

# Clinical Flow Cytometry for the perplexed

## Part 3: Lymphomas

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# Recap from Parts 1 & 2

- What is Flow Cytometry
- Gating Strategy(ies)
- Technical Issues/Artifacts

# Goals for Part 3

- Understand the major diagnostic flow cytometric division in B cell lymphomas
- Recall the phenotype of abnormal plasma cells
- Recognize the maturational pathway of immature B and T cells

# Phenotypes you should care about...

- CLL vs MCL
- Follicular Lymphoma
- Things that are big
- Plasma Cell Myeloma
- T cells...???

# CD5+ B cell populations (CLL/MCL)

## CLL

- Decreased "B cell" ness
- Decreased CD20, CD22, light chains
- Absence of FMC7
- Expression of CD200 and/or CD23

## MCL

- Retained B cell phenotype
- Normal to increased CD20 and CD22
- Retained CD19 and FMC7
- No CD200 or CD23
- Aberrant CD43

# *Nota Bene*

Not everything that is CD5+ is CLL or MCL

- CD5+ Splenic Marginal Zone Lymphoma
- CD5+ DLBCL
- CD5+ B-PLL

# CD10+ Follicular Lymphoma

- CD10 is frequently lost in high grade FL
  - Benign light chain restricted GC populations
  - Cytoplasmic bcl-2 by flow cytometry
- 
- Other things that are CD10+
    - » CD10+ HCL
    - » CD10+ MZLs

# Large Cell Lymphomas

- Gating Strategy must include evaluation of high FS events

Typical phenotype:

Preserved or increased CD19, CD20, CD22

Light chain restricted

Can be CD5+, CD10+, or both



# Large Cell Lymphoma

- Mimics
  - » Burkitts
  - » B-LBL
- High Grade B cell Lymphoma (Double/Triple Hit)
  - » Often decreased CD45, decreased CD20
  - » CD10+, increased CD38
- Other things to note:
  - » ALK+ DLBCL
  - » Plasmablastic Lymphoma

# CD5-/CD10- Lymphomas

- HCL
- HCL-v
- MZL (SMZL, NMZL, MALToma)
- LPL
- CD5- CLL/MCL
- CD10- FL

# Hairy Cell Leukemia (CD103omas)

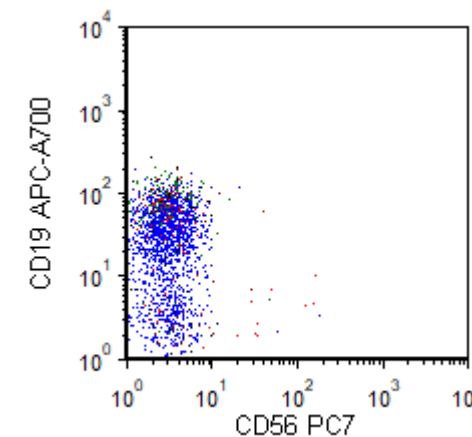
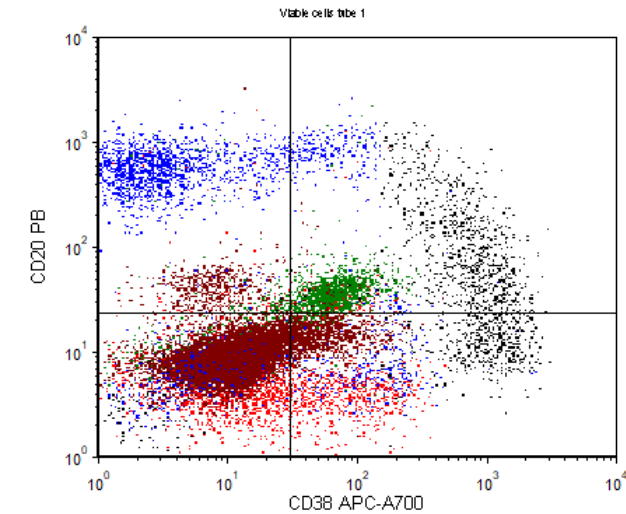
- CD103, CD11c, (CD123, CD25)
- Increased CD20, CD22, Light chains
- 10% with CD10
  
- HCL-v
  - » Similar except without CD25 and/or CD123
  
- Splenic Marginal Zone
  - » Similar to HCL-v

# Utility of flow in CD5-/CD10- lymphomas

- MZL vs LPL
  - » Can't be determined by flow alone (perhaps bright IgM?)
  - » CD25 expression
  - » CD13 expression
- HCL vs HCL-v
  - » Slam dunk
- HCL-v vs SMZL
  - » Little utility
- CD10-negative FL vs MZL
  - » FL typically associated with higher grade
  - » FL will lack CD11c
  - » FL with generally have lower CD19 (retained or increased CD20)

# B cell lymphomas with plasmacytic differentiation

- B cell LPD
  - » Can be anything!
  - » MCL, FL, CLL, MZL, LPL
- Plasma Cell
  - » Looks 'normal'
  - » LC restricted
  - » Retained CD19, gain of CD20
  - » No aberrancies in CD56 or CD117
  - » Occasionally CD13+



# Plasma Cell Neoplasm

- Gating strategy: CD38 high events with variable or decreased CD45
  - Cytoplasmic Kappa/Lambda-key to diagnosis
  - CD138 isn't a great marker by flow due to antigen loss/shedding
  - Phenotypes:
    - » CD19-negative 80-90%
    - » CD56-positive 50-70%
- \*Normal polytypic populations of CD19-/CD56+ PC

# Other plasma cell markers

- CD27
  - » Similar issues with CD138
- CD81
  - » Normally intermediate, can be increased or decreased
- CD200
  - » Normally negative, but occasional reactive cases are positive and polytypic
- CD117
  - » Specific for disease, only 20% of cases

# T cells

T cell abnormalities are a dime a dozen and PCR and TCR clones are frequent  
Aberrancy:

- CD4:CD8 as a surrogate “clonality” marker – n.b. New Marker T-cell receptor (TCR)  $\beta$ -chain constant regions (TRBC1) published shows high specificity for clonal processes
- Loss/decreased of CD2, CD3, CD5, CD7

Numerous (normal) T cell subsets exist:

Memory T cells (CD4+/CD5-)

Naïve T cells (CD5++/CD4+)

Gamma-delta T cells (decreased CD8, absent CD5, increased CD3)



# T cells

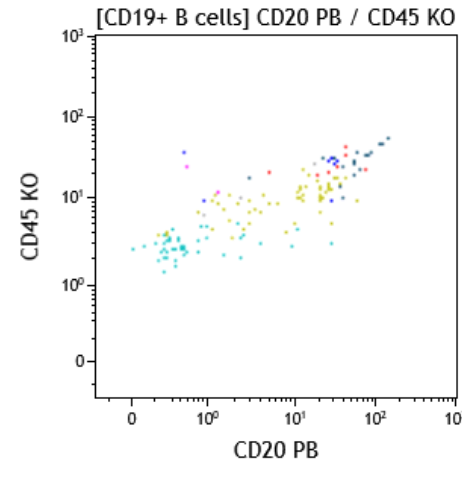
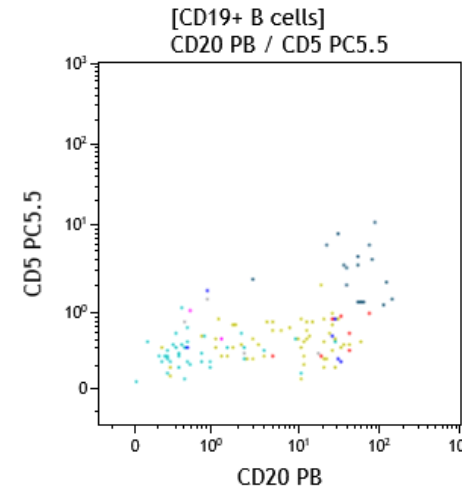
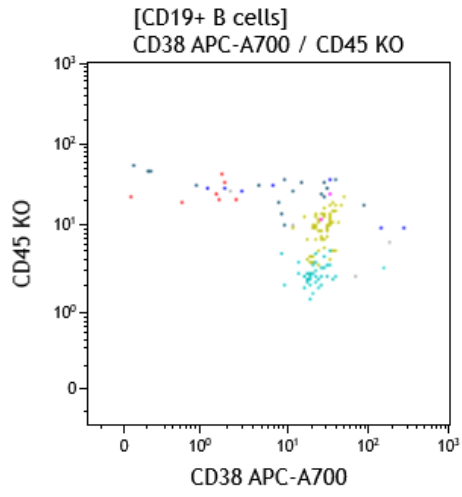
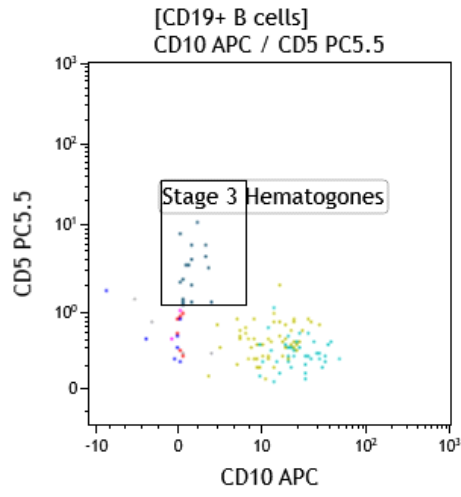
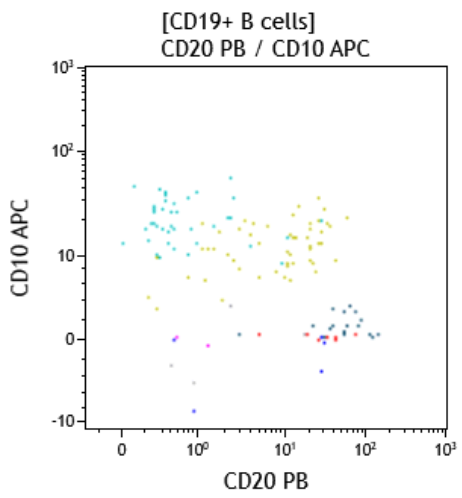
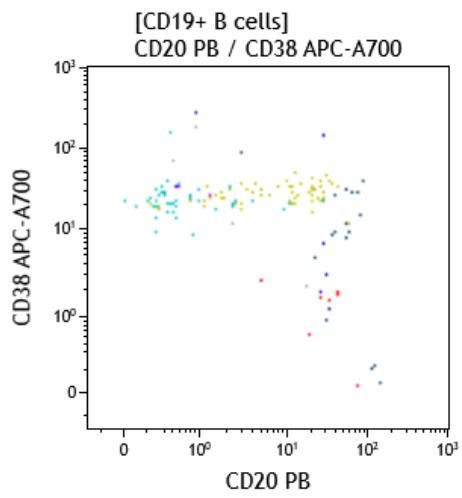
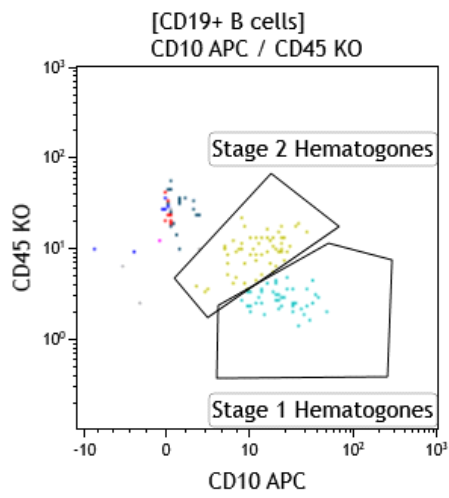
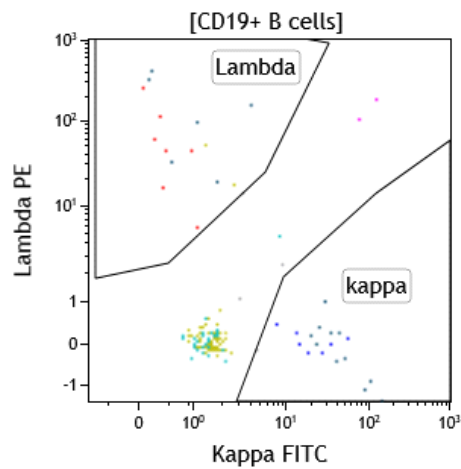
- Large Granular Lymphocytes
  - » They happen, is it lymphoma?
  - » CD3+, CD57+, CD8+, CD16+, CD56+/-
  - » Does it matter? Cytopenias?
  - » 6 months persistent LGLs
  - » STAT3 mutation may help
- NK cells
  - » Morphology like LGLs
  - » sCD3-negative, cCD3-maybe+,
  - » CD8+
  - » CD16+, CD56+/-

# Lymphoblastic Leukemias

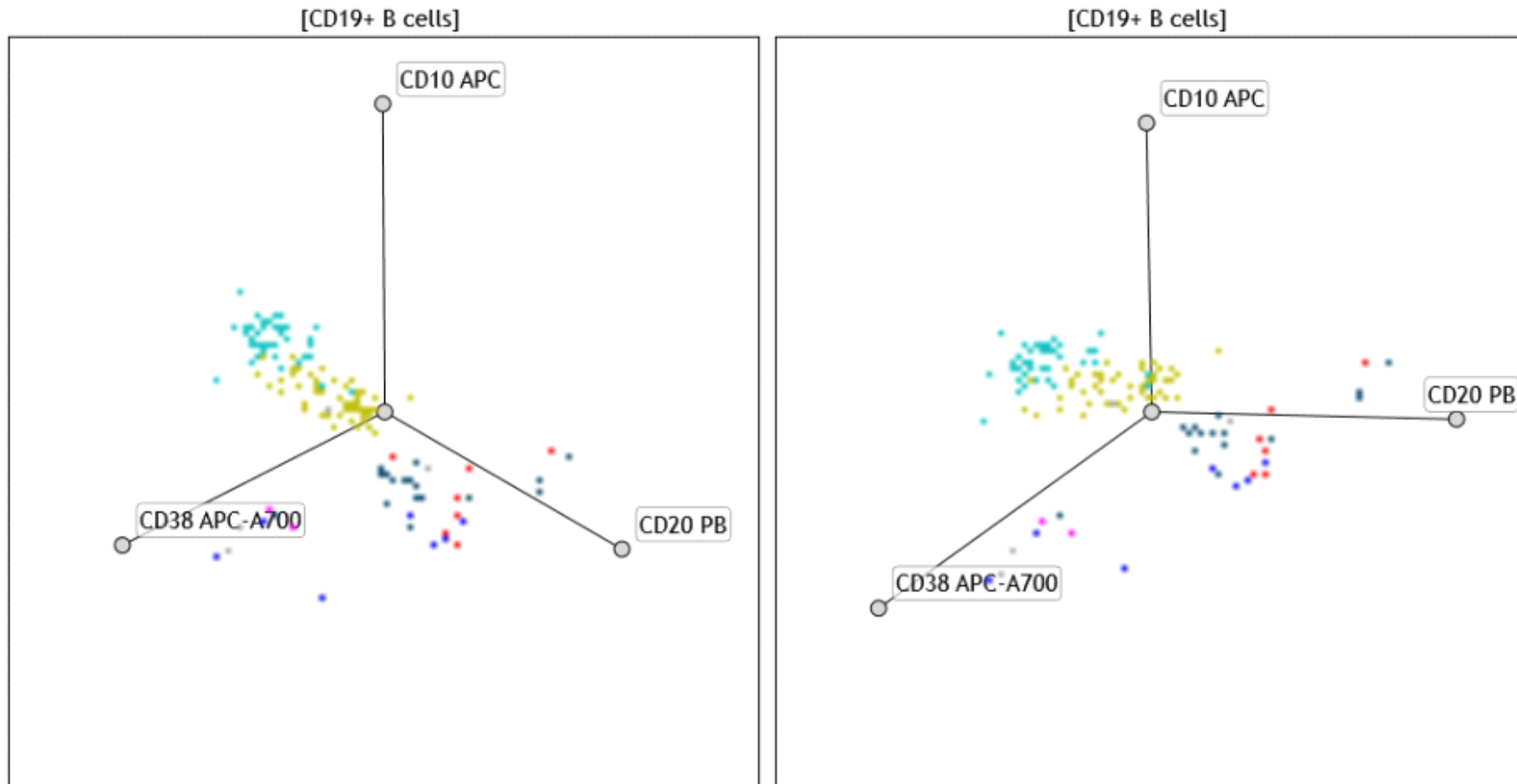
- B >> T
- Maturation Patterns are the key!
  - » Arrested or Dysregulated

# Normal B cell Maturation

- Hematogones (all CD19+)
  - » Stage 1 – high CD10, CD38, low CD45, absent CD20
  - » Stage 2 – gaining CD20 and CD45, retained CD10, high CD38
  - » Stage 3 – normal CD20, picks up CD5, some light chains, lose CD10 and CD38, near lymphocyte CD45



# 3D projections



# B-LBL

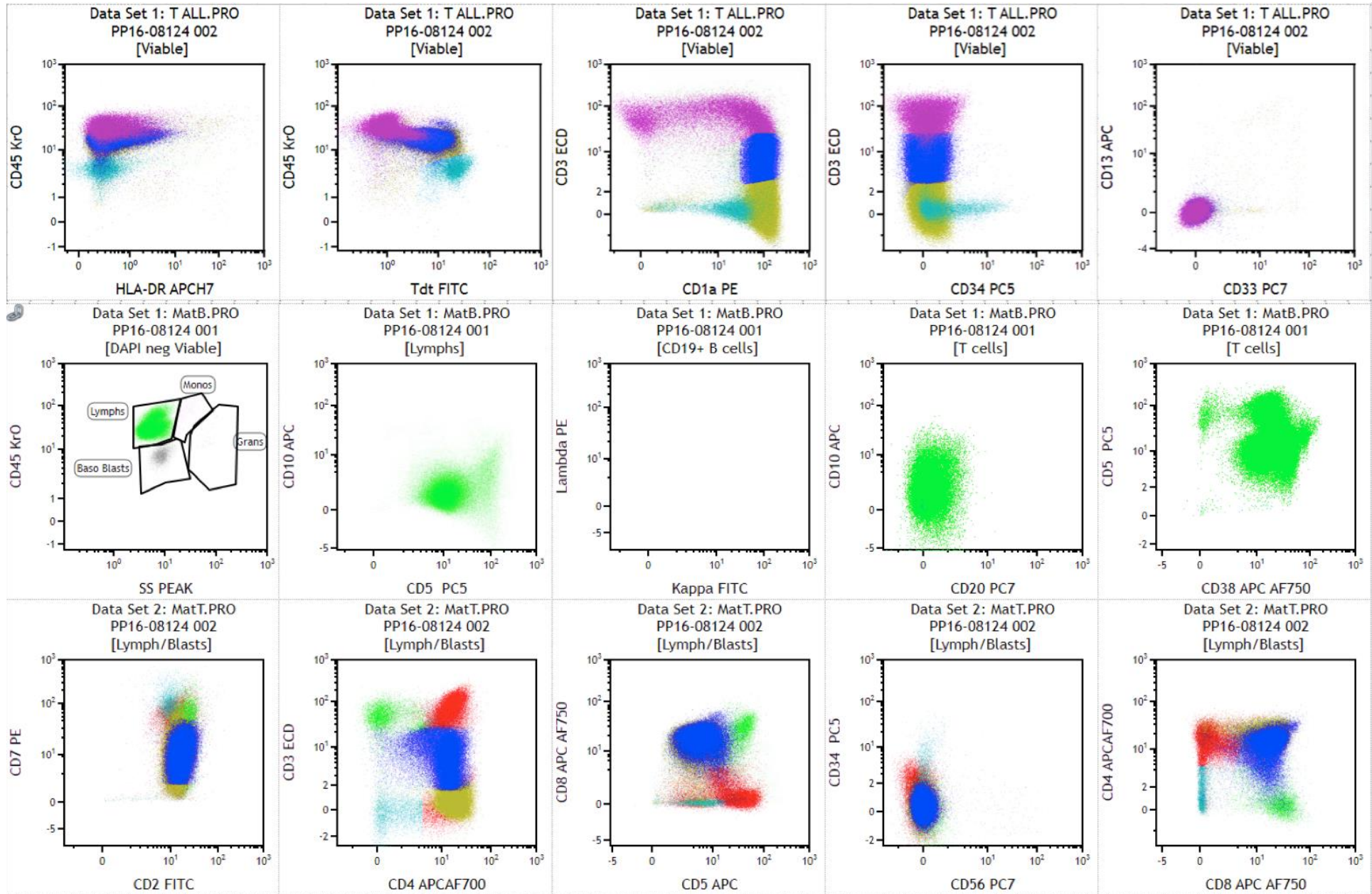
- CD19, CD22, CD79a
- Decreased CD45 (compared to lymphocytes)
- CD34
- CD10 (aberrant loss)
- CD38 +/-
  
- Aberrant CD13, CD33, CD20 (low to intermediate)
- Aberrant CD15

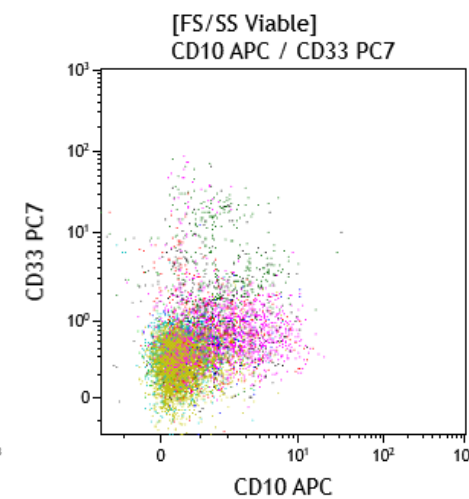
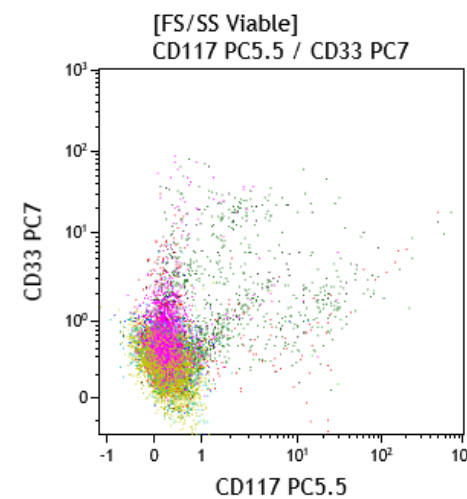
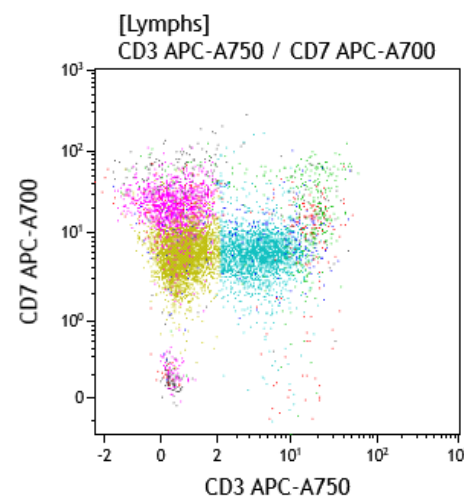
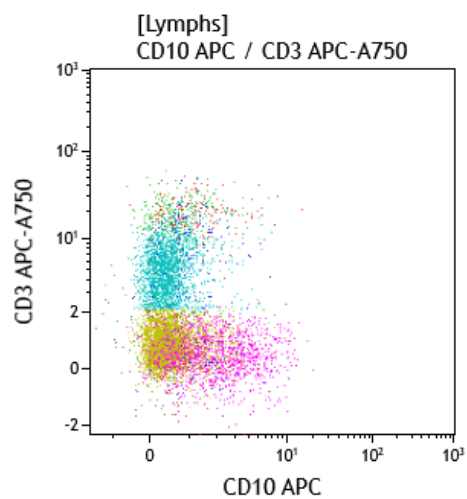
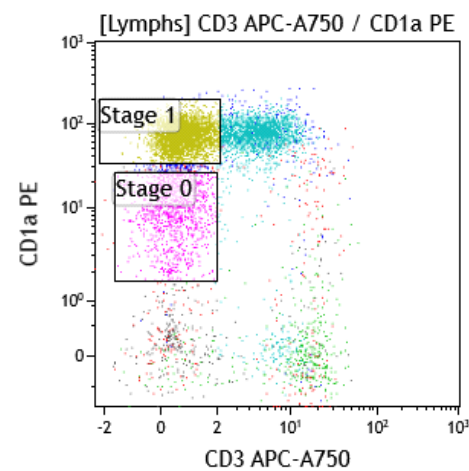
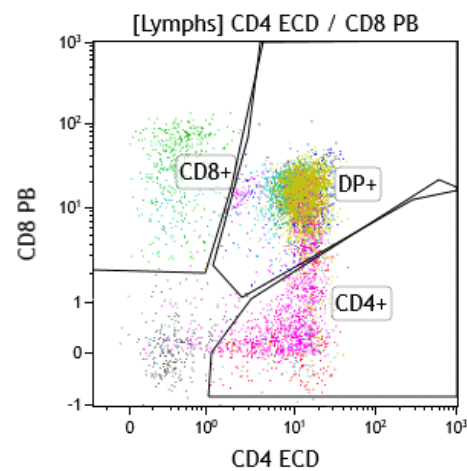
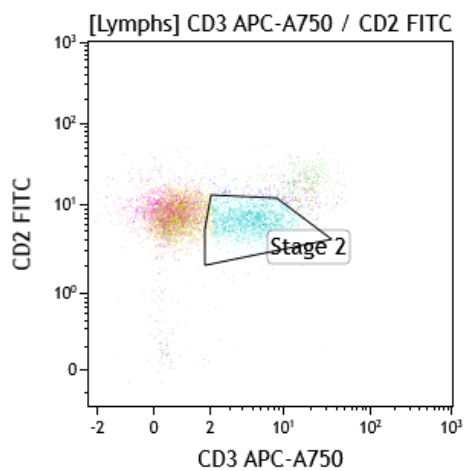
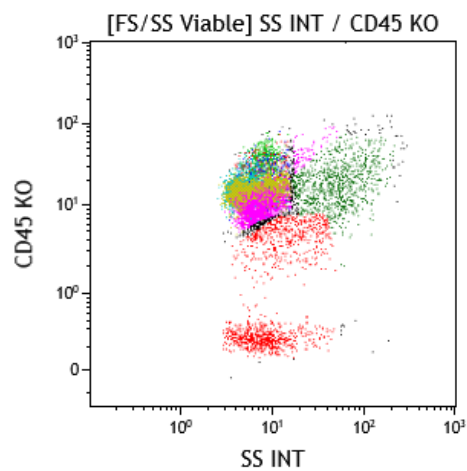
Marker	Prevalence
CD10	89%
CD13	5%
CD19	100%
CD20	24%
CD22	69%
CD33	31%
CD34	76%
CD45 (bright)	2%
CD45 (moderate)	33%
CD45 (dim)	36%
CD45 (negative)	29%
CD56	36%
CD79a	88%
CD117	0%
cytoplasmic IgM	22%
HLA Dr	98%
TdT	91%

# Normal T cell Development

- Normally in the thymus
- Can happen in extrathymic sites
- Thymoma vs normal thymus – not possible by flow alone
  
- Maturational patterns are preserved
  - » Several “subpopulations”







# T-LBL

- Arrested maturation
- Aberrant expression of myeloid markers
  - » CD13, CD33, CD117, CD15

# Theranostics (targeted therapy)

- CD20 -rituximab
- CD22 - inotuzumab ozogamicin
- CD19 – blinatumomab (BITE)
- CD30 - brentuximab vedotin
- CD38 - daratumumab

# And next time...

## Myeloid Neoplasms



*ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.*