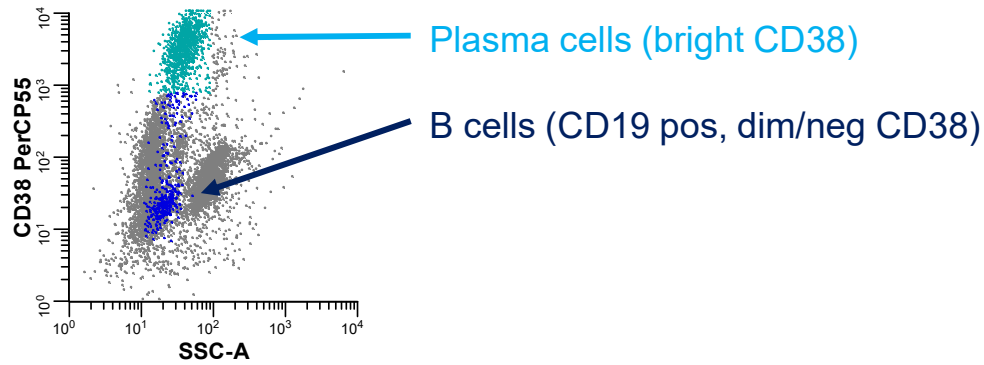
Approach

- Permeabilized cells – to detect intracellular kappa and lambda.
- Bulk lysis (LSW) and increased number of cells for analysis – percentage of plasma cells is often low.

Markers

- Identification
 - CD38
 - CD138
- Clonality
 - Kappa
 - Lambda
- Phenotype
 - CD19
 - CD45
 - CD56

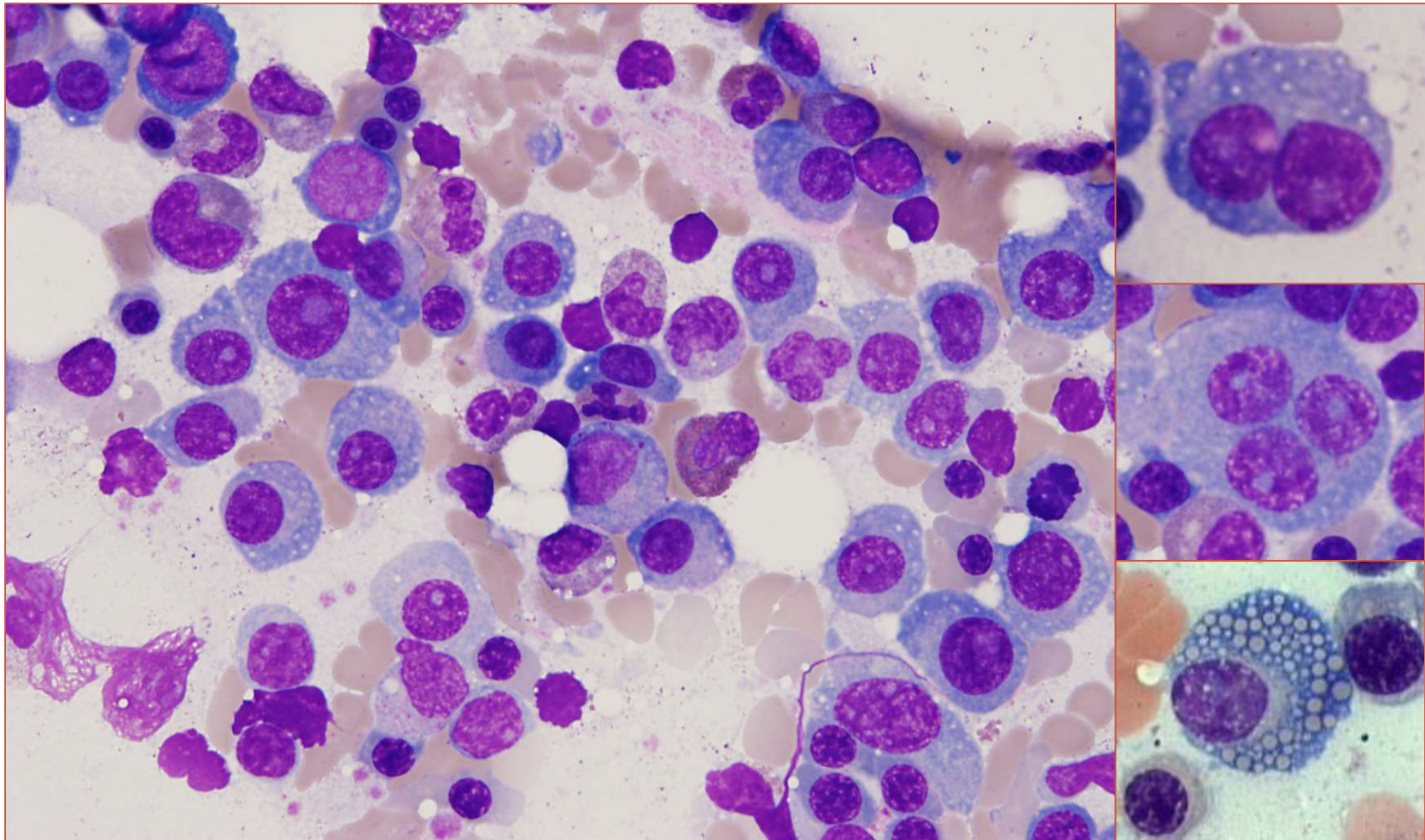
Normal Plasma Cells



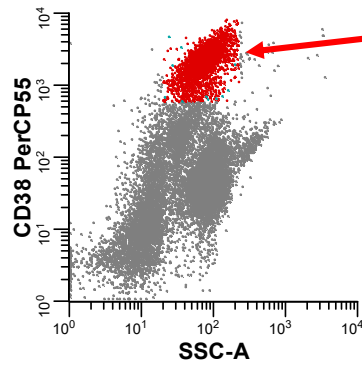
Plasma Cell Myeloma

- Proliferation of monoclonal plasma cells in bone marrow and/or tissue.
- Clinical manifestations:
 - Mostly a disease of older patients (median age 65-69), more common in males and African Americans
 - Plasma cells generate monoclonal immunoglobulin (IgG > IgA >> IgM)
 - Clinical consequences include lytic bone lesions, hypercalcemia, renal impairment, and anemia

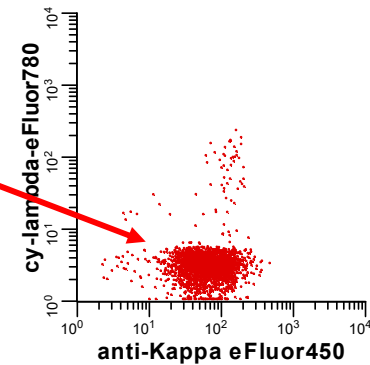
Plasma Cell Myeloma



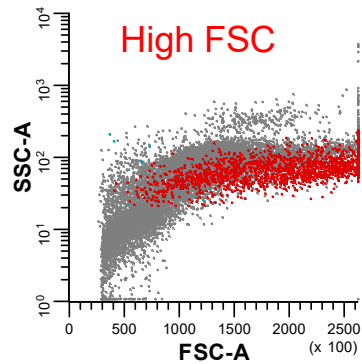
Abnormal Plasma Cells in Myeloma



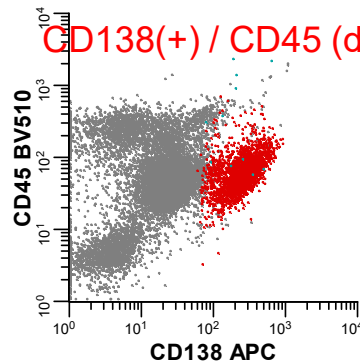
Bright CD38



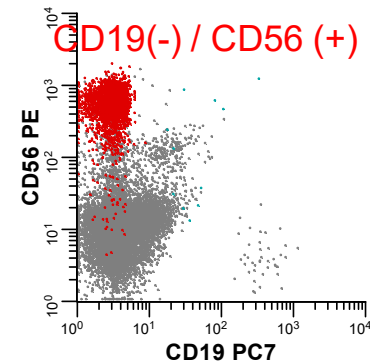
Monotypic
Kappa



High FSC



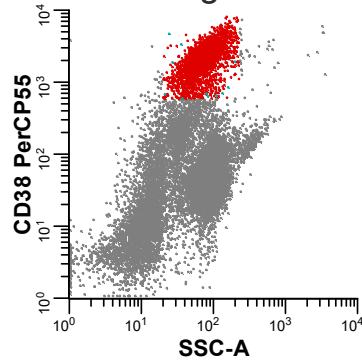
CD138(+) / CD45 (dim)



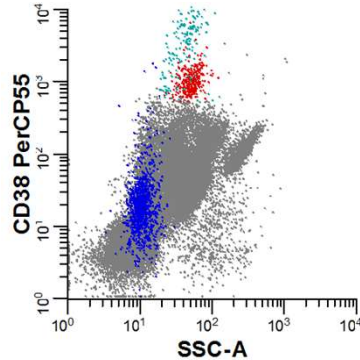
CD19(-) / CD56 (+)

Challenges: CD38

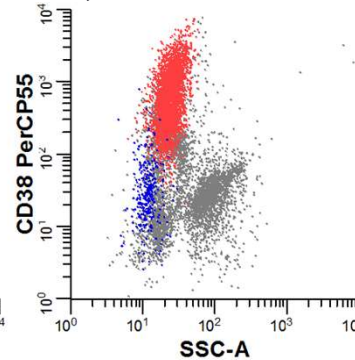
Normal: bright CD38



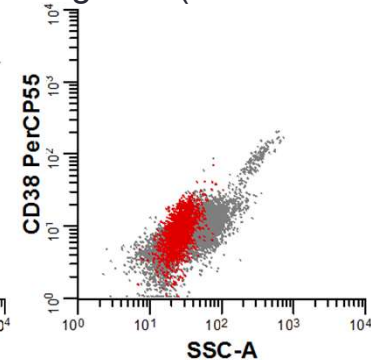
Abnormal: dim CD38 dim, variable



dim, variable

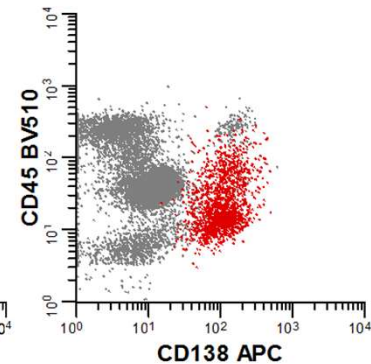
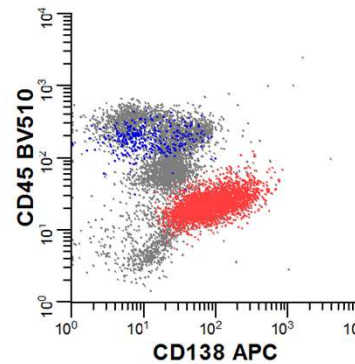


negative (daratumumab)



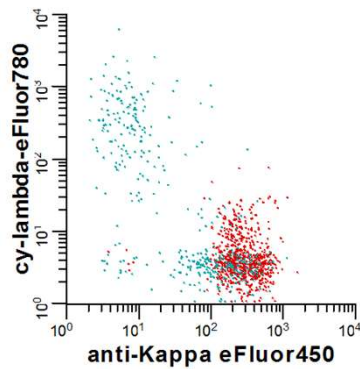
Take home:

- Don't just focus on CD38/SSC
- Make sure your panel has a way to identify plasma cells that doesn't rely exclusively on CD38.

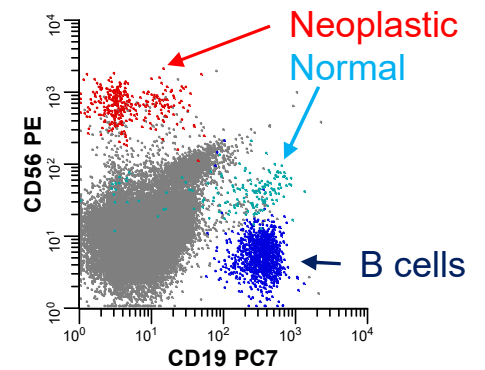
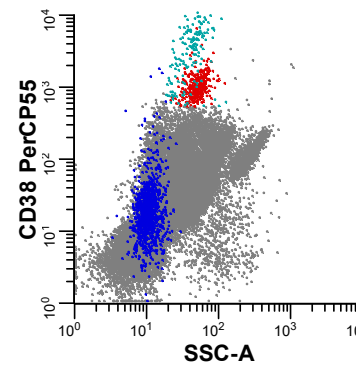


Options include CD138, p63, CD229

Challenges: Light chain



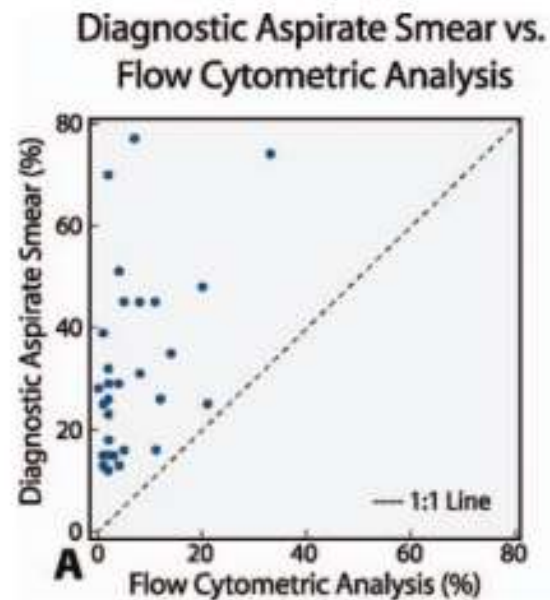
Monotypic plasma cells
on a background of
normal polytypic plasma
cells



Take home: Neoplastic plasma cells can be separated from normal by identifying aberrant expression of other markers.

Challenges: Plasma Cell Enumeration

- Plasma cell numbers are consistently lower by flow cytometry than by aspirate smear differential counts or immunohistochemistry.



Immunophenotypic Aberrancy

Marker	Normal Plasma Cells	Plasma Cell Myeloma	Diagnostic/Clinical Significance
CD19	Positive	Negative (95%)	Myeloma vs. lymphoma
CD20	Negative	Positive (10-20%)	t(11;14), better prognosis
CD27	Positive	Dim/negative	Neg = worse prognosis
CD28	Negative	Positive (40%)	↑ with disease progression
CD33	Negative	Positive	Pos = worse prognosis
CD38	Bright	Dimmer	
CD45	Moderate/dim	Dim/negative	Neg = worse prognosis
CD52	Negative	Positive (8-14%)	
CD56	Negative	Positive (75-80%)	Neg = more circulating PCs
CD81	Positive	Dim/negative	
CD117	Negative	Positive (20-35%)	Pos = better prognosis
CD200	Negative	Positive (60-75%)	Pos = worse prognosis

B-cell Lymphoma with Plasmacytic Differentiation

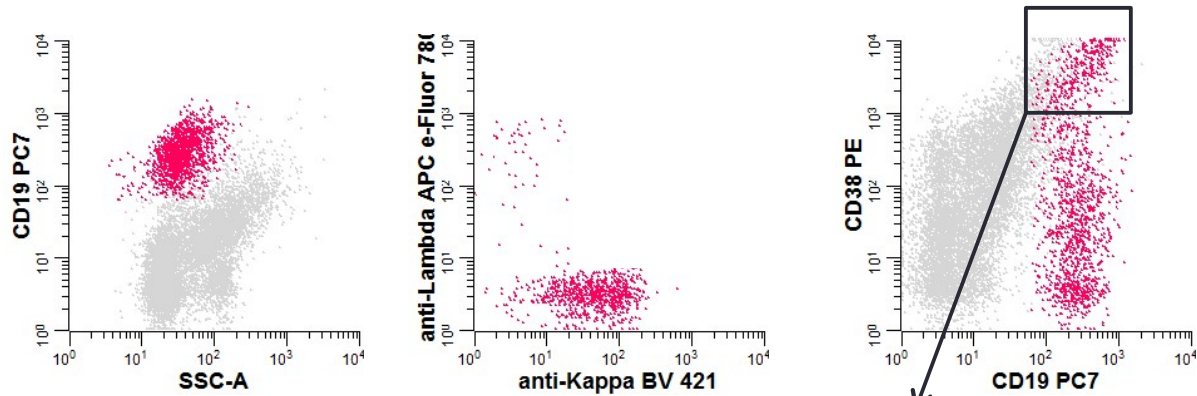
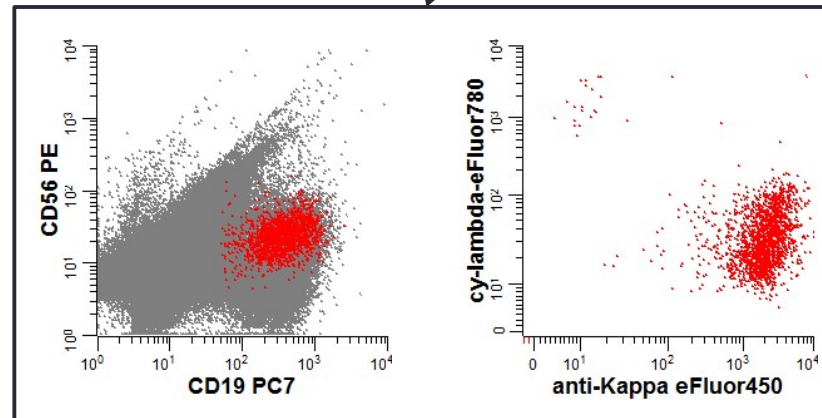


Table 1
Expression of Cell Surface Markers on Neoplastic PCs in B-Cell Non-Hodgkin Lymphomas and PC Myelomas

Antigen/Disease	No. (%) Positive	P ^a
CD19		
Lymphoma (n = 41)	39 (95)	<<.001
Myeloma (n = 41)	4 (10)	
CD20		
Lymphoma (n = 41)	13 (32)	.81
Myeloma (n = 41)	11 (27)	
CD45		
Lymphoma (n = 33)	30 (91)	<<.001
Myeloma (n = 41)	17 (41)	
CD56		
Lymphoma (n = 18)	6 (33)	.01
Myeloma (n = 41)	29 (71)	
Surface immunoglobulin		
Lymphoma (n = 41)	31 (76)	.006
Myeloma (n = 41)	18 (44)	

PC, plasma cell.
^a By the Fisher exact test.





QUESTIONS?
