

Clinical Flow Cytometry for the Perplexed

Part 4: Leukemias and Myeloid Neoplasms

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Recap from parts 1-3

- Gating Strategies
- Flow Cytometry Artifacts
- Lymphoma Phenotypes
- Maturation pathways in immature lymphoid populations

Goals

- Recognize important flow cytometric phenotypes among acute myeloid leukemias
- Understand the gating strategies needed for diagnosing monocytic and myelomonocytic neoplasms
- Understand the normal maturational pathways for maturing myeloid precursors and their potential derangements

Terminology

- Myeloid Lineage
 - » Granulocytic
 - » Monocytic
 - » Eosinophils
 - » Basophils
 - » Plasmacytoid Dendritic Cells

Acute Leukemias

- B cell
- T cell
- Myeloid
- Mixed

APML - Classical

- CD34-/CD117+ blast population
- Low intermediate CD45
- High CD9 and CD33
- Negative for HLA-DR
- Variable CD13
- Increased side scatter

- Key – HLA-DR-/CD34-/CD33++

APML - Microgranular

- Positive for CD34 and CD2
 - Low side scatter
 - Negative for HLA-DR
-
- N.B. – MRD assessment by flow and molecular is impossible in ATRA treated APL

AML with t(8;21)

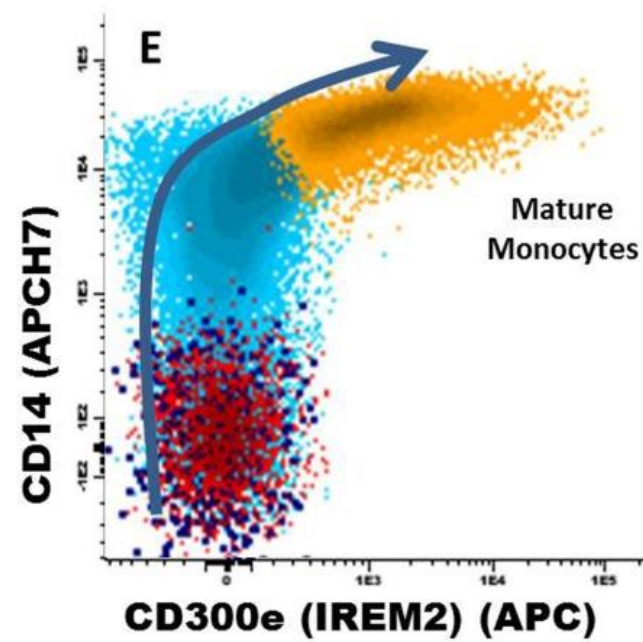
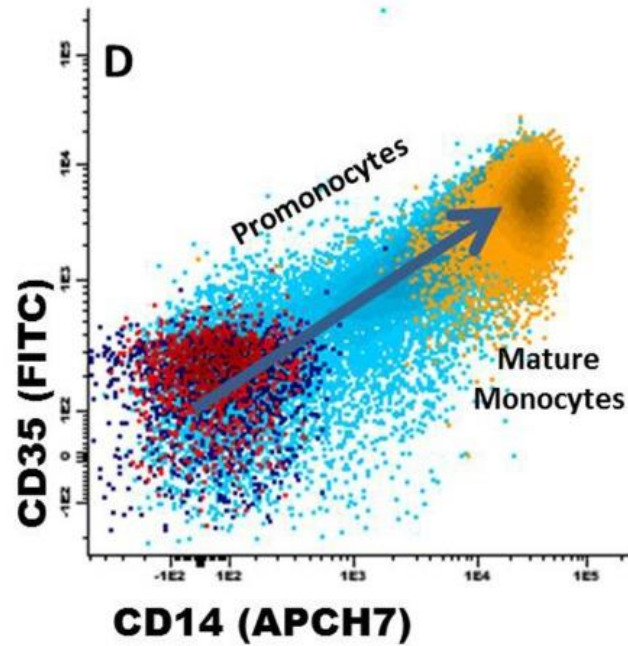
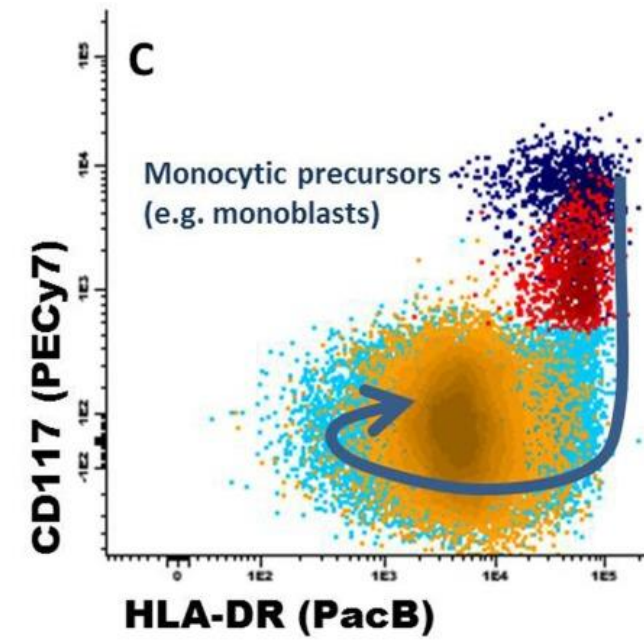
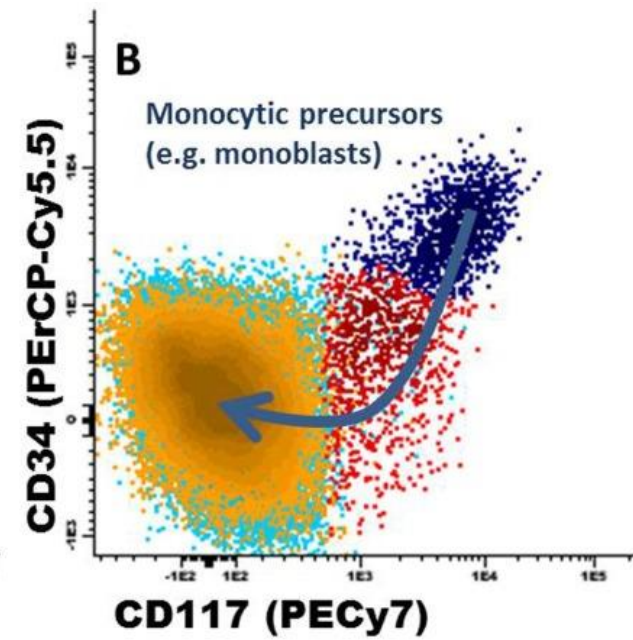
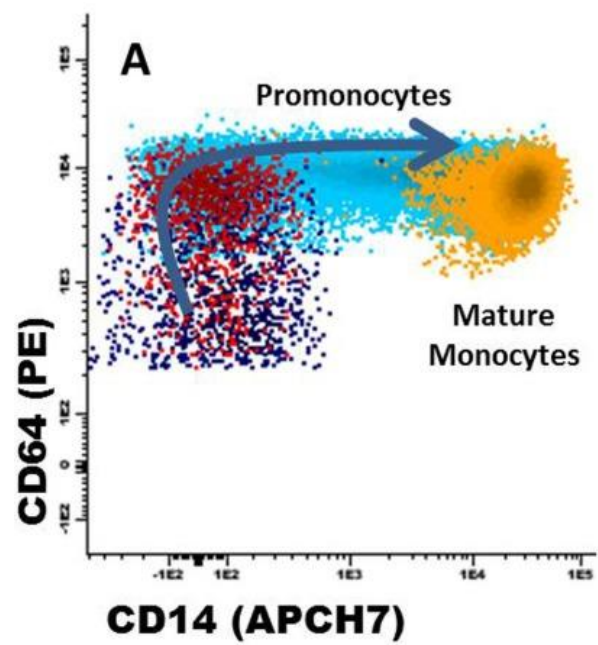
- MPO+/CD19+
- Pax5+
- Expression of CD56 is correlated with kit mutation

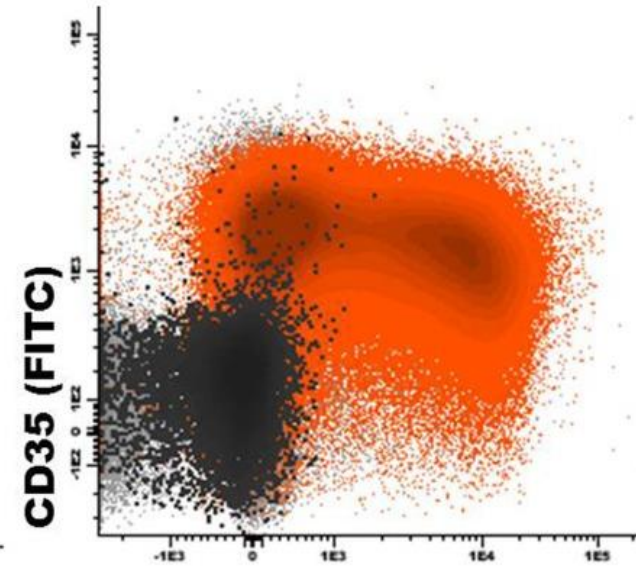
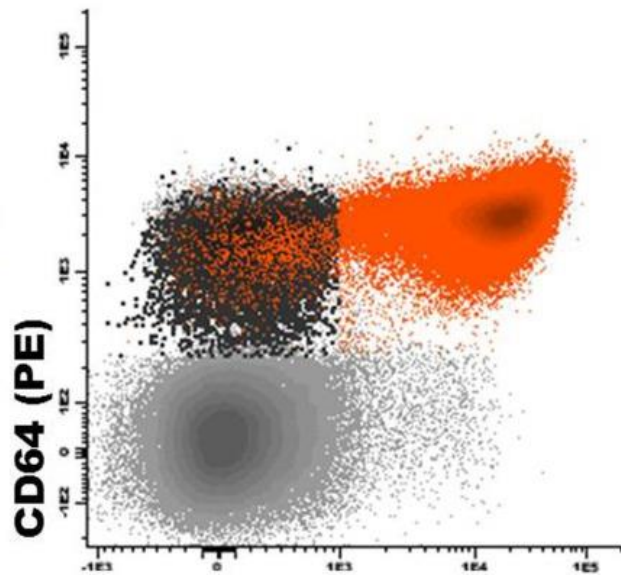
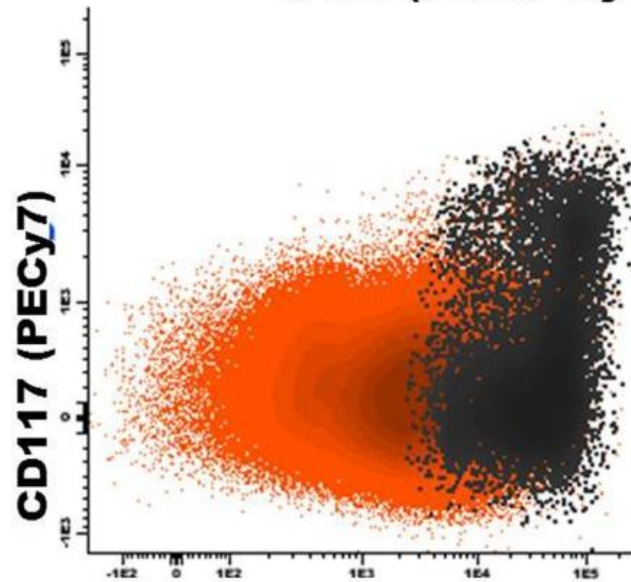
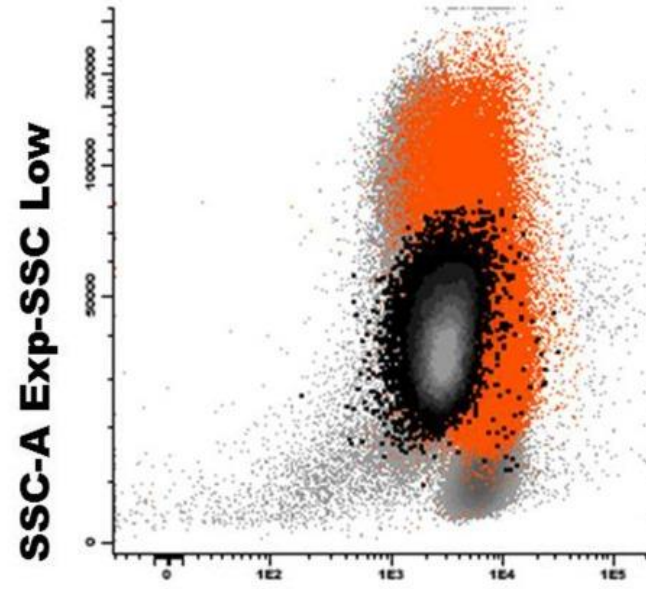
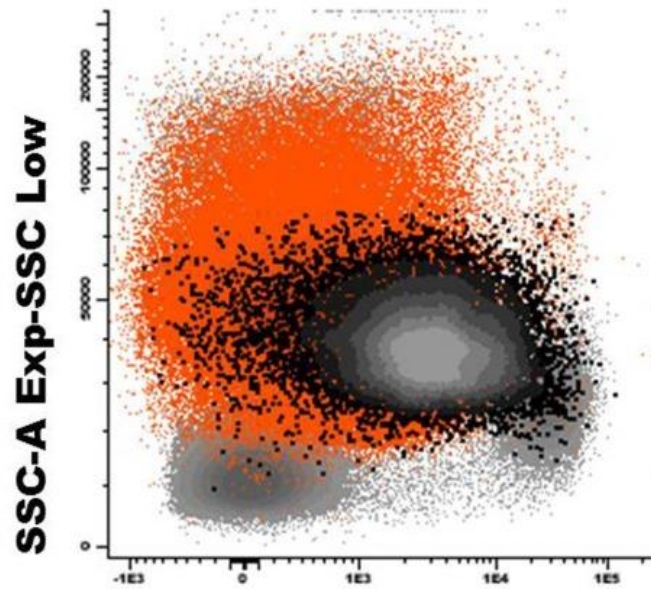
AML with monocytic differentiation

- Acute Myelomonocytic Leukemia (M4)
 - » Presence of a myeloid blast population
 - » Blast+Blast Eq > 20%
- Acute Monoblastic Leukemia (M5a)
 - » >80% monoblasts
- Acute Monocytic Leukemia (M5b)
 - » <80% monoblasts and promonocytes

Blasts/Blast Equivalents

- Blasts
 - » Myeloid Blasts - CD34+, MPO+
- Blast Equivalents:
 - » Generally higher CD45 (between blast and monos)
 - » CD13/CD33/CD11c/CD36/HLA-DR/CD64 pan monocytic markers
 - » Promonocytes (M4/M5)
 - CD64+/CD14-
 - » Monoblasts (M5)
 - CD64+/CD117+/CD34+/CD14-

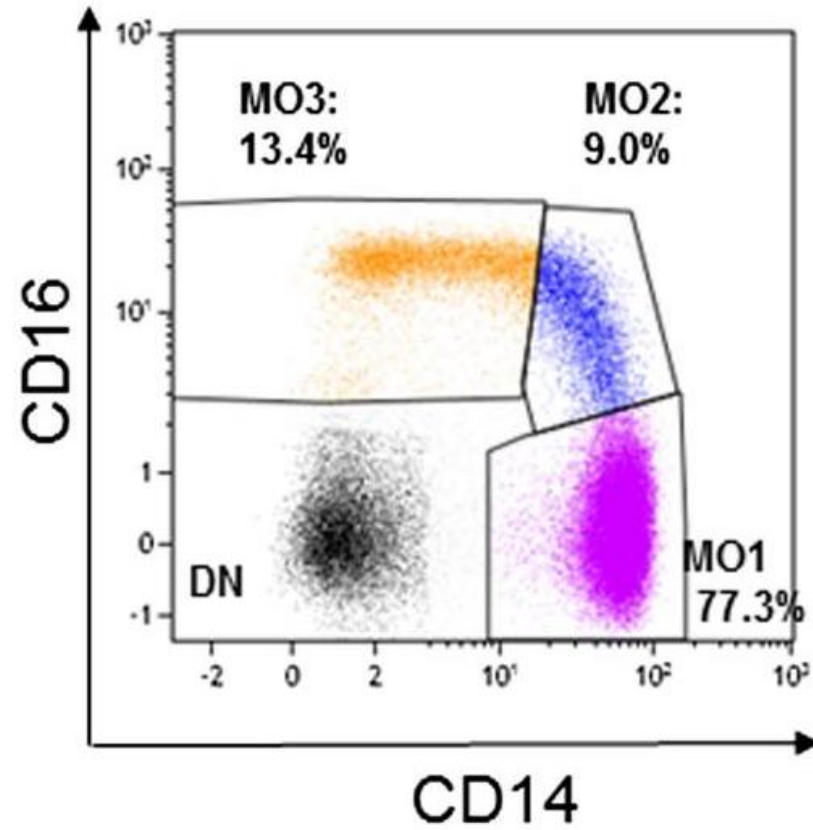




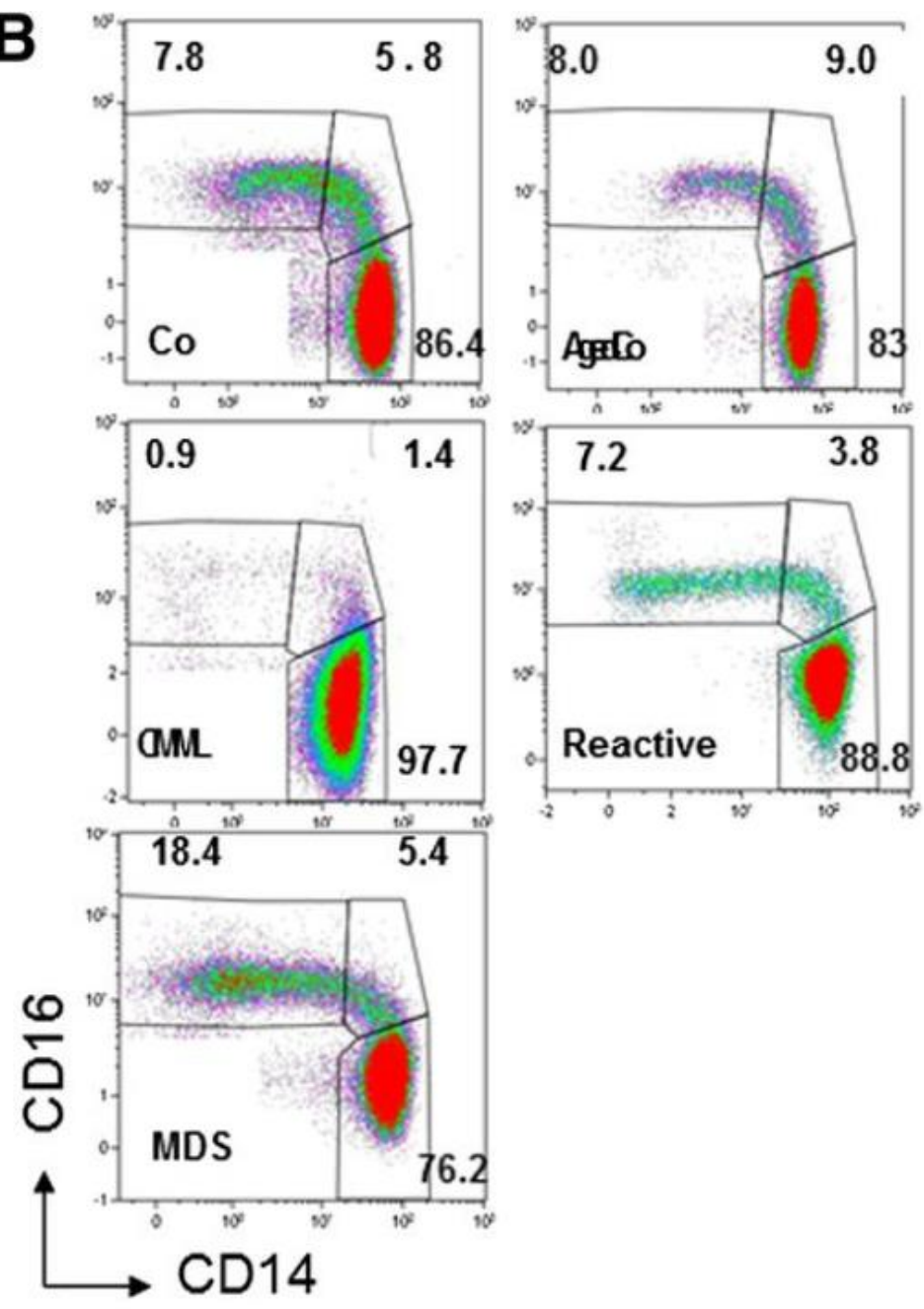
CMML/AMML

- Promonocytes
 - » CD64-positive (pan-monocyte marker)
 - » CD14-negative (mature monocyte marker)
- Other things to note:
 - » CD14/CD16 distribution of M1, M2 and M3 monocytes
 - » >90% M1 Classical Monocytes (CD14++/CD16-)

A



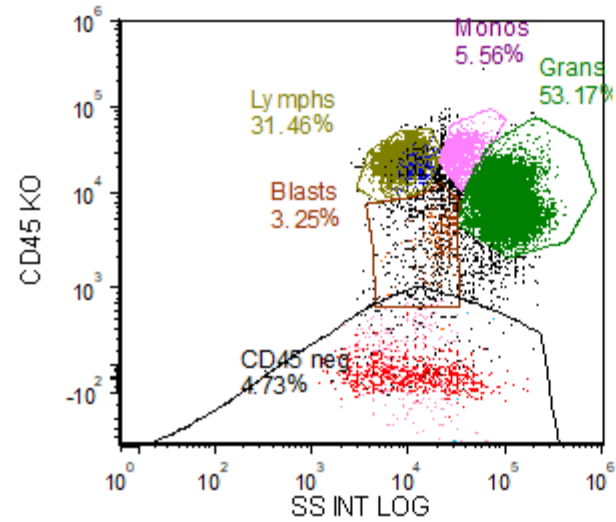
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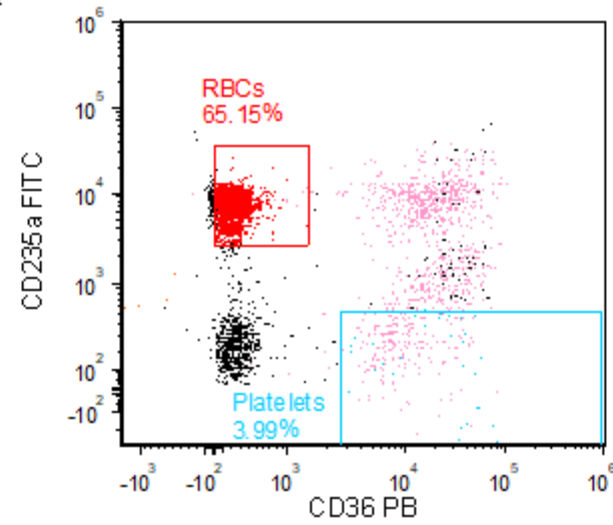
Erythroid Leukemias

- Low CD45
 - CD117 – blasts and early normoblasts
 - CD235a – pan erythroid (++ on mature RBCs)
 - CD71 – not specific (+ on all active cells)
 - CD36 – (+ on a monos, megs, erythroids)
-
- CD49d – just early normoblasts

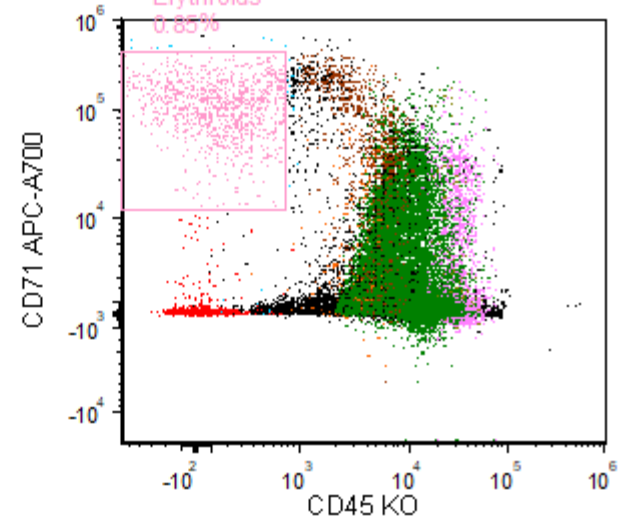
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Acute Megakaryocytic Leukemia

- CD117 – blasts and early megakaryoblasts
- CD42b
- CD41
- CD61
- CD36

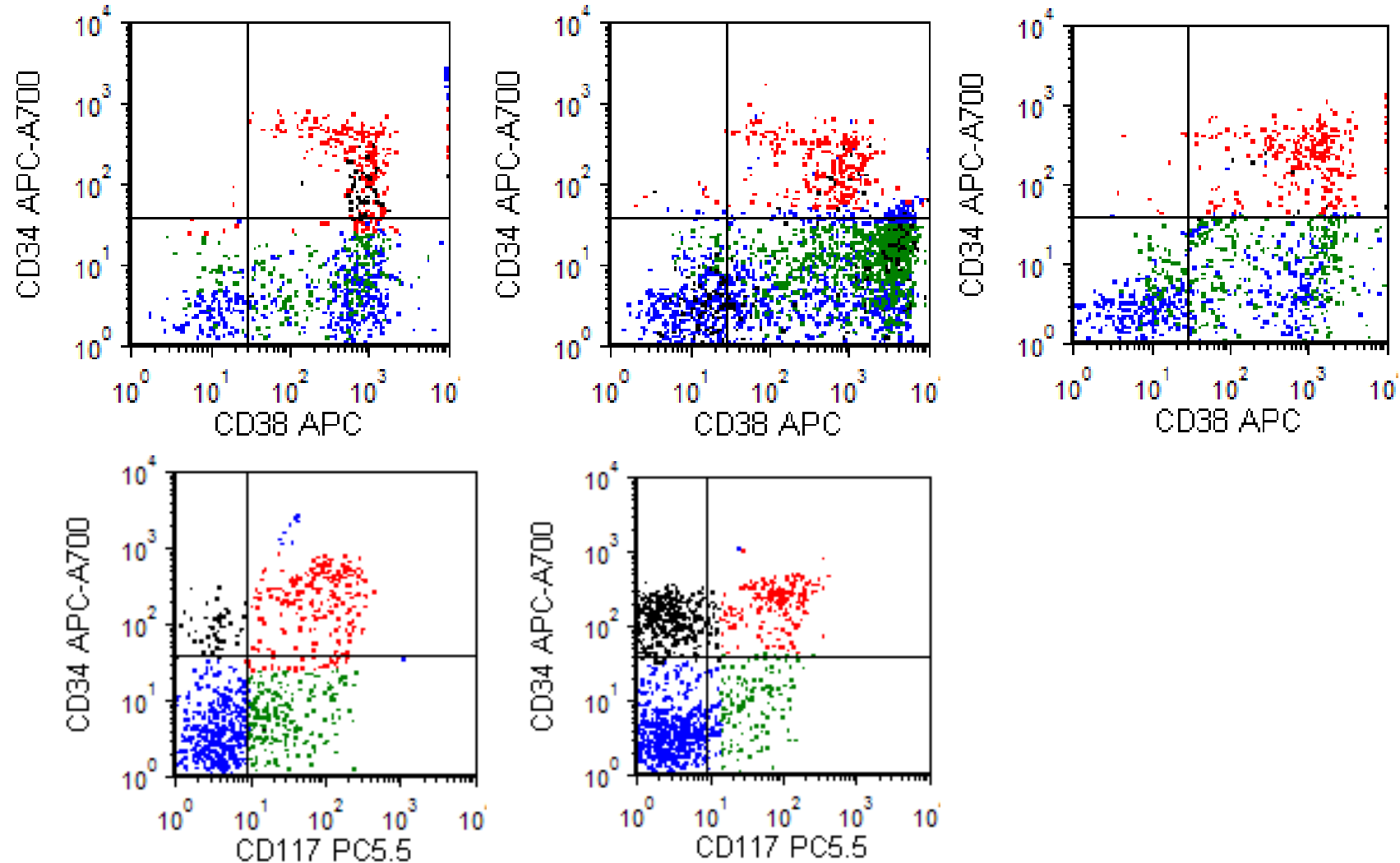
Myeloid Neoplasms

- <20% Blasts
 - » Flow Quantification not the standard
 - » $Blasts\% = \frac{\#blasts}{\#total\ cells} = \frac{\#blasts}{\#mye+\#lym+\#blasts+\#ery}$
 - » Erythroid Lysis
 - Older specimens = less lysis

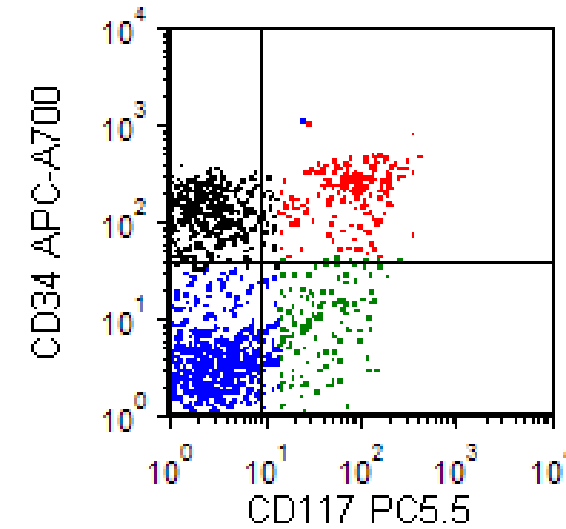
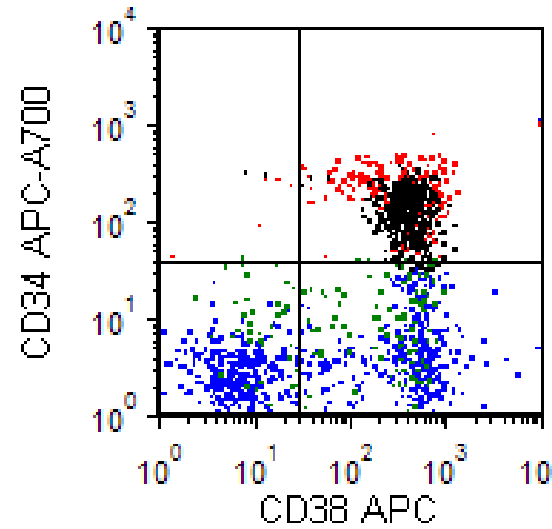
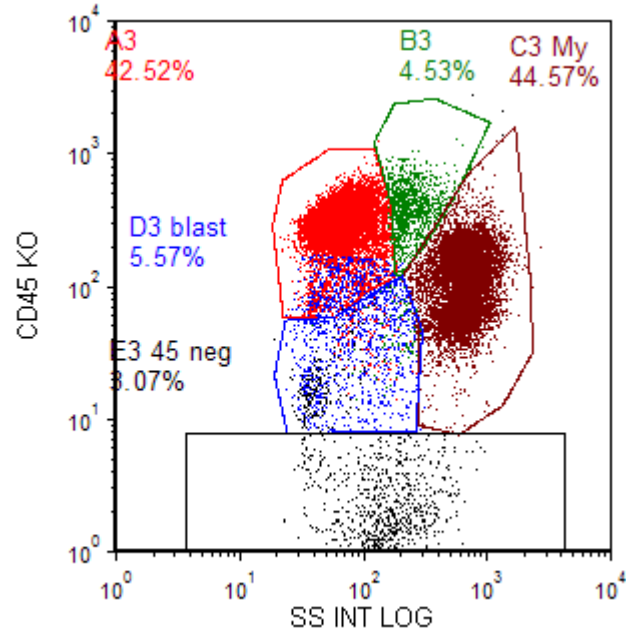
Cell Development is tightly regulated

- Cells turn on and turn off proteins during their maturation
- Maturational Patterns are highly conserved
- Myelodysplastic Syndrome (MDS) shows a derangement of maturation
 - » Morphologic abnormality
 - » Immunophenotypic abnormality
 - » Driven by genetic changes

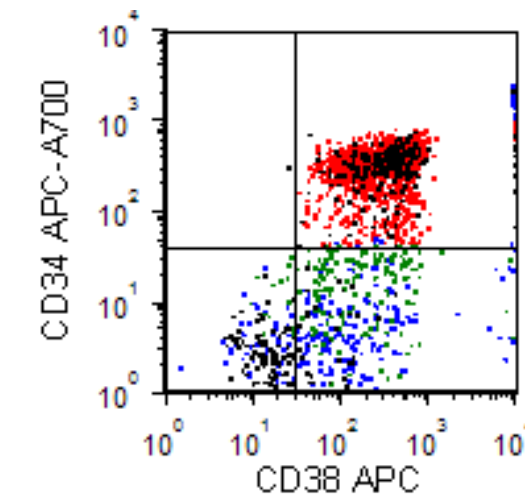
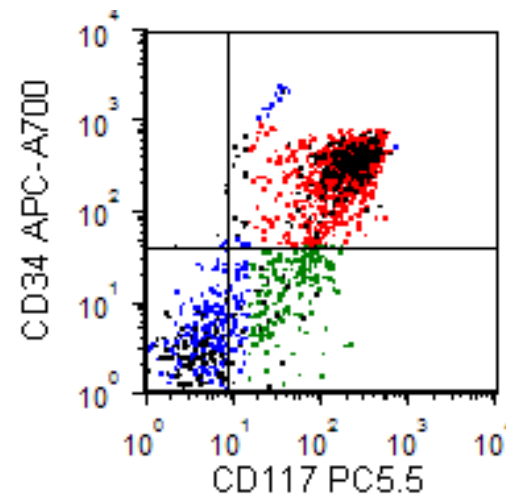
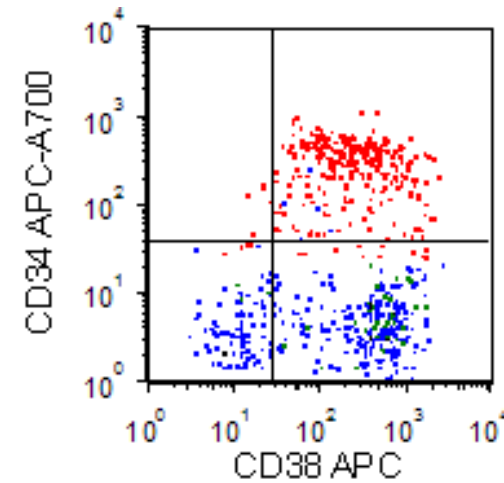
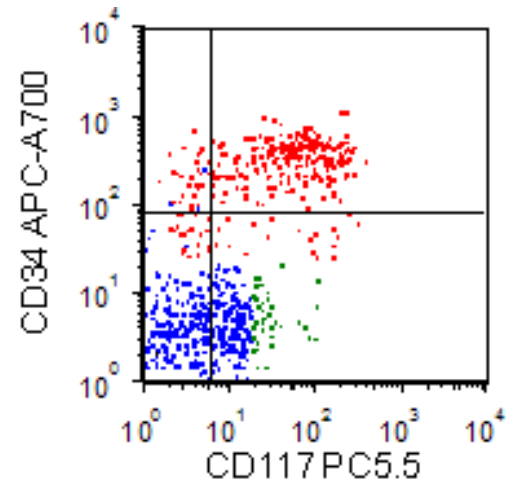
Normal Blast Maturation Patterns



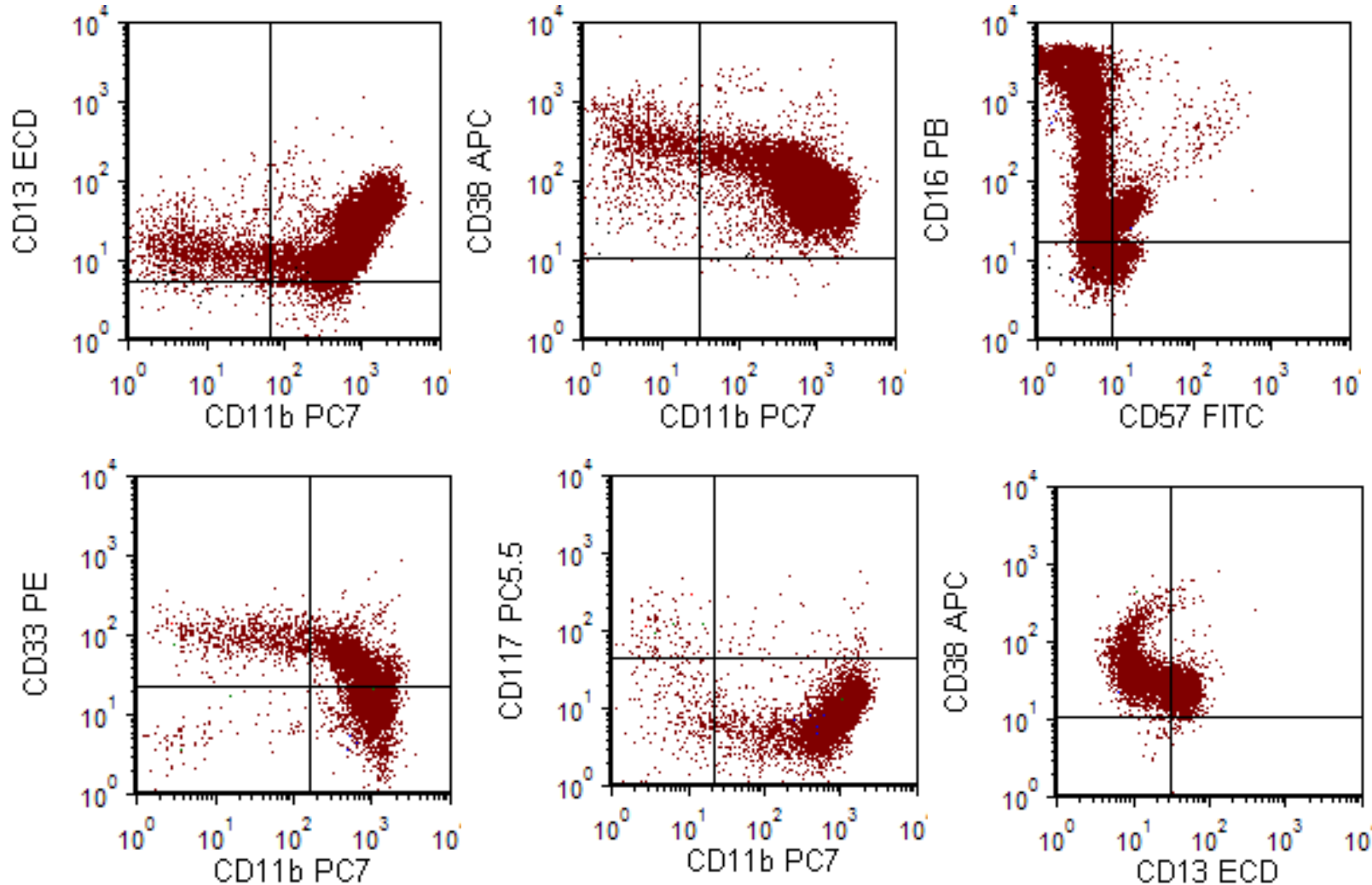
Hematogones



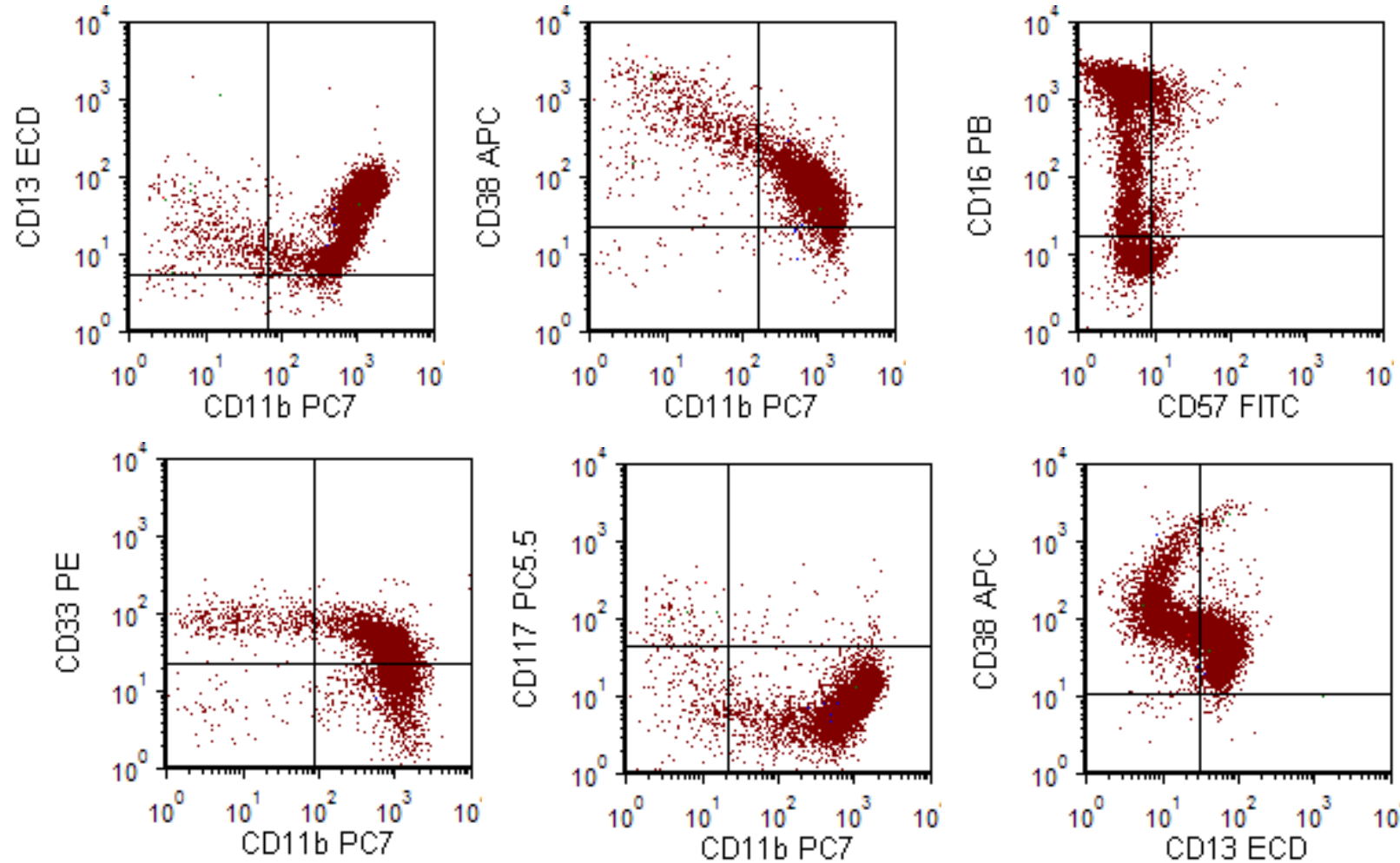
Blast Atypia



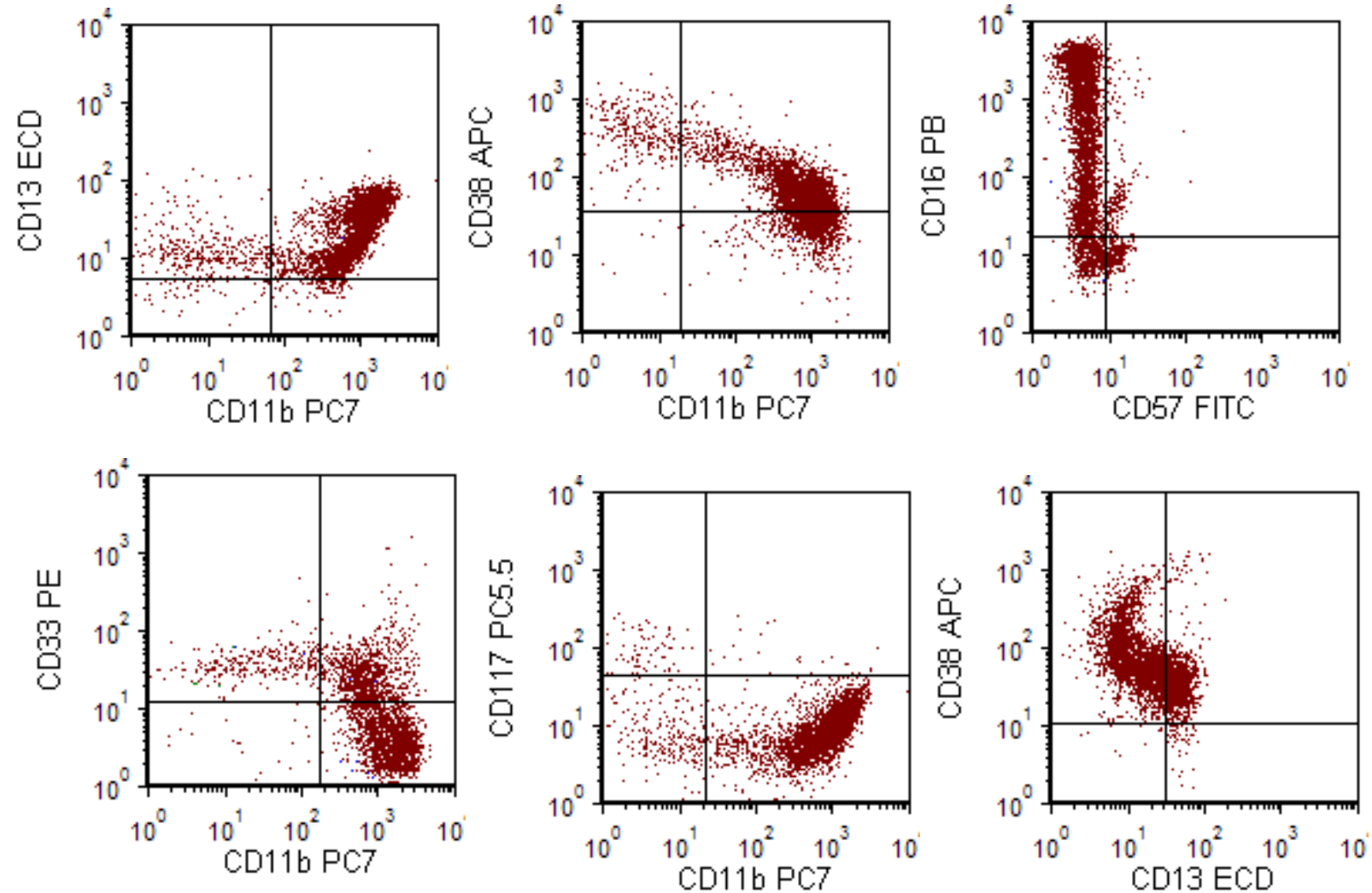
Granulocytic Maturation (normal)



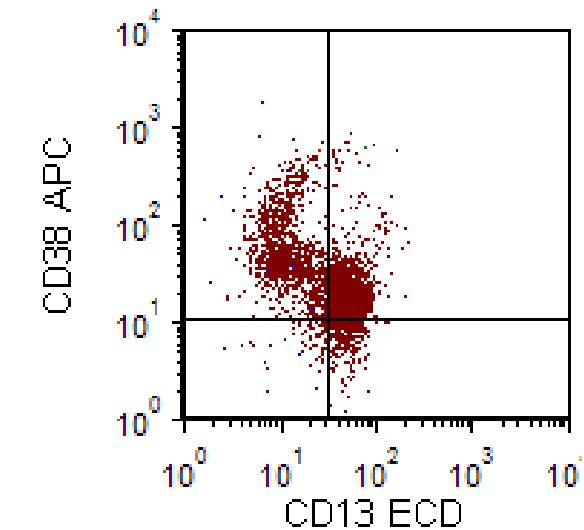
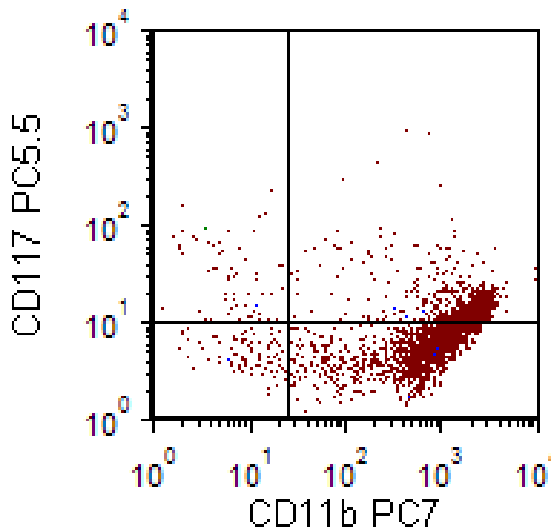
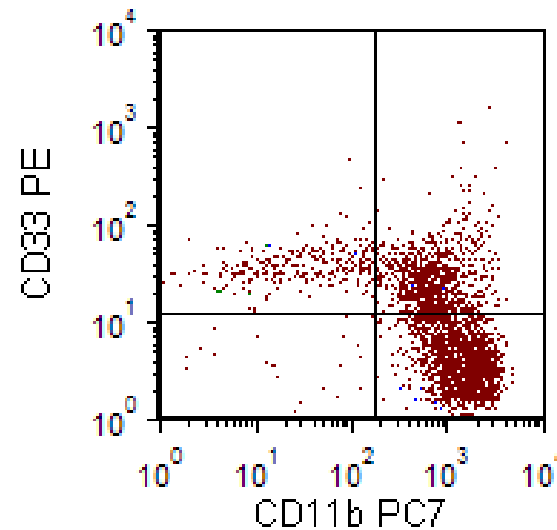
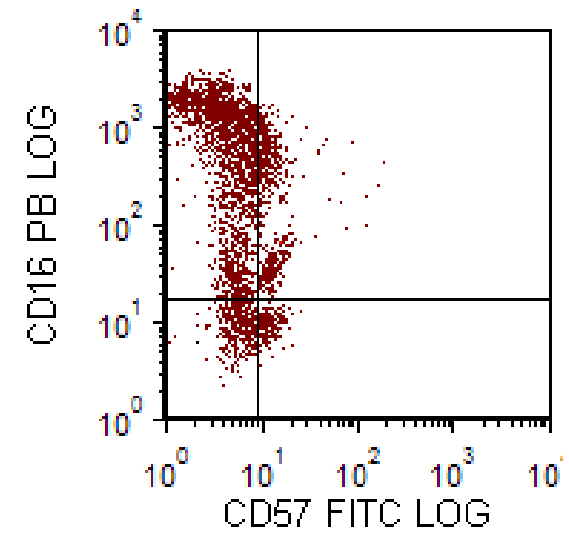
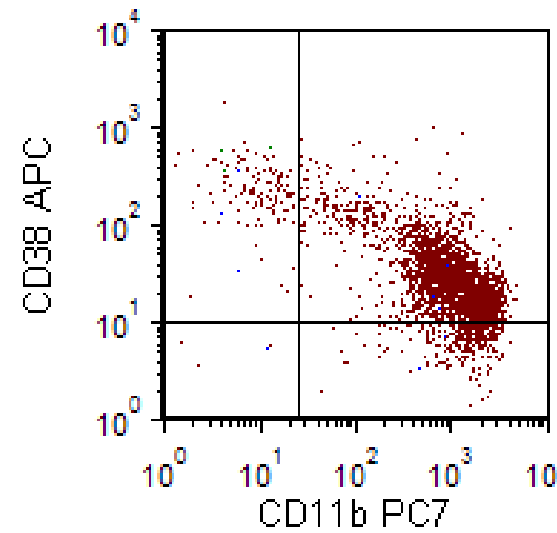
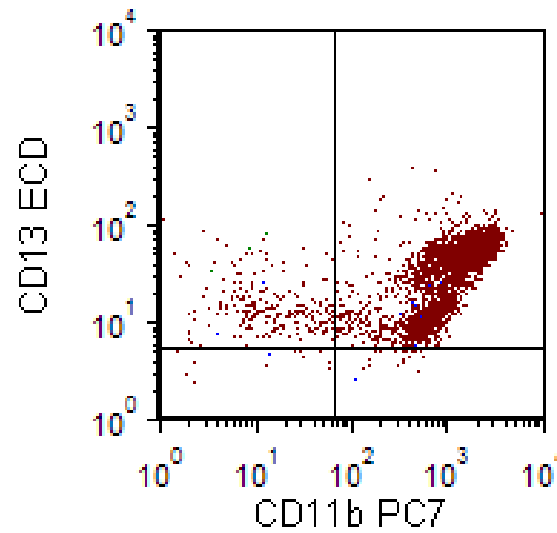
More Examples (normal)



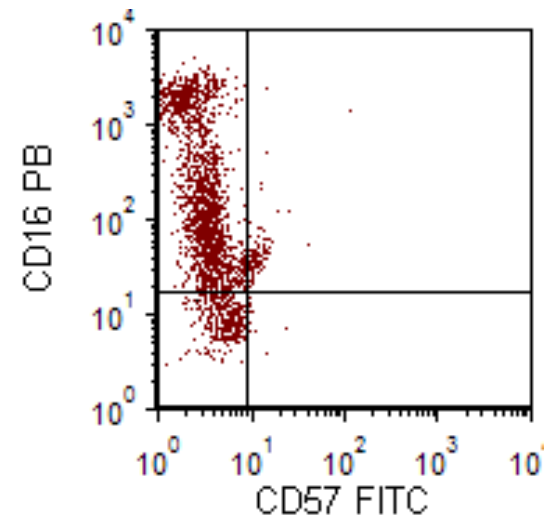
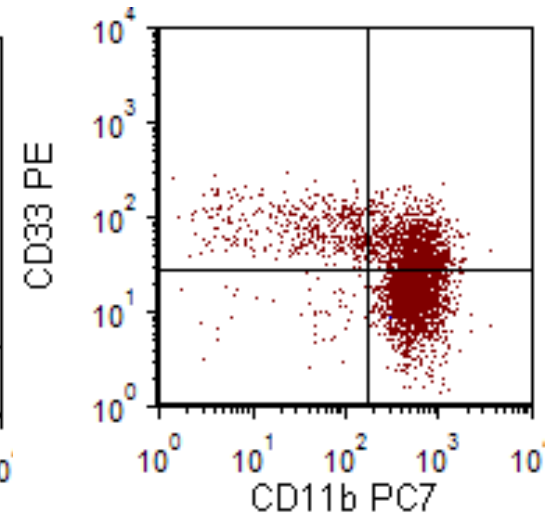
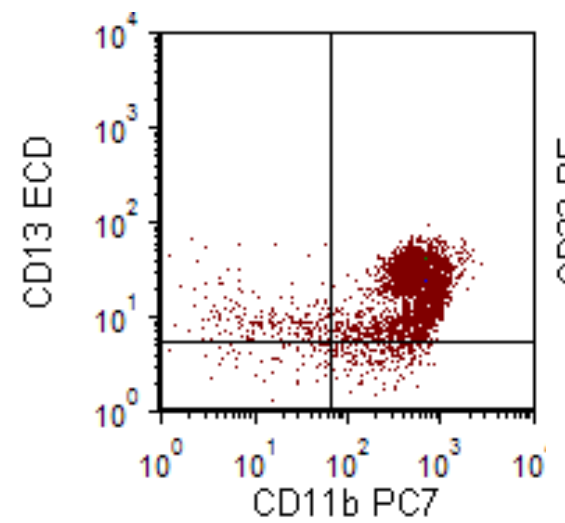
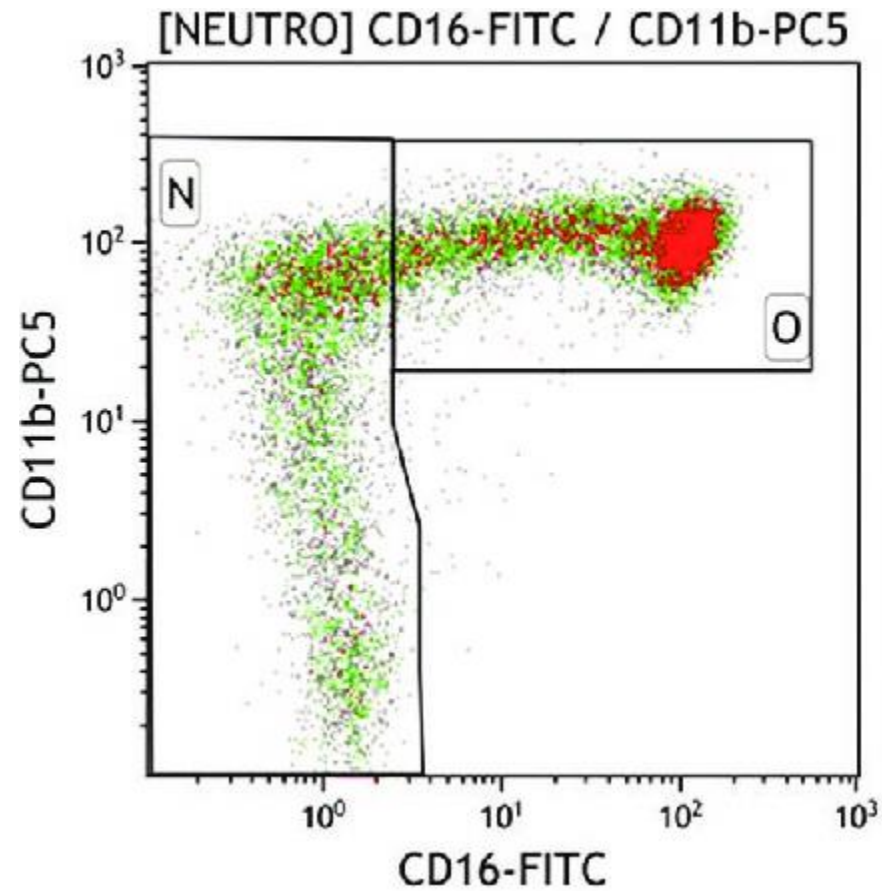
More Examples



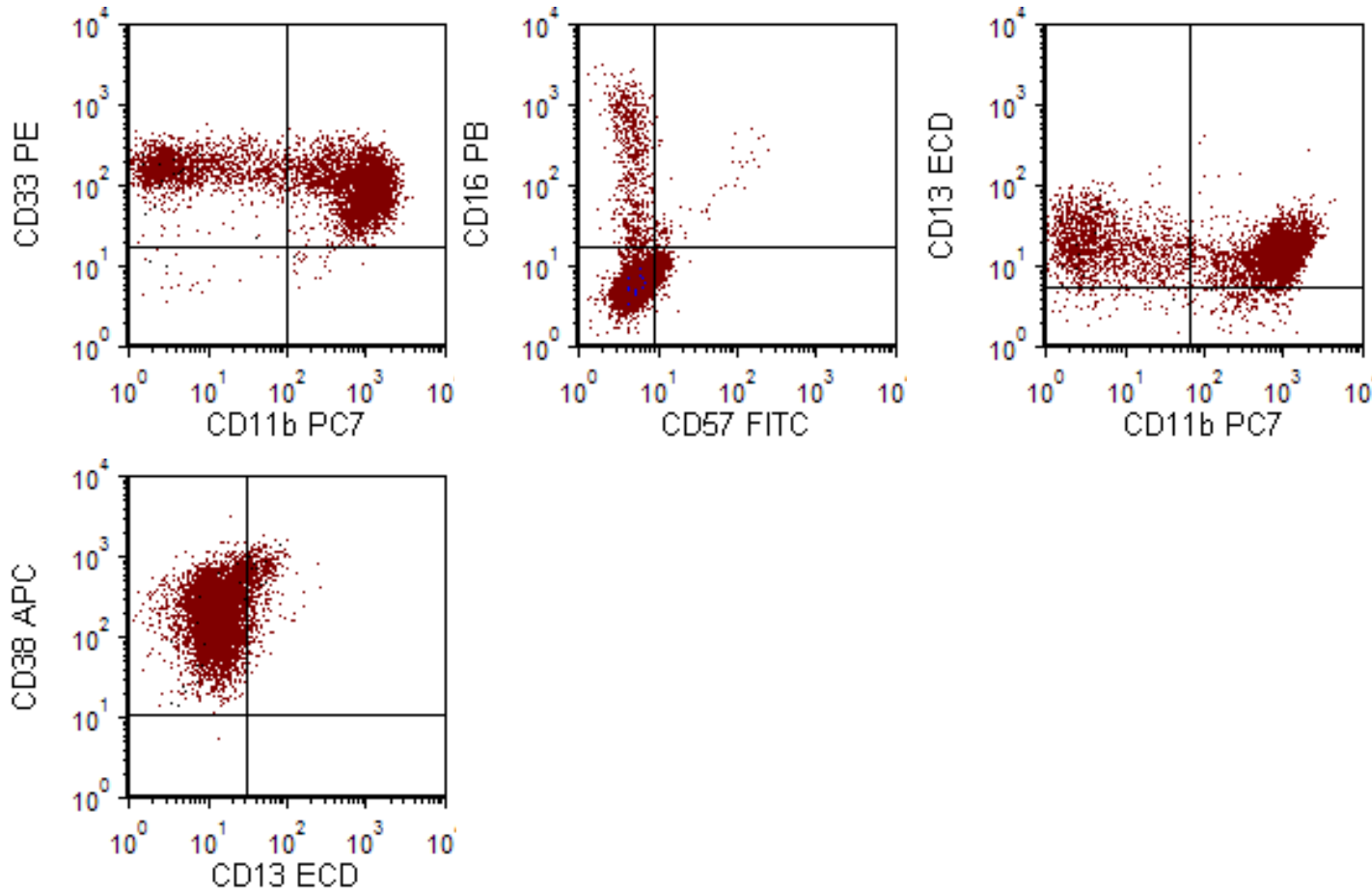
More Examples (normal)



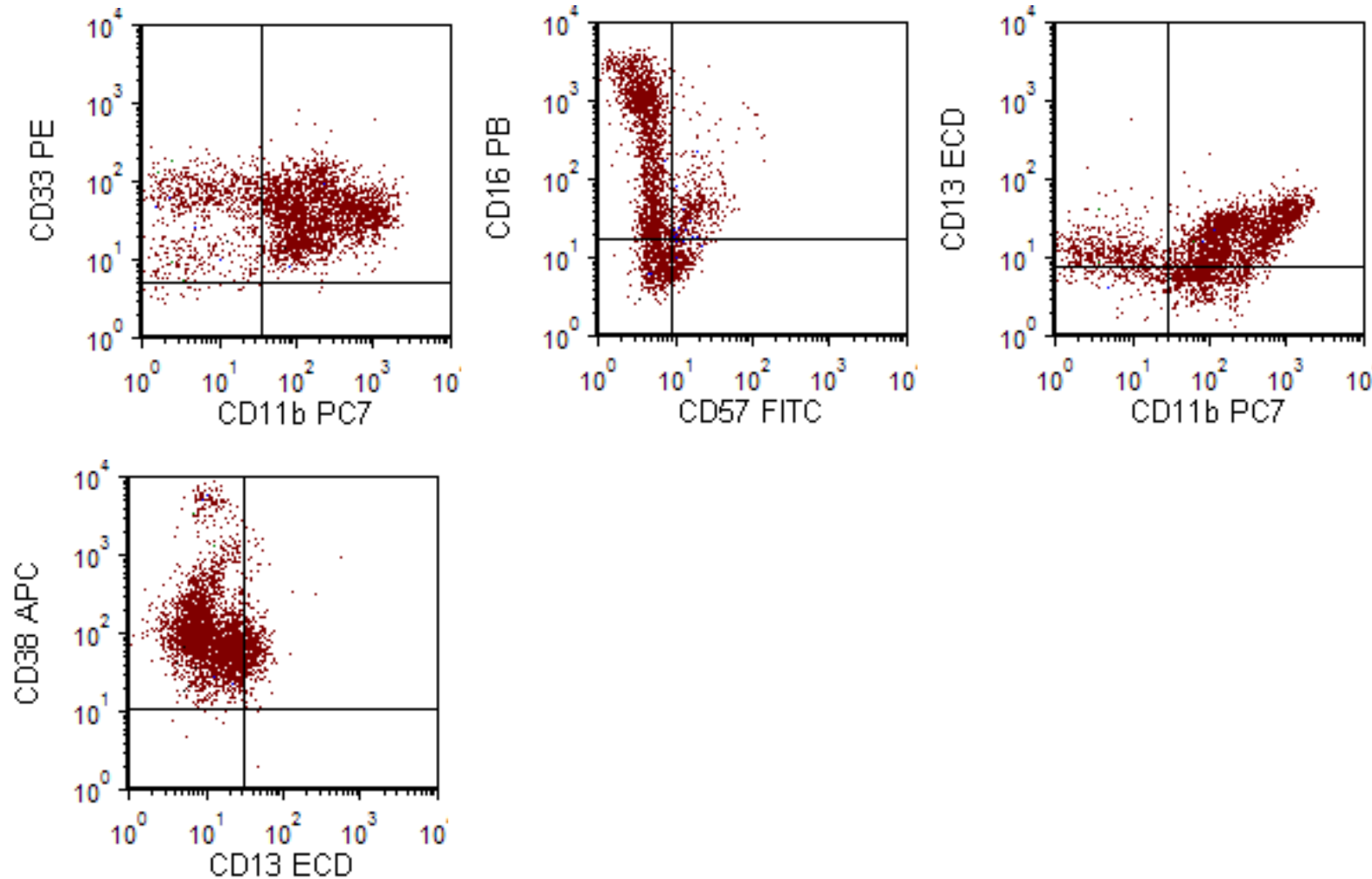
Granulocytic Atypia



More atypical patterns



More atypical patterns



Mixed Phenotype

- Who knows? (wastebasket diagnosis)
- Typically do poorly
- Biggest categories are transformed CML
 - » CML into B-ALL/AML

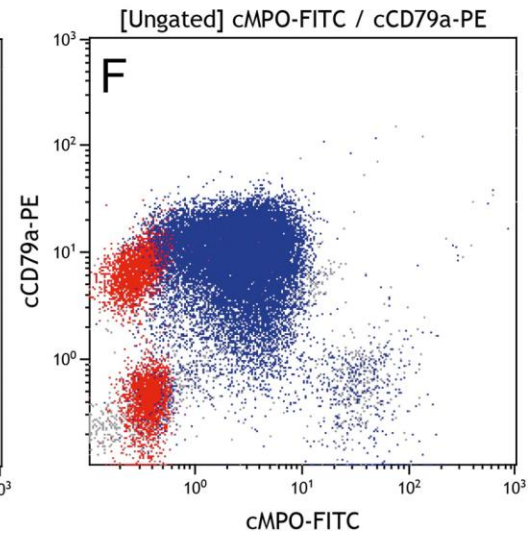
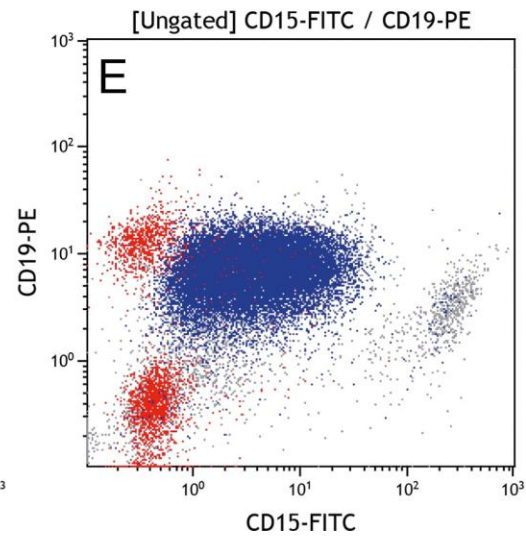
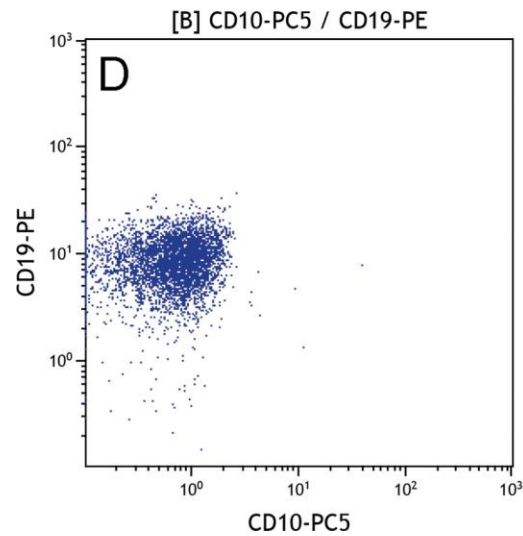
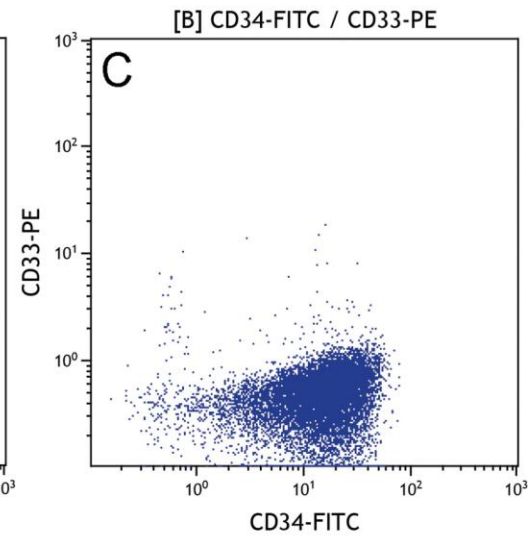
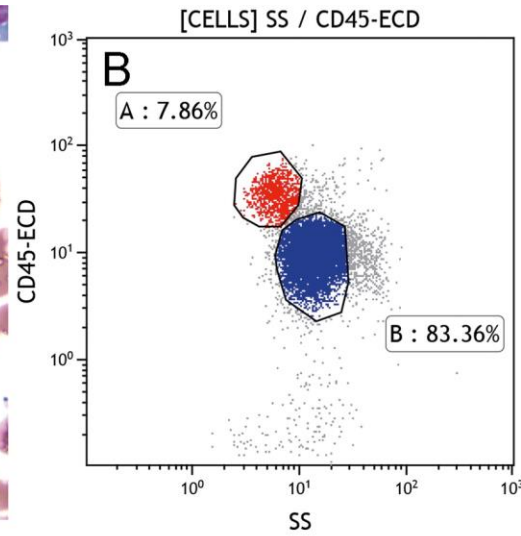
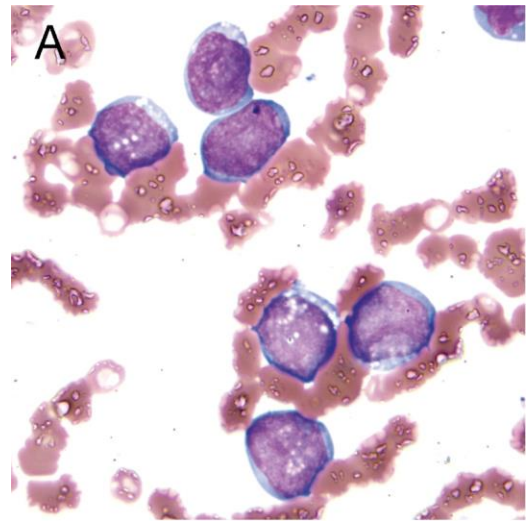
Lineage	Markers
Myeloid	MPO (flow cytometry, immunohistochemistry, or enzyme cytochemistry) -OR- Monocytic differentiation (at least 2 of the following: NSE cytochemistry, CD11c, CD14, CD64, lysozyme)
T lineage	Strong ^b cytoplasmic CD3 -OR- Surface CD3
B lineage	Strong ^b CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10 -OR- Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10

Abbreviations: MPO, myeloperoxidase, NSE, nonspecific esterase.

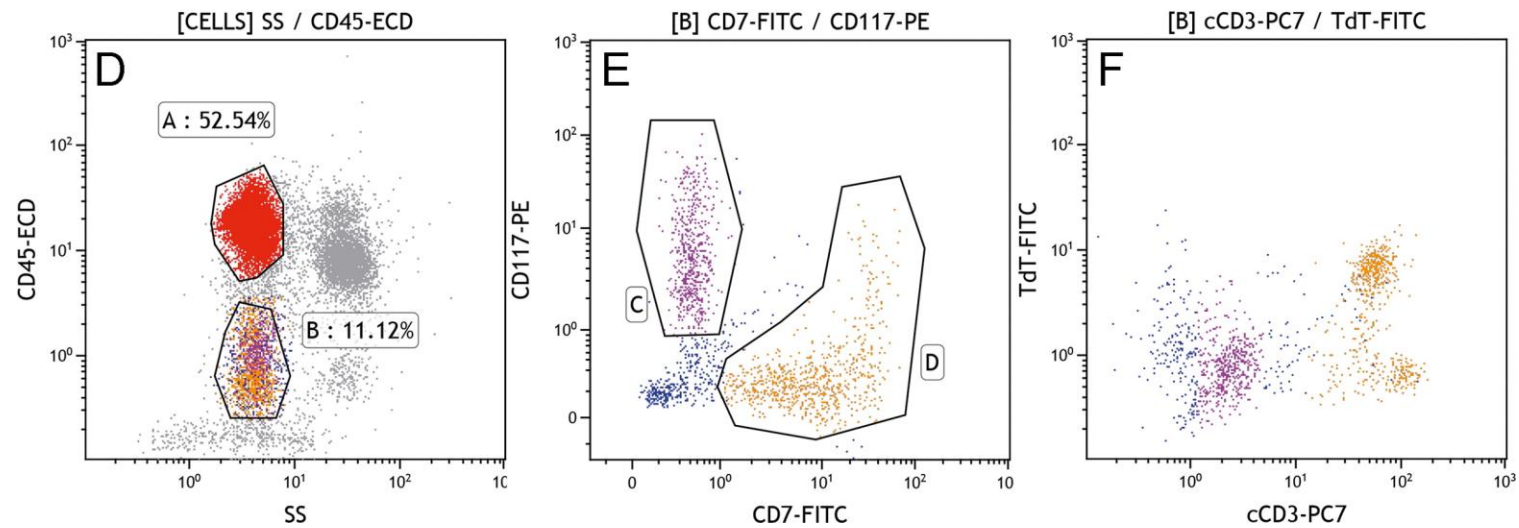
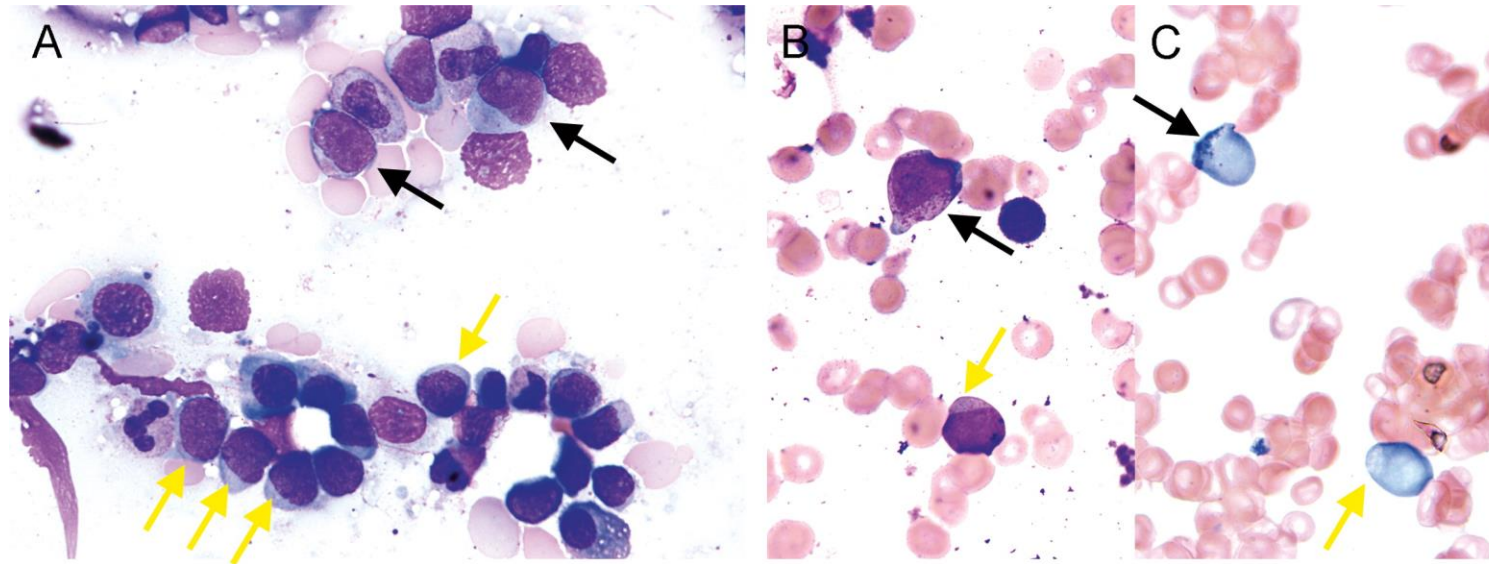
^a Data derived from Borowitz et al.⁶ and Arber et al.⁷

^b Strong = at least as intense as in normal B or T cells.

Biphenotypic



Bilineal



TdT

- Not lineage specific
 - 10% of AML will have TdT (FAB M0 and M1)
 - Burkitt Lymphoma can express TdT
 - » CD10+/CD19+/Decreased CD45/Increased Cell Size
- ...but* Express Light Chain and Negative for CD34

Mixed Phenotype take home points

- Lineage assignation table is useful but not the end all
- M0 and M1 may not meet myeloid assignment (not enough MPO)
- MPAL may be underdiagnosed...



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