THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Flow Cytometry Applications in Hematological Diseases

Case Study

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Objectives

- Using case presentation format to illustrate flow cytometry applications in hematological disease diagnosis, and classification.
- Demonstrate multi-color (Canto II) flow cytometry assays (panels and analysis)
- Incorporate recent updates in relevant entities

Case illustration (in Clusters/categories)

- **Clusters I:** Analysis of plasma cells
- **Clusters II:** Analysis of T-cell and NK-cell
- **Clusters III:** Acute myeloid leukemia

Case Cluster I- #1:
Analysis of Plasma Cells

Clinical presentation:

- 69 year old man with anemia and thrombocytopenia
- Serum immunofixation: IgA kappa
- Bone survey: diffuse osteopenia with multiple ill-defined lucencies in the calvarium.

Case Cluster I-case #1:
Bone Marrow Examination

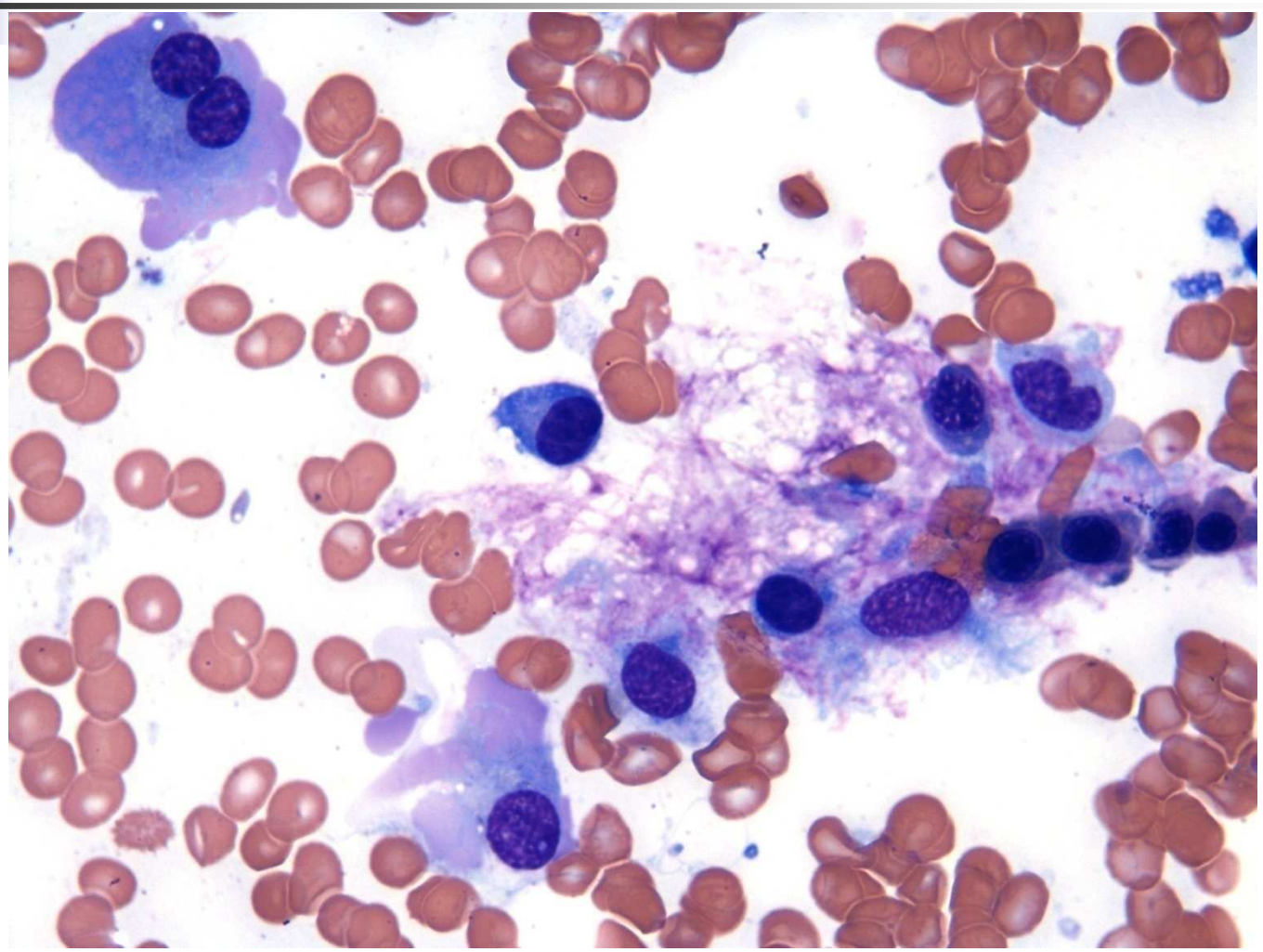

Table 1. Consensus medical indications of multiparametric flow cytometry immunophenotyping in the study of multiple myeloma and other monoclonal gammopathies

Clinical application	*Parameters measured by flow cytometry*
Differential diagnosis between myeloma, MGUS and reactive conditions	(i) Plasma cells as a percentage of total leucocytes. (ii) Plasma cell immunophenotype (see Table 2) (iii) Plasma cell clonality (iv) Abnormal plasma cells as a percentage of total plasma cells
Prognostic markers in myeloma	Expression of specific antigens by abnormal plasma cells, e.g. CD45/CD56/CD117/CD28
Prediction of outcome for patients with MGUS and asymptomatic myeloma	Abnormal plasma cells as a percentage of total plasma cells
Detection of minimal residual disease in myeloma patients after treatment and determination of a stringent complete response	Abnormal plasma cells, identified by immunophenotype and cytoplasmic κ/λ, as a percentage of either total leukocytes or as a percentage of total plasma cells; requires high sensitivity assessment

Rawstron, A. C. et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Haematologica 2008;93:431-438

haematologica
the hematology journal

Table 2. List of most useful antigens for the detection of aberrant plasma cells in multiple myeloma.[5-10;20-44]

Antigen	*Normal expression profile (percentage expression on normal plasma cells)*	*Abnormal expression profile*	*Percentage of myeloma cases with abnormal expression*	*Requirement for diagnosis and monitoring*
CD19	Positive (>70%)	Negative	95%	Essential
CD56	Negative (<15%)	Strongly positive	75%	Essential
CD117	Negative (0%)	Positive	30%	Recommended
CD20	Negative (0%)	Positive	30%	Recommended
CD28	Negative/weak (<15%)	Strongly positive	15-45%	Recommended
CD27	Strongly positive (100%)	Weak or negative	40-50%	Recommended
CD81	Positive (100%)	Weak or negative	Not published	Suggested
CD200	Weakly positive	Strongly positive	Not published	Suggested

Rawstron, A. C. et al. Haematologica 2008;93:431-438

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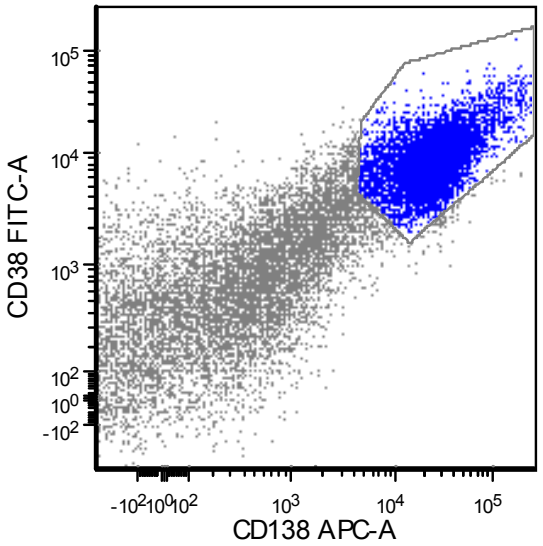
Plasma Cell Neoplasm

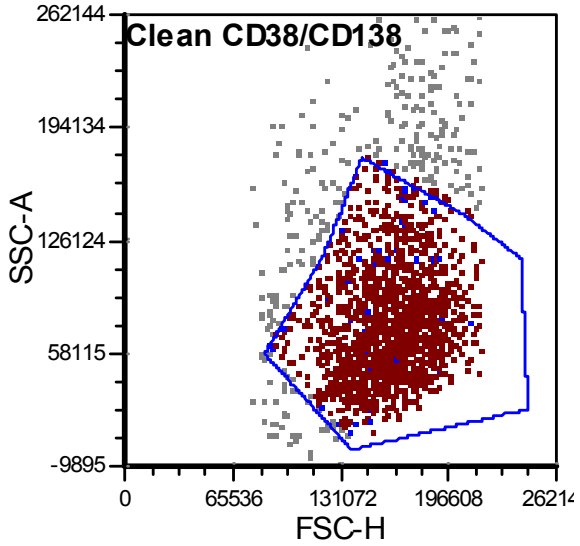
A-7-color Panel

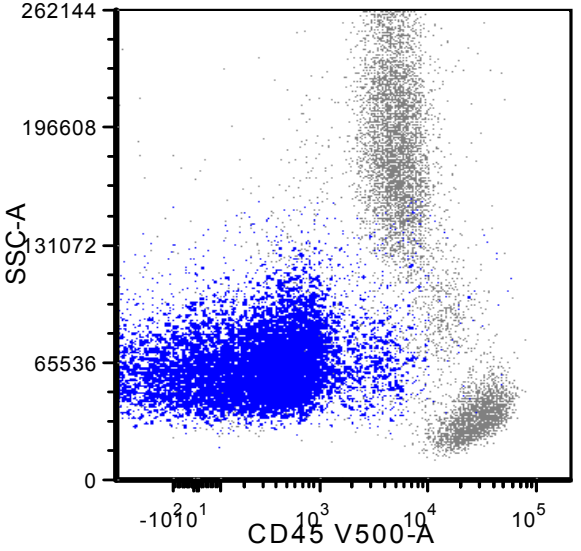
FITC	PE	PerCP-Cy5-5	PE-Cy7	APC	V450	V500
CD38	CD28	CD19	CD117	CD138	CD56	CD45
Kappa cyto	Lambda cyto	CD38	-	CD138	CD20	CD45

Plasma cell Neoplasm

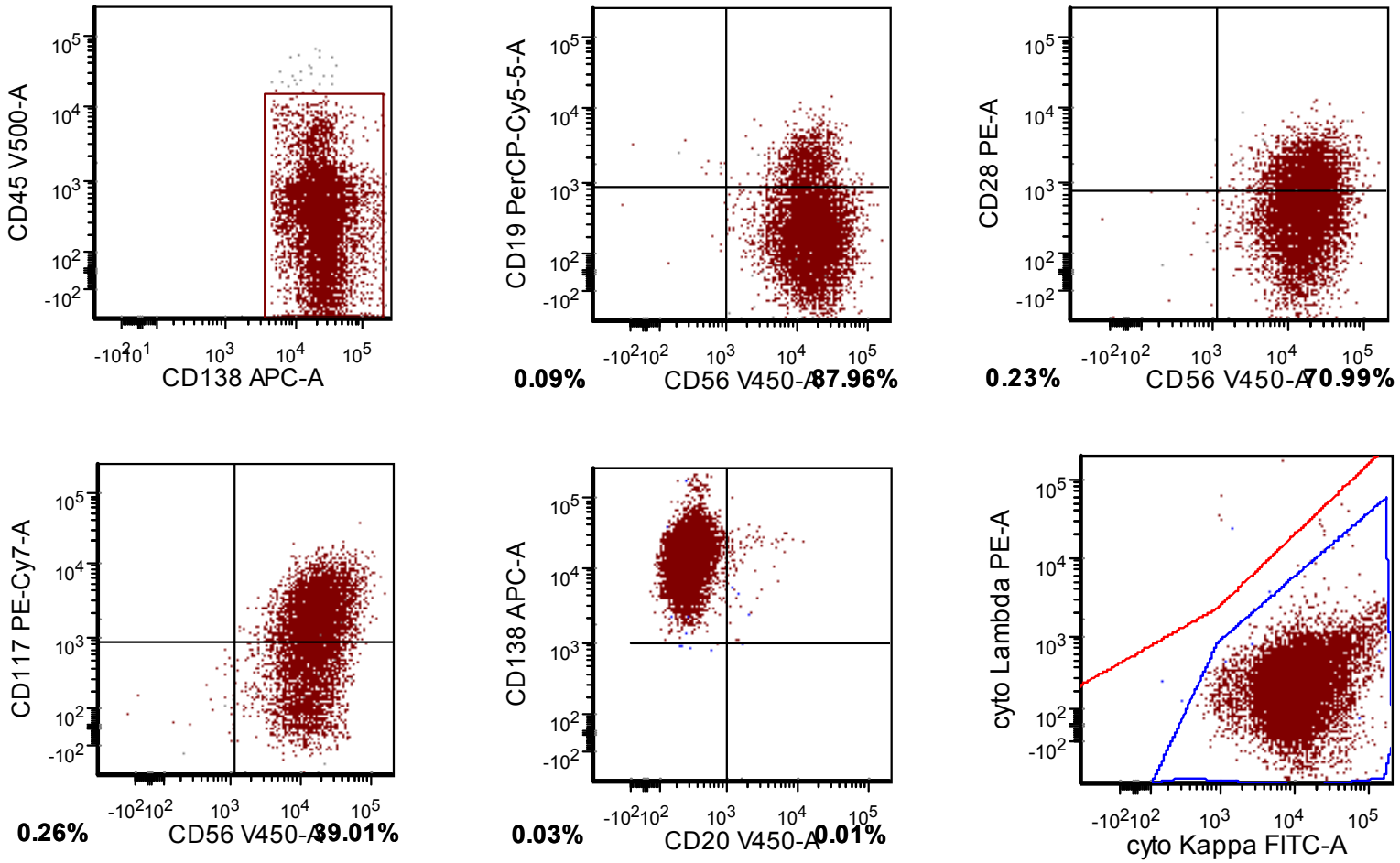
Flow Cytometry Analysis





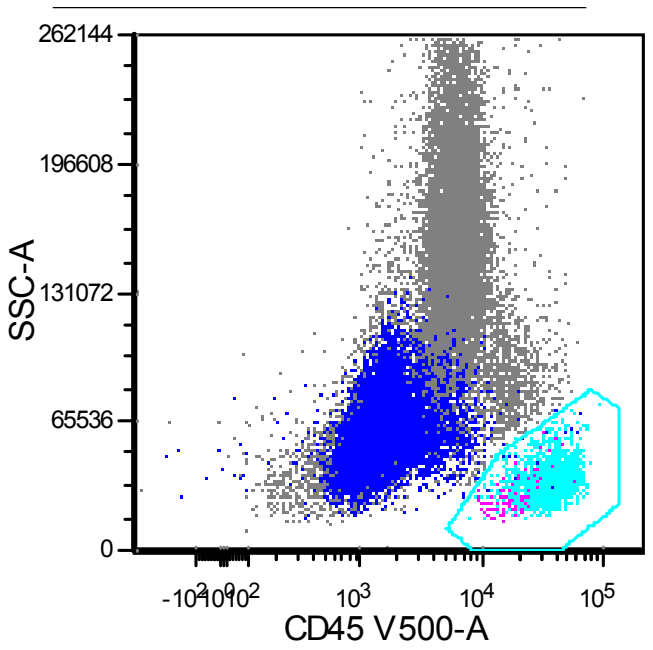


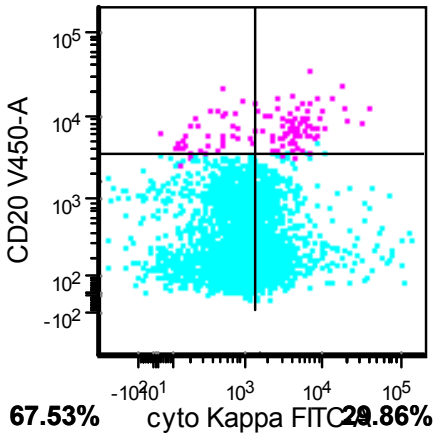
Plasma cell Neoplasm
Aberrant immunophenotype

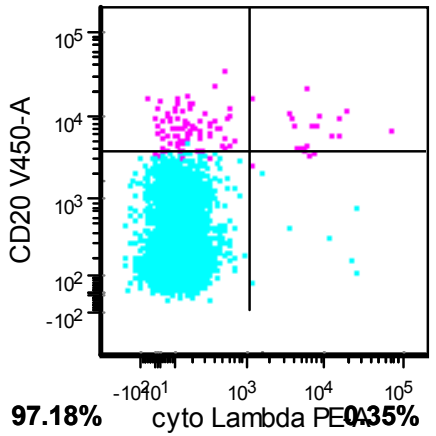


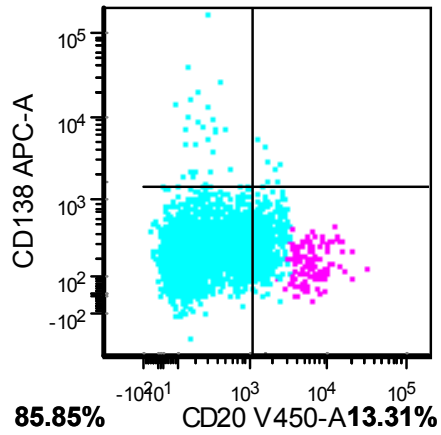
Plasma cell Neoplasm

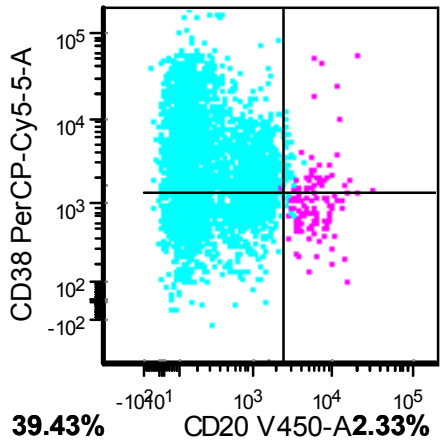
B-cell clonality











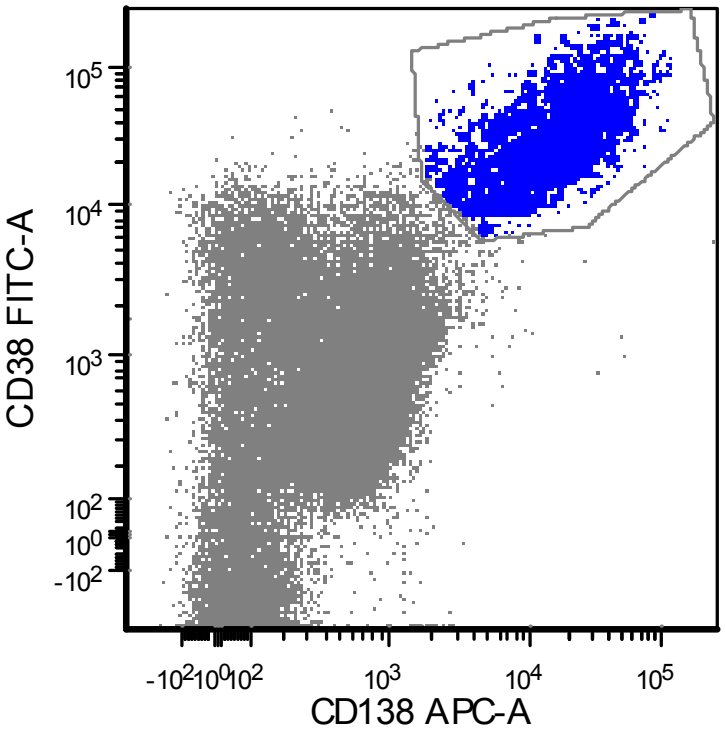
Case Cluster I-Case #2
FCI Analysis of Plasma Cells

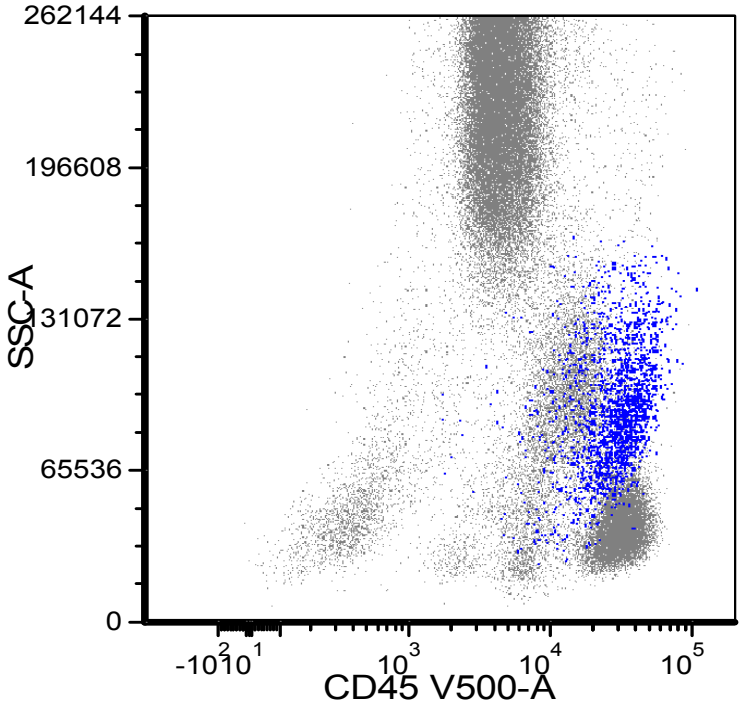
Clinical presentation:

- 64 year old man with hypertension, found with increased creatinine, then found monoclonal paraprotein kappa light chain on UPEP

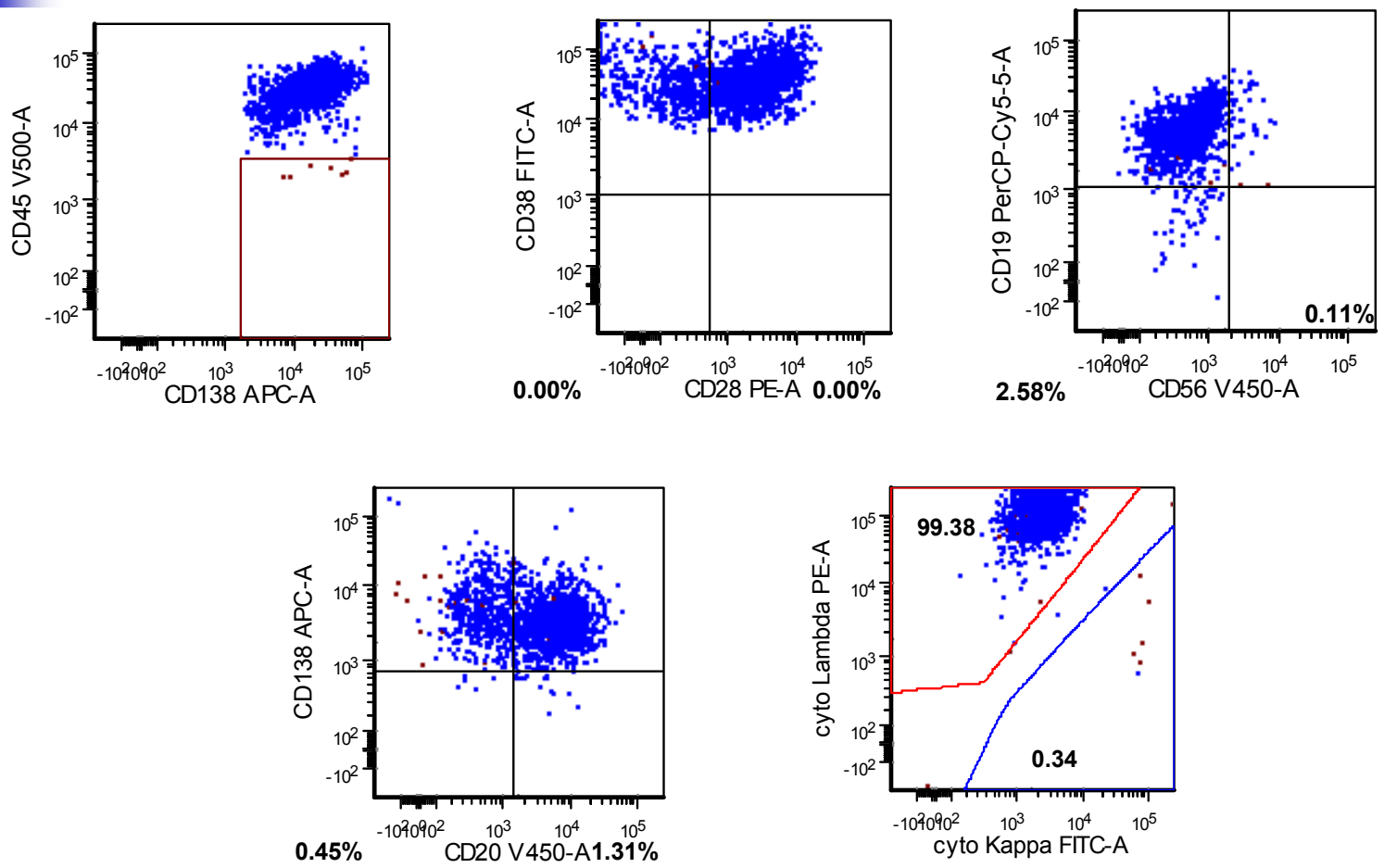
- Immunofixation:
  - Kappa free light chain

Case Cluster I-Case #2
FCI Analysis of Plasma Cells



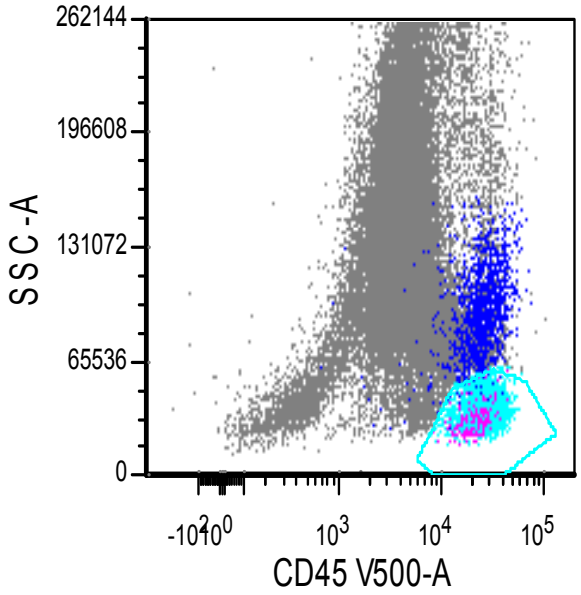


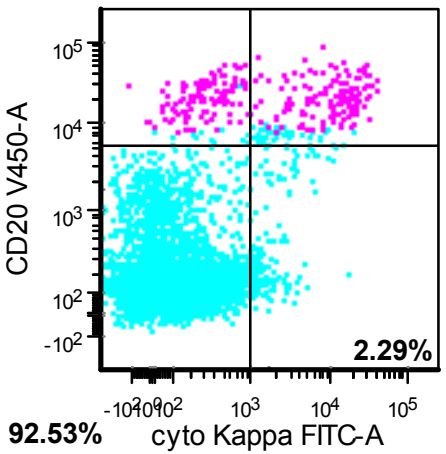
Case Cluster I-Case #2
FCI Analysis of Plasma Cells

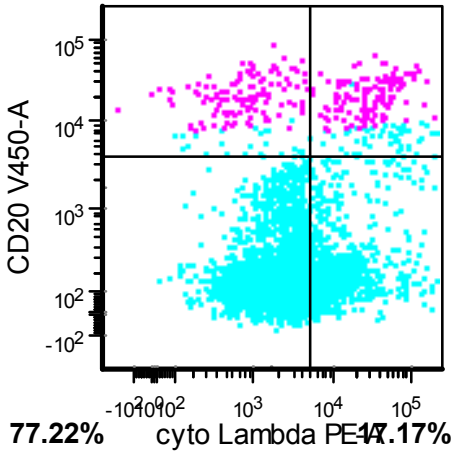


Case Cluster I-Case #2

B-cell clonality

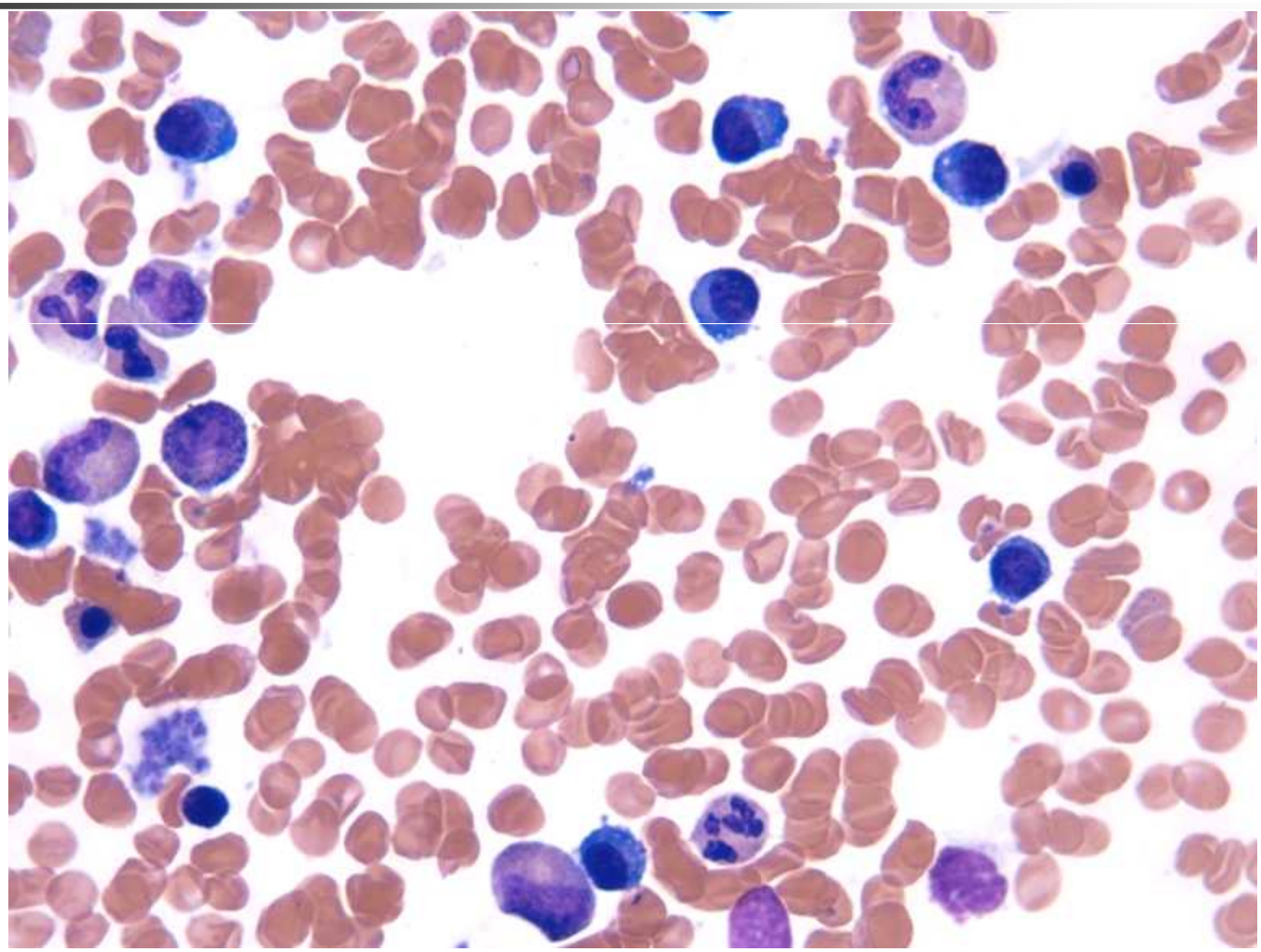




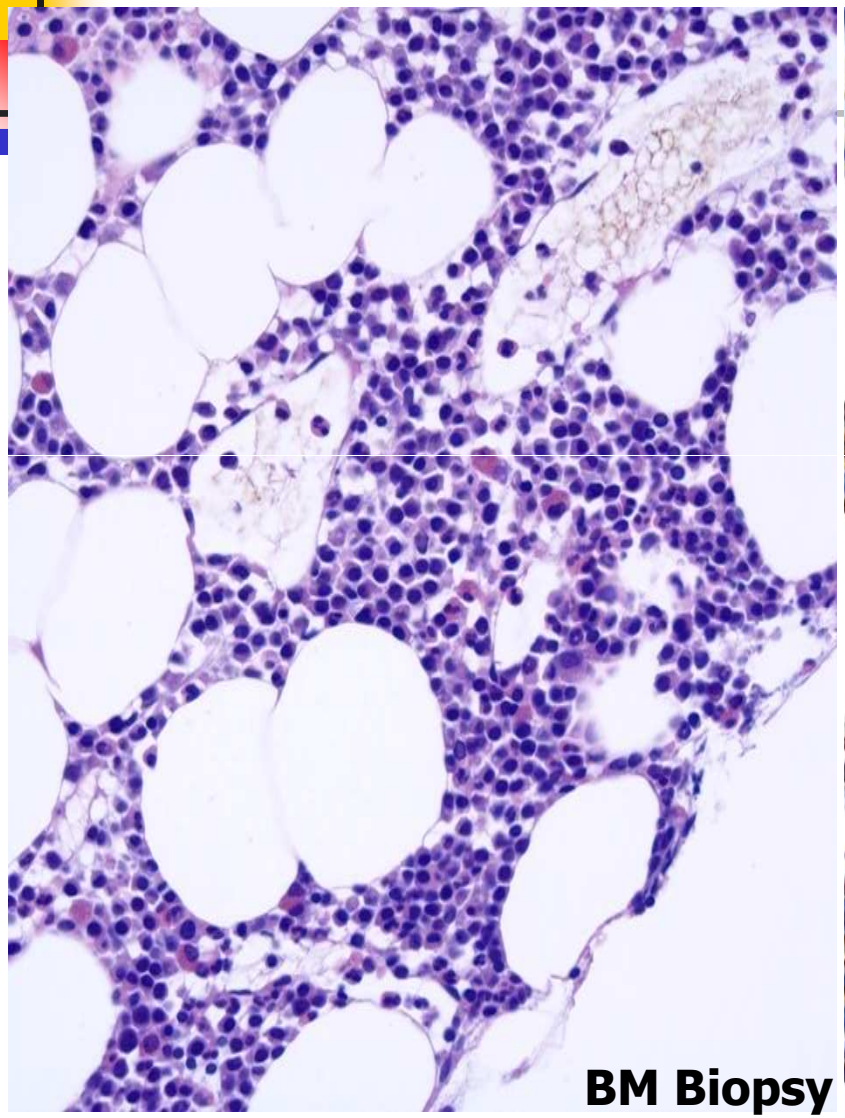


Case Cluster I-Case #2

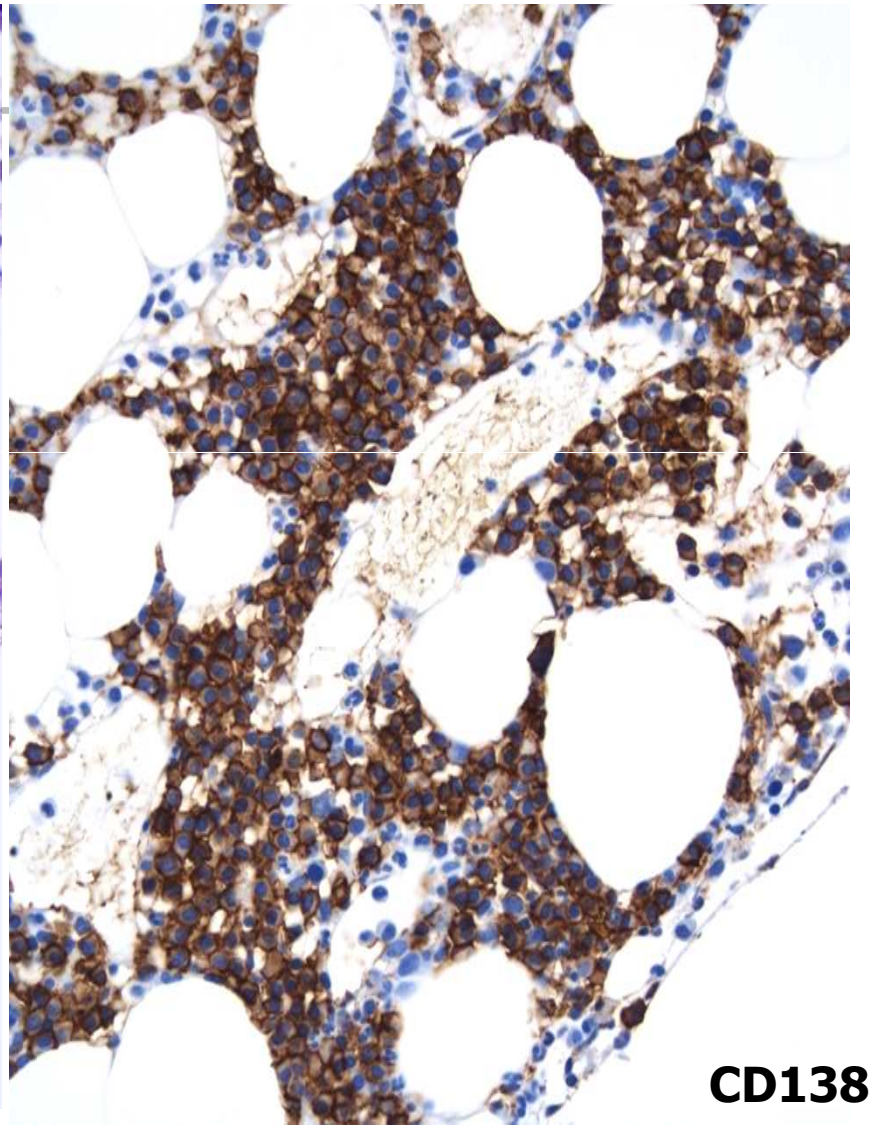
BM Aspirate Smear



Case Cluster I-Case #2
FCI Analysis of Plasma Cells



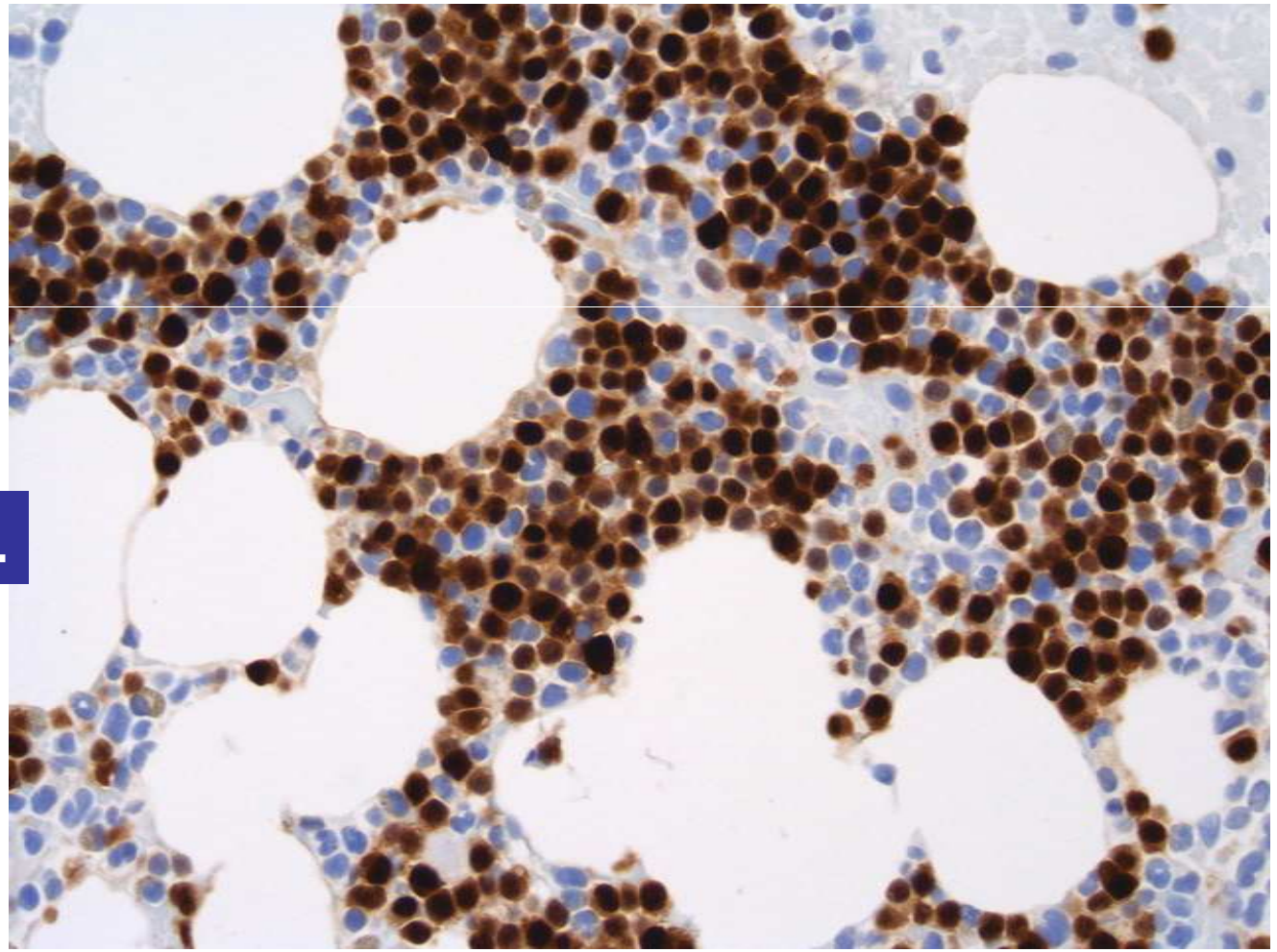
BM Biopsy



CD138

Case Cluster I-Case #2

BM Aspirate Smear



Cyclin D1

Case Cluster I-Case #2

Small Cell Variant Plasma cell Myeloma

Immunophenotypic and Molecular Characteristics of Small Lymphocyte–Like Plasma Cell

	Flow Cytometric Plasma Cell Immunophenotype							
Case No.	CD45	CD19	CD20	CD56	CD38	CD138	CD117	Cytogenetics
1	–	–	–	ND	+	+	–	46,XY[cp20]
2	–	–	+	–	+	+	–	46,XY,t(6;10)(p12;p13)[20]
3	–	ND	–	–	+	+	+	46,XX[20]
4	–	ND	–	–	+	+	–	46,XX[19]/45,XX,–20[1]
5	–	ND	–	+	+	+	+	46,XY[20]
6	–	ND	–	–	+	+	+	46,XX[20], t(11;14)(q13;q32) (positive by FISH)
7	–	ND	–	–	+	+	–	46,XY,t(11;14)(q12~13.1;q32), del(13)(q14q22),?del(17)(p12)[cp2]/46,XY[18]
8	–	ND	+	–	+	+	+	45,XY,t(11;14)(q12~13.1;q32), –13[1]/46,XY[cp19]
9	–	ND	+	–	+	+	–	46,XY,der(14)t(11;14)(q13;q32)[4]/46,XY[8]
10	–	ND	+	–	+	+	–	46,XY[20]
11	–	ND	+	–	+	+	–	46,XX[20],t(11;14)(q13;q32) (positive by FISH)
12	ND	ND	ND	ND	ND	ND	ND	46,XY[20]

Heerema-McKenney A et al Am J Clin Pathol. 2010 Feb;133(2):265-70.

Case Cluster I-Case #3

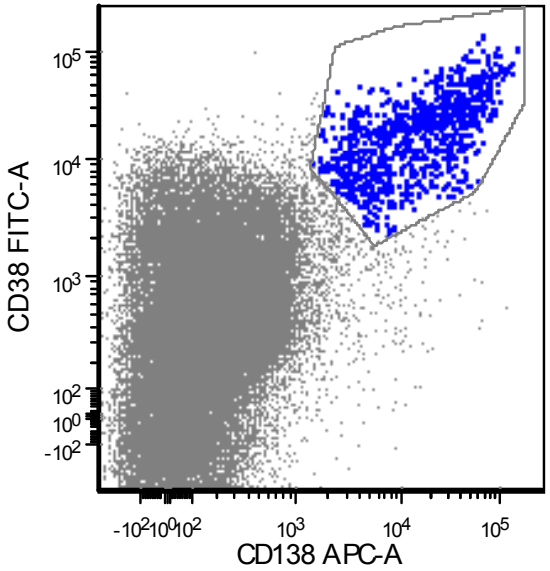
FCI Analysis of Plasma Cells

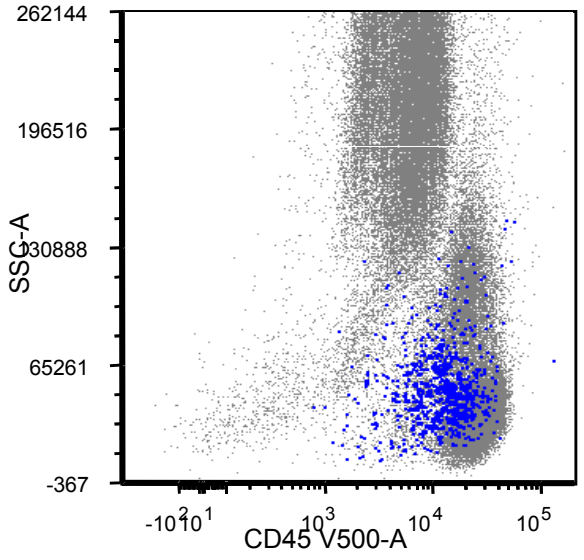
Clinical presentation:

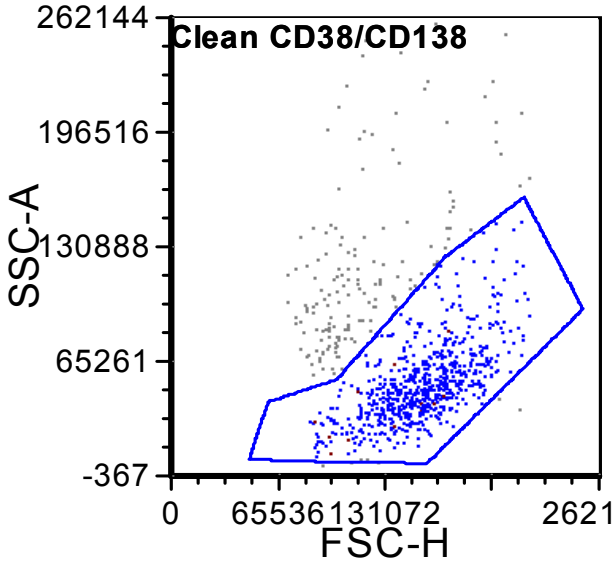
- 49 year old man with anemia, work-up revealed a peptic ulcer, treated
- However, persistent anemia, then found increased IgM

Case Cluster I-Case #3

FCI Analysis of Plasma Cells

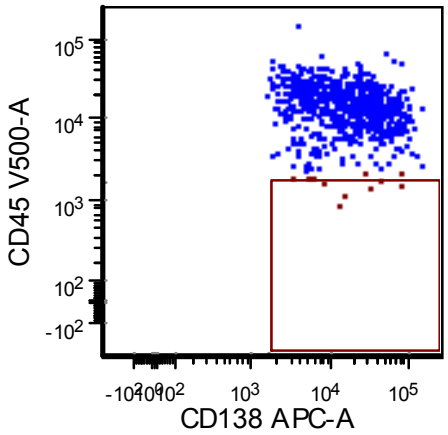


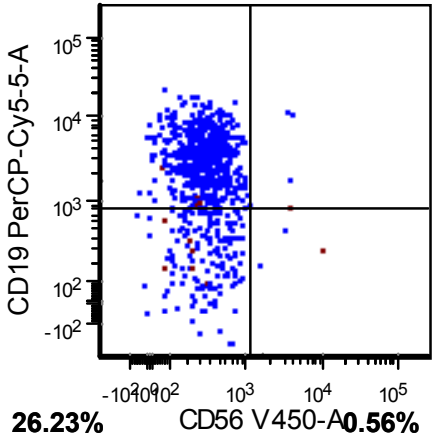


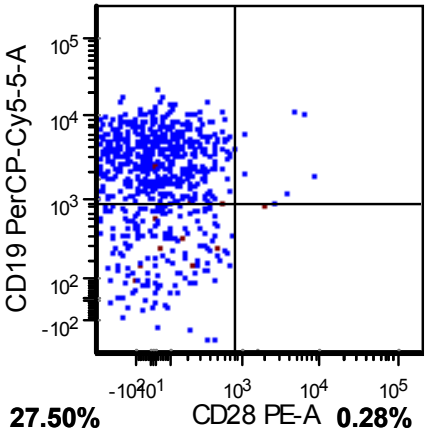


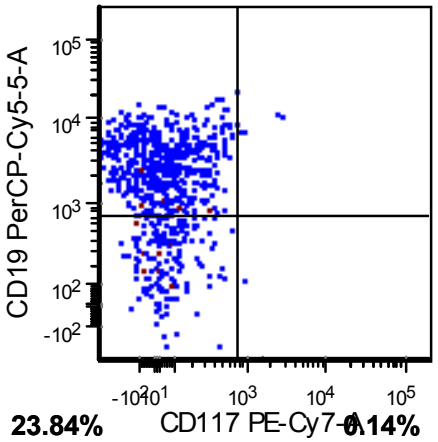
Case Cluster I-Case #3

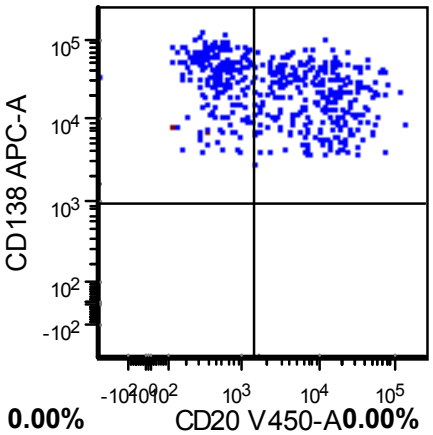
FCI Analysis of Plasma Cells

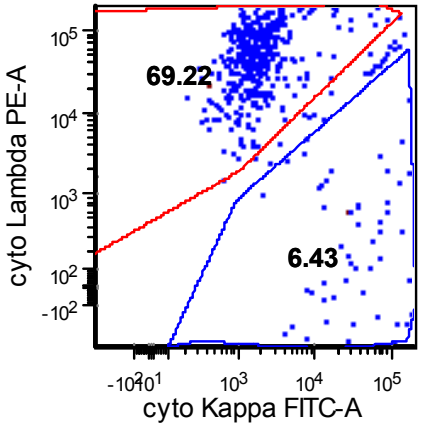






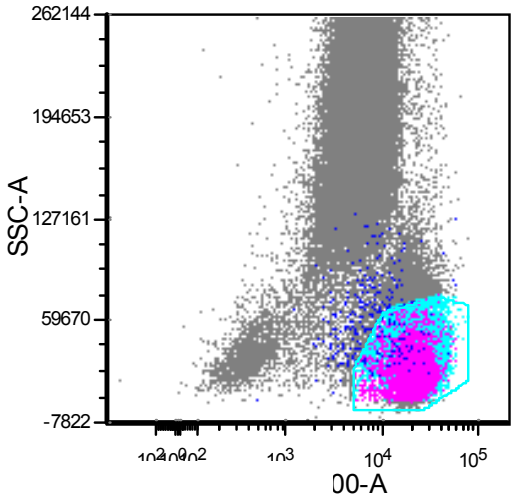


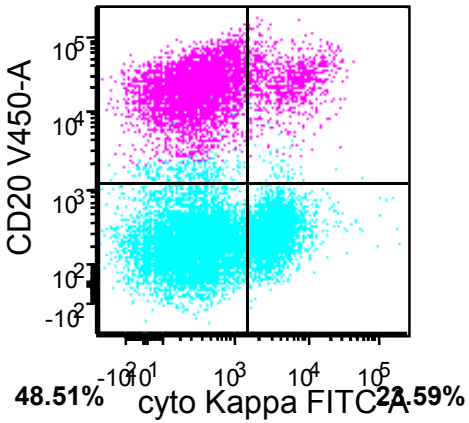


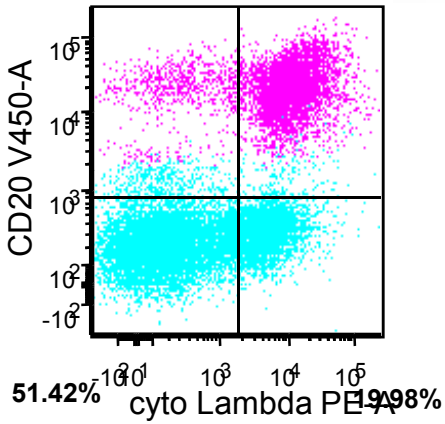


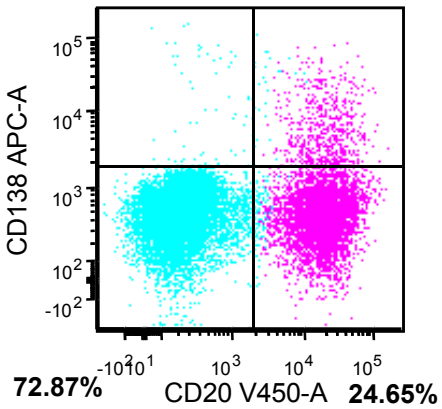
Case Cluster I-Case #3

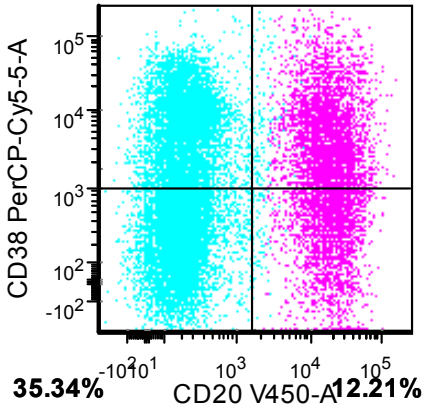
FCI Analysis of Plasma Cells

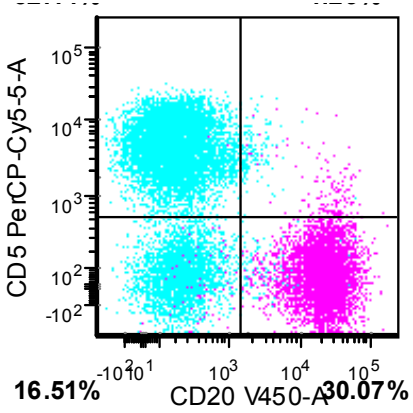


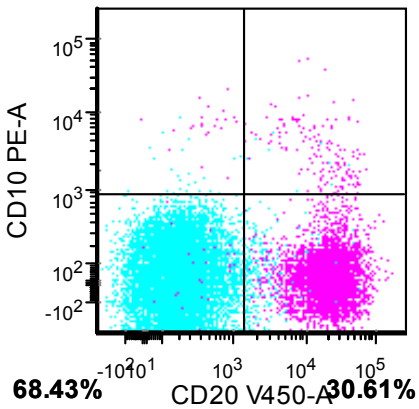






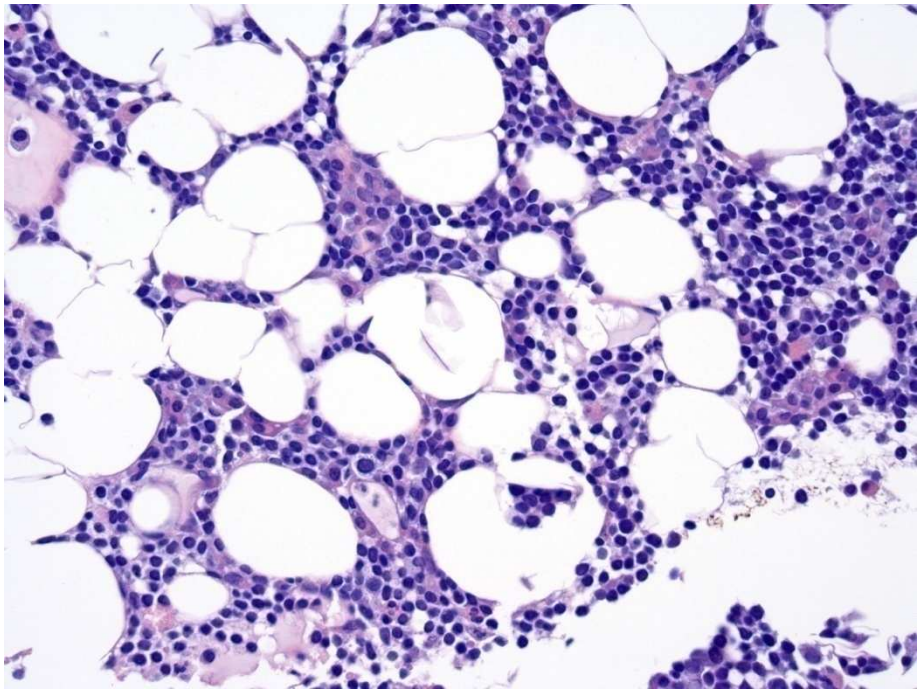


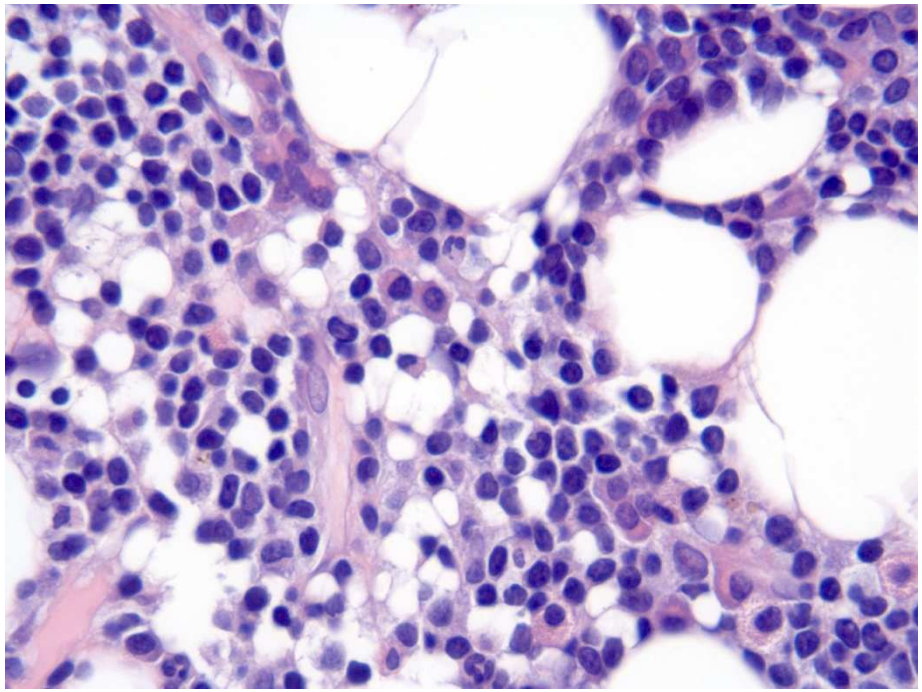




Case Cluster I-Case #3

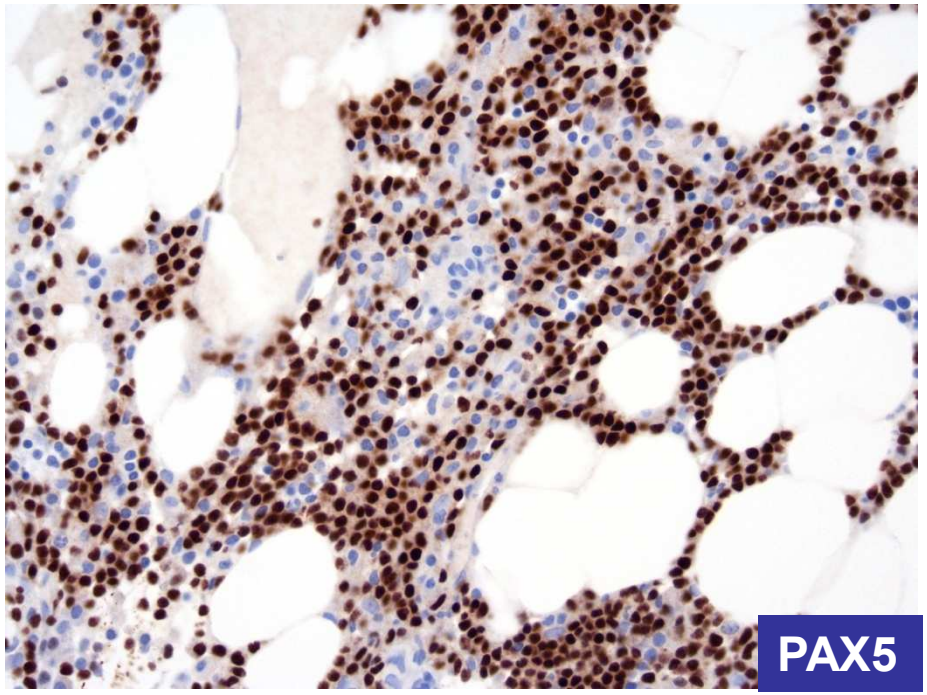
Bone Marrow Biopsy

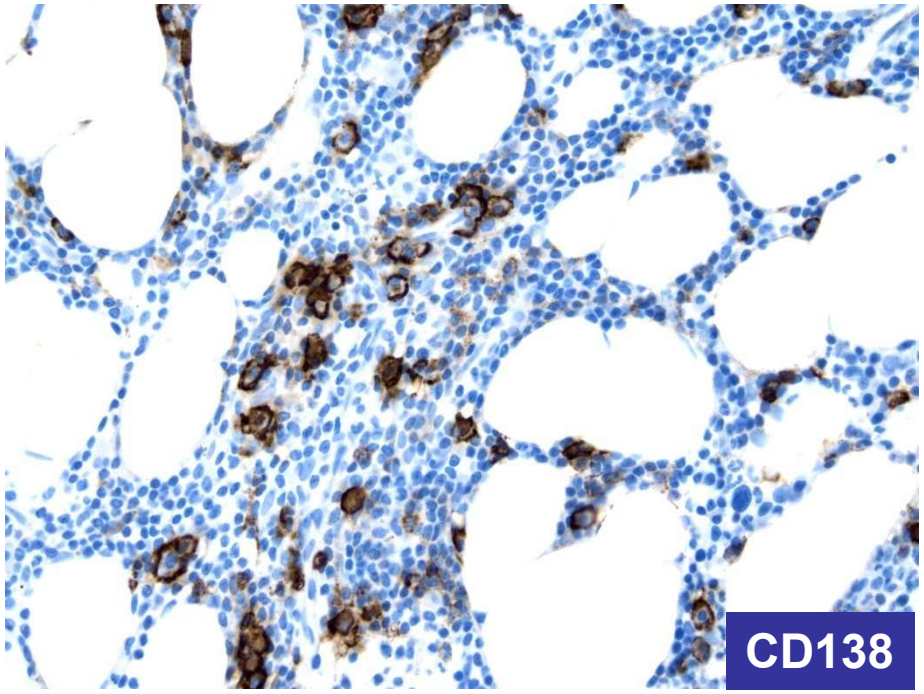




Case Cluster I-Case #3

Immunohistochemistry





Plasma cells
in Plasma cell neoplasia versus in lymphoma

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

Antigen/Disease	No. (%) Positive	*P**
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.
* By the Fisher exact test.

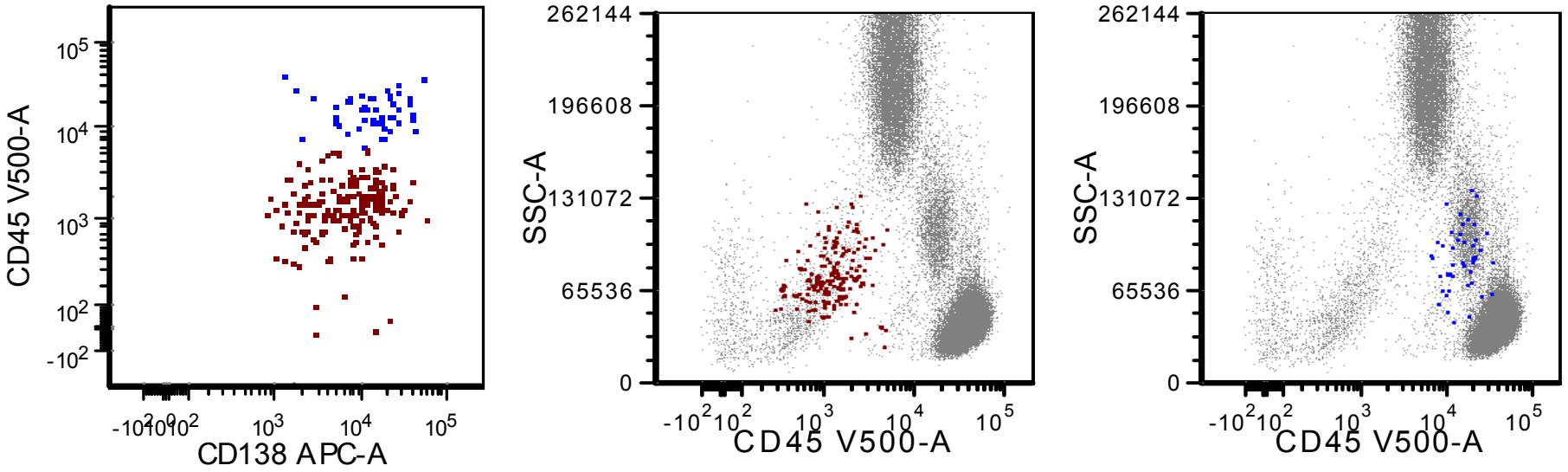
Seegmiller AC et al Am J Clin Pathol. 2007;127(2):176-81.

Case Cluster I-Case #4
FCI Analysis of Plasma Cells

- 55 yo woman, at routine check-up found with mild anemia
- Lab work revealed 0.5gm IgG kappa
- Bone marrow biopsy was performed and showed 8% plasma cells, kappa+
- FCI performed

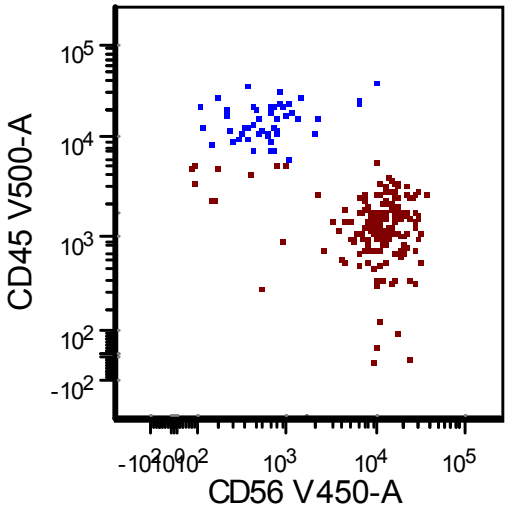
Case Cluster I-Case #4

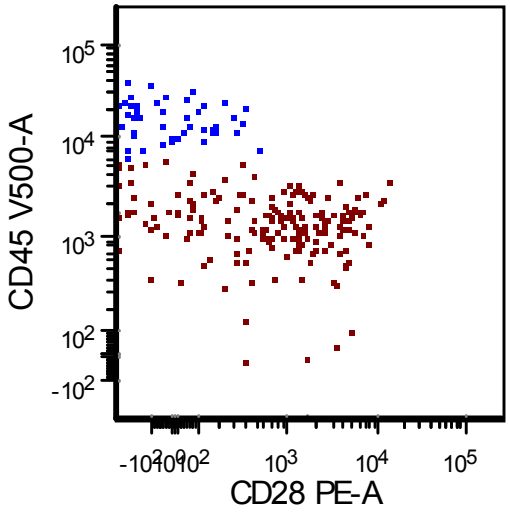
FCI Analysis of Plasma Cells

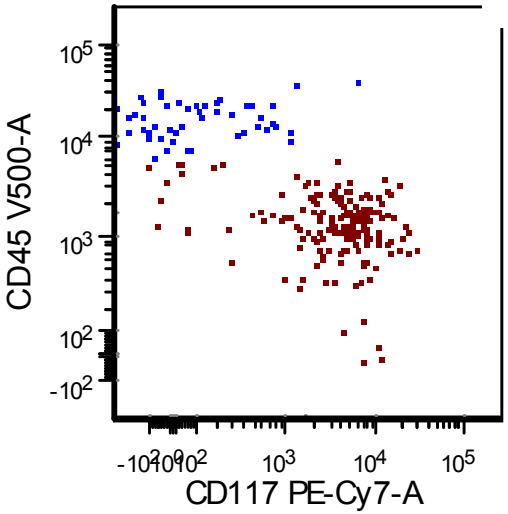


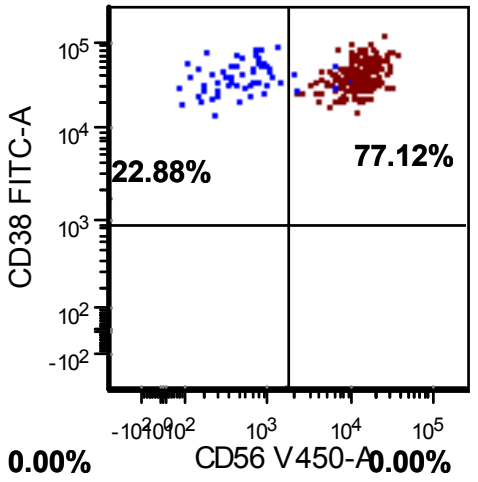
Case Cluster I-Case #4

FCI Analysis of Plasma Cells









Monoclonal Gammopathy of Uncertain Significance (MGUS)

Immunophenotypically, the neoplastic plasma cells are similar to other myeloma

However, in MGUS, normal plasma cells are often present coexisting with neoplastic myeloma cells

- MGUS: abnormal plasma cells
  - 73 (0–100) %
- Smoldering myeloma: abnormal plasma cells
  - 95 (11–100)%

Pérez-Persona E et al Br J Haematol. 2010 Jan;148(1):110-4.
Pérez-Persona E et al. Blood 2007;110:2586-2592

blood
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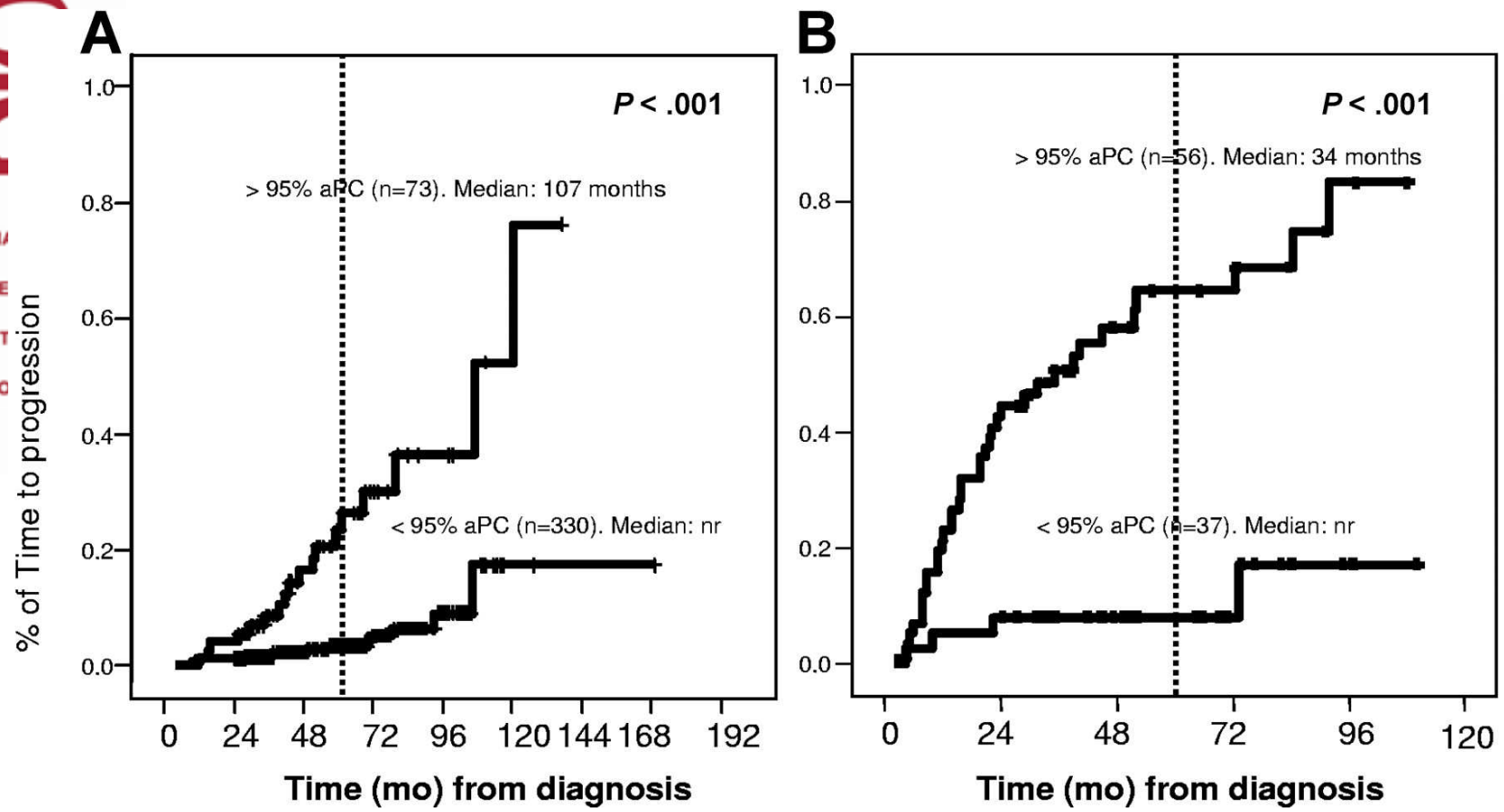
Time to progression in MGUS and SMM according to the percentage of immunophenotypically aberrant plasma cells.



Pérez-Persona E et al. Blood 2007;110:2586-2592

Summary
FCI in Plasma cell Neoplasm

Initial Diagnosis

- Characterize aberrant immunophenotype and light chain restriction

- Reporting Neoplastic Plasma/Normal Plasma cell ratio

Reporting

- **1. Aberrant plasma cells detected, __% of total nucleated cells and __% of total plasma cells, consistent with Plasma Cell Neoplasm**
  - The aberrant plasma cells CD38+, CD138+, CD19(-), CD20(-), CD28( ), CD56( ), CD117( ), cyto-Kappa( ), cyto-Lambda( )
- **2. B-cells are polytypic**

Disclaimer: the number of plasma cells detected by flow cytometry does not reflect the actual number of plasma cells in the bone marrow/or tissue.

Summary
FCI in Plasma cell Neoplasm

Minimal Residual Disease (MRD)

Current Complete Remission (CR) criteria

- <5% plasma cells in BM;
- Absence of M protein by IFX

Stringent CR:

Plus normalization of light chain ratio and BM negative by IHC

Application of FCI in Post Treatment Assessment

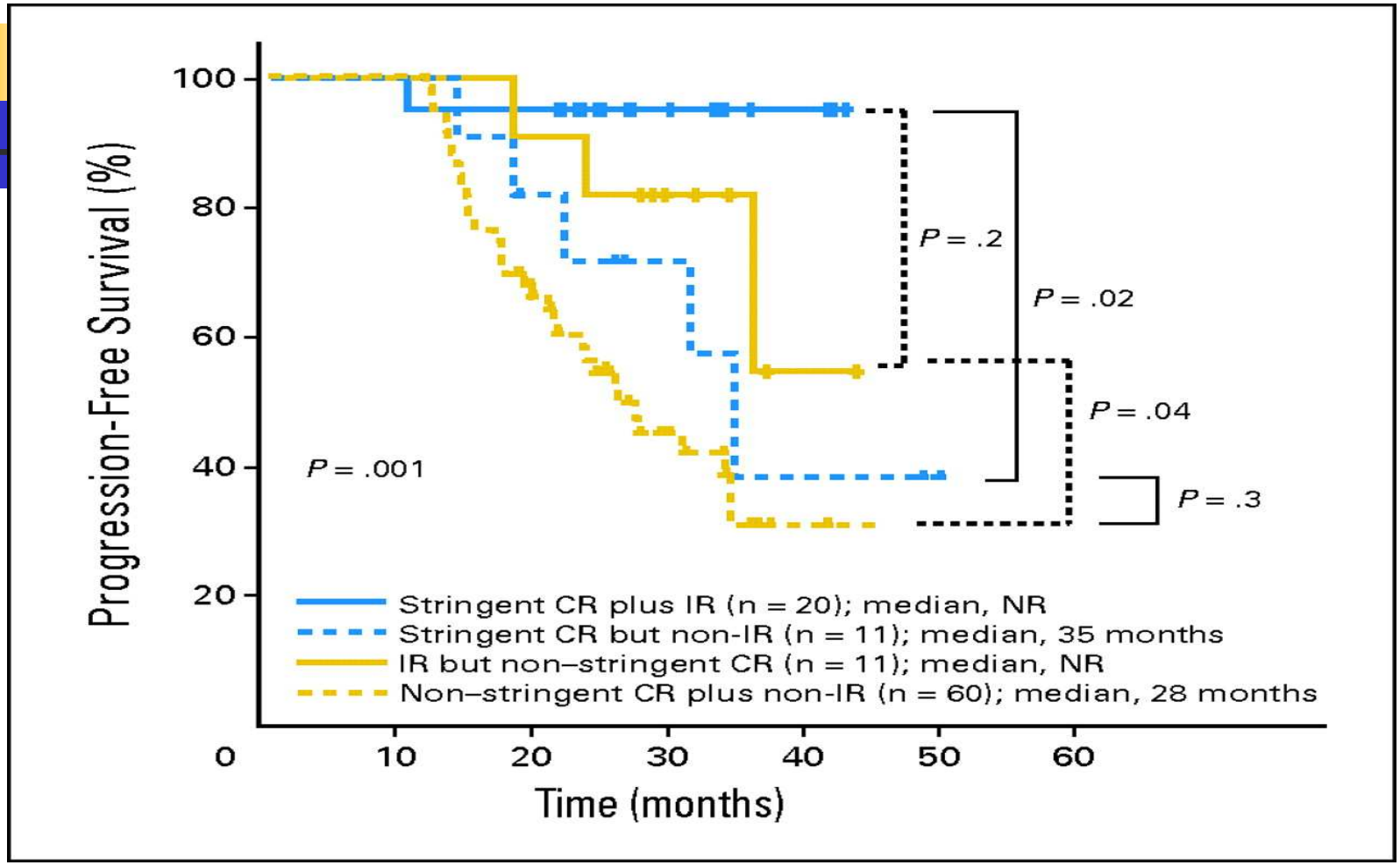
7% (n =7) immunofixation-**Pos** but flow-**Neg**

- In all patients, immunofixation become negative in subsequent analysis.

20% (n=20), immunofixation-**neg** but flow- **Pos**

- Early reappearance of the M-component in 3 months (1 to 12 months) in all patients

Paiva B, et al. J Clin Oncol. 2011 Apr 20;29(12):1627-33.



JOURNAL OF CLINICAL ONCOLOGY

Paiva B et al. JCO 2011;29:1627-1633

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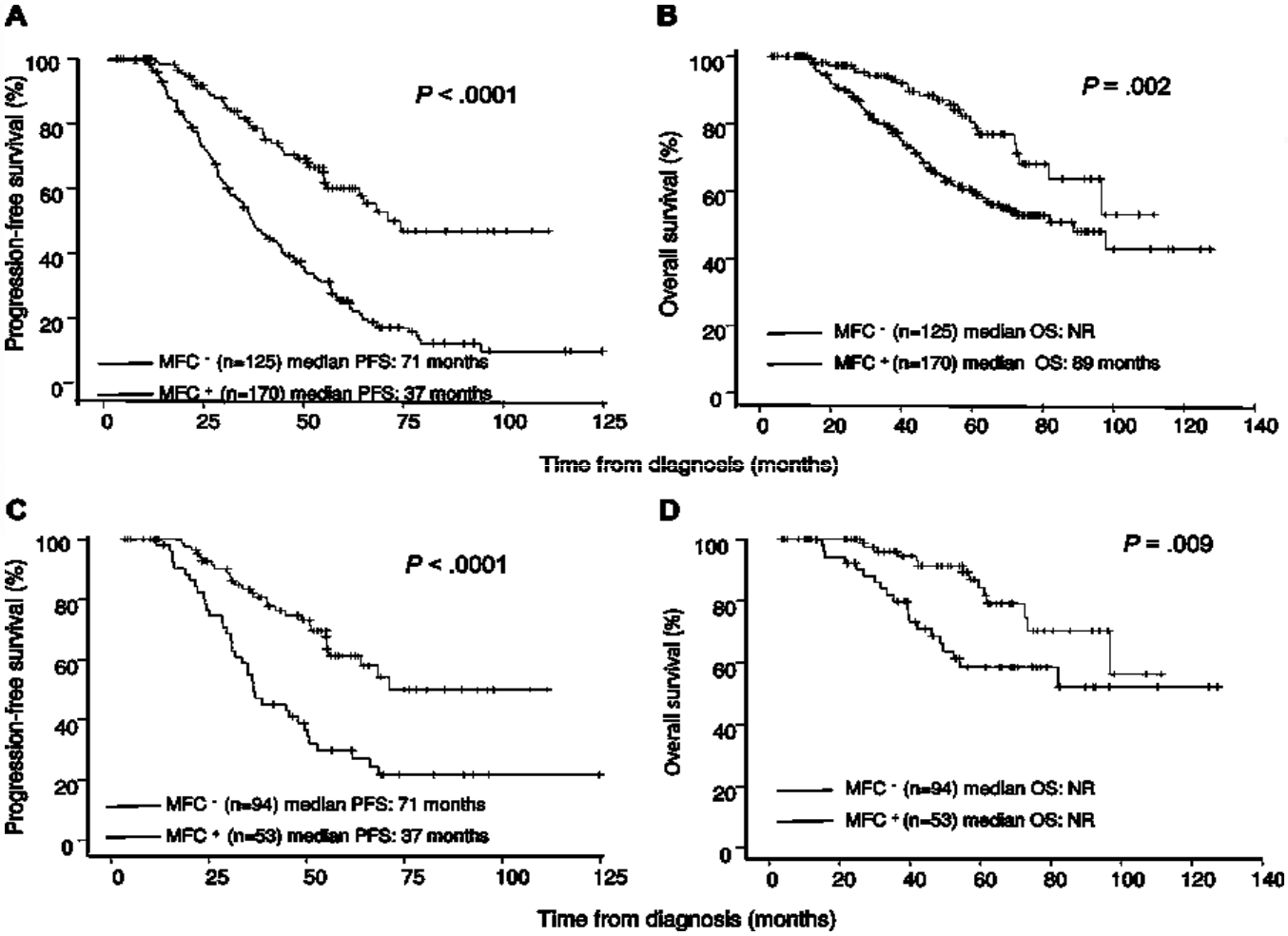
Progression-free survival and overall survival according to the presence or absence of MM-PCs in the bone marrow at day 100 after ASCT.

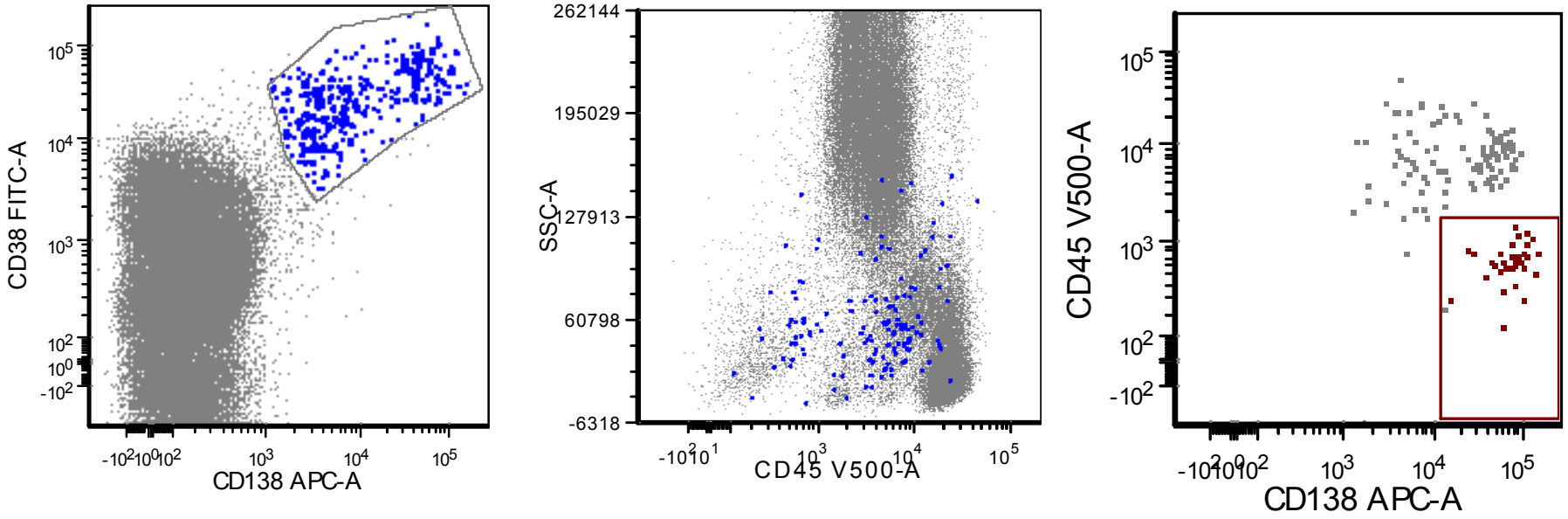


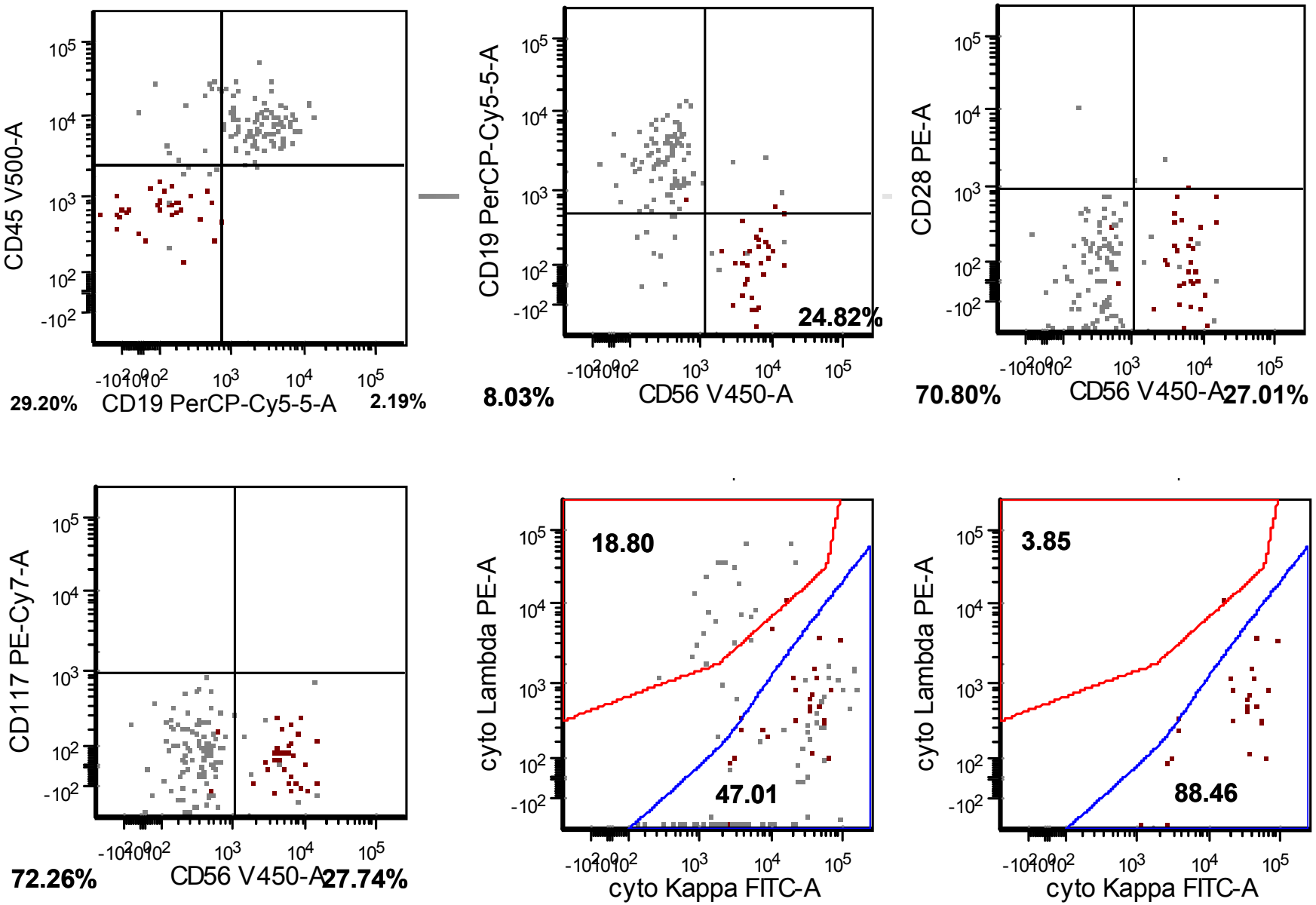
All patients

CR patients

Paiva B et al. Blood 2008;112:4017-4023

FCI in Detection of Minimal Residual Disease




CD45 V500-A
29.20%
CD19 PerCP-Cy5-5-A
2.19%
CD19 PerCP-Cy5-5-A
24.82%
8.03%
CD56 V450-A
CD28 PE-A
70.80%
CD56 V450-A
27.01%
CD117 PE-Cy7-A
72.26%
CD56 V450-A
27.74%
cyto Lambda PE-A
18.80
47.01
cyto Kappa FITC-A
cyto Lambda PE-A
3.85
88.46
cyto Kappa FITC-A

Case Cluster II

Analysis of T-cells and NK-cells

Mature T- and NK-cell Neoplasms

T-cell prolymphocytic leukaemia

T-cell large granular lymphocytic leukaemia

Chronic lymphoproliferative disorders of NK cells

Aggressive NK cell leukaemia

EBV+ T-cell lymphoproliferative disorders of childhood

Adult T-cell leukaemia/lymphoma

Extranodal NK/T cell lymphoma, nasal type

Enteropathy-associated T-cell lymphoma

Hepatosplenic T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Mycosis fungoides

Sézary syndrome

Primary cutaneous CD30 positive T-cell lymphoproliferative disorders

Primary cutaneous gamma-delta T-cell lymphomas

Peripheral T-cell lymphoma, NOS

Angioimmunoblastic T-cell lymphoma

Anaplastic large cell lymphoma (ALCL), ALK positive

Anaplastic large cell lymphoma (ALCL), ALK negative

Case Cluster II
Analysis of T-cells and NK-cells

Flow cytometry needs to answer:

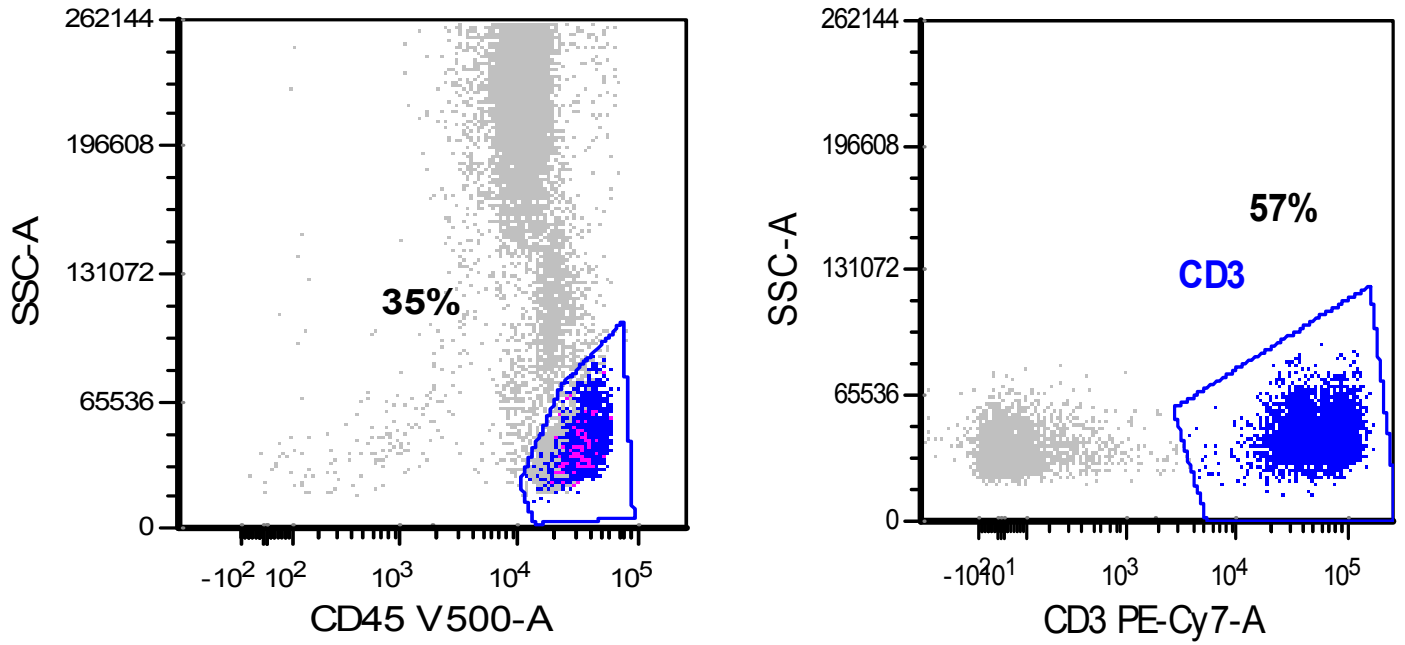
- Mature, immature?
- CD4, CD8?
- Immunophenotypical aberrancies
- Alpha/beta versus Gamma/delta?
- Target therapy markers
- B-cell clonality

Case Cluster II

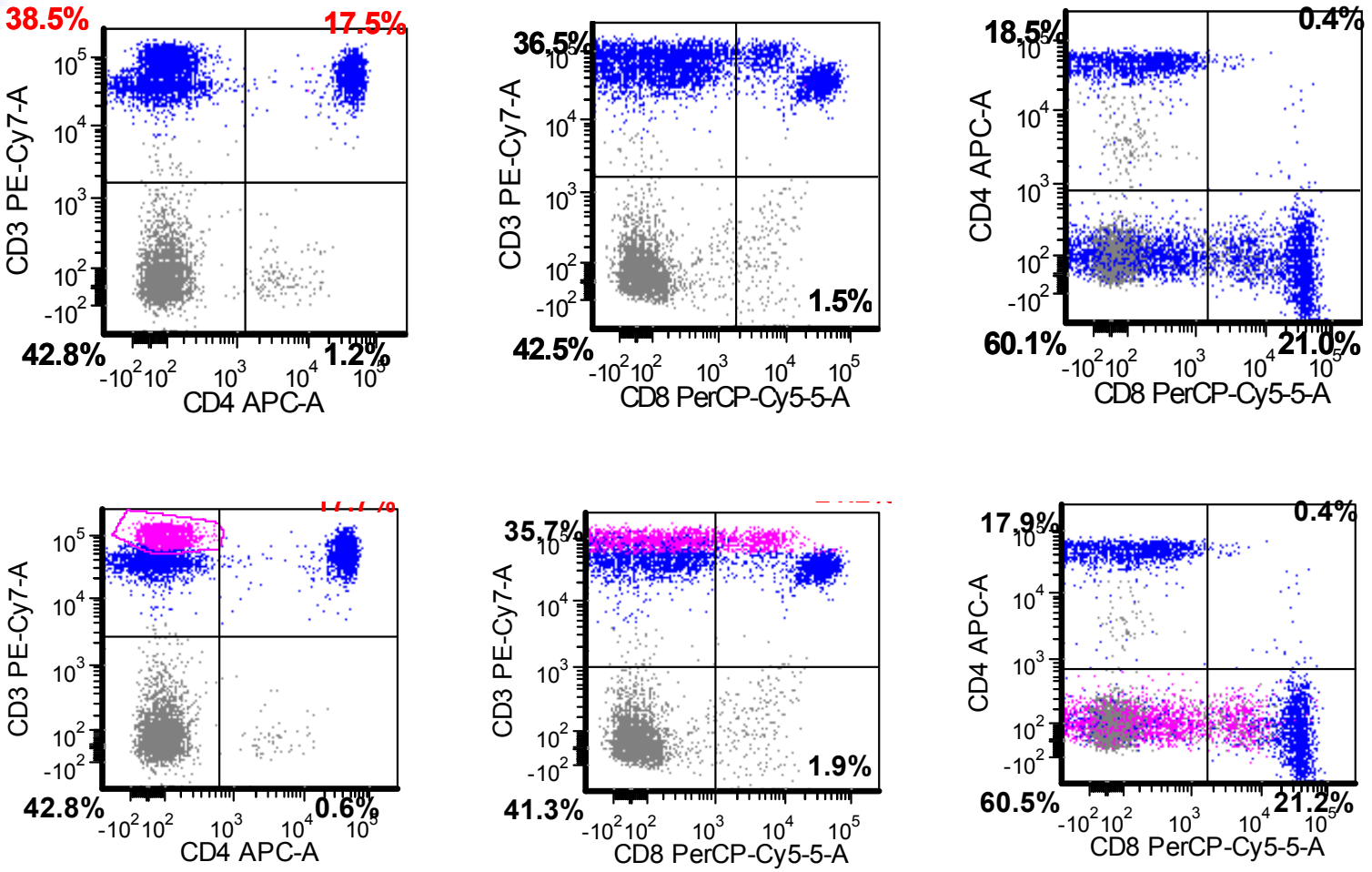
Analysis of T-cells and NK-cells

FITC	PE	PerCP-Cy5-5	PE-Cy7	APC	V450	V500
CD7	CD26	CD8	CD3	CD4	CD14	CD45
CD57	CD94	CD16	CD3	CD56	CD8	CD45
A/B	D/G	CD5	CD3	CD4	CD8	CD45
CD52	CD2	CD4	CD3	CD10	CD25	CD45
Kappa	Lambda	CD5	CD19	CD10	CD20	CD45

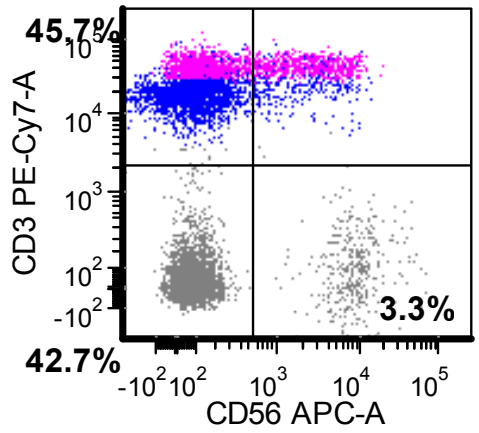
FCI Analysis of T-cells and NK-cells

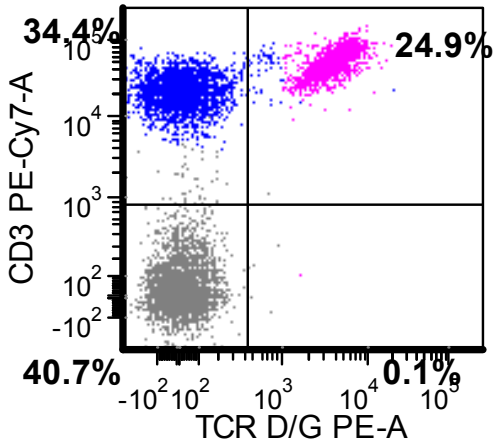


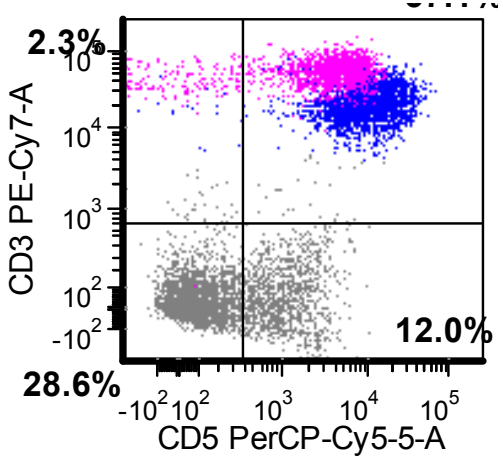
FCI Analysis of T-cells and NK-cells

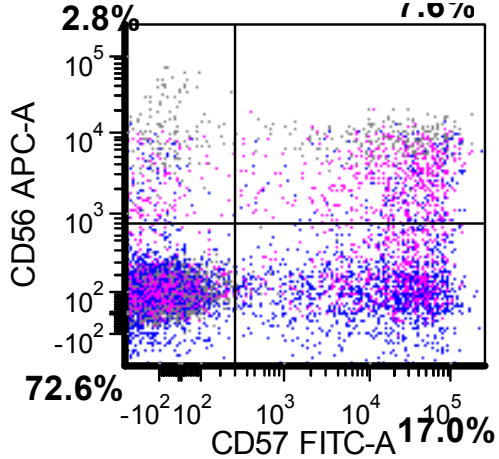


Normal Gamma/delta T cells









Normal Gamma/delta T-cells

Immunophenotype:

- CD3bright+, CD2+, CD7+
- CD4-, CD8subset/dimer+,
- CD5dimer/neg
- CD56subset+, CD57subset+
- TCRalpha/beta-, TCR gamma/delta+

Normally: 5% of total T cells

- Increased in a number of reactive conditions: mycobacteria, viral; post- transplant…

Case Cluster II
Analysis T cells and NK cells

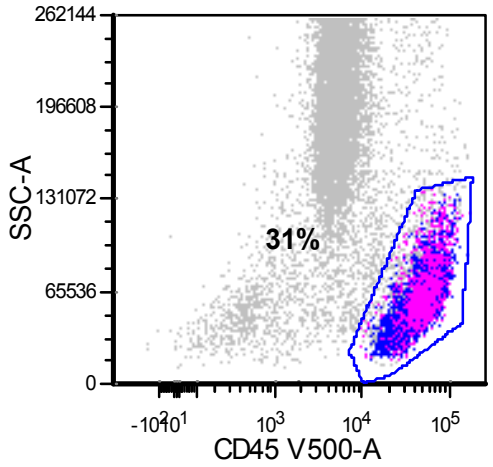
Case #1

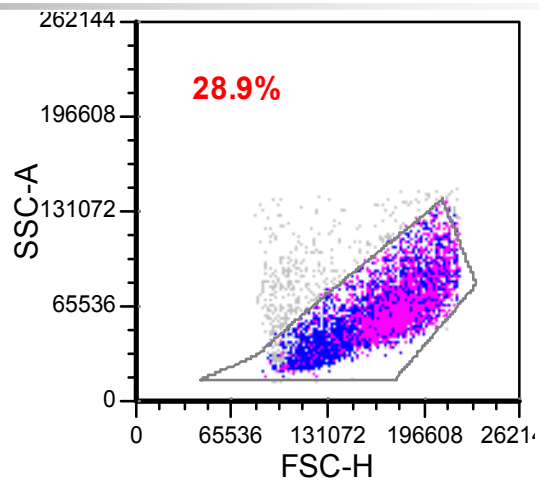
Clinical Presentation:

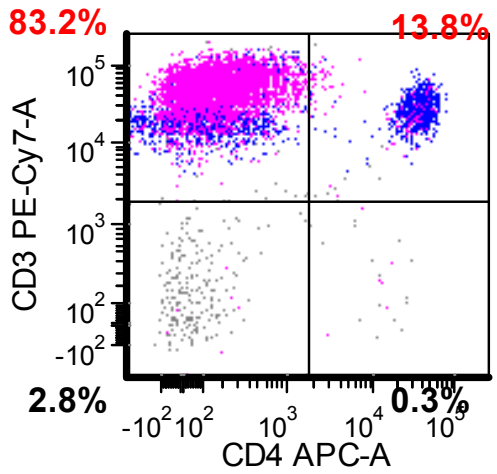
- 39 year old female was noted with an abdominal mass at regular GYN check-up, confirmed with massive splenomegaly
- Anemia and leukopenia

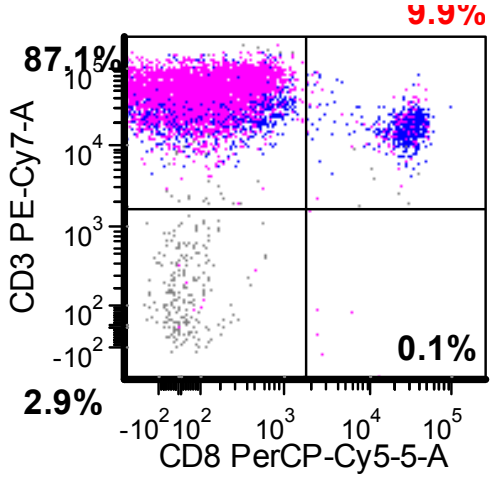
BM biopsy performed and sent for FCI

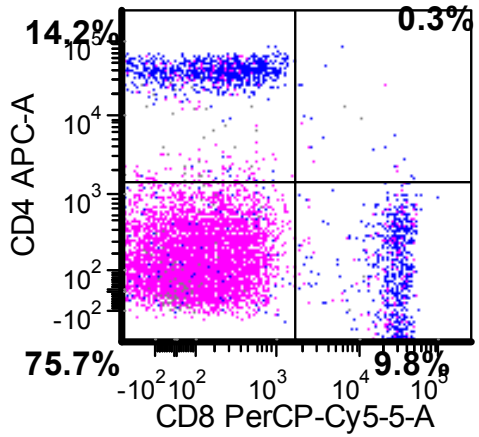
Case Cluster II-#case 1
Analysis T cells and NK cells



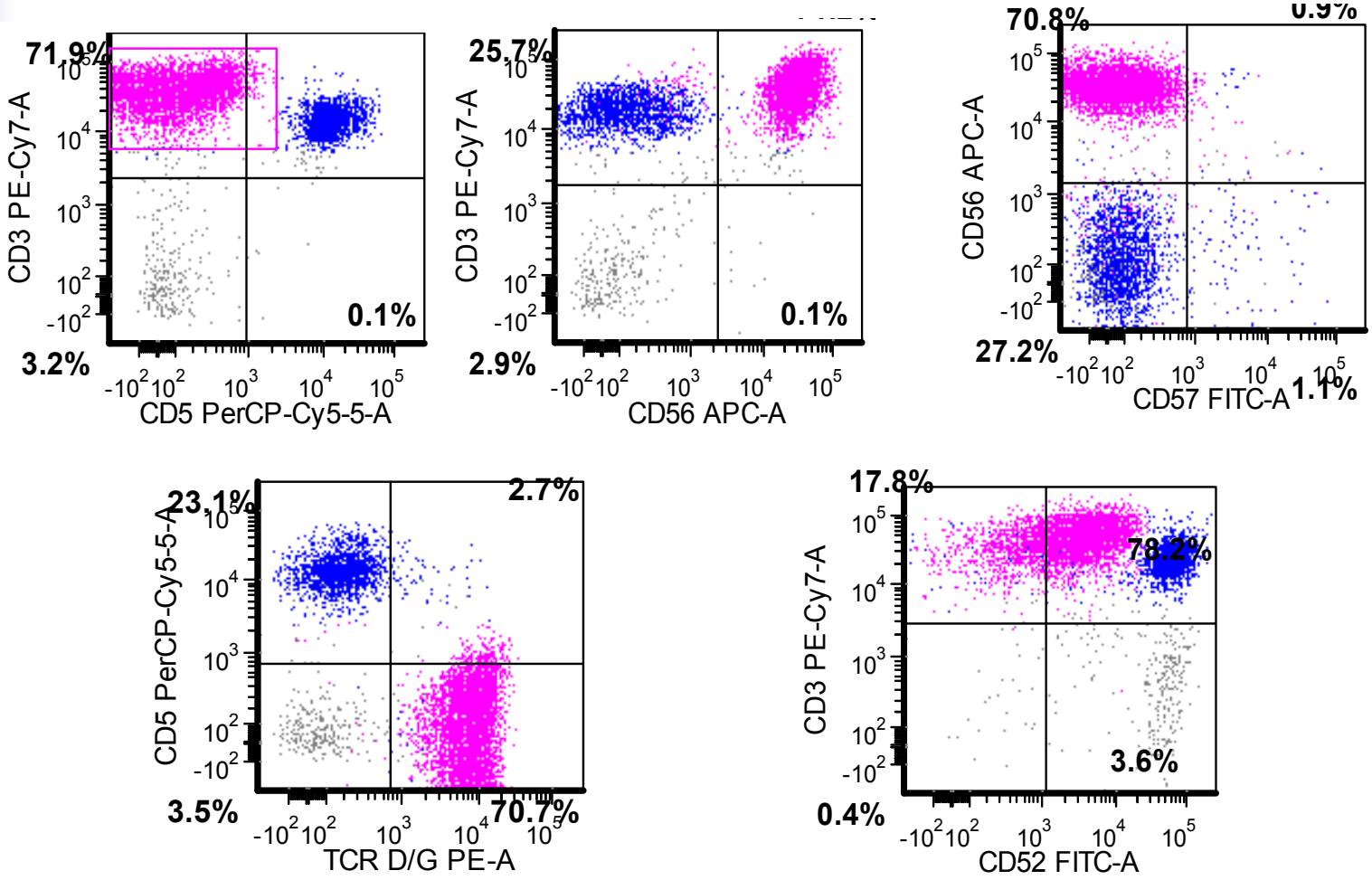




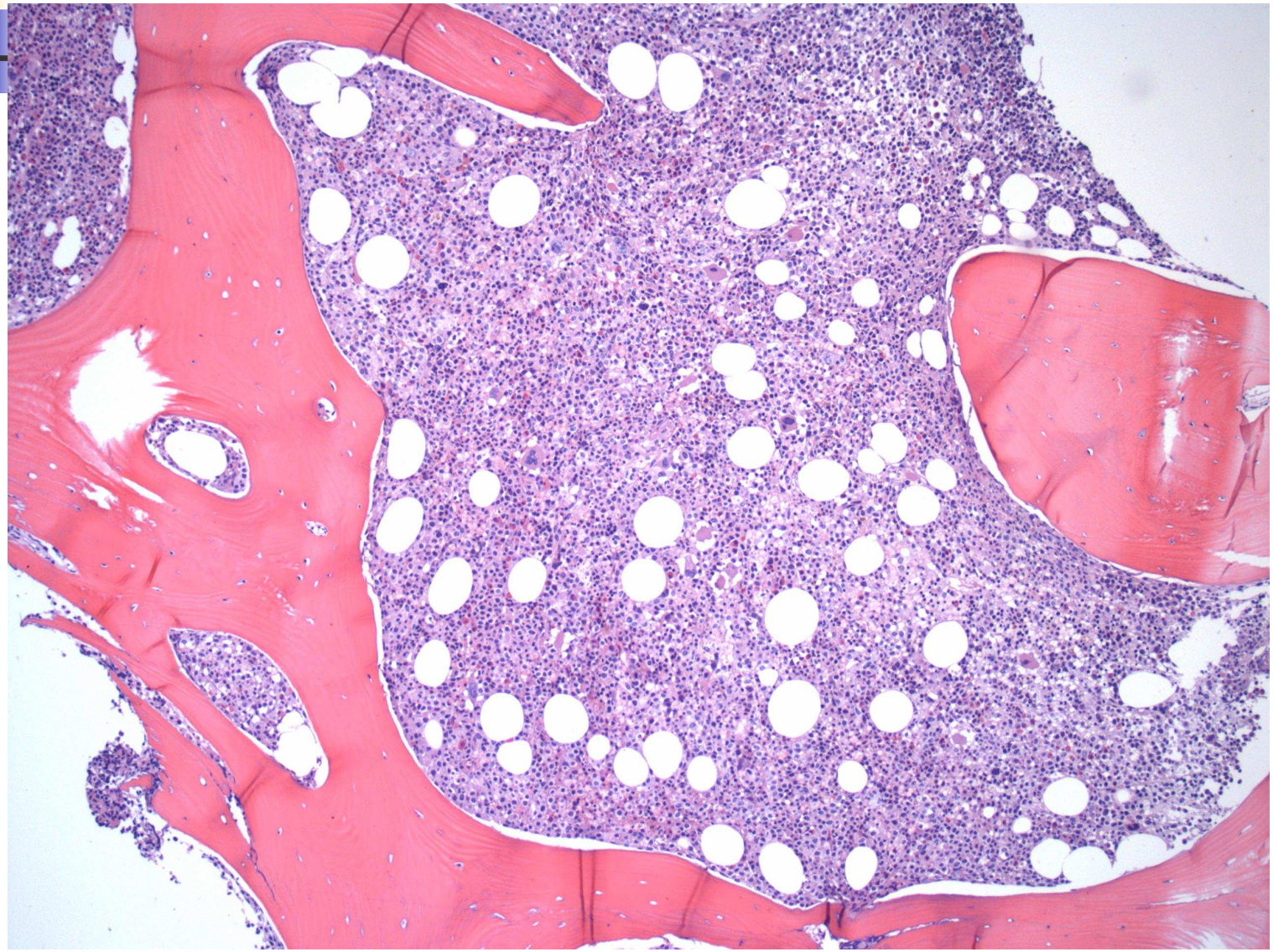




Case Cluster II-#case 1
Analysis T cells and NK cells

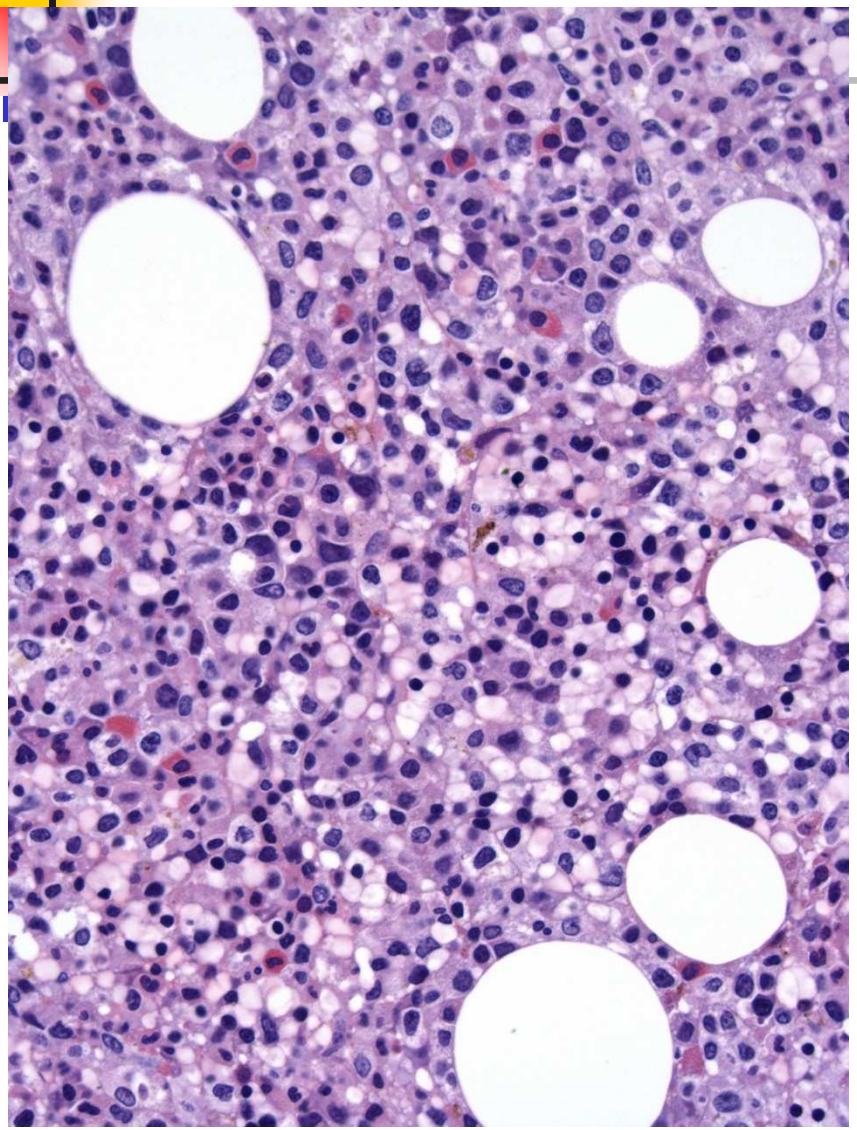


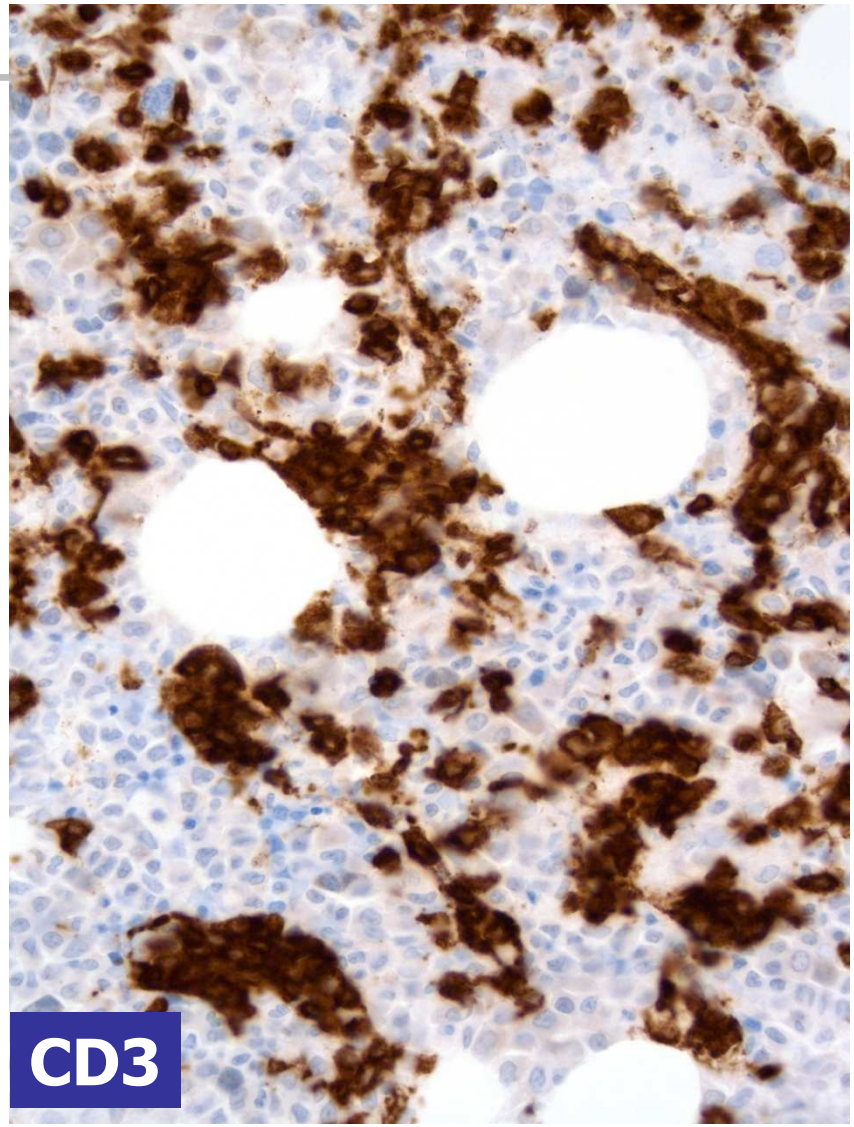
Case Cluster II-#case 1
Bone Marrow Biopsy



Case Cluster II-#case 1

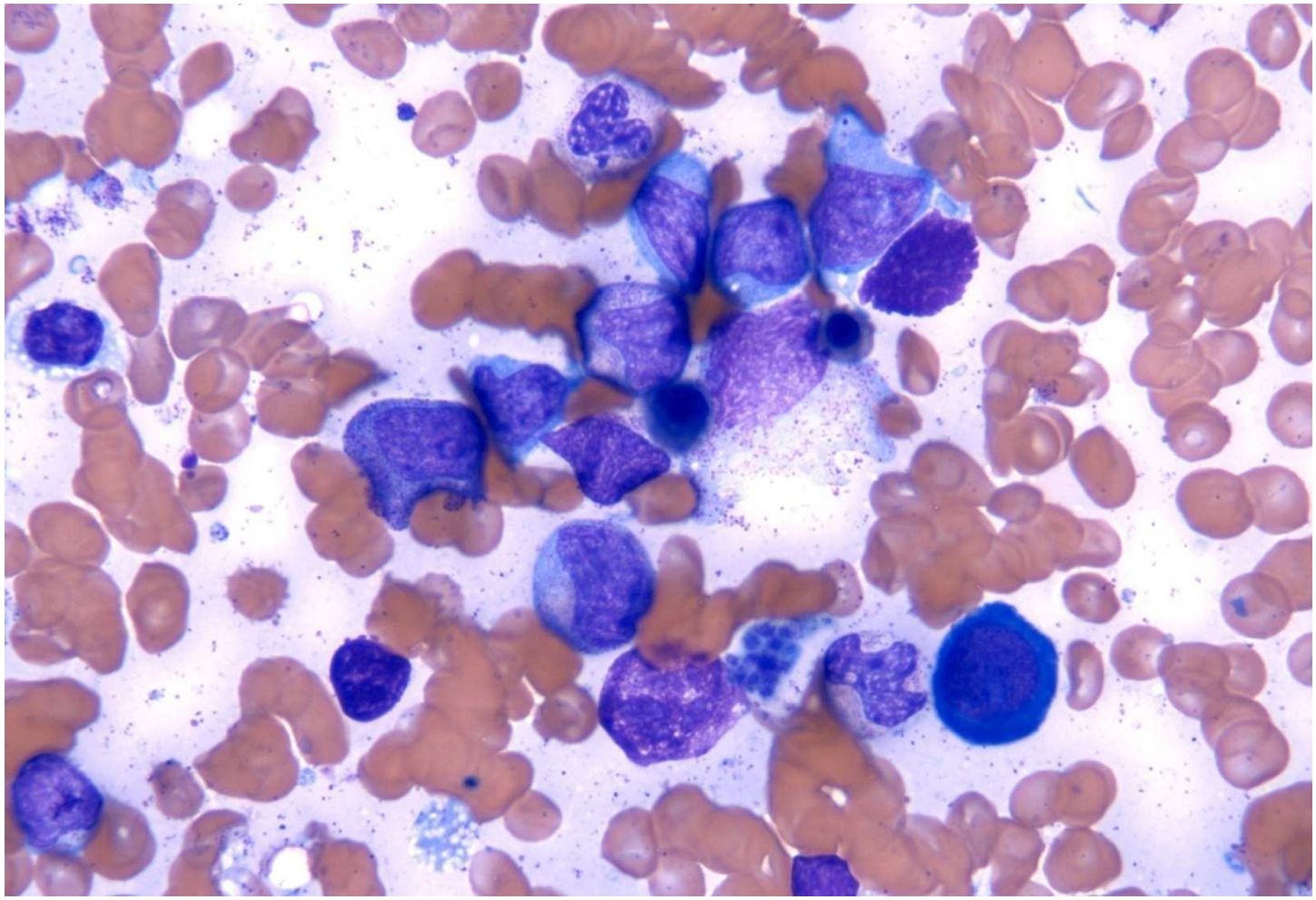
Bone Marrow Biopsy





Case Cluster II-#case 1

Bone Marrow Aspirate



Case Cluster II-#case 1
Bone Marrow Aspirate

Diagnosis

- Hepatosplenic gamma/delta T-cell lymphoma

Case Cluster II-case #2

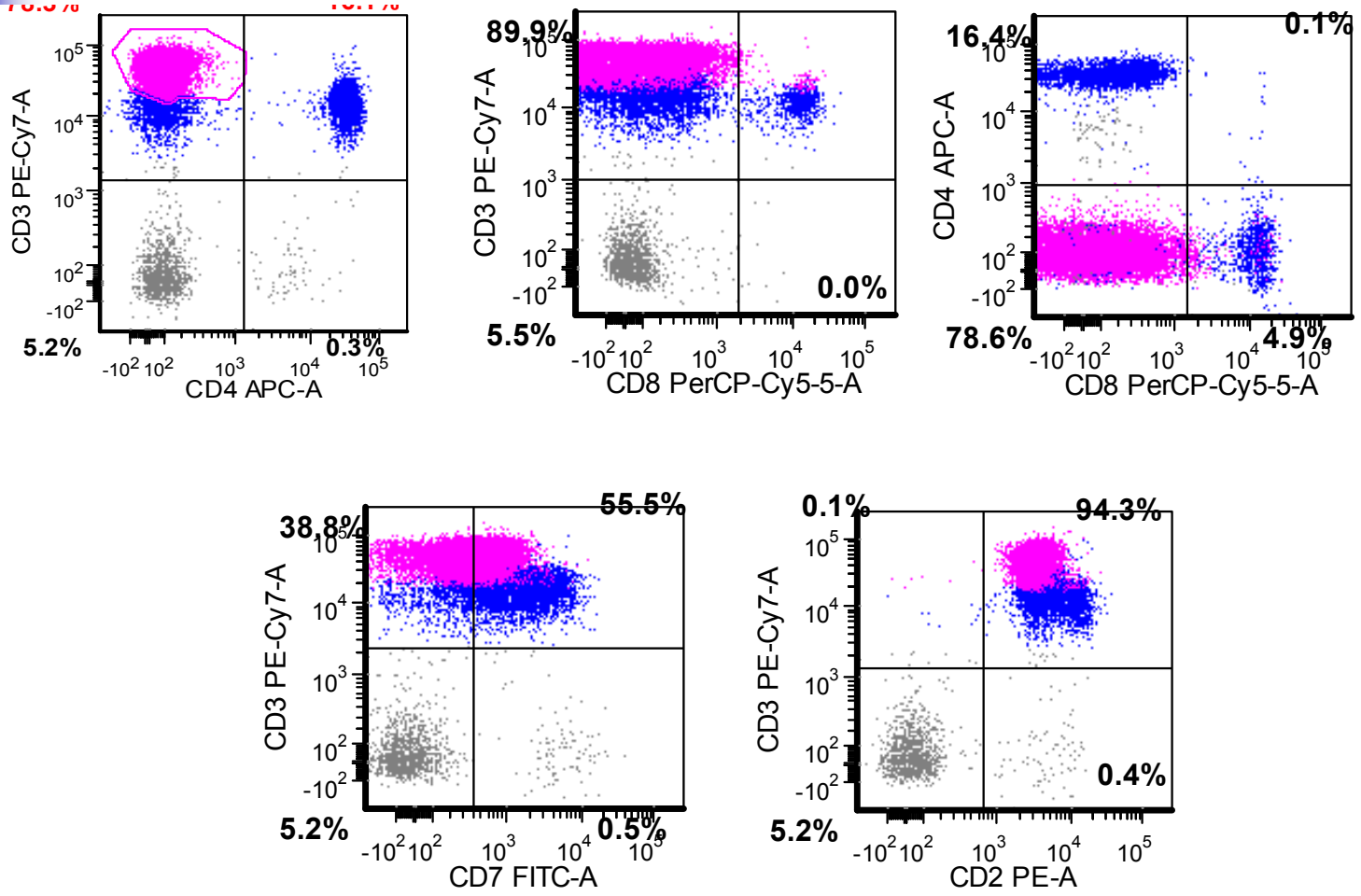
Analysis T cells and NK cells

60 year old woman, 10 years history of rheumatoid arthritis, treated with methotrexate, nonsteroidals, Enbrel, Femara, and Orencia etc

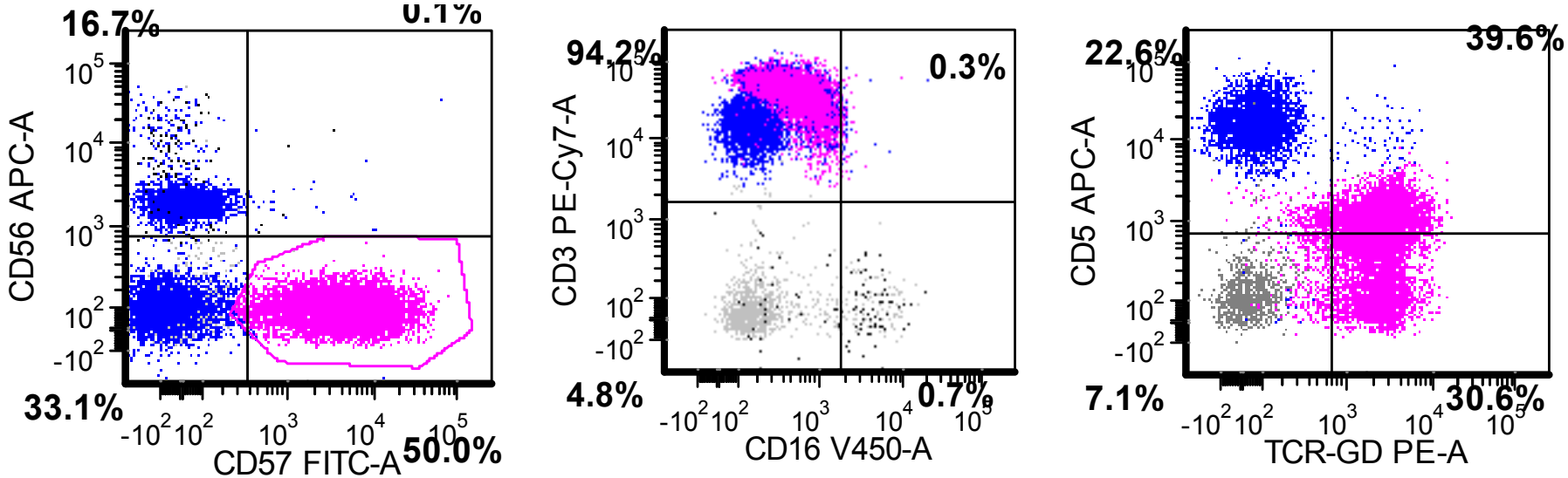
She has developed isolated leukopenia

- WBC 5.8 with 13% neutrophils and 73% lymphocytes

Case Cluster II-case #2
Analysis T cells and NK cells



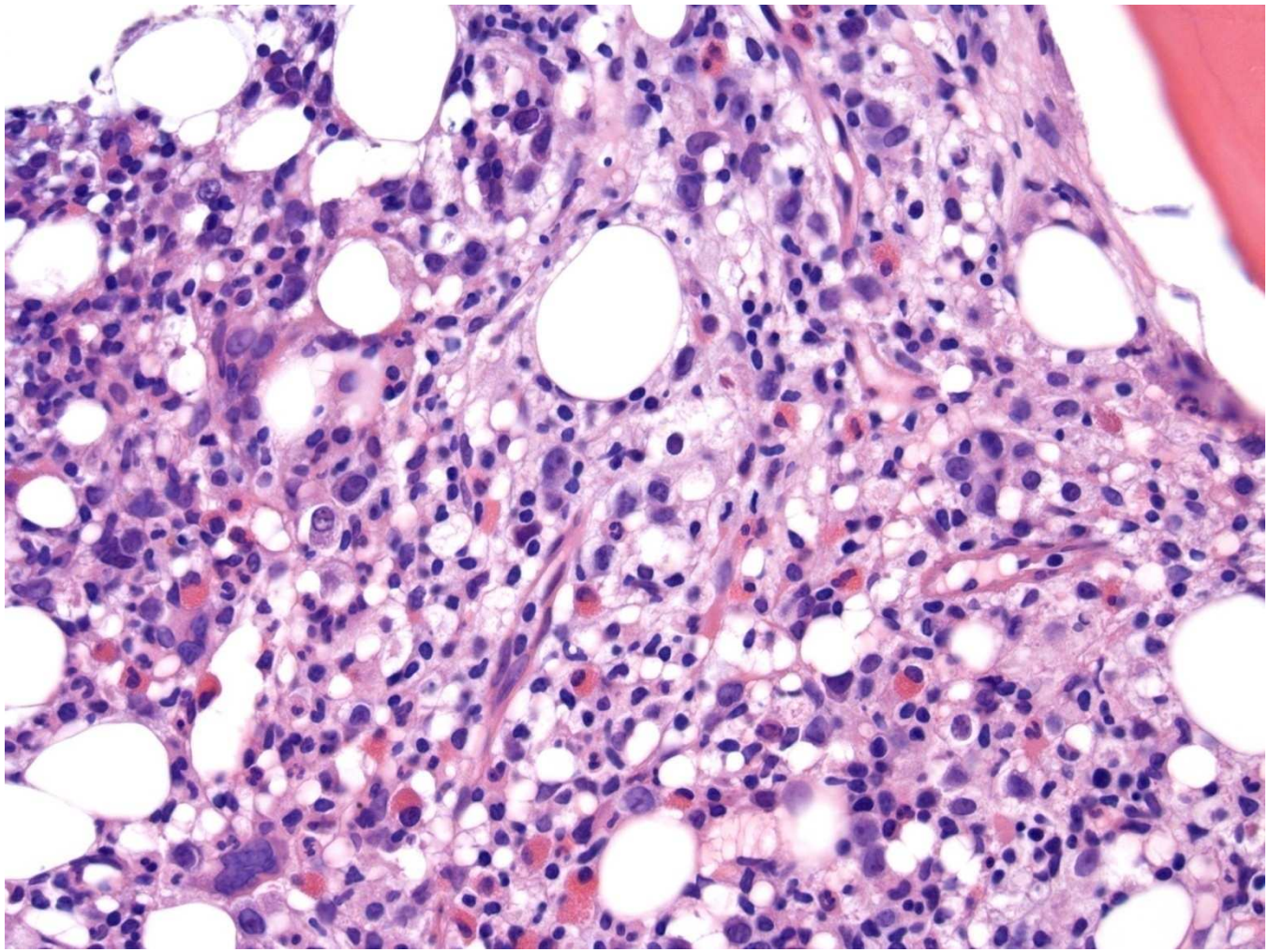
Case Cluster II-case #2
Analysis T cells and NK cells



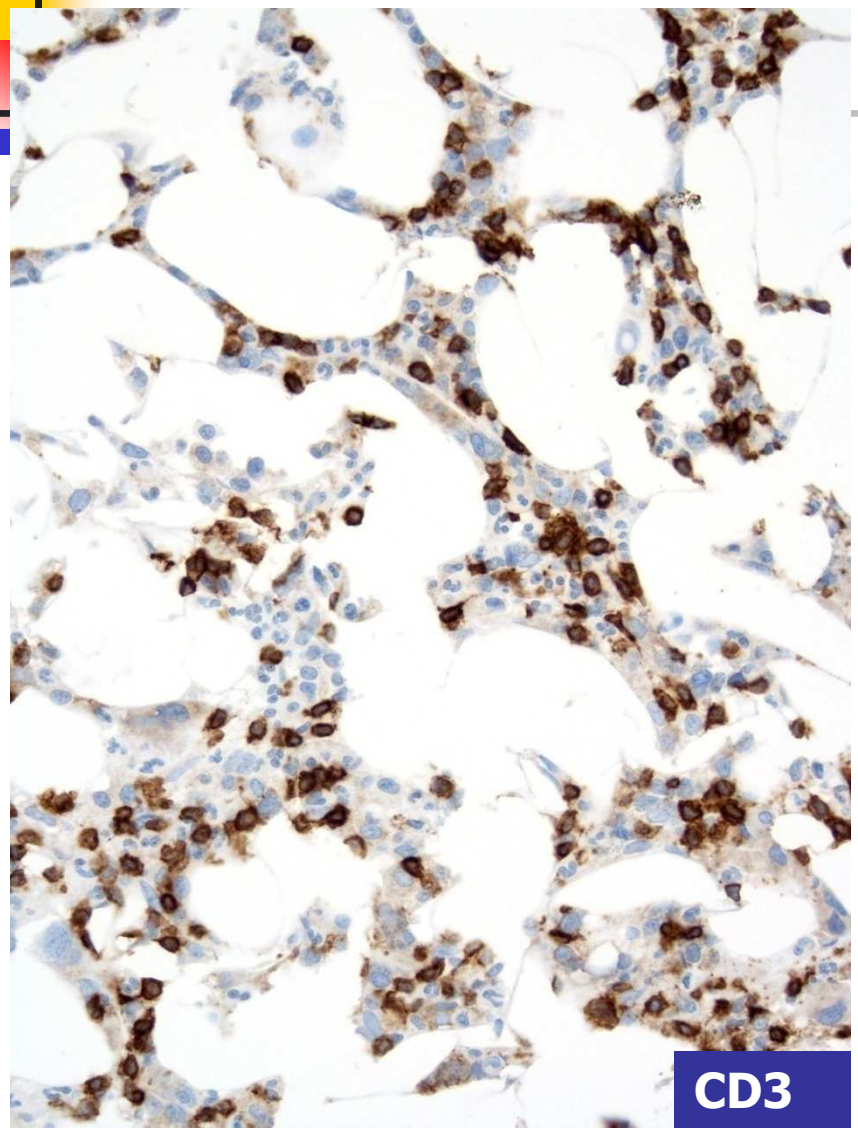
CD3+, CD2+, CD4-, CD8-, CD7dim+, CD16dim+, CD5dim/neg,

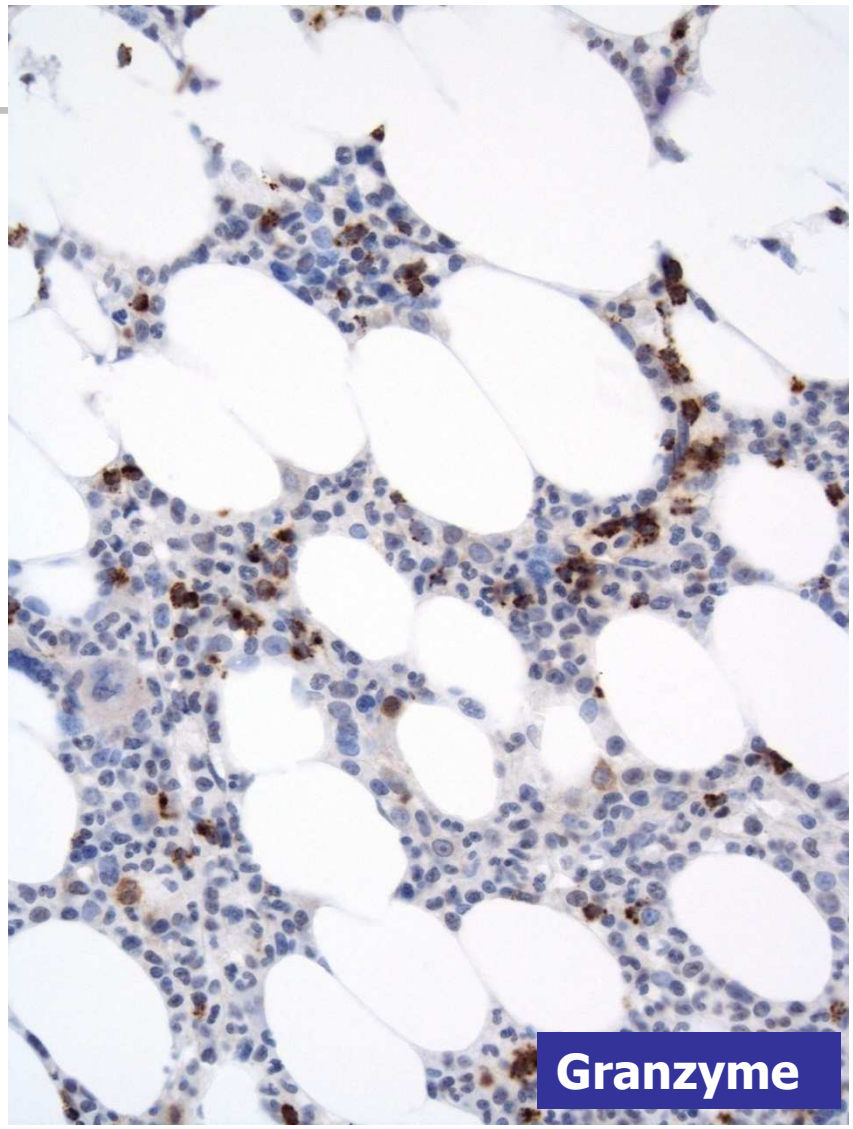
TCRgamm/delta+, TCRalpha/beta-, CD56-, CD57+

Case Cluster II-case #2
Analysis T cells and NK cells



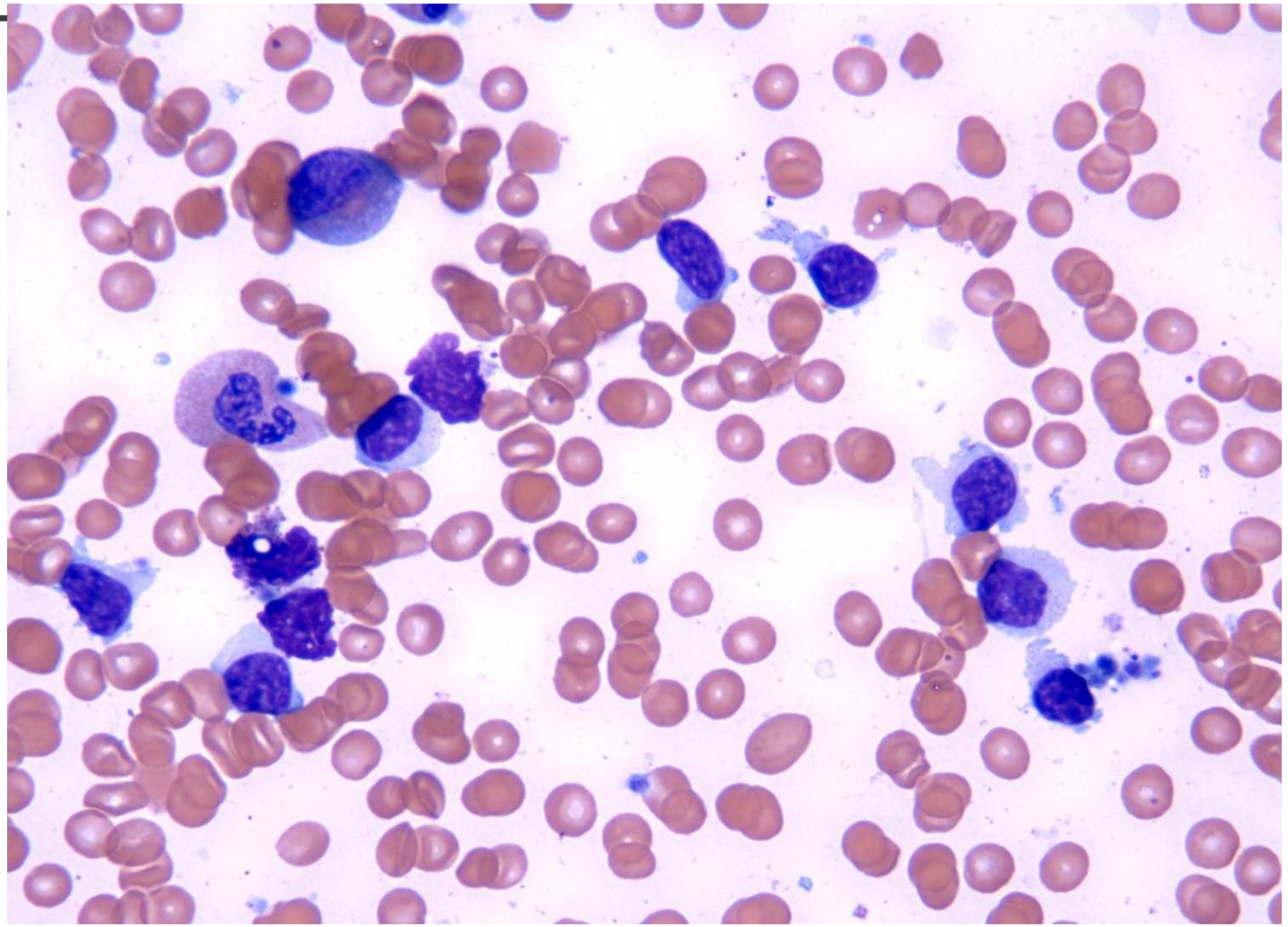
Case Cluster II-case #2
Immunohistochemistry





Case Cluster II-case #2

Bone Marrow Aspirate



Case Cluster II-case #2
Analysis T cells and NK cells

Diagnosis

- Large granular lymphocytic leukemia, gamma/delta variant

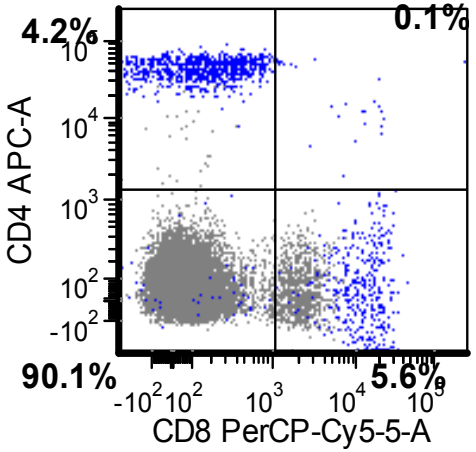
Case Cluster II-Case 3
Analysis T cells and NK cells

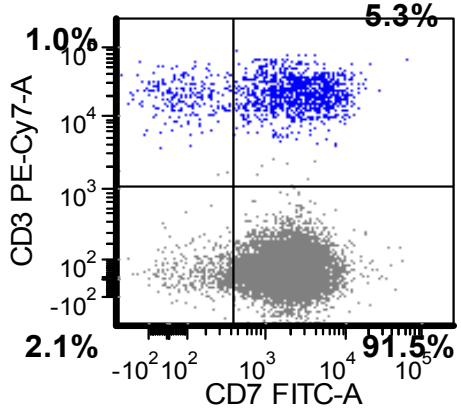
Clinical Presentation

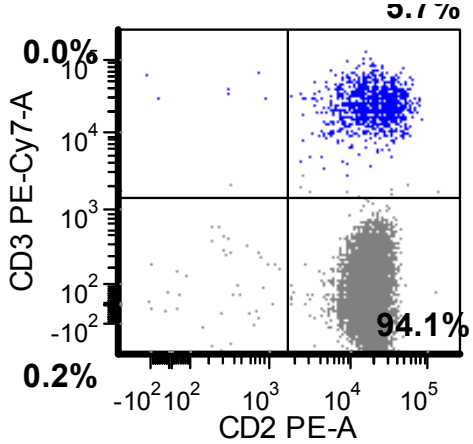
- 60 years old with a history of chronic lymphocytic leukemia (CLL), treated with rituximab and prednisone. He was found to be anemic.

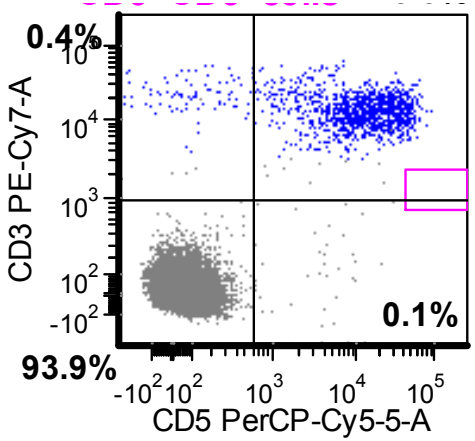
- He underwent a BM biopsy, which showed no morphological or immunophenotypic evidence of CLL

Case Cluster II-Case 3
Analysis T cells and NK cells

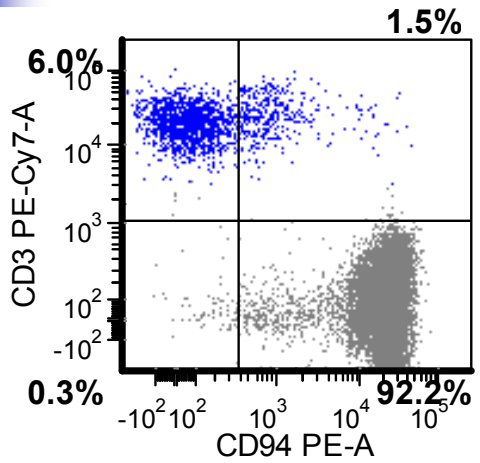


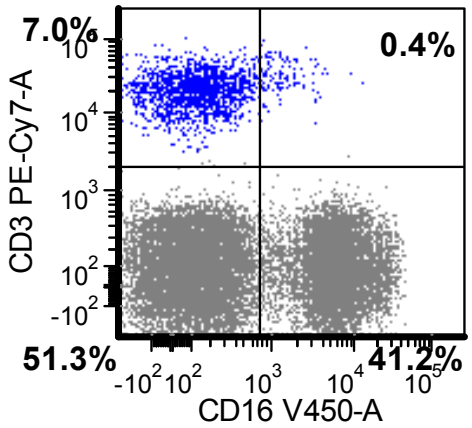


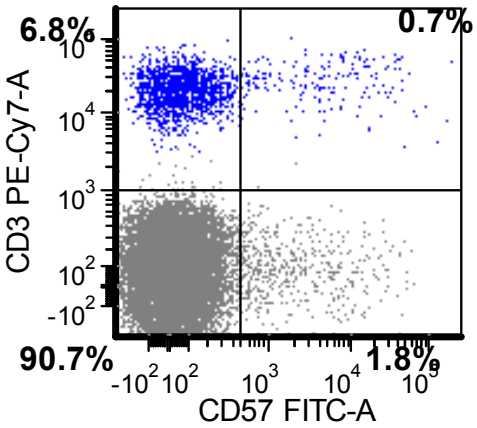


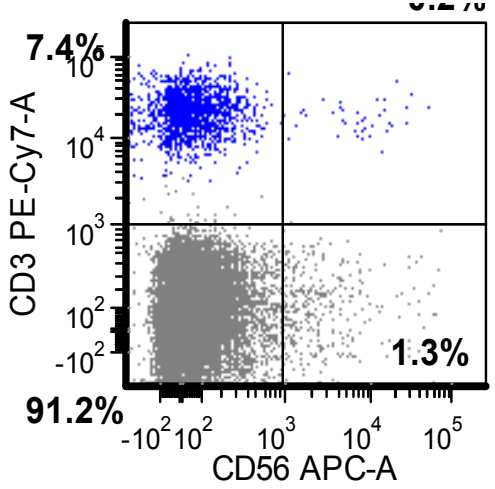


Case Cluster II-Case 3
Analysis T cells and NK cells





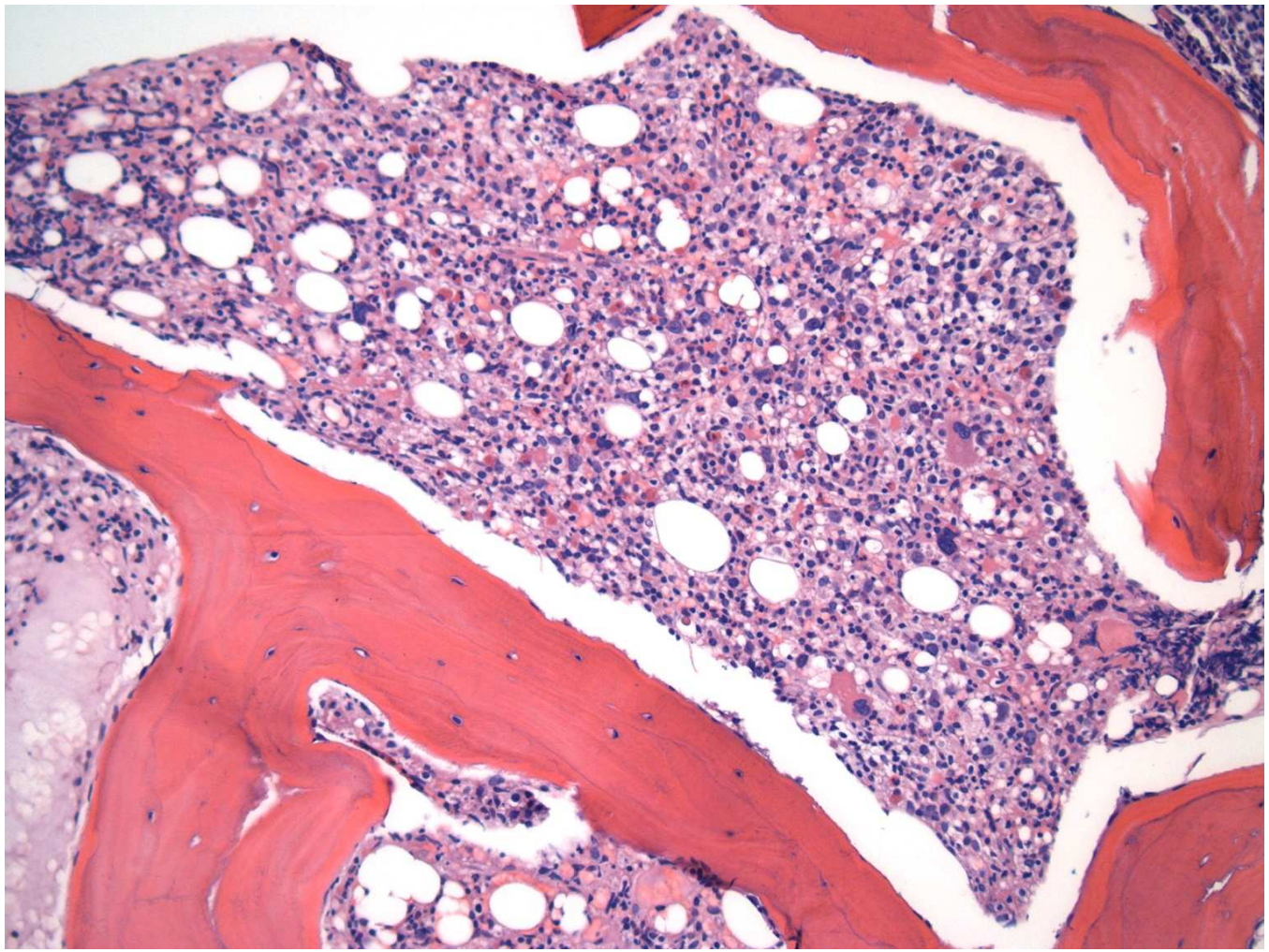




Summary:
sCD3-, CD2+, CD7+, CD5-, TCR-, CD94+, CD16subset+, CD56-, CD57-

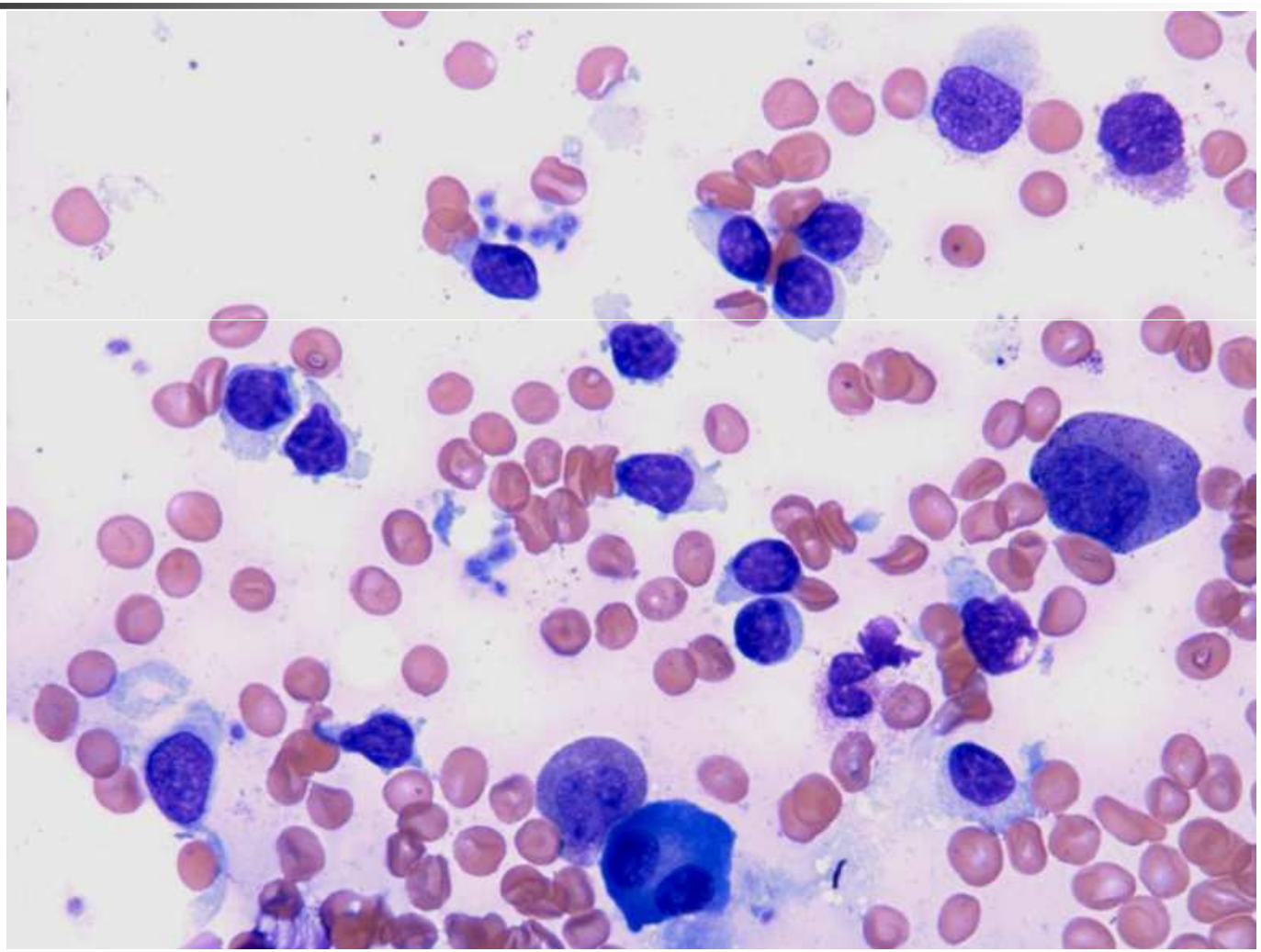
Case Cluster II-Case 3

Bone Marrow Biopsy



Case Cluster II-Case 3

Bone Marrow Aspirate



Case Cluster II-Case 3

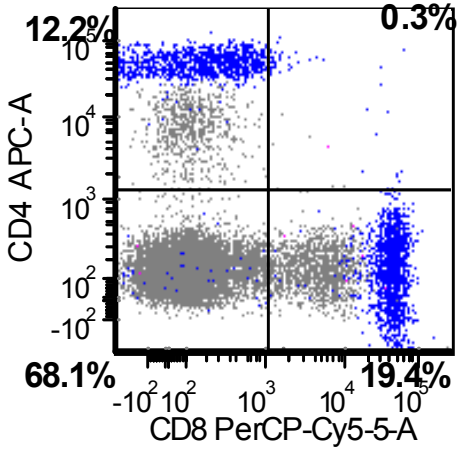
Diagnosis:

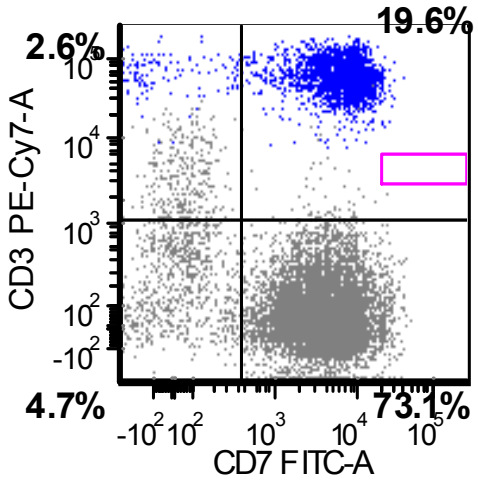
Chronic lymphoproliferative disorder of NK-cells (LGL leukemia, NK cell subtype)

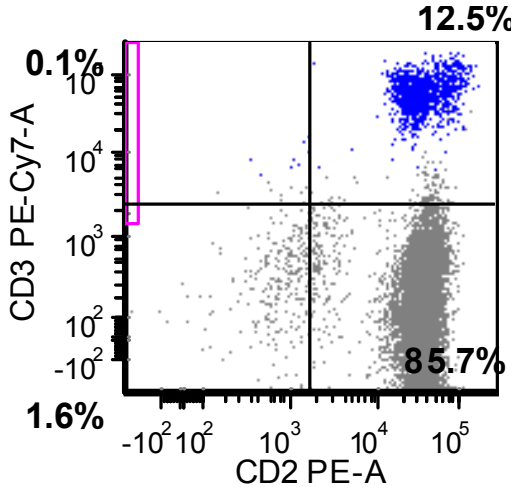
Case Cluster II-Case 4
Analysis of T cell and NK cells

- 28 year old male with focal segmental glomerulonephritis, treated with cyclosporin
- He was found to have abnormal liver function
- He has peripheral lymphocytosis
  - WBC 12K with 51% lymphocytes

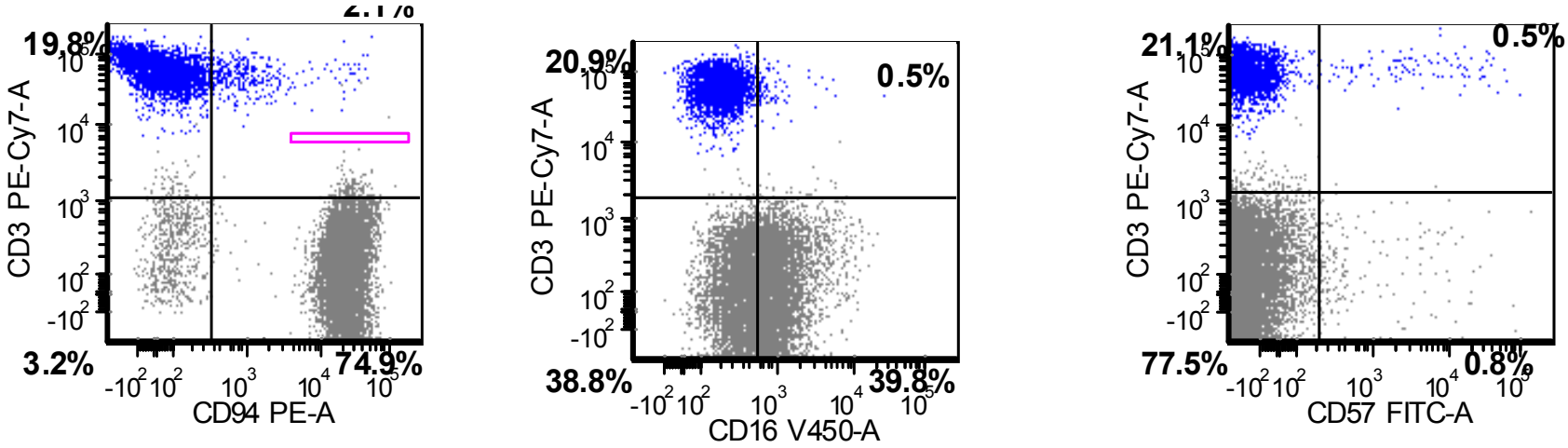
Case Cluster II-Case 4
Analysis of T cell and NK cells





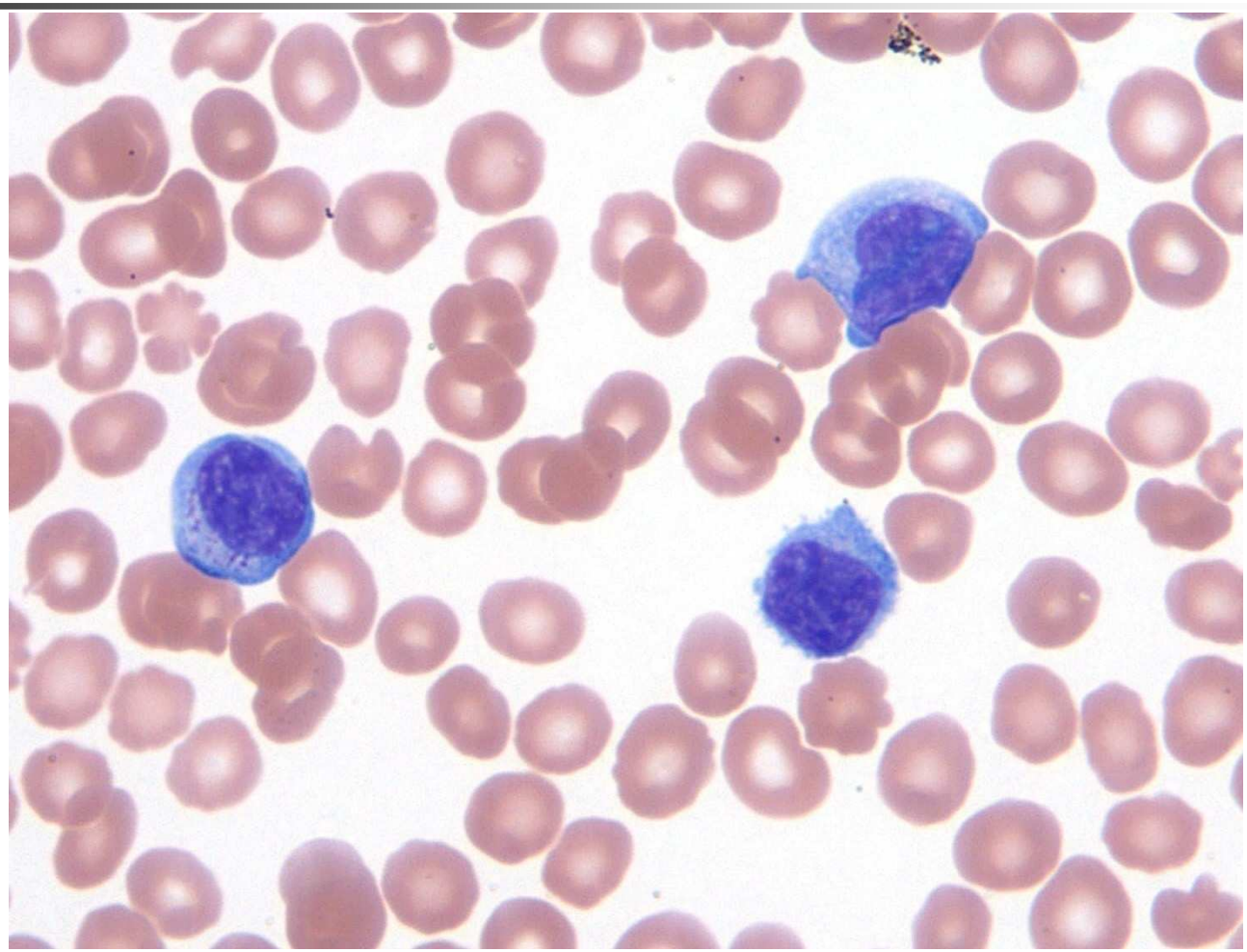


Case Cluster II-Case 4
Analysis of T cell and NK cells

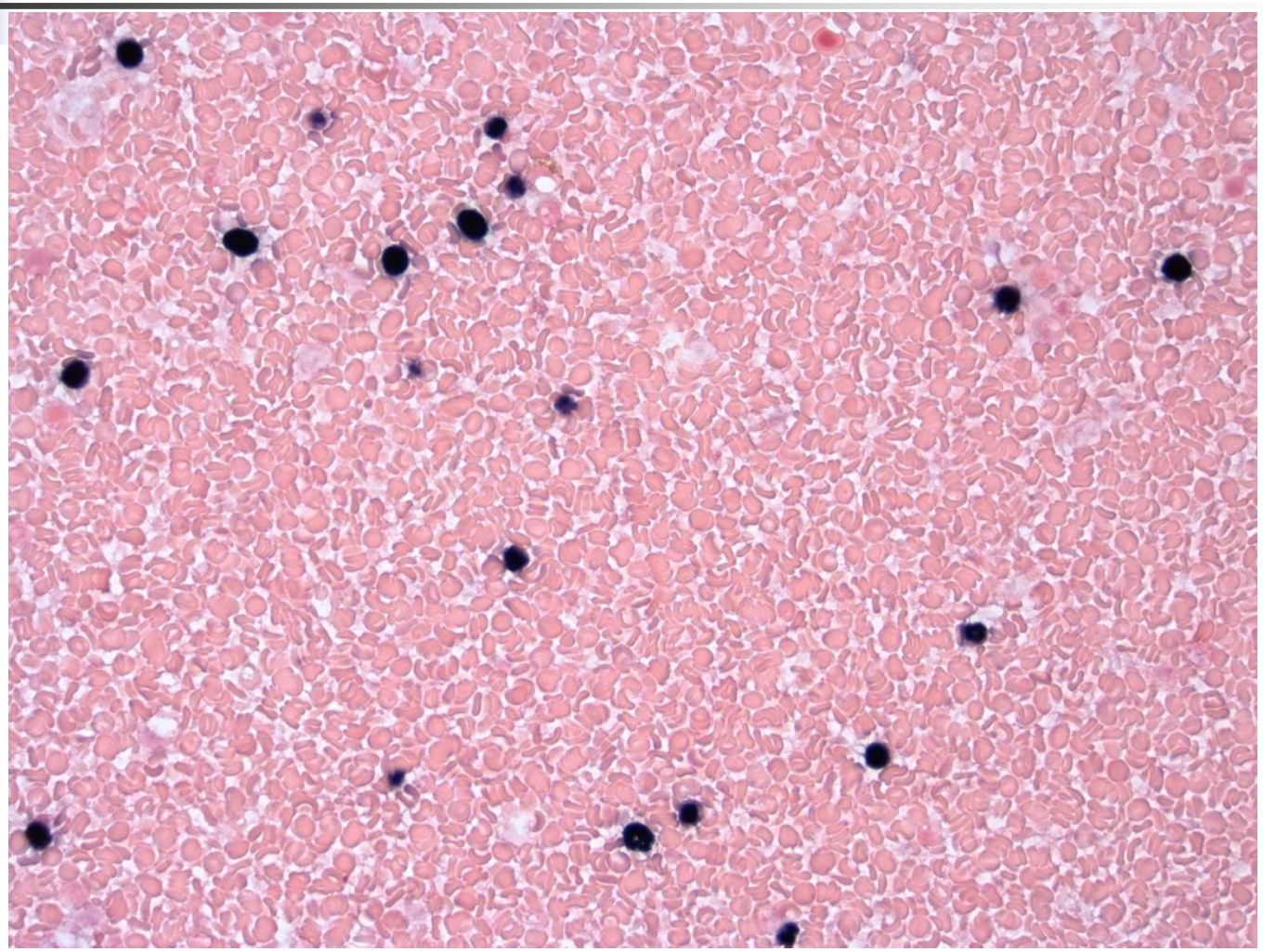


Summary:
sCD3-, CD2+, CD7+, CD5-, TCR-, CD94+, CD16+, CD56+, CD57-

Case Cluster II-Case 4
Peripheral Blood



Case Cluster II-Case 4
Peripheral Blood



Case Cluster II-Case #4
Analysis of T cells and NK cells

Diagnosis

- Aggressive NK-cell leukemia

Summary of LGL leukemia

Typically, T-cell, TCR alpha/beta

CD3+, CD2+, CD5+, CD7+,
CD8+, CD57+, CD16+, CD94dim+, CD56-

Variants:

CD56+ variant

- TCRalpha/beta+, CD3+, CD4-, CD8-, CD56+, CD57-, CD94+, CD16+
- Younger patients, aggressive variant
- Are some hepatosplenic T cell lymphoma?

CD56+ Aggressive Variant LGL

TABLE I. Features of Reported Cases of Aggressive T-cell LGL Leukemia

Patient	Absolute WBC (1×10^9/l)	Immunophenotype	Karyotype
1	14.8	$CD3^+CD8^+CD56^+CD57^-$	46XX
2	29.9	$CD3^+CD8^+CD56^+CD57^-$	46XY
3	19.0	$CD3^+CD8^+CD56^+CD57$	NS
4	17.9	$CD3^+CD8^+CD56^+CD57^-$	46XY
5	2.0	$CD3^+CD8^+CD56^+CD57^-$	46XY,i(7),t(8;14), + 13
6	18.2	$CD3^-CD8^+CD56^+CD57^-$	46XX,t(2;17), + 14
7	6.0	$CD3^+CD8^+CD56^+CD57^-$	46XY,i(7), + 12
8	3.0	$CD3^+CD8^+CD56^+CD57^-$	NS
9	2.6	$CD3^+CD8^+CD56^+CD57^-$	NS
10	55.0	$CD3^+CD4^-CD8^-CD56^+CD57^-$	i(7q)
11	9.0	$CD3^+CD8^+CD56^-CD57^-$	NS
12	106.0	$sCD3^-cCD3^+CD8^+CD56^-CD57^-$[a] $sCD3^-cCD3^+CD4^+CD8^+CD56^-CD57^-$[b]	NS
13	53.3	$CD3^+CD8^+CD56^+CD57^-$	46XY

Alekshun, T.J. et al. (2007) American Journal of Hematology, 82, 481–485.

Summary of LGL leukemia

- **CD4+ variant**
  - CD4+, CD8dimer/neg, CD56+, CD57+
  - often associated with other malignancies
  - do not show cytopenias and autoimmune phenomena
    - Lima M et al. Am J Pathol. 2003 Aug;163(2):763-71.

- **TCRgamma/delta+ variant**
  - CD3+, CD4-, CD8-/+, CD57+, CD56 variablely+, CD16variable+, CD94+
  - Indolent

    - Sandberg Y, te al. Leukemia 2006, 20: 505-513.

Summary of LGL Leukemia

- Chronic lymphoproliferative disorder of NK-cells (LGL leukemia, NK cell subtype)

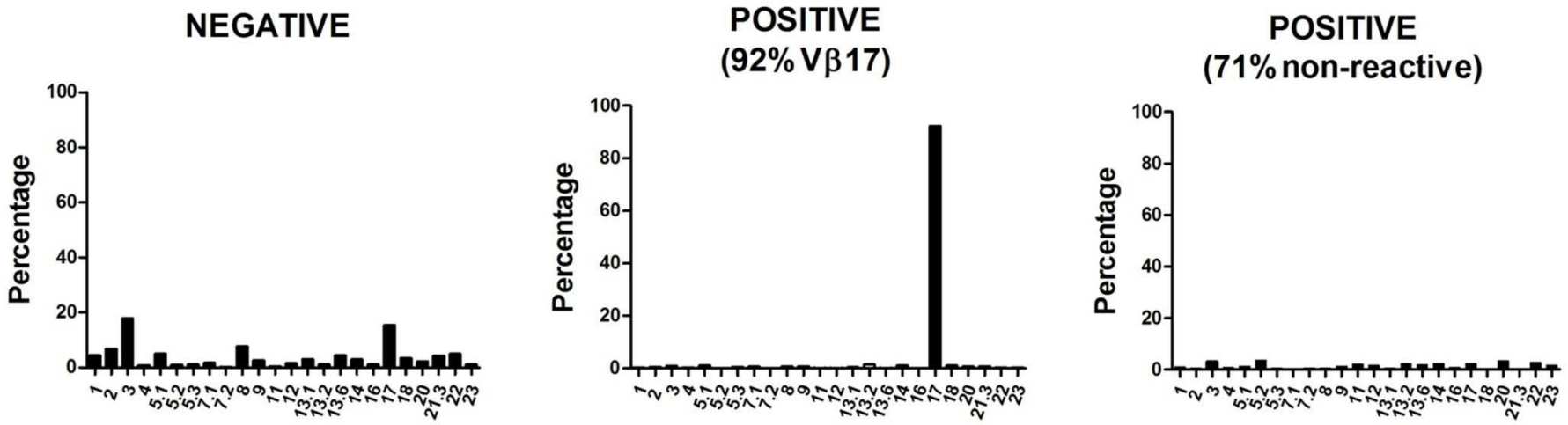
Large Granular Lymphocytic (LGL) Proliferations

Table I. Features of T- and NK- cell large granular lymphocyte cell leukaemias.

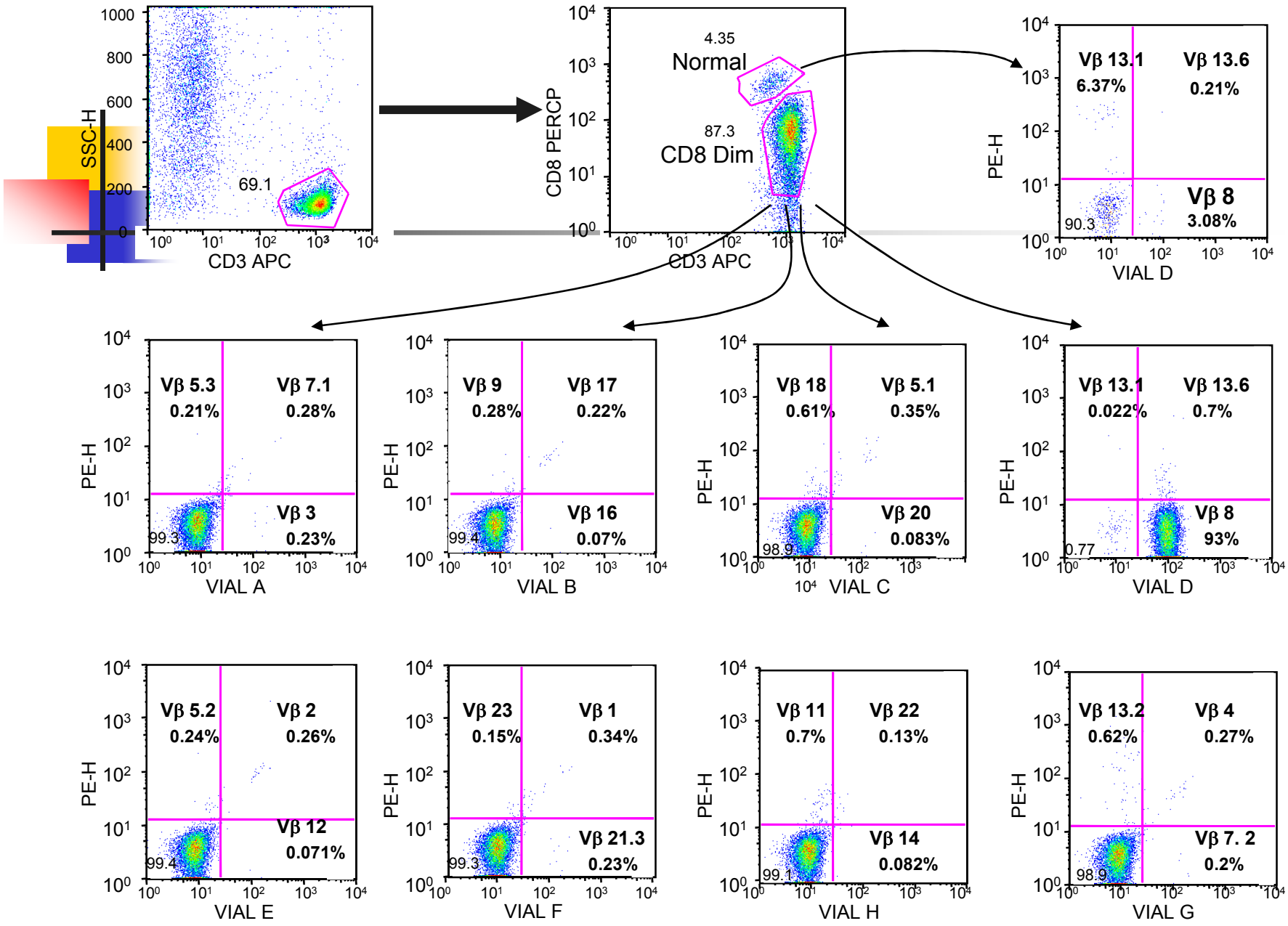
	Clinical features	Associated diseases	Phenotype	TCR	Treatment	OS
Indolent						
Reactive lymphocytosis (T or NK)	Transient benign	Viral infection Splenectomy	$CD3^+$ $CD8^+$, $CD57^+$ or $CD3^-$ $CD16^+$ $CD56^+$	Polyclonal	None	
T-LGL leukaemia	T-LGLs 2–20 × 10^9/l > 6 months Often Asymptomatic Cytopenias, infection, splenomegaly, liver, BM	RA other AID	$CD3^+$ $CD8^+$ CD 16^+ $CD57^+$ KIR (50%) TIA1, Granzymes	Clonal	Observation MTX, CSA, PAs, MoAbs	>10 years
CLPD-NK	NK cells > 2 × 10^9/l for >6 months Often Asymptomatic Cytopenias BM	Malignancy, AID	$CD3^-$, $CD3\varepsilon^+$, $CD16^+$ $CD56w^+$, KIR^+ $CD94/NKG2A^+$ $CD161^-$ TIA1, Granzymes	Germ-line	Observation MTX, CSA, PA, MoAbs	Good
Aggressive						
T-LGL variant	Progressive cytopenias and systemic features		$CD3^+$, $CD56^+$	Clonal	ALL-like SCT	Poor
NK-cell leukaemia	Fulminant Blood, BM, Liver, spleen Haemo-phagocytic syndrome	EBV	$CD3^-$ $CD16^+$ $CD56^+$	Germ-line (EBV clonal integration)	ALL-like SCT	2 months

Dearden C et al. Br J Haematol. 2011 Feb;152(3):273-83

T-cell Clonality Assessment



Feng B et al. Mod Pathol. 2010 Feb;23(2):284-95.

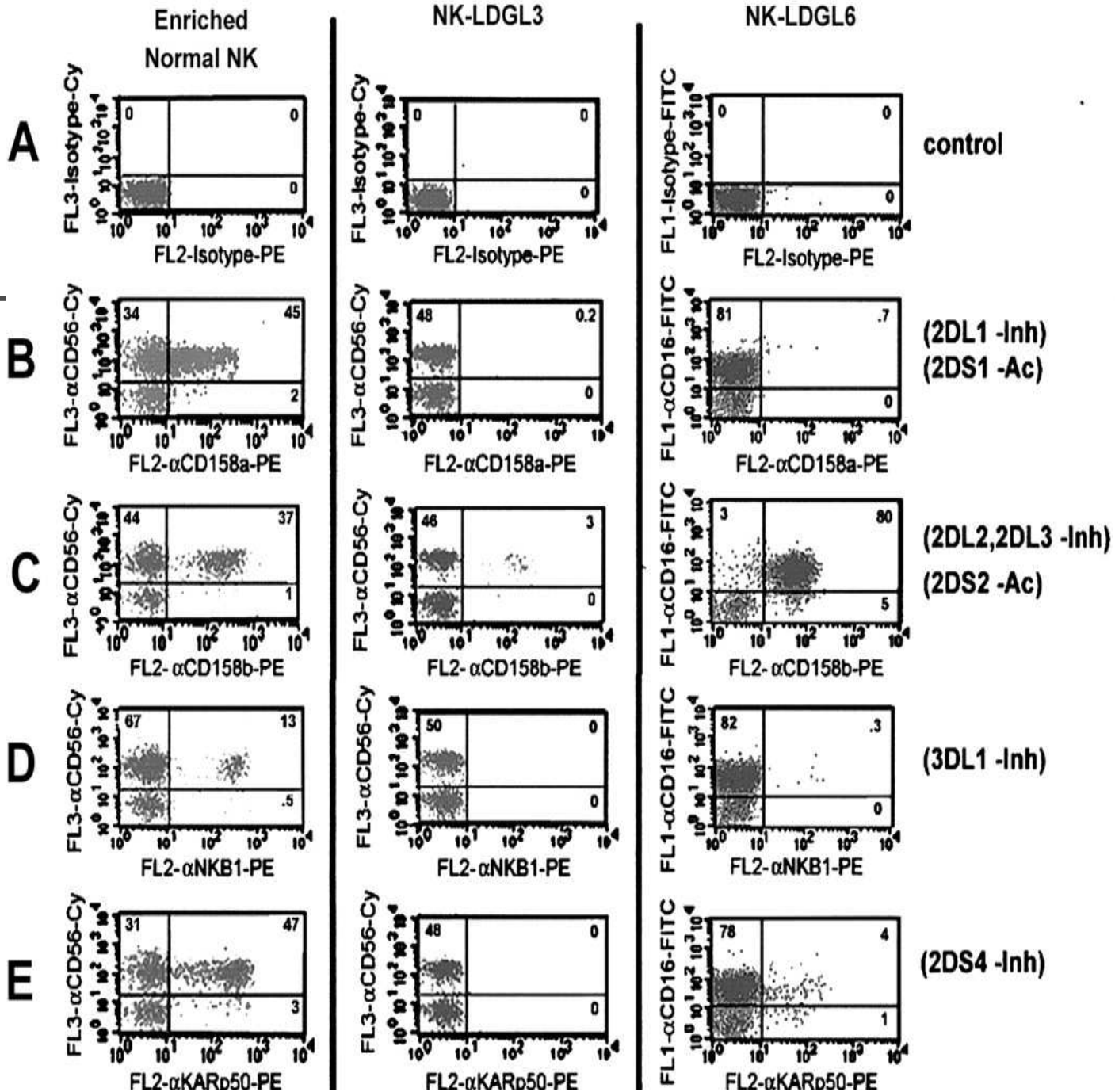


Feng B et al, J Clin Pathol. 2010 Feb;63(2):141-6.

NK-cell Clonality Assessment

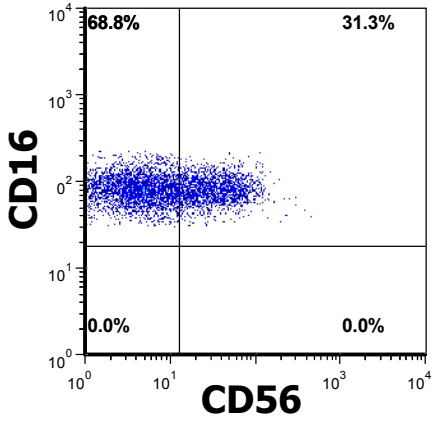
- KIR-killer cell immunoglobulin-like receptor
- Recognition of self-HLA alleles, thereby limiting the lysis of autologous cells
- NK cells from healthy individuals express between 2 and 8 KIRs per cell
- CD158a (KIR2DL1, KIR2DS1), CD158b (KIR2DL2, KIR2DL3, KIR2DS2), CD158e (KIR3DL1)

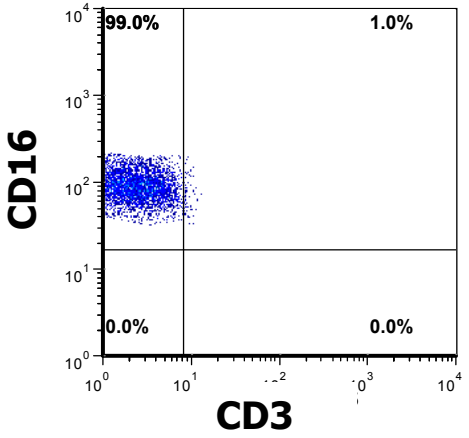
blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

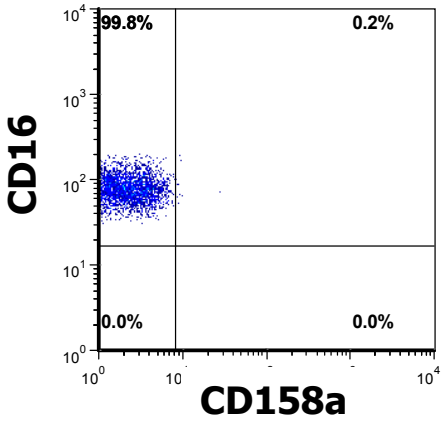


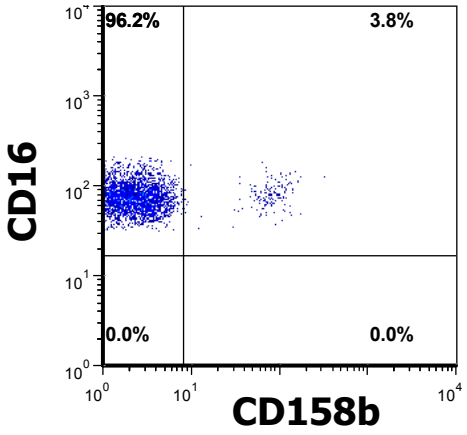
Epling-Burnette P K et al. Blood 2004;103:3431-3439

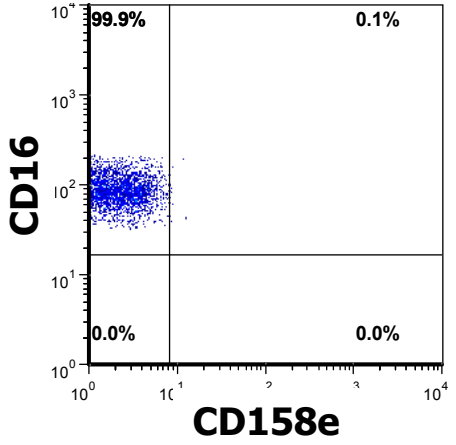
NK-cell Clonality Assessment











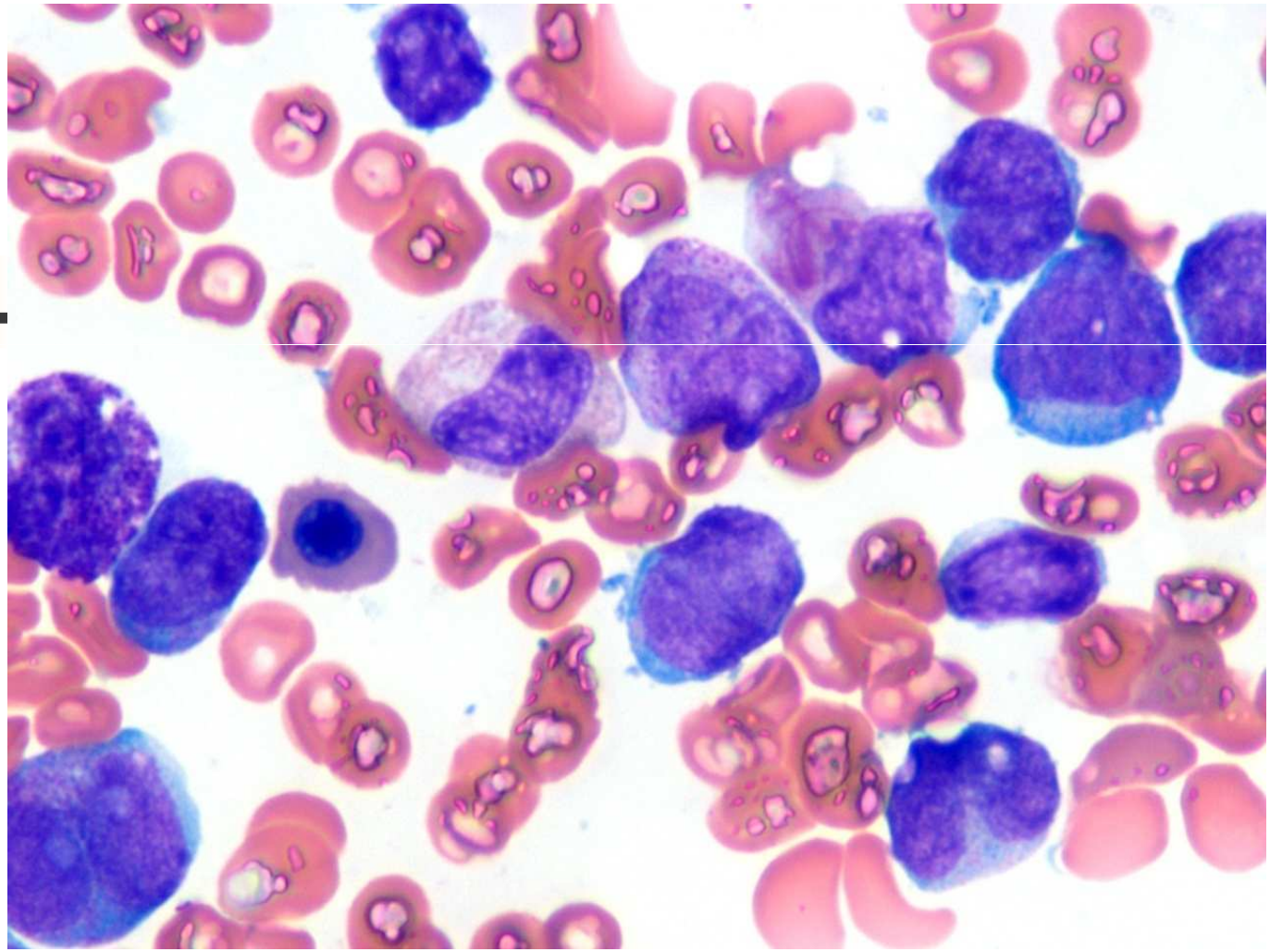
Case cluster III
AML-APL or Not

Case #1

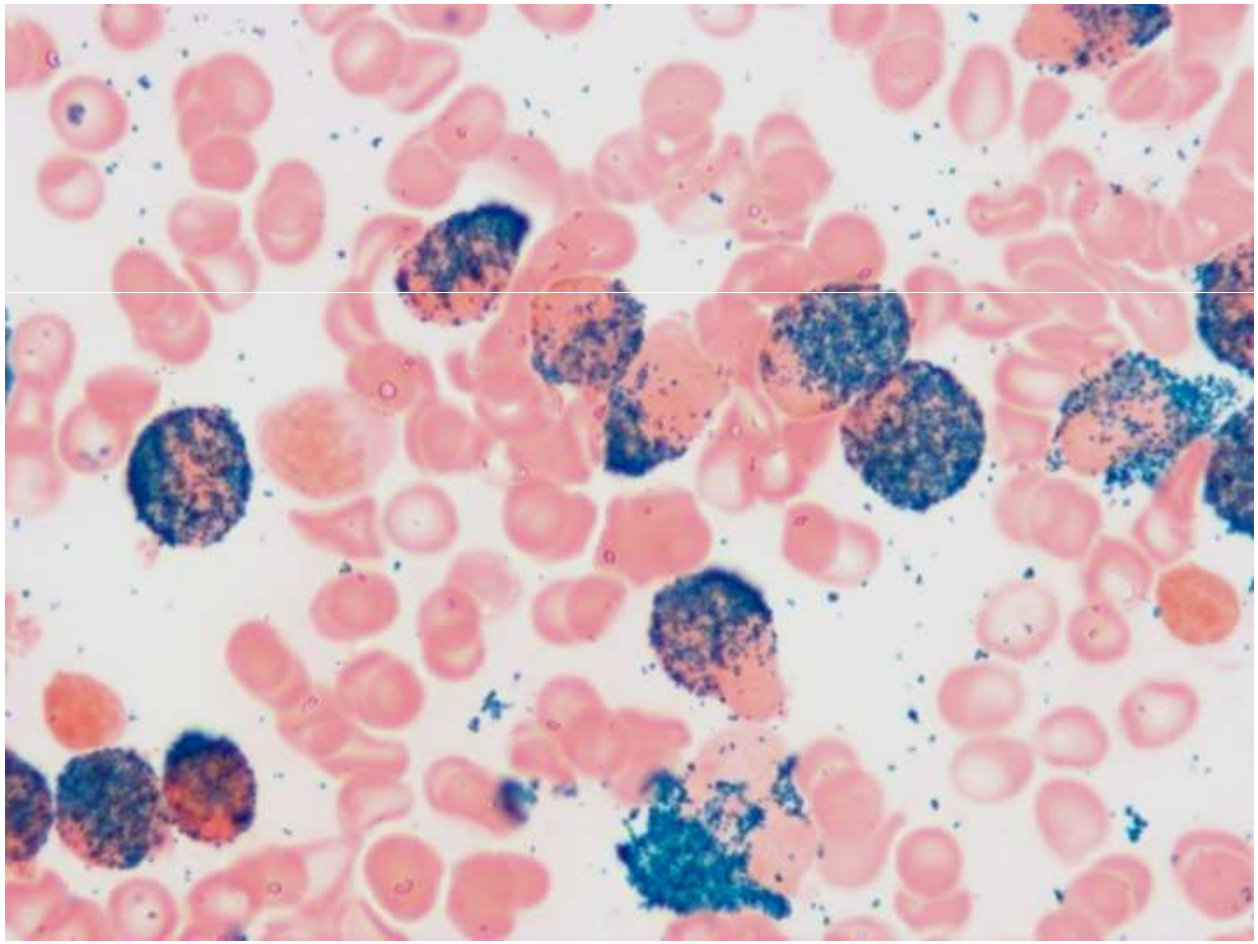
- 56 year old woman with a remote history of breast cancer, complained "not feeling well", and found to have pancytopenia

- BM biopsy performed

Case Cluster II-case #2
Bone Marrow Aspirate

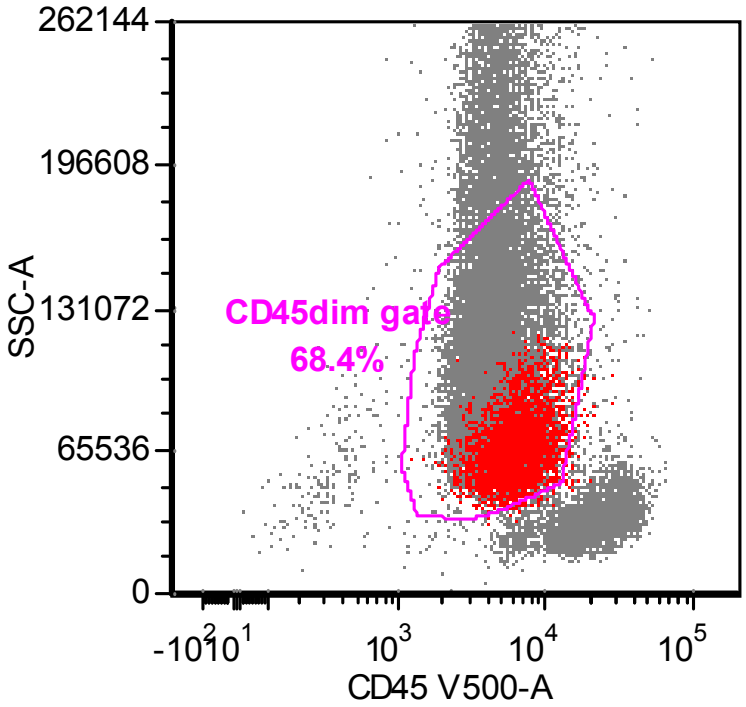


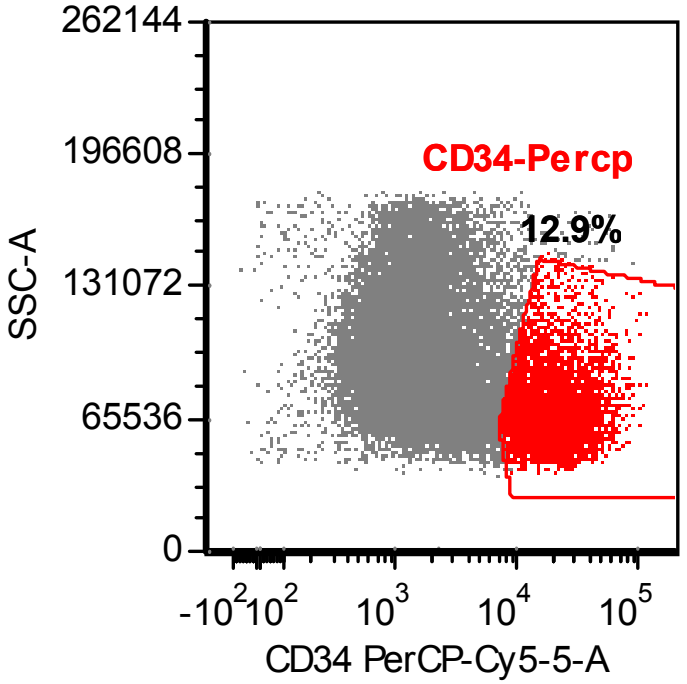
Case Cluster II-case #2
Bone Marrow Aspirate MPO



Case Cluster II-case #2

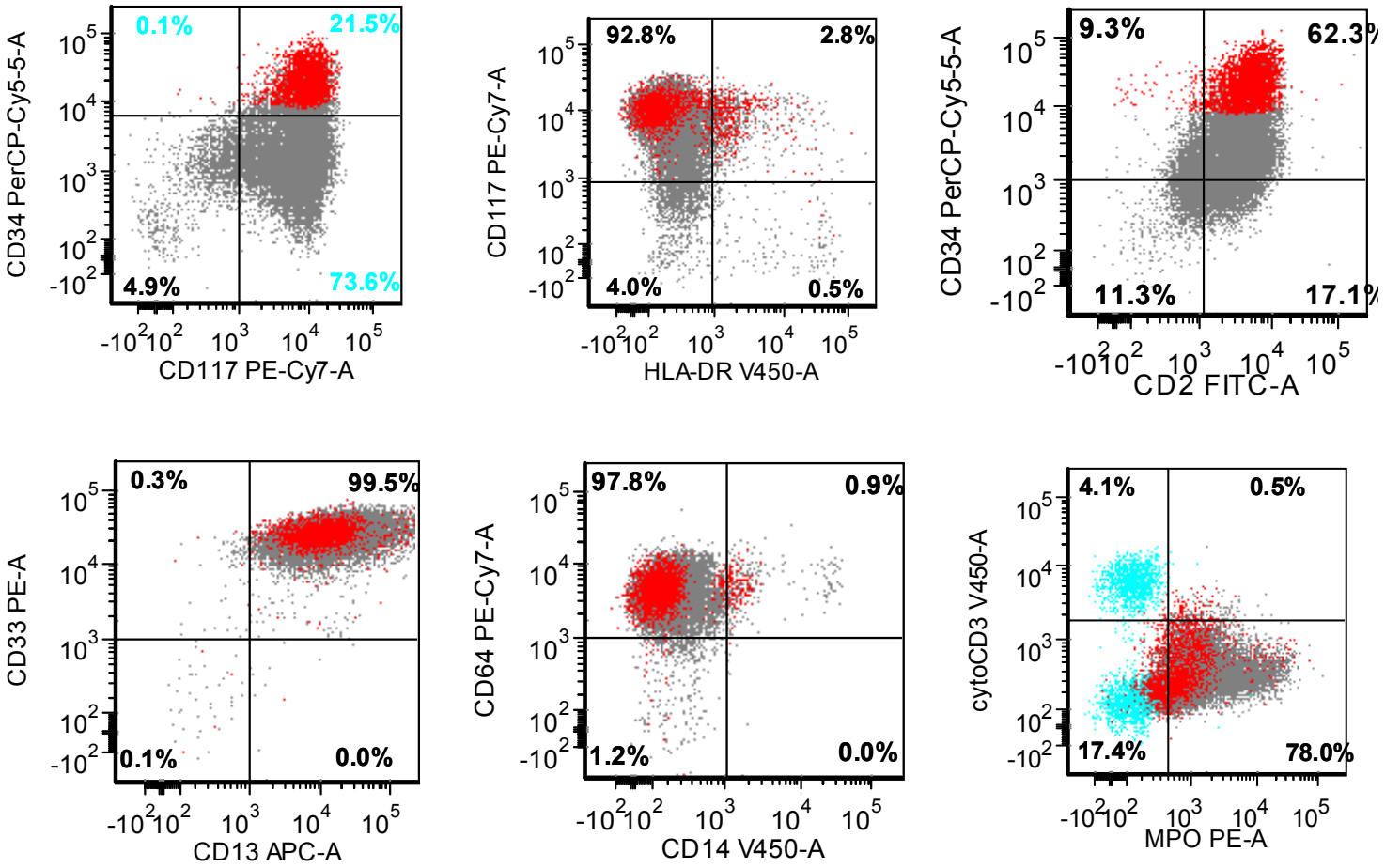
Flow Cytometry Analysis





Case Cluster II-case #2

Flow Cytometry Analysis



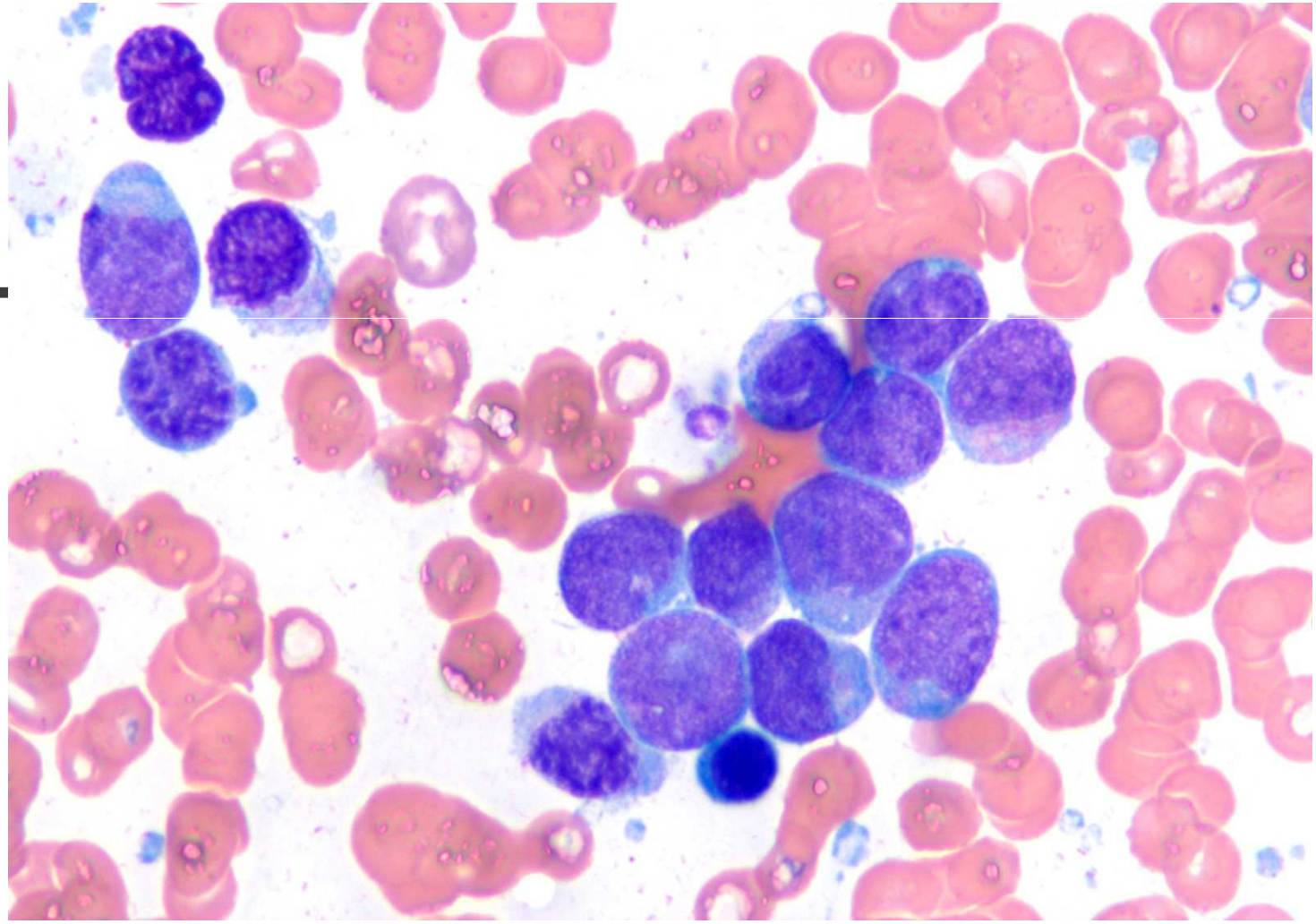
Case clusters III-Case 2# APL or not?

Case #2

- 49 year old with fever, chill, headache and found to have leukocytosis

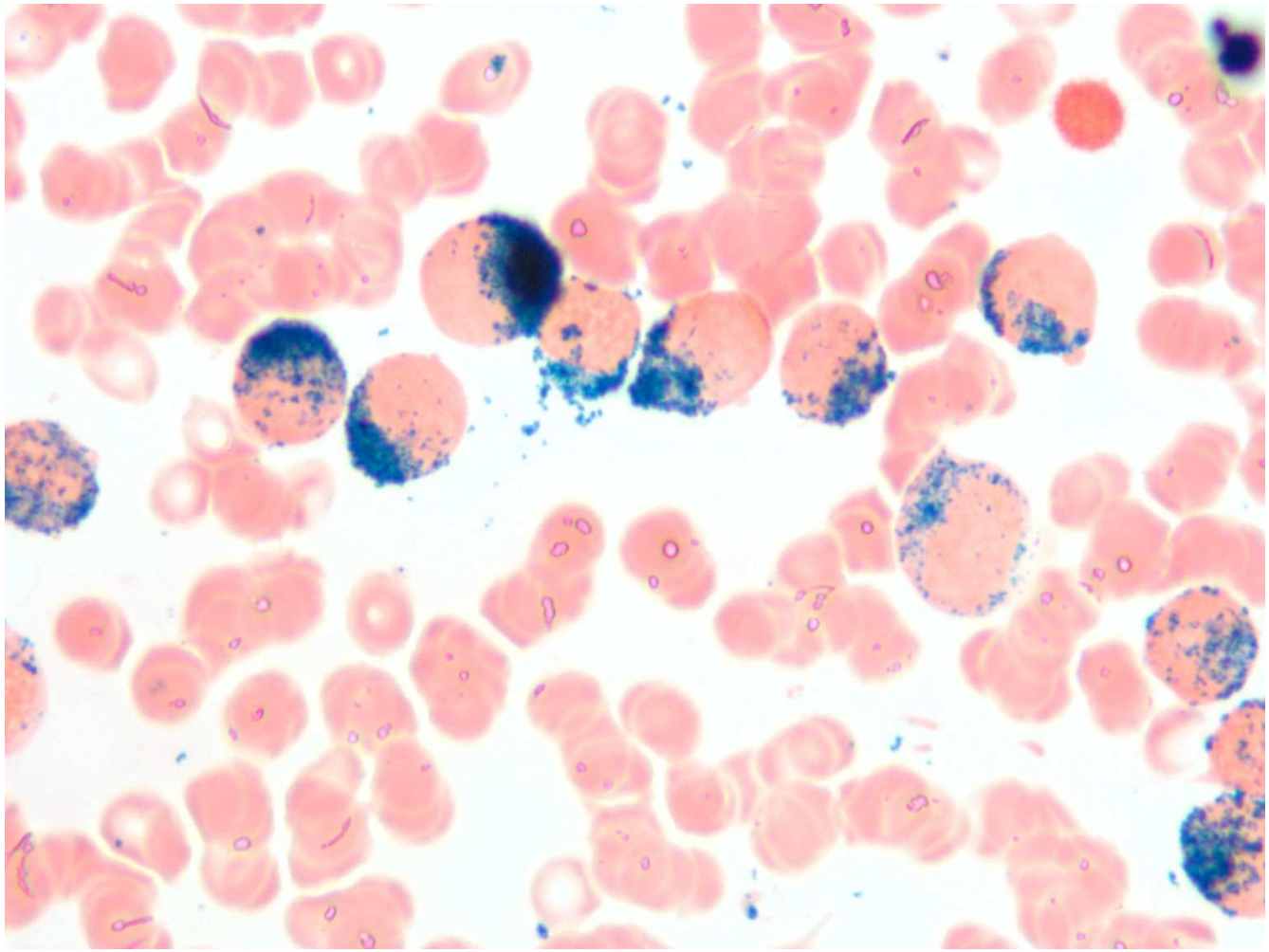
Case Cluster II-case #2

Bone Marrow Aspirate



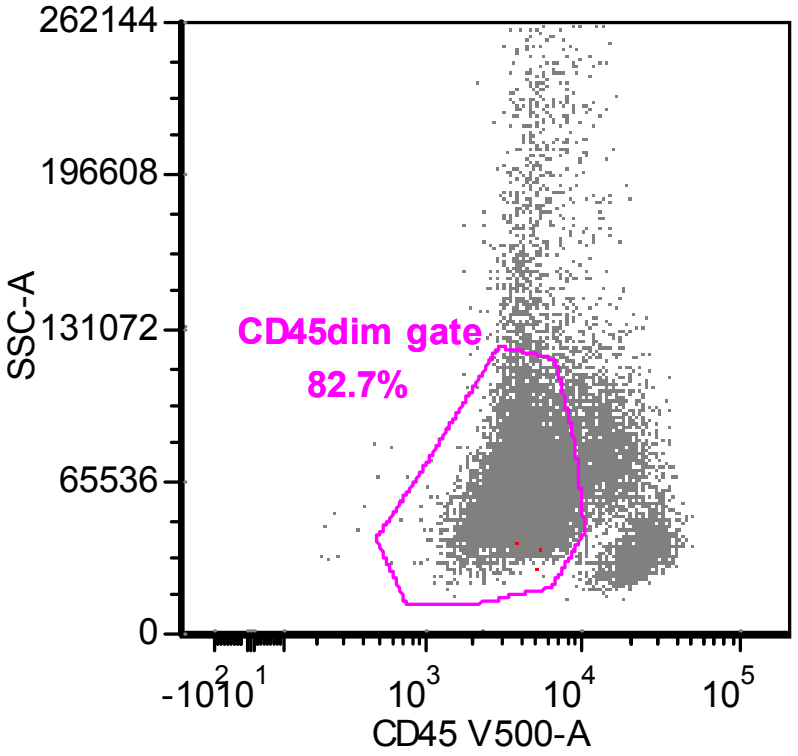
Case Cluster III-case #2

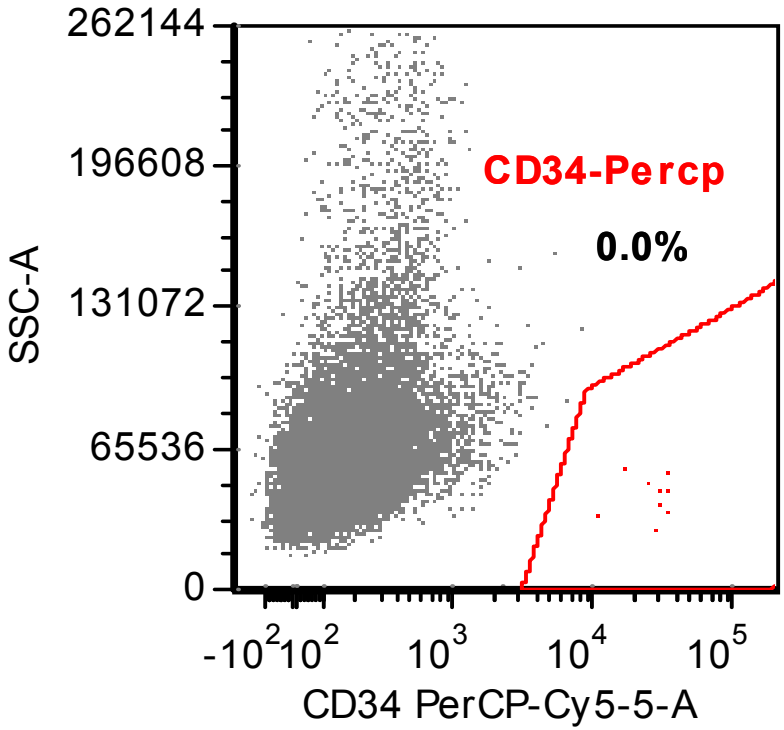
Bone Marrow Aspirate MPO



Case Cluster III-case #2

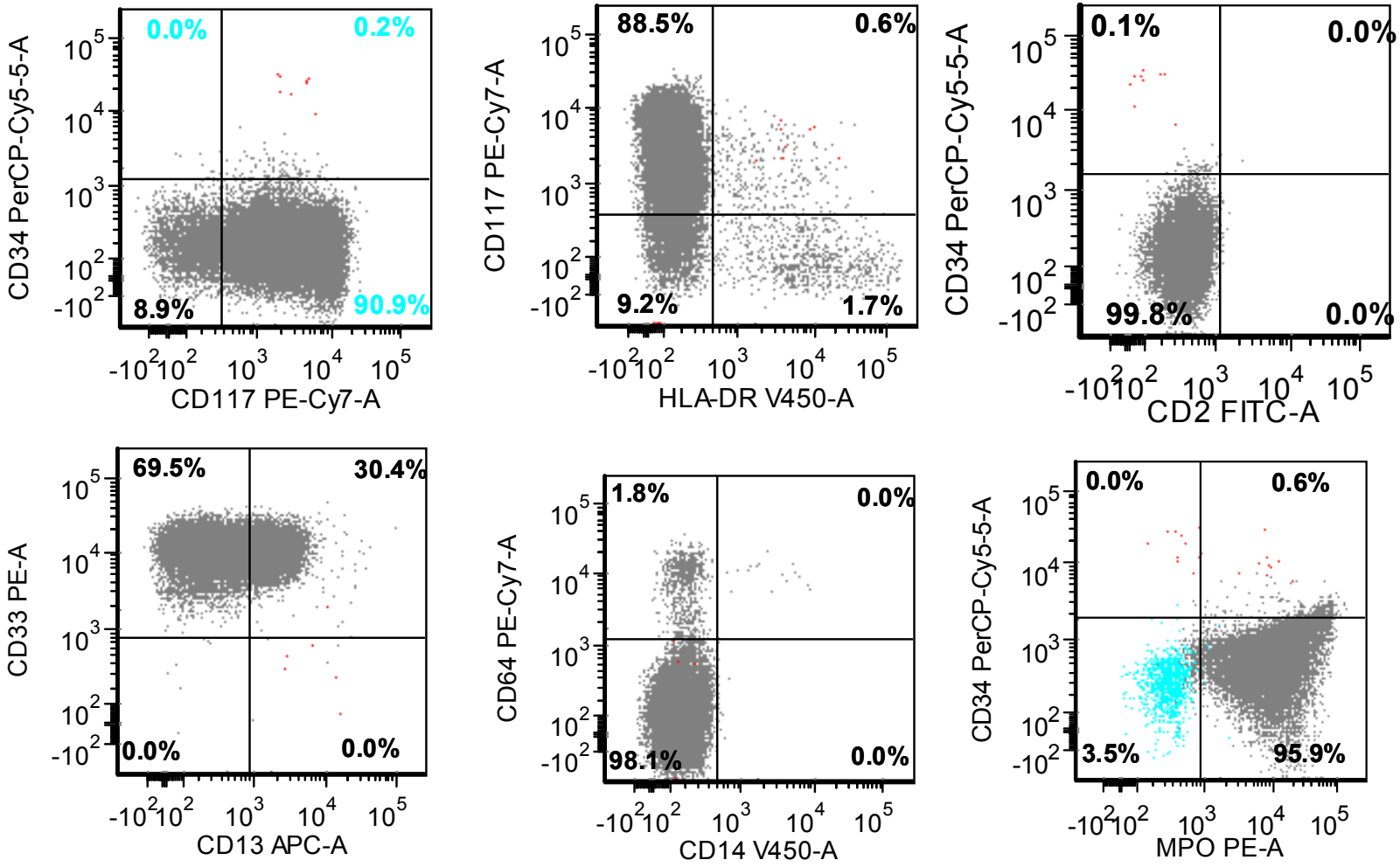
Flow Cytometry Analysis



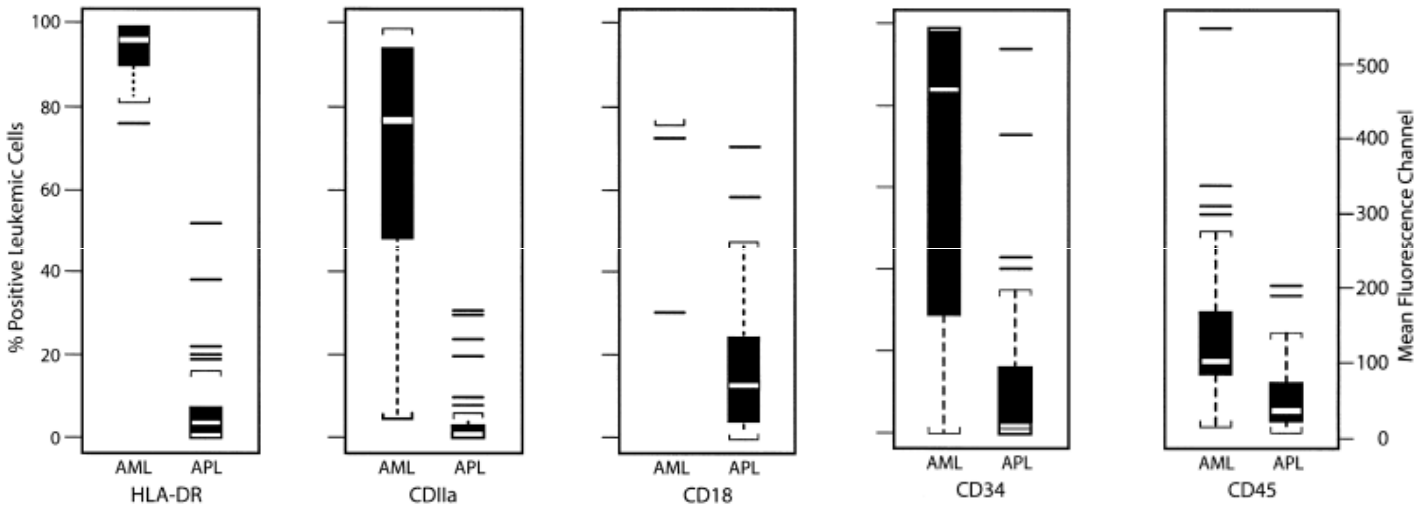


Case Cluster III-case #2

Flow Cytometry Analysis



Acute Promyelocytic Leukemia (APL)

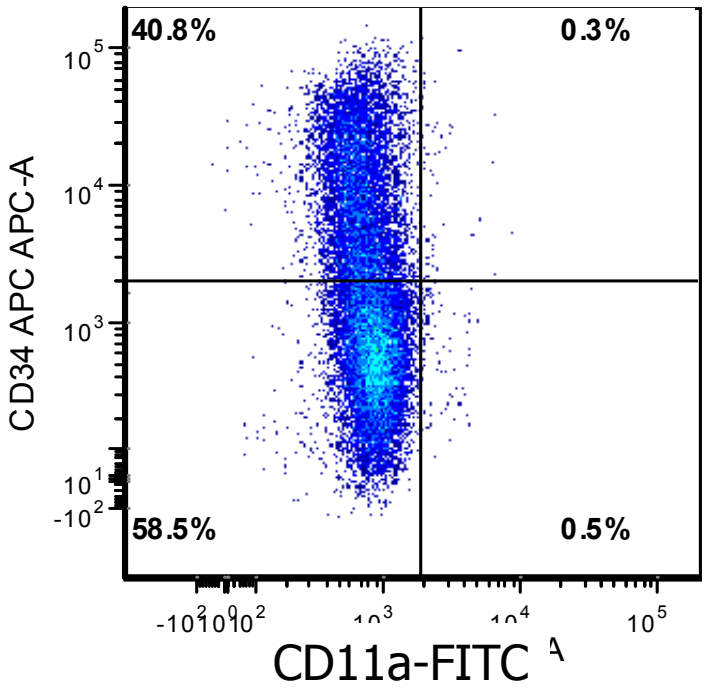


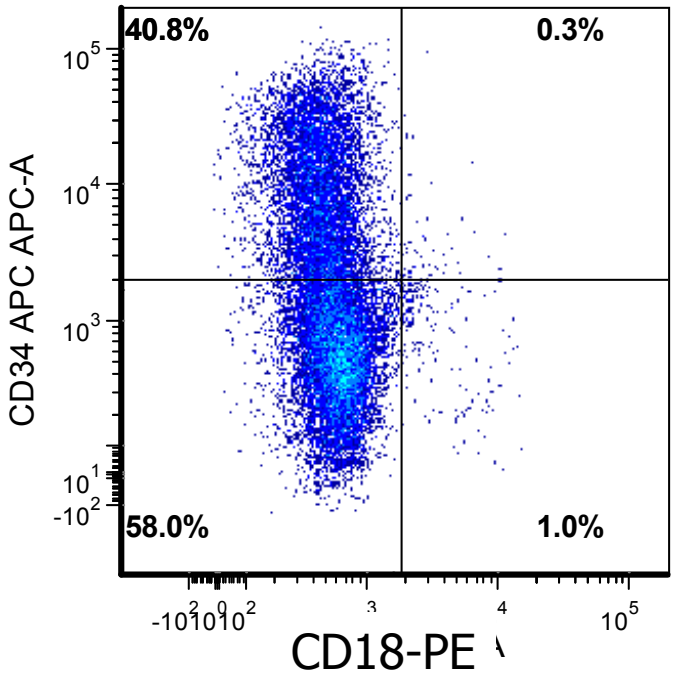
Surrogate immunophenotypic profile for M3
HLA-DRlow, CD11aLow, CD18Low

Paietta E et al. Cytometry B Clin Cytom. 2004 May;59(1):1-9.

Case Cluster III-case #2

Flow Cytometry Analysis





	APL		non-APL		
	(n)	*(%)*	*(n)*	*(%)*	*specificity*
DR-	35	95%	15	47%	56%
CD34-	31	84%	18	56%	41%
CD117+	29	78%	26	81%	21%
DR-/CD34-/CD117+	23	62%	11	34%	68%
CD11a-	34	92%	12	38%	62%
CD18-	34	92%	14	44%	53%
CD11a -or CD18-	37	100%	20	63%	35%
CD11a-/CD18-	31	84%	6	19%	79%
CD11a-/CD18 or CD2+/(CD11a or CD18-)	35	95%	7	22%	
DR-/(CD11a- /or CD18-)	35	95%	13	41%	62%
DR-/CD11a-/CD18-	29	78%	4	13%	88%
DR-/CD11a-/CD18- or DR-/CD2+/(CD11a- or CD18-)	33	89%	5	16%	85%

Summary (take home message)

FCI is an important tool in hematological Diseases

- Diagnosis
- Classification
- Prognostic markers
- Target therapy
- Minitor treatment response

Difficult cases in clinical flow cytometry

2011 ASCP Annual Meeting/ WASPaLM XXVI World Congress

Jo-Anne Vergilio, MD

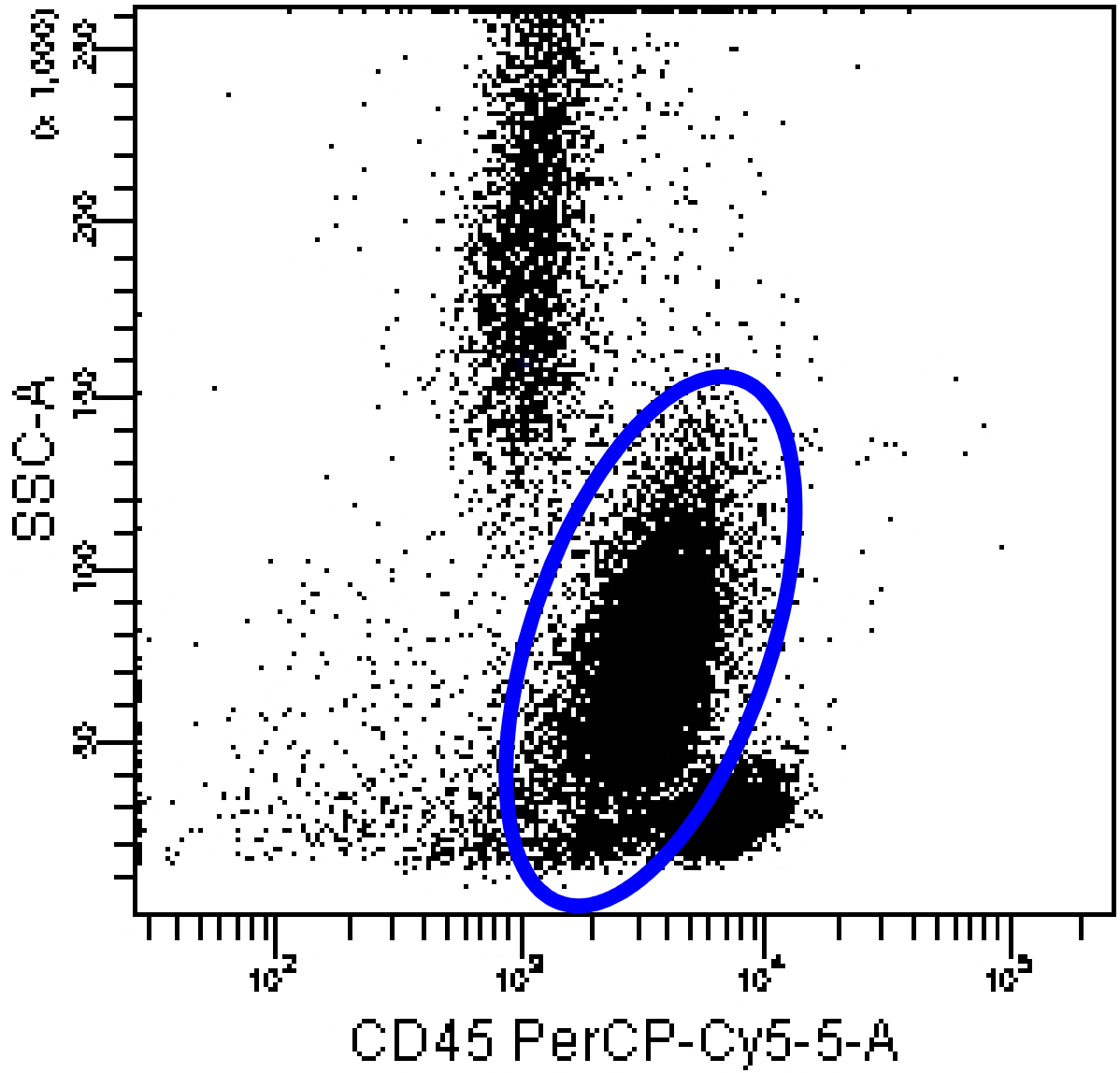
Children's Hospital Boston
Harvard Medical School

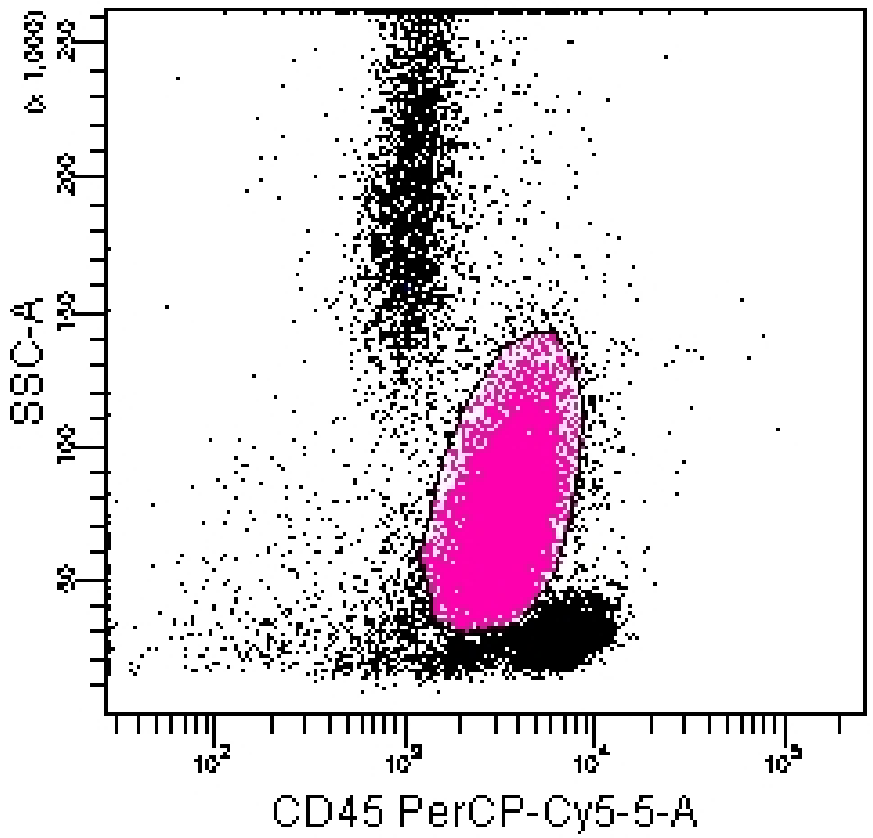
Principles and foundations

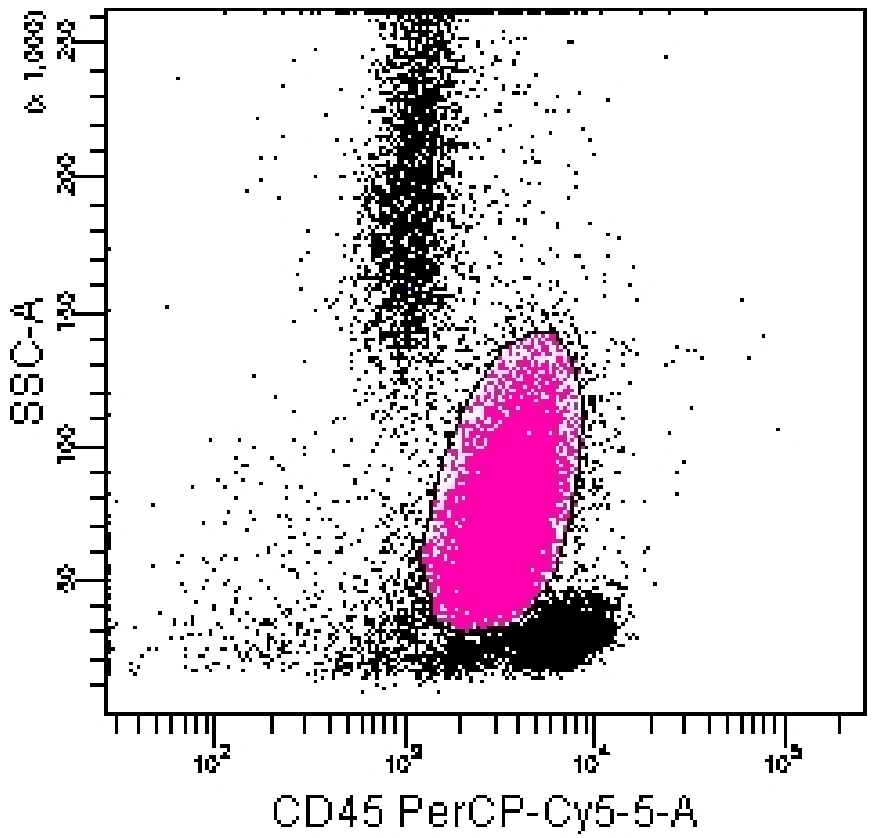
- Different approaches are acceptable
- Be open-minded (have a broad differential)
- Know thy audience (ie. patient population)
- Phenotype predicts genotype (so speak up)

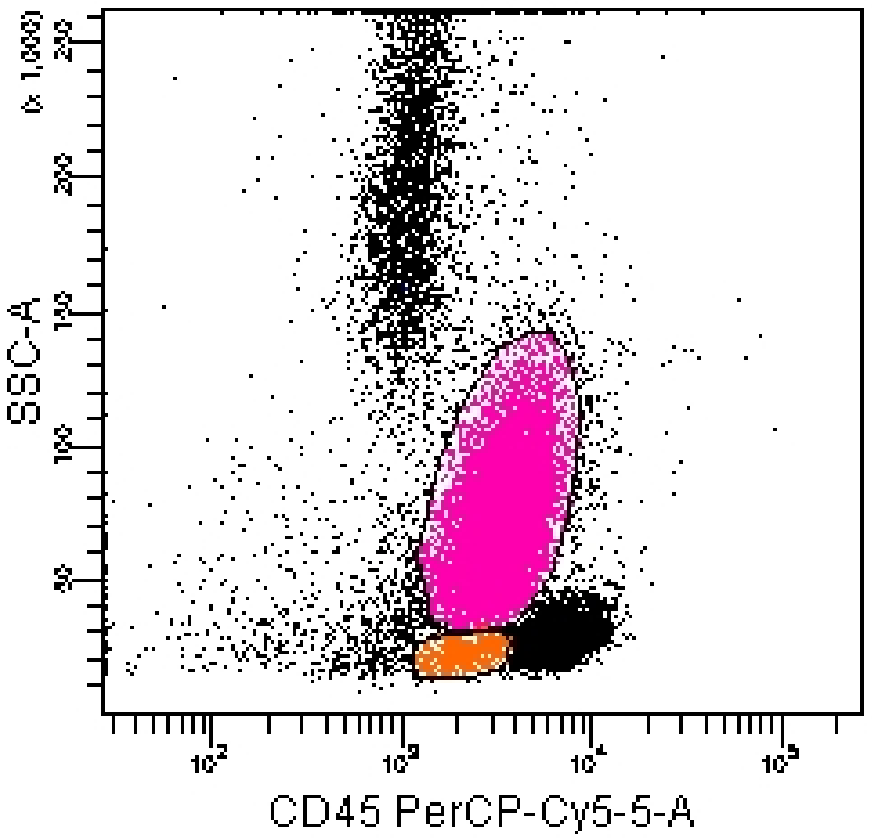
Case 1

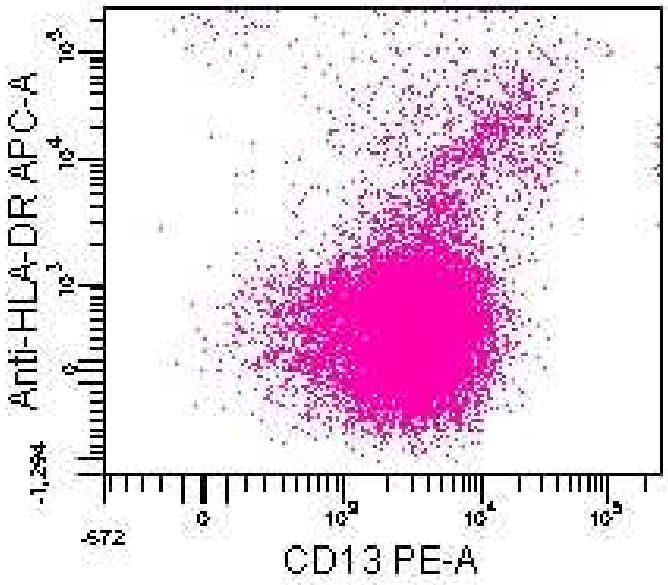
Bone marrow analysis




SSC-A
(x 1,000)
250
200
150
100
50
10^2
10^3
10^4
10^5
CD45 PerCP-Cy5-5-A


SSC-A
(x 1,000)
250
200
150
100
50
10^2
10^3
10^4
10^5
CD45 PerCP-Cy5-5-A


SSC-A
(x 1,000)
250
200
150
100
50
10^2
10^3
10^4
10^5
CD45 PerCP-Cy5-5-A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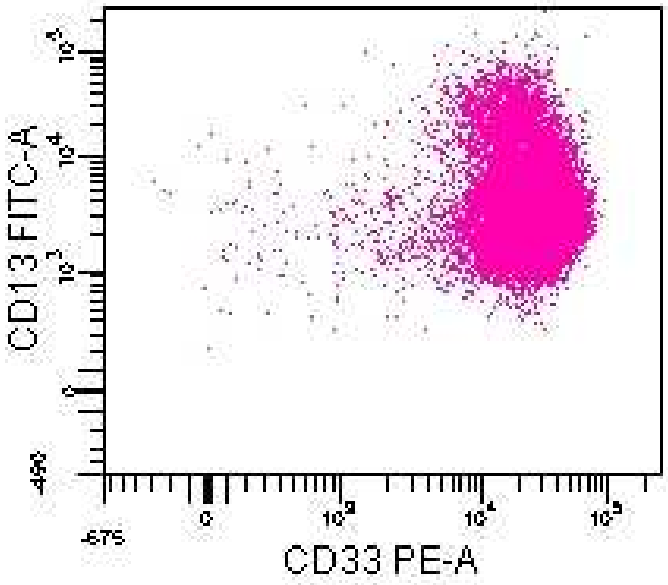
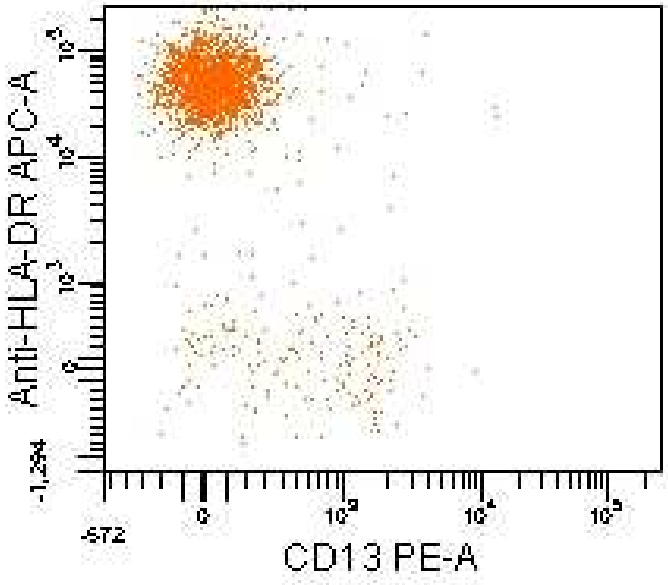
CD13 PE-A


CD13 FITC-A
CD33 PE-A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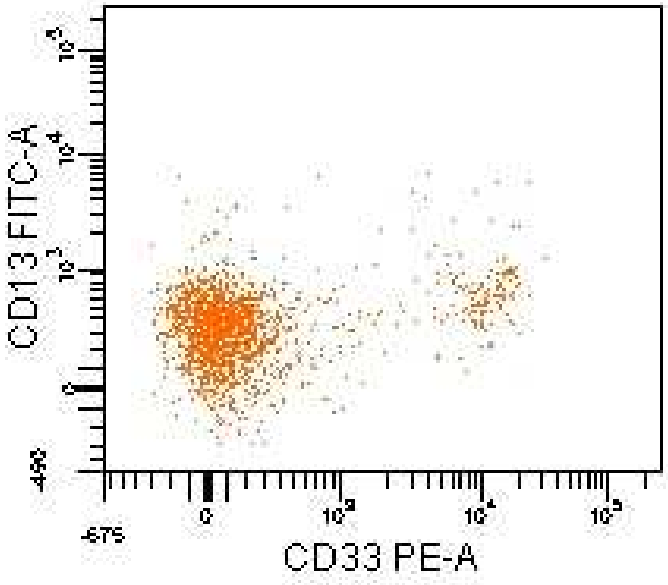
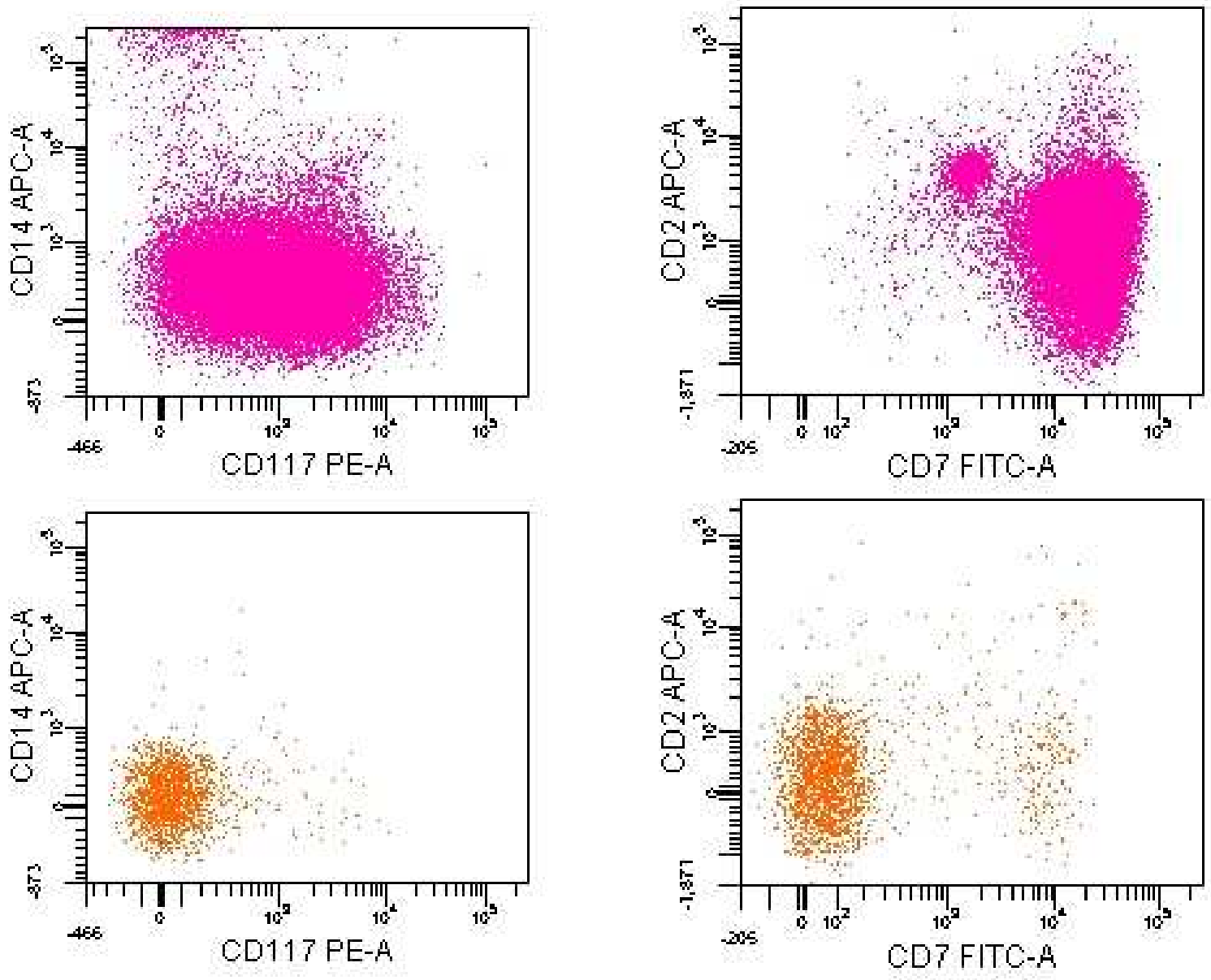
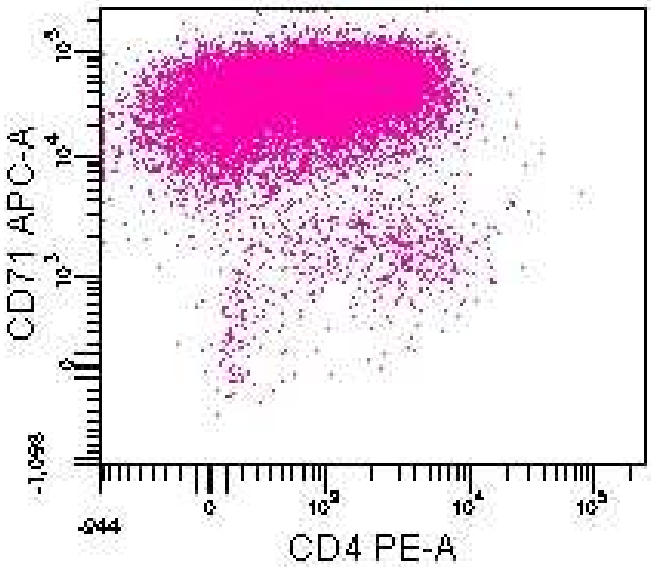
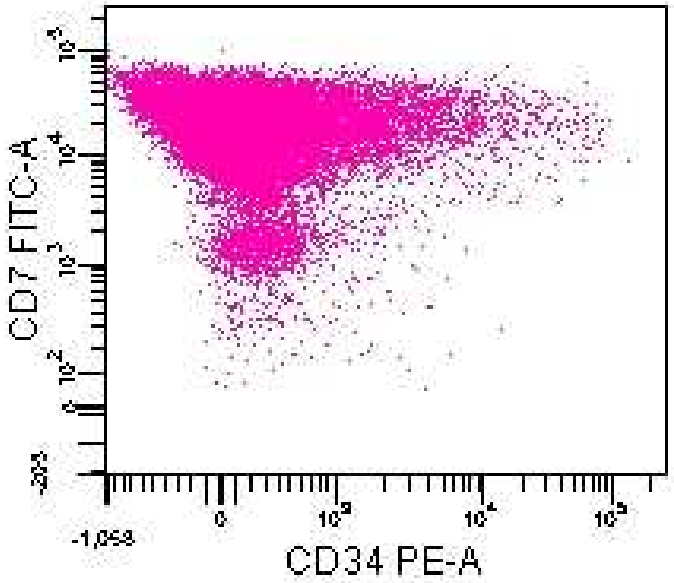
CD13 PE-A

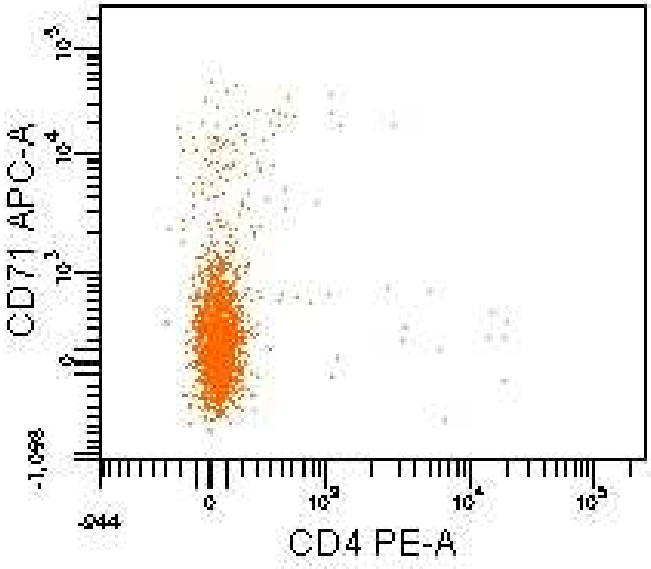

CD13 FITC-A
CD33 PE-A

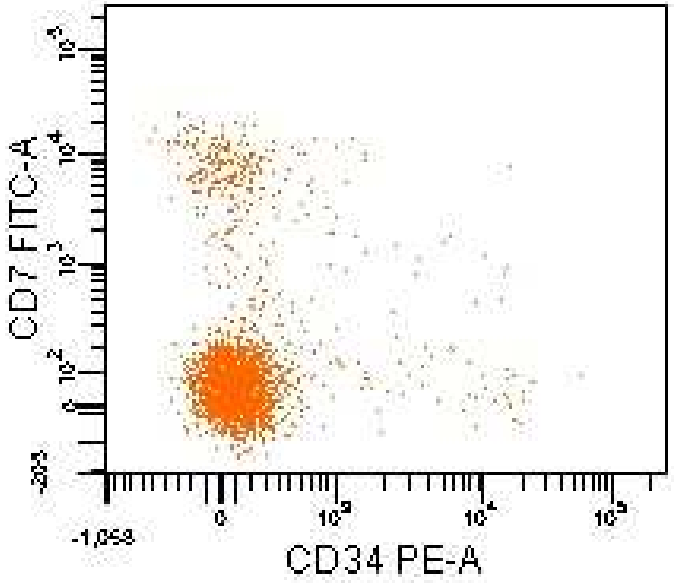


CD64, CD15 negative



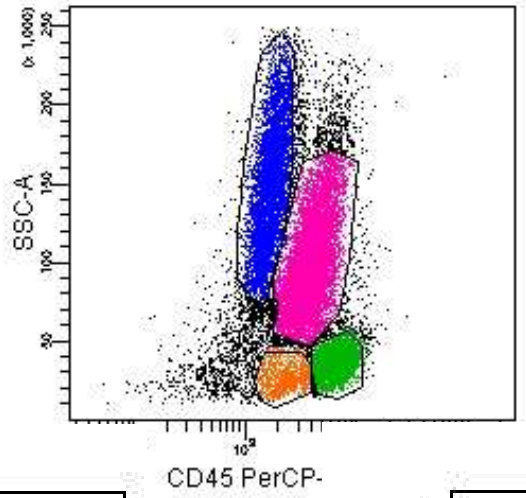


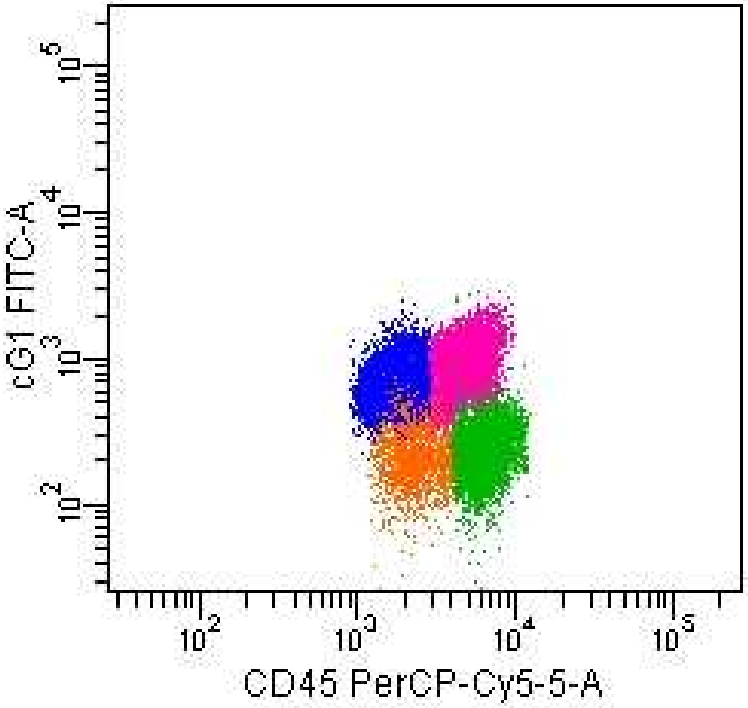


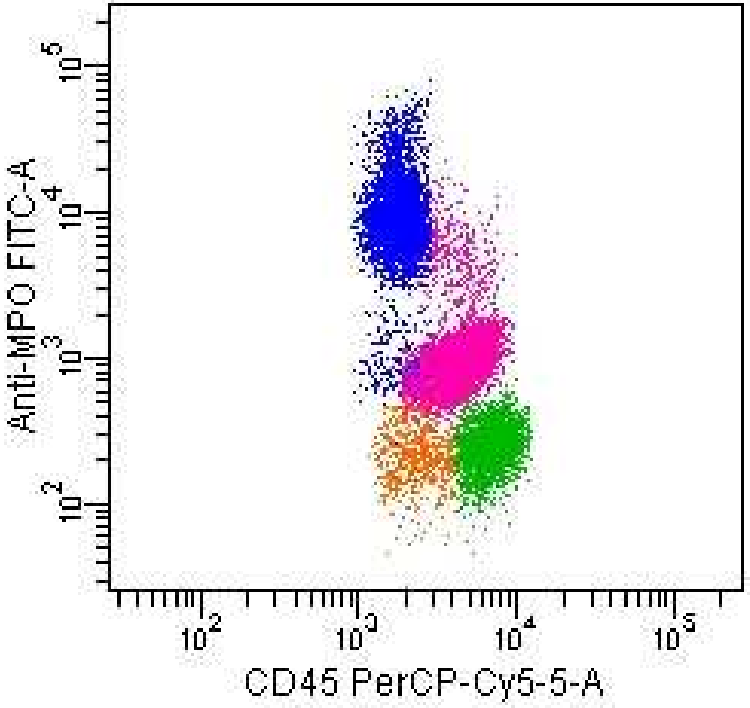


CD19, CD20, CD22 negative

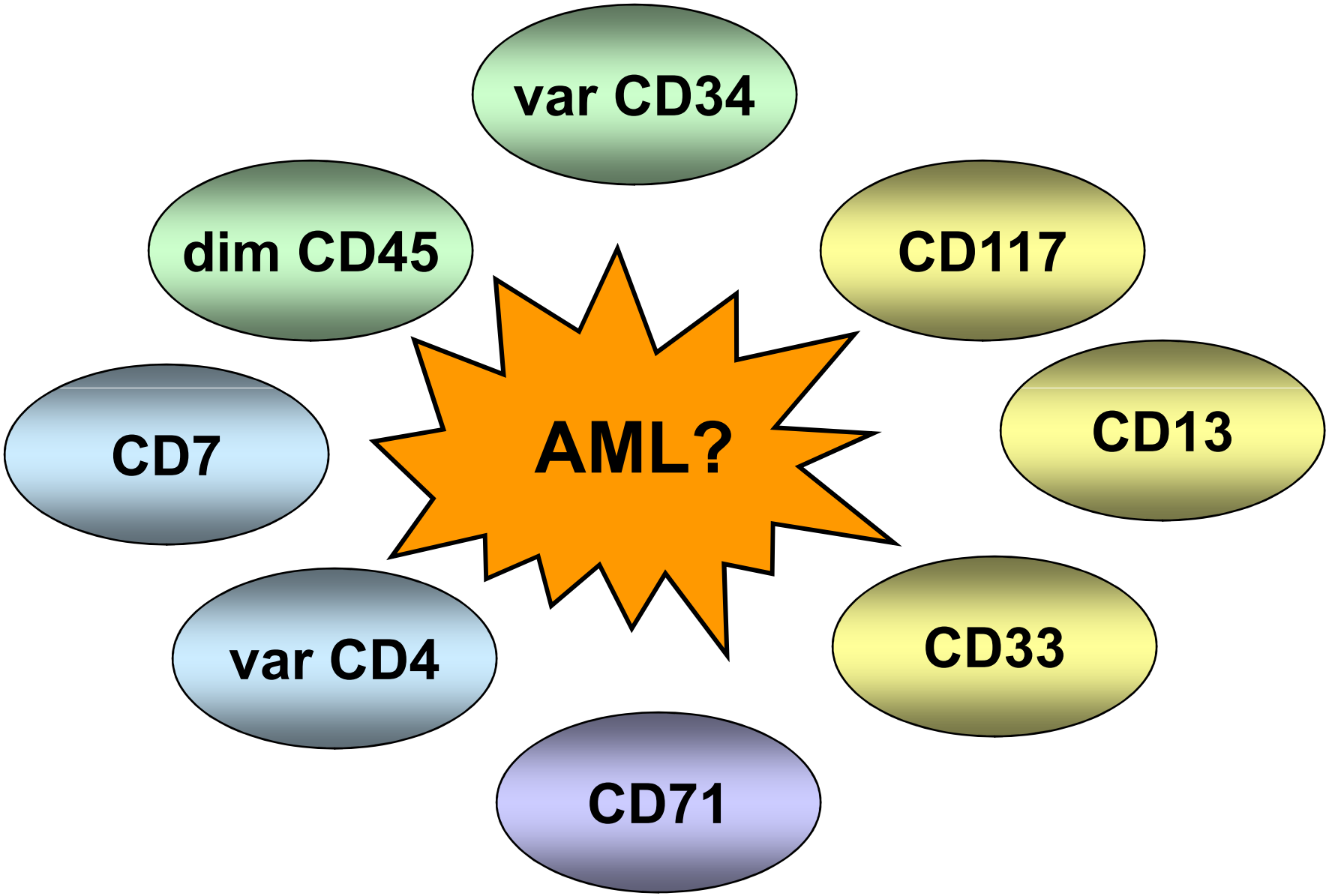
Myeloperoxidase ?







Immunophenotypic summary



Lineage … "infidelity"?

- CD7 expressed in 15-30% AMLs
  - CD2 more common in microgranular APML
- CD4 positivity in 25% of AMLs
  - Myelomonocytic and myeloblastic subtypes
- CD71 = transferrin receptor
  - Not erythroid lineage specific; expressed in replicating cells of all hematopoietic lineages

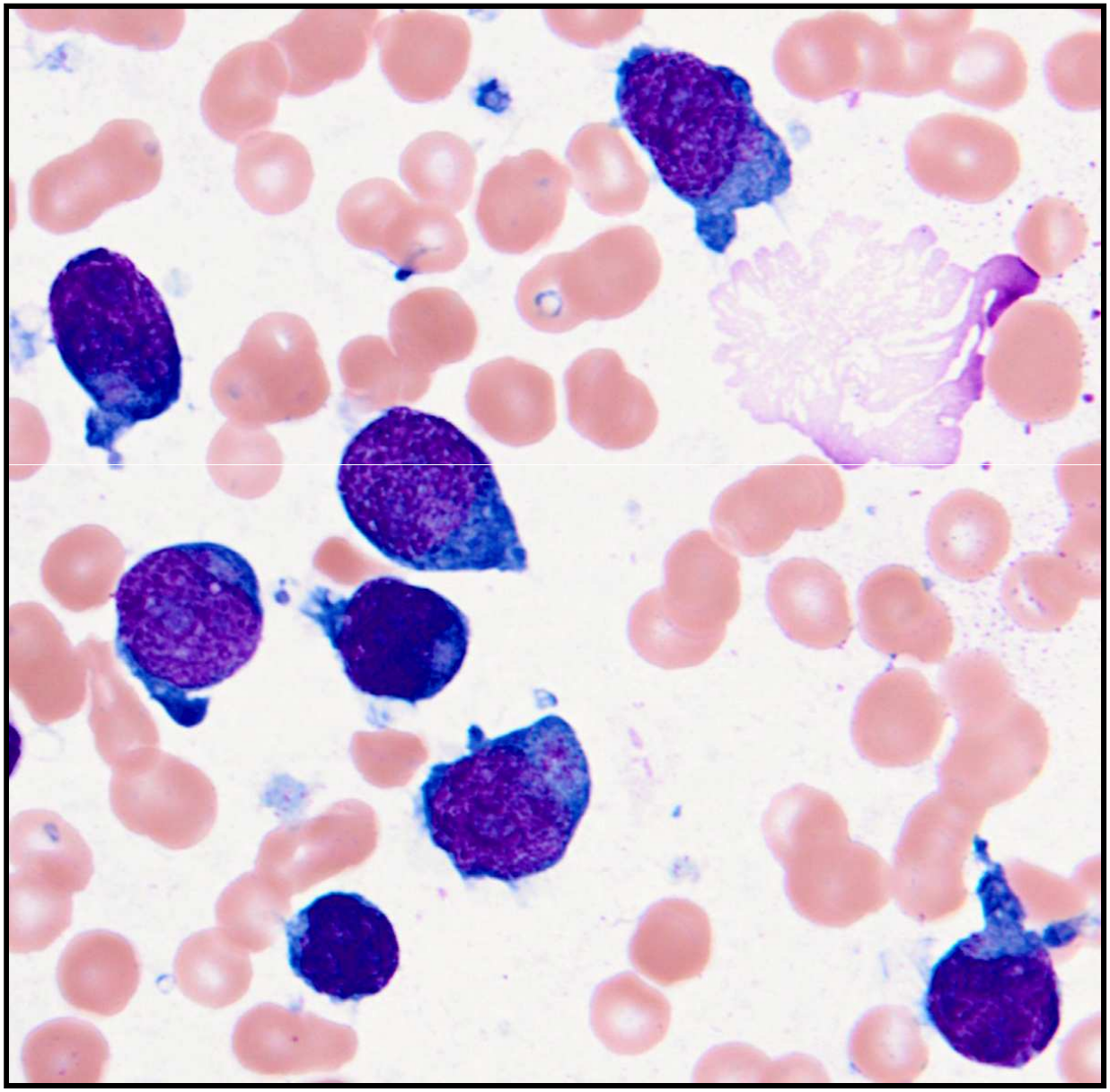
Karandikar, *AJCP* 2001; Venditti, *Leukemia*, 1998; Khalidi, *AJCP*, 1998; Drexler, *Leukemia*, 1993

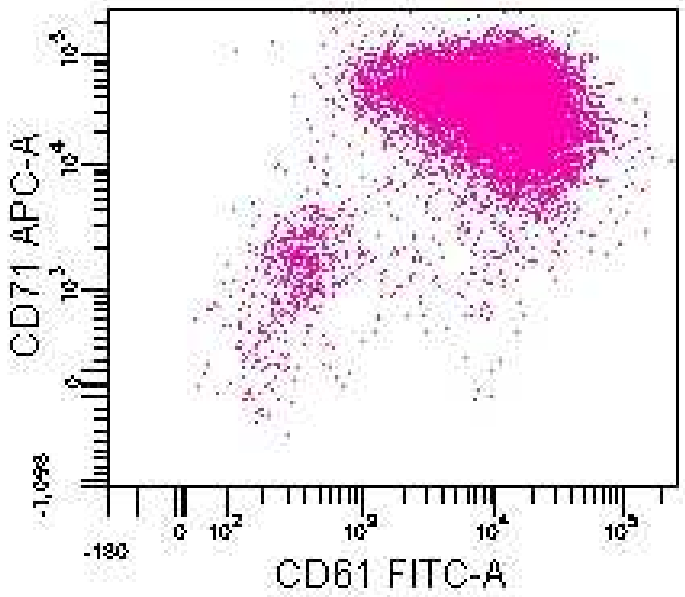
Acute myeloid leukemia … sufficient?

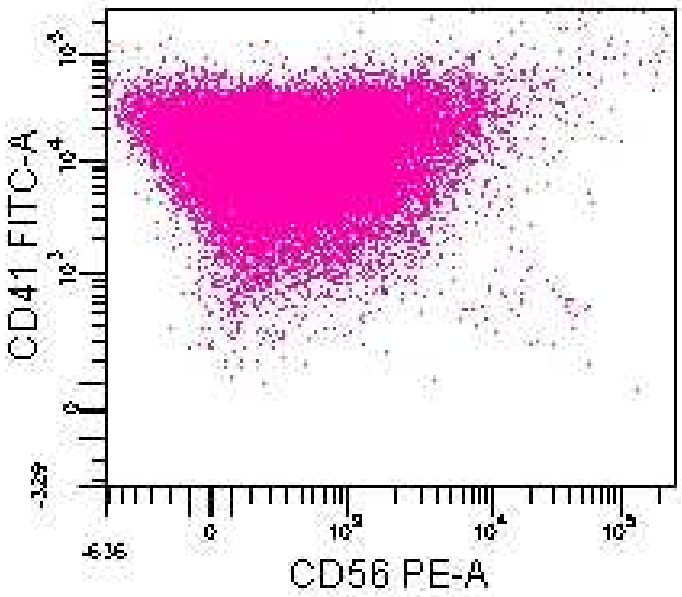
- Subtypes:
  - Minimally differentiated (expect DR+)
  - With(out) maturation (expect MPO+, DR+)
  - Myelomonocytic/monoblastic (expect CD14+, CD64+)
  - Erythroid?
  - Megakaryoblastic?

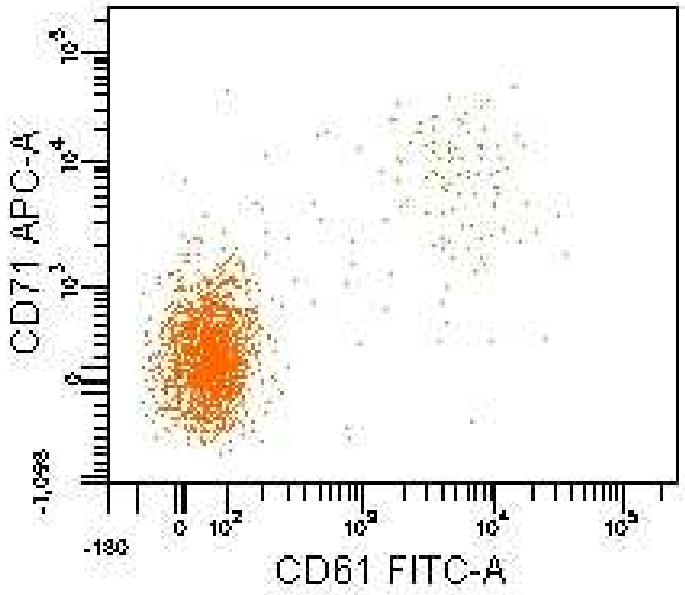
- Other?

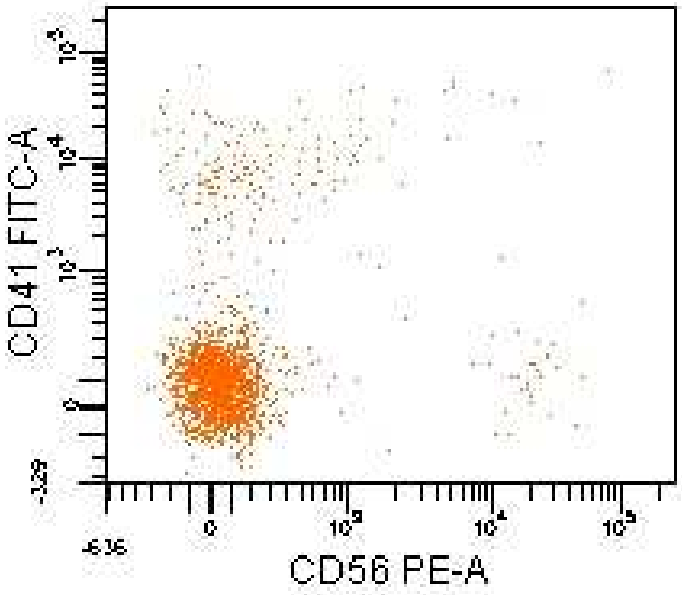
Marrow aspirate cytology




CD71 APC-A
CD61 FITC-A


CD41 FITC-A
CD56 PE-A

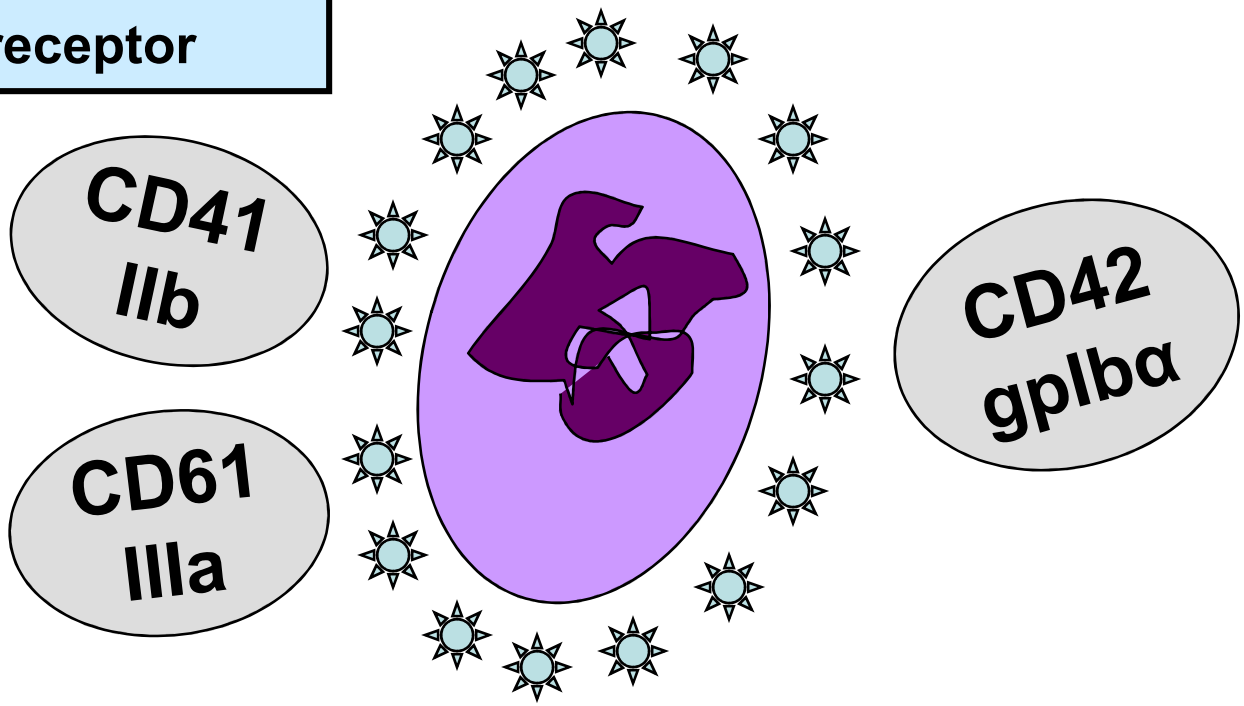

CD71 APC-A
CD61 FITC-A


CD41 FITC-A
CD56 PE-A

Megakaryocytic markers - review

***GP IIb/IIIa complex*:**

Fibrinogen receptor



***GP Ibα*:**

Von Willebrand factor receptor

Platelet-endothelial & platelet-platelet interactions in hemostasis & thrombosis

AMKL - WHO 2008 *[AML, NOS]*

- Comprises less than 5% of AMLs

- Associated features
  - Cytopenias (esp. thrombocytopenia)
  - Rarely hepatosplenomegaly
  - Trilineage dysplasia not uncommon

- Criteria
  - ≥20% blasts (≥50% blasts of megakaryocytic lineage)

- Immunophenotype
  - CD41, CD61, vCD42, CD13, CD33, CD7 positive
  - CD45, CD34, HLA-DR often negative

AML, further specified - WHO 2008

- AMKL w/ t(1;22)(p13q13) [*RBM15-AMKL1*]
- AML w/ inv(3)(q21q26.2) or t(3;3)(q21q26.2) [*RPN1-EVI1*]
- Megakaryoblastic crisis of any underlying myeloproliferative neoplasm
- Myeloid proliferations related to Down's syndrome

Potential clinical scenarios

Scenario 1:

1 year old female with persistent thrombocytopenia

AMKL w/ t(1;22)(p13q13) [*RBM15-MKL1*]

- <1% AMLs
- Most common in non-DS infants and young children (<3 yrs of age, median age onset = 6 mos)
- Leukocytosis, bicytopenias, organomegaly
- Stromal pattern of BM infiltration (not solid tumor)
- Complex karyotype more often in older children
- Variable prognosis, responsive to intensive chemotherapy

Bernstein*, Leukemia (1999)*

Potential clinical scenarios

Scenario 2:

50 year old male with thrombocytosis

AML w/ inv(3)(q21q26.2) or t(3;3)(q21q26.2)

- 1-2% AMLs
- Most common in adults
- Develops de novo or in setting of myelodysplasia
- Normal PLT count (but, 10-20% with thrombocytosis)
- Often with monosomy 7 and/or 5q deletions
- Poor prognosis, aggressive course, short survival

Actual clinical scenario

Scenario 3:

3 year old male

- +21
- Transient abnormal myelopoiesis as neonate

Cancer in DS

- 10-20x increased risk of developing acute leukemia
- Lower risk of solid tumors of childhood and adult non-hematopoietic cancers
- 500x increased risk of AMKL in affected children
- Spectrum of preleukemic and leukemic disease in the first 5 years of life

Vyas and Crispino*, Curr Opin Pediatr (2007)*

Transient abnormal myelopoiesis (TAM)

- 10% of neonates with DS develop TAM
  - Transient myeloproliferative disorder, transient leukemia
  - True incidence not well-defined

- 25% pts are asymptomatic (often incidental finding)
  - Occasional bruising, hepatomegaly or respiratory distress

- Typically leukocytosis & circulating (megakaryo)blasts
  - Often with thrombocytopenia

- Self-limited disease (spontaneous resolution within 3 months)
  - Low-dose chemotherapy sometimes required
  - Aberrant down-regulation of fetal liver hematopoiesis?

Massey*, Blood (2006)*

DS-AMKL – more common sequela of TAM

- 30% of patients with TAM develop AMKL
- Typically within first 5 years of life
- Low WBC
- Hepatosplenomegaly common
- Progressive marrow fibrosis
- Develop additional cytogenetic abnormalities (+8, -7)

Hitzler*, Nat Rev Cancer (2005)*

DS-AMKL – other unique features

- Increased chemosensitivity of blasts
  - Cytarabine and anthracyclines
  - Less than 10% of standard doses are effective

- 70-100% cure rate (better than non-DS AMKL)

- Cytidine deaminase (cytarabine-catabolizing enzyme)
  - Decreased gene transcription in DS
  - Diminished intracellular drug metabolism?

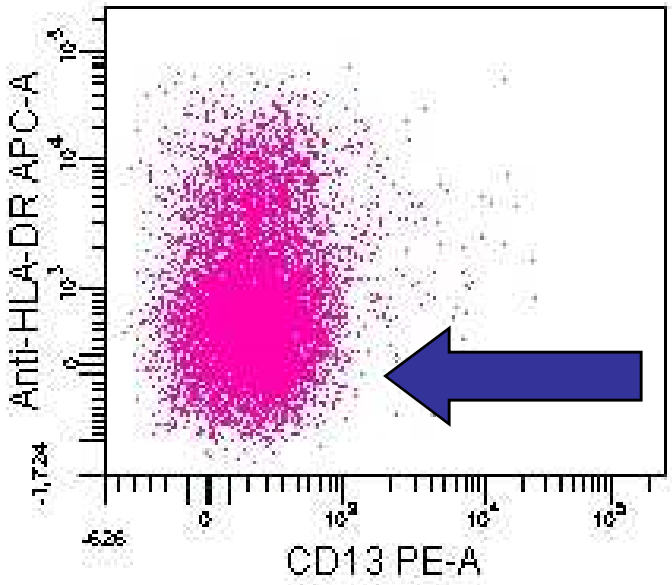
Ge, *Cancer Res (2004)*

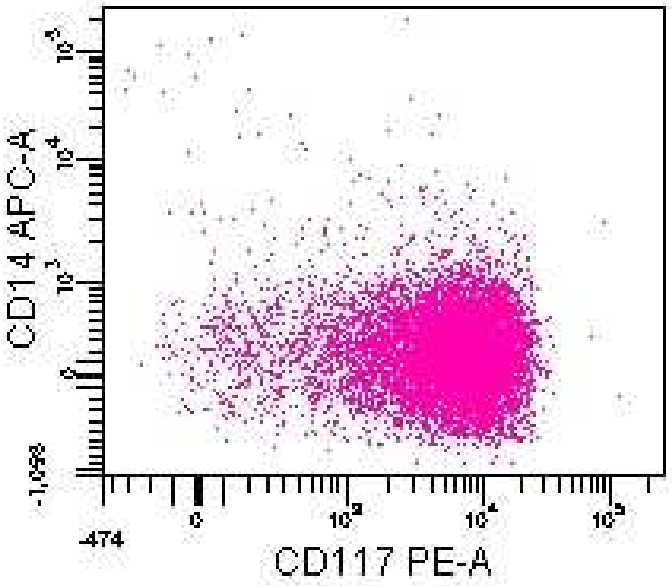
Other potential clinical scenario

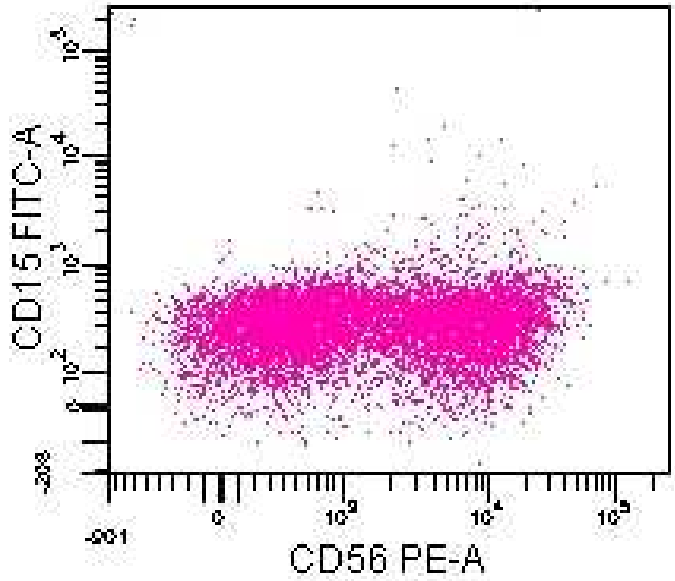
Scenario 4:

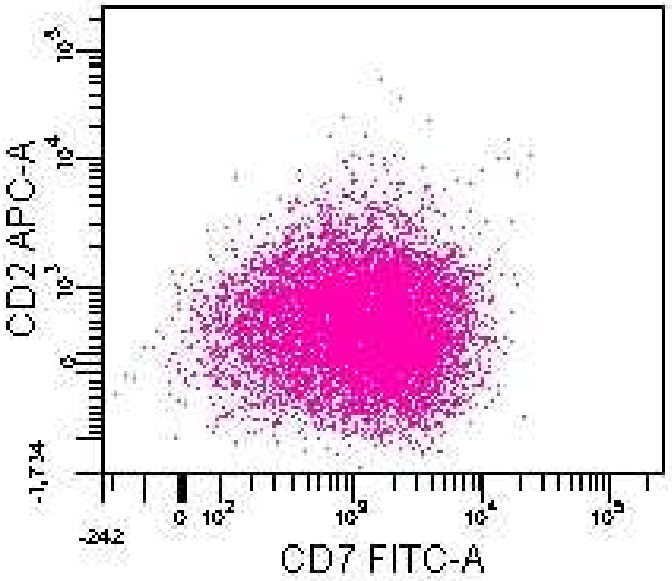
2 day old male, ex-38 wk, WBC 85 K/ul

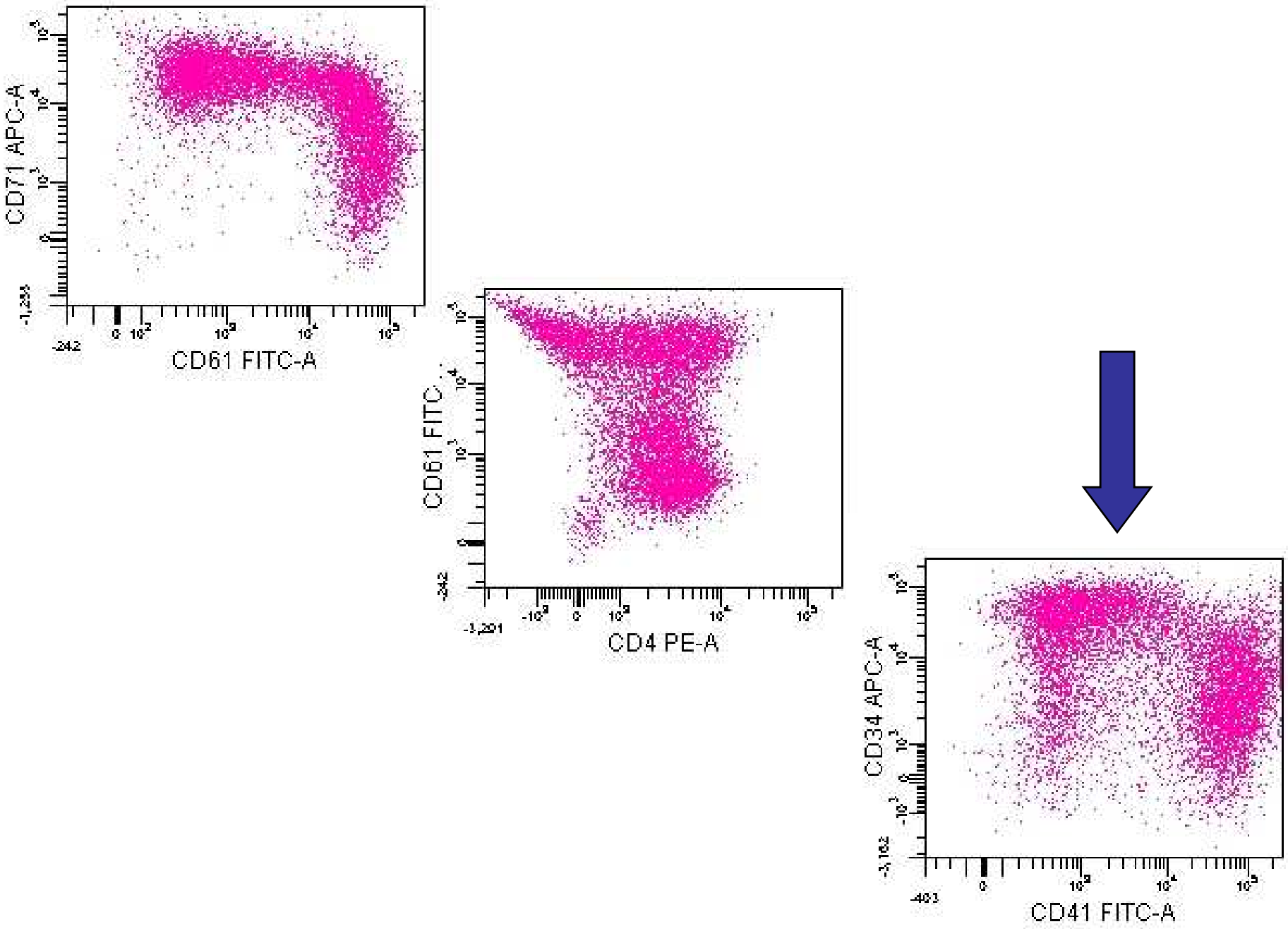
Flow cytometric analysis of peripheral blood


Anti-HLA-DR APC-A
CD13 PE-A


CD14 APC-A
CD117 PE-A


CD15 FITC-A
CD56 PE-A


CD2 APC-A
CD7 FITC-A


CD71 APC-A
CD61 FITC-A
CD61 FITC-A
CD4 PE-A
CD34 APC-A
CD41 FITC-A

Not necessarily AMKL

- Clinical history is important
  - Otherwise healthy child?
  - Classic dysmorphic features?
  - Maternal age?
  - Cytogenetic karyotypic analysis?
- Must exclude trisomy 21 / Down's syndrome

TAM

- Neonates
- High WBC (PB>BM blasts)
- Isolated thrombocytopenia
- Organomegaly uncommon
- Increased CD34 positivity
- Isolated T21

AMKL

- Median age onset = 2 yrs
- Low WBC (PB<BM blasts)
- Bi- and tri-cytopenias
- Organomegaly common
- Decreased CD34 positivity
- Other abnormalities: +8, -7

GATA1 mutations

Final diagnosis

Acute megakaryoblastic leukemia,

arising in a 3 year old with trisomy 21,

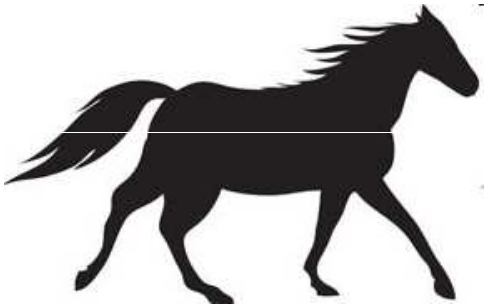
and

transient abnormal myelopoiesis as a newborn

Difficulties

Old adage:

If you hear hoof beats, think horse



Flow cytometric adage:

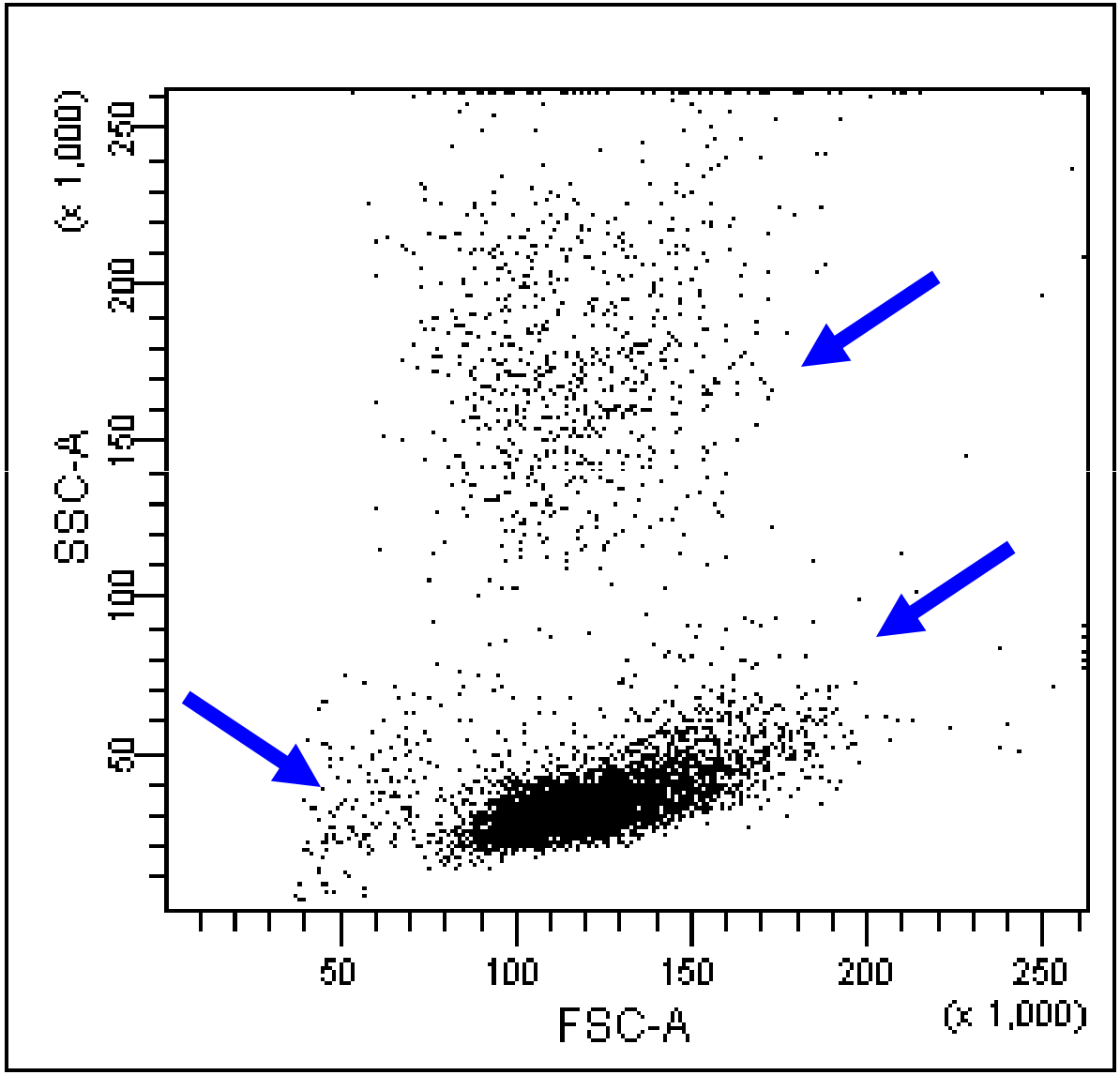
If you hear hoof beats, atleast consider the zebra

Other considerations

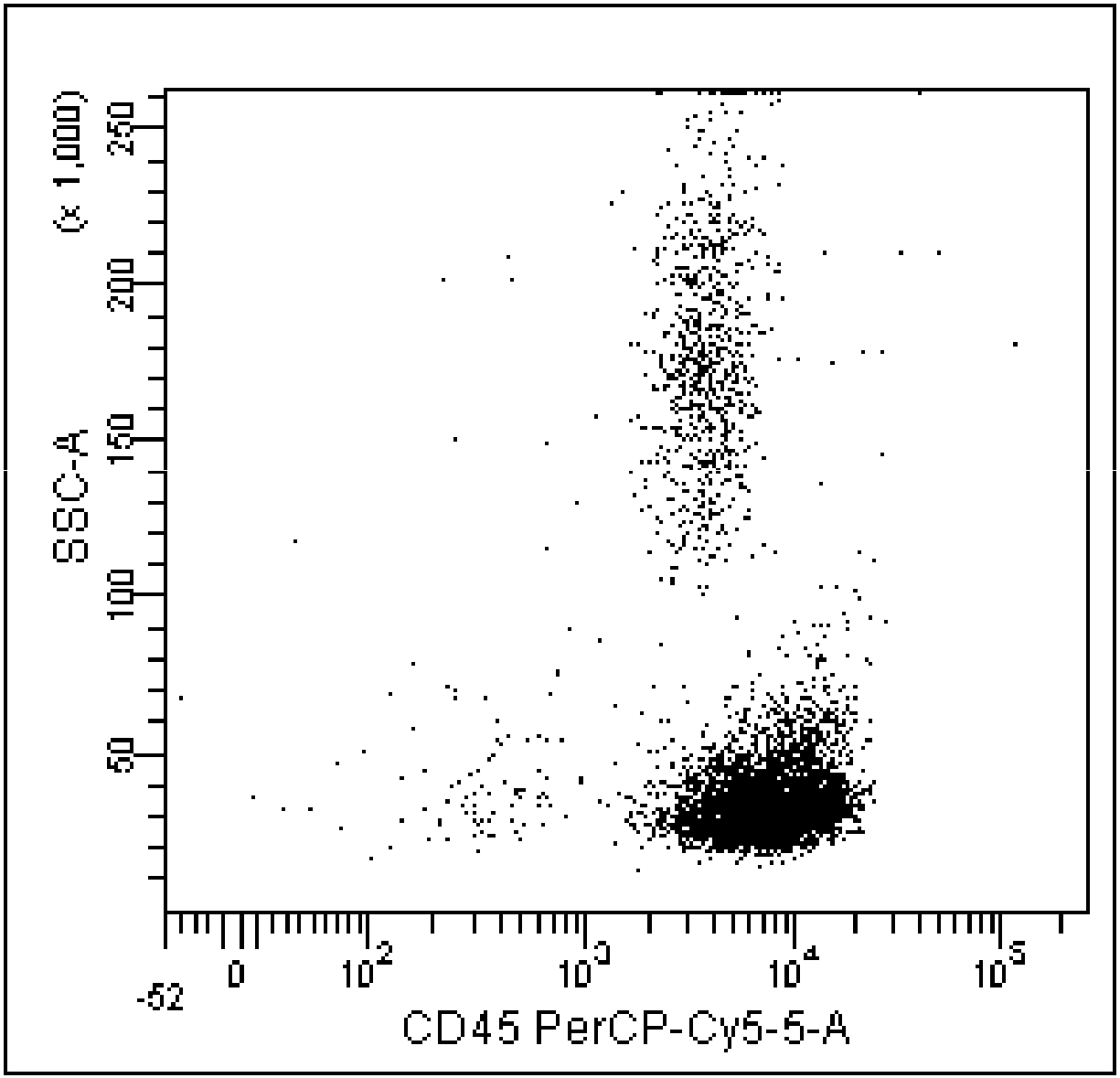
- Lineage infidelity
- Clinical context is key
- Circulating blasts (≥20%) don't always equate with acute leukemia
- Phenotypic-genotypic correlates

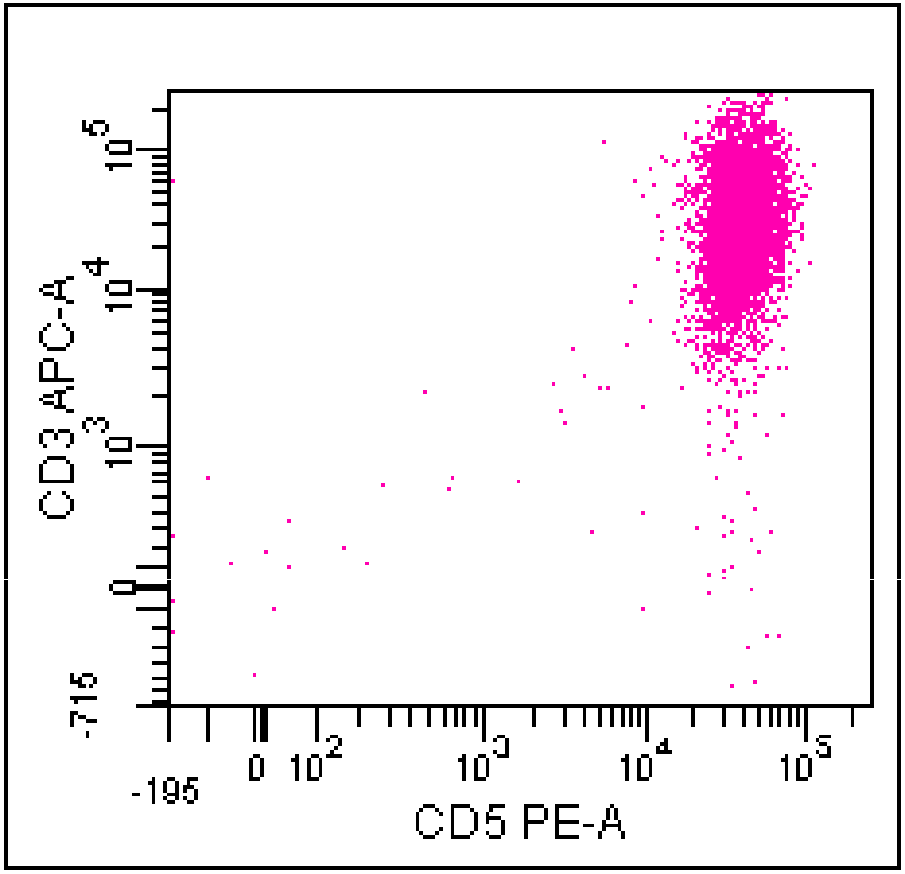
Case 2

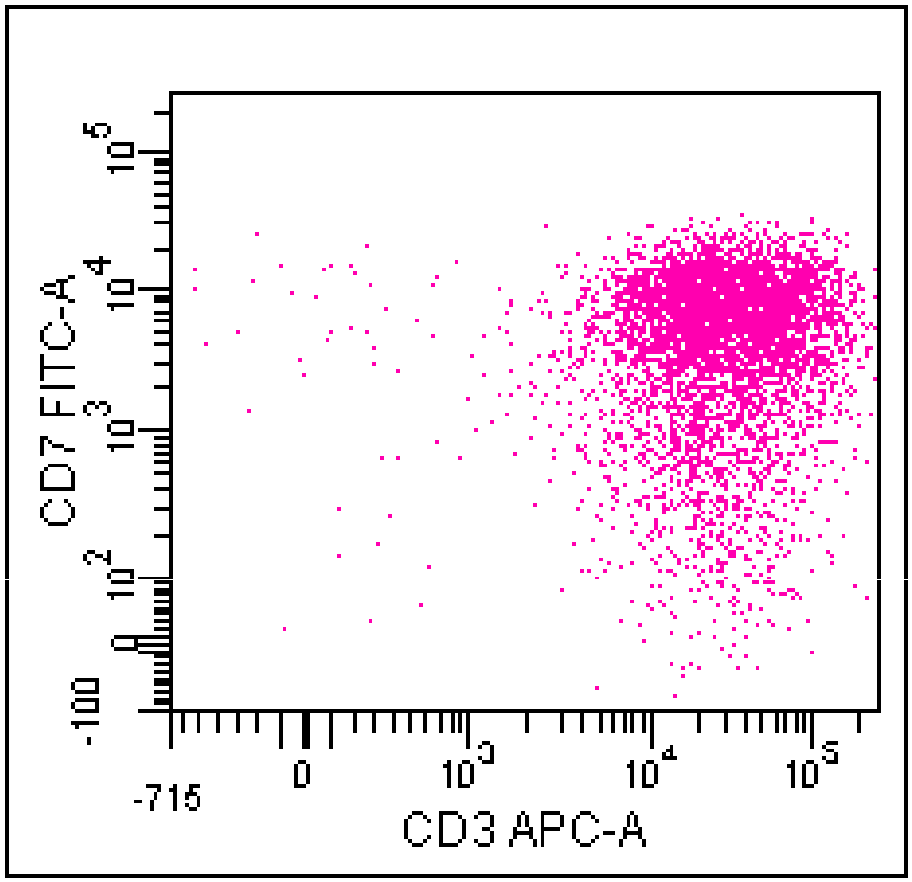
Pleural fluid analysis

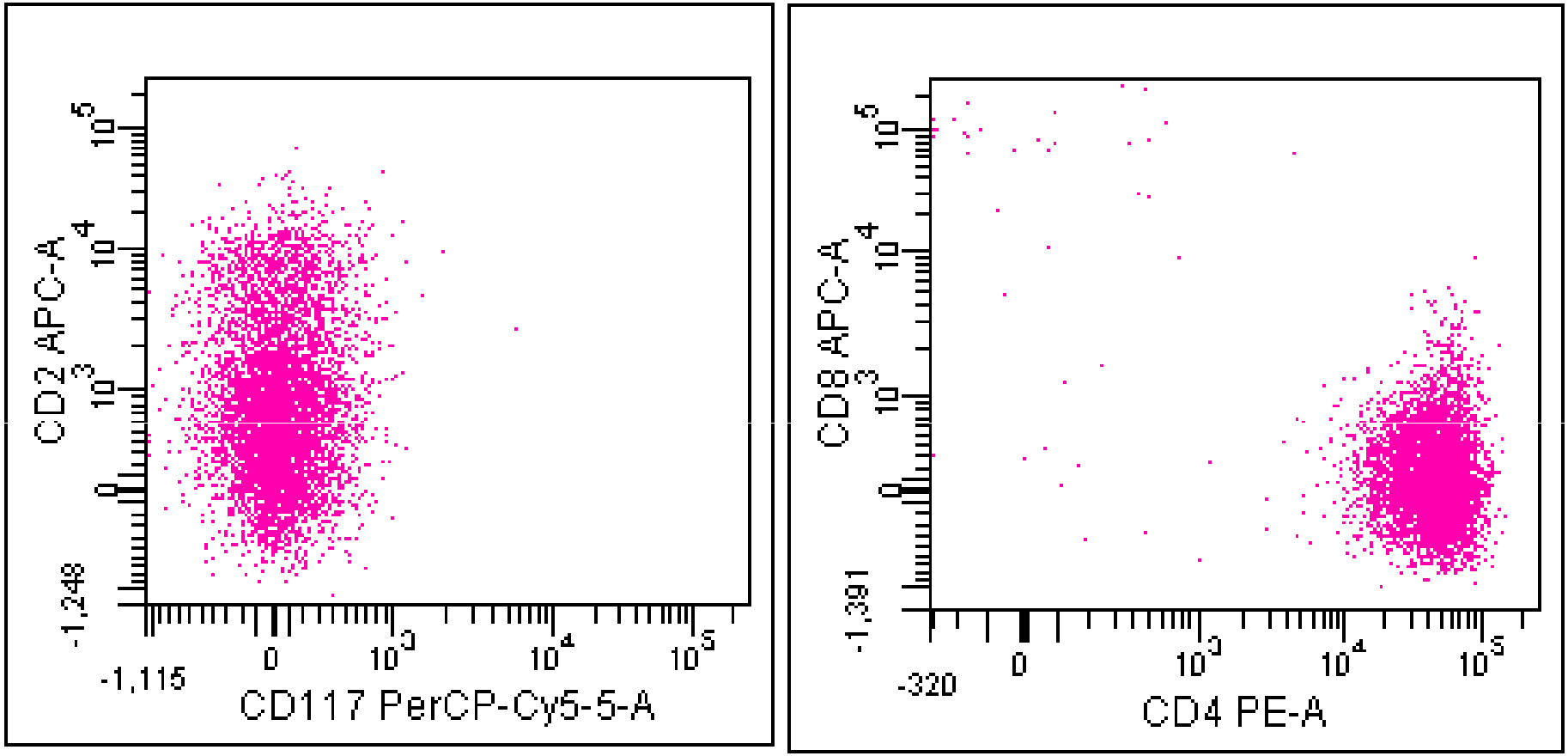


Pleural fluid analysis



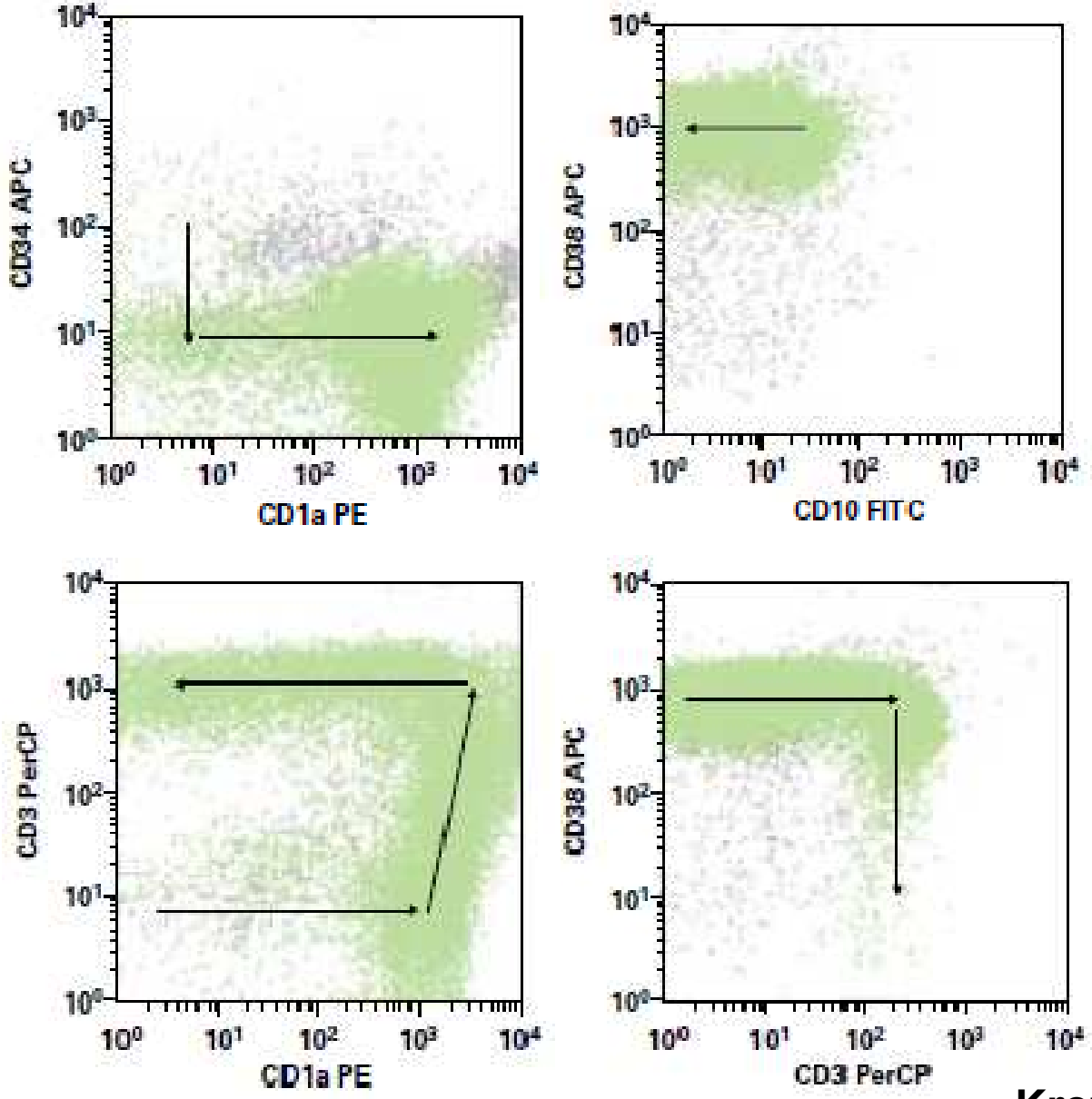

CD3 APC-A
-715
0
10^3
10^4
10^5
-195
0
10^2
10^3
10^4
10^5
CD5 PE-A


CD7 FITC-A
-100
0
10^2
10^3
10^4
10^5
-715
0
10^3
10^4
10^5
CD3 APC-A


CD2 APC-A
-1,248
0
10^3
10^4
10^5
-1,115
0
10^3
10^4
10^5
CD117 PerCP-Cy5-5-A
CD8 APC-A
-1,391
0
10^3
10^4
10^5
-320
0
10^3
10^4
10^5
CD4 PE-A

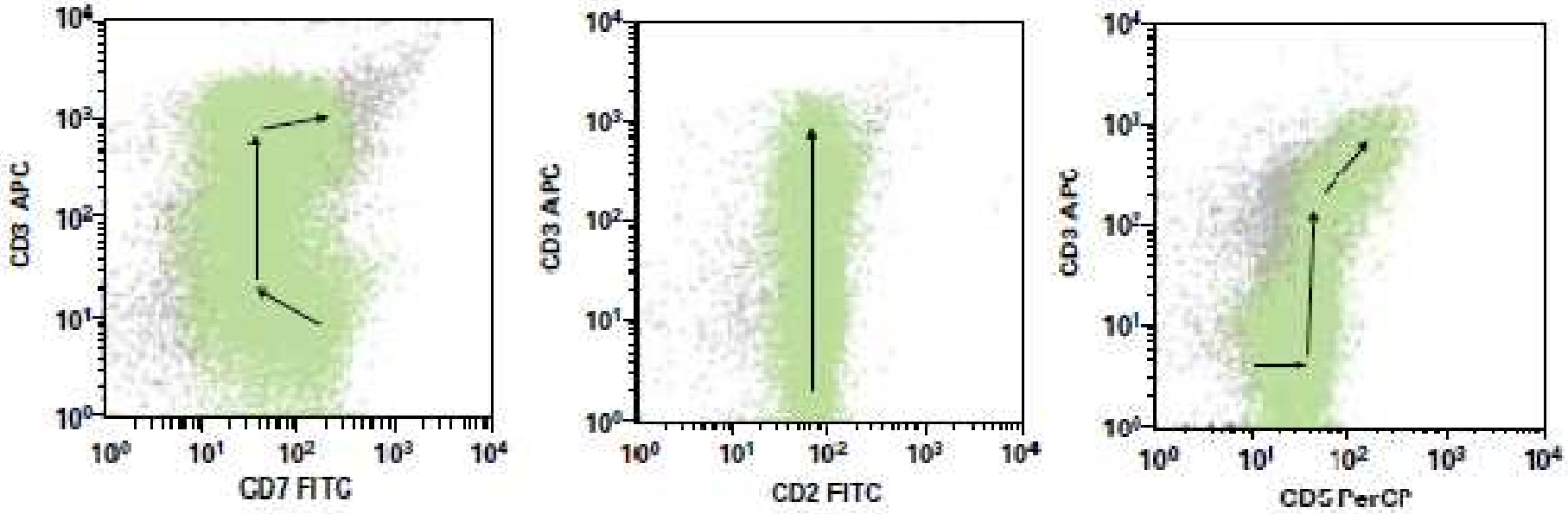
Review of T-cell maturation

T-cell maturation – the early years



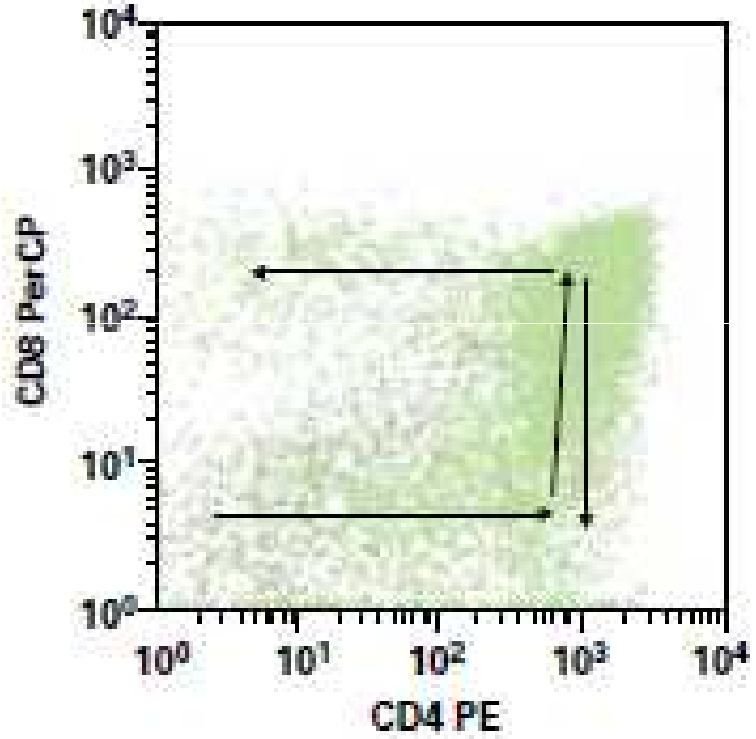
Kroft, *AJCP*, 2004

T-cell maturation - adolescence



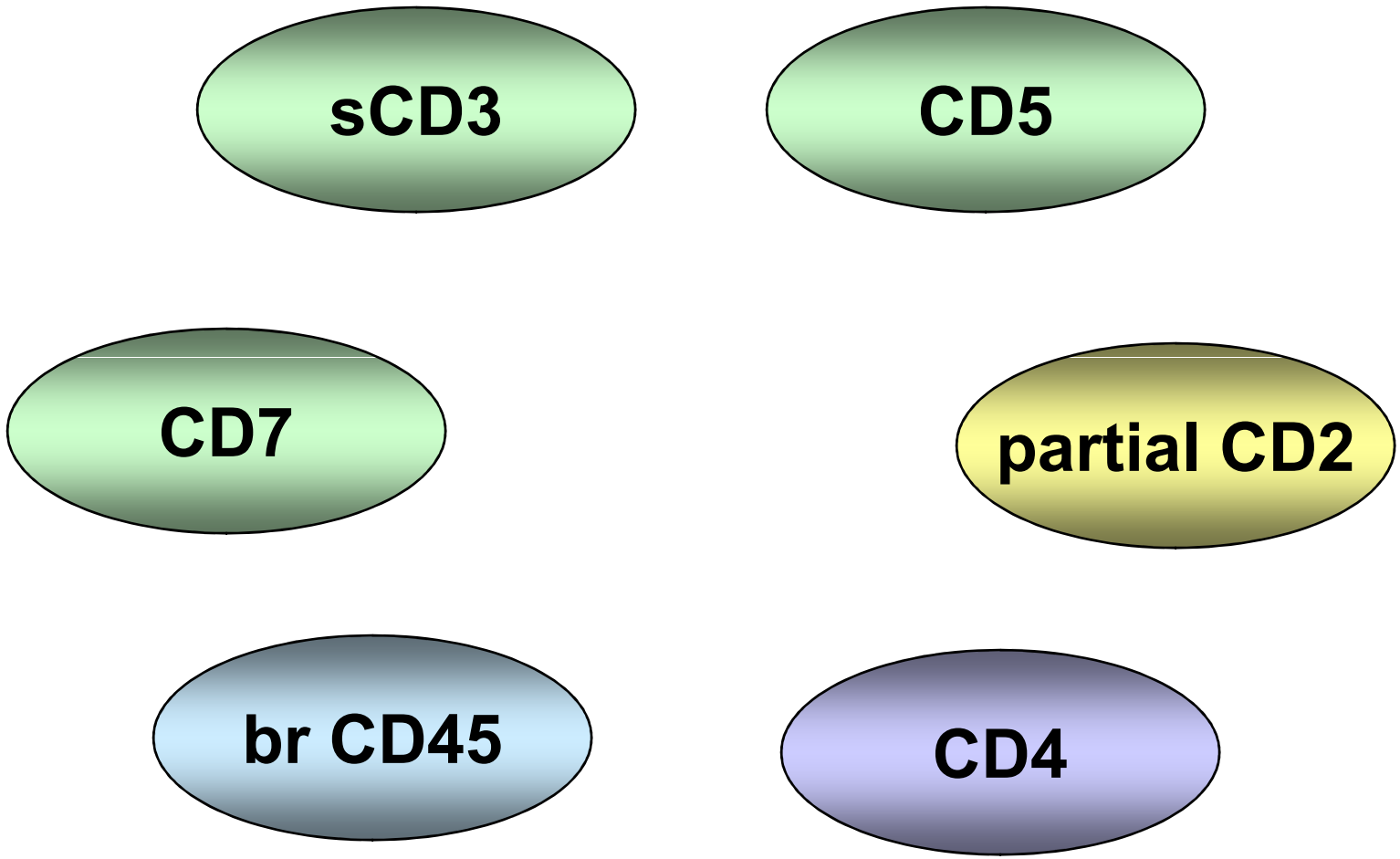
Kroft, *AJCP*, 2004

T-cell maturation - adulthood



Kroft, *AJCP*, 2004

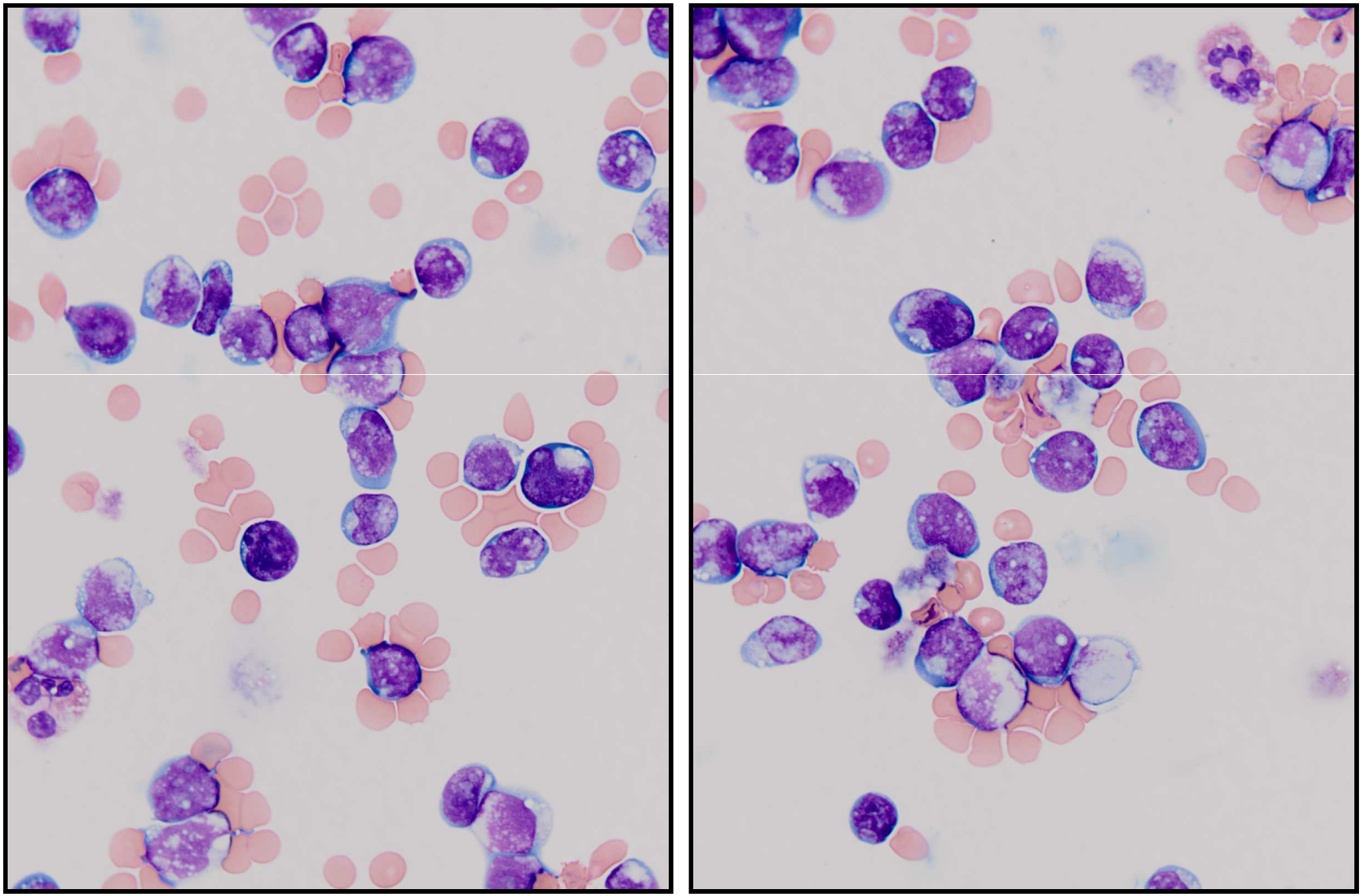
Immunophenotype



Differential diagnosis

- Mature T-cell lymphoma (no PB involvement)
  - Angioimmunoblastic T-cell lymphoma
  - Adult T-cell lymphoma/leukemia (CD2+, CD7-)
  - Anaplastic large cell lymphoma (CD30+, ALK+/-)
  - PTCL, NOS
- Thymoma
  - Pleural fluid involvement?
  - Not homogeneous cell population [Li, *AJCP*, 2004]
- Not NK or plasmacytoid dendritic cells
- Other?

Cytology



Clinical

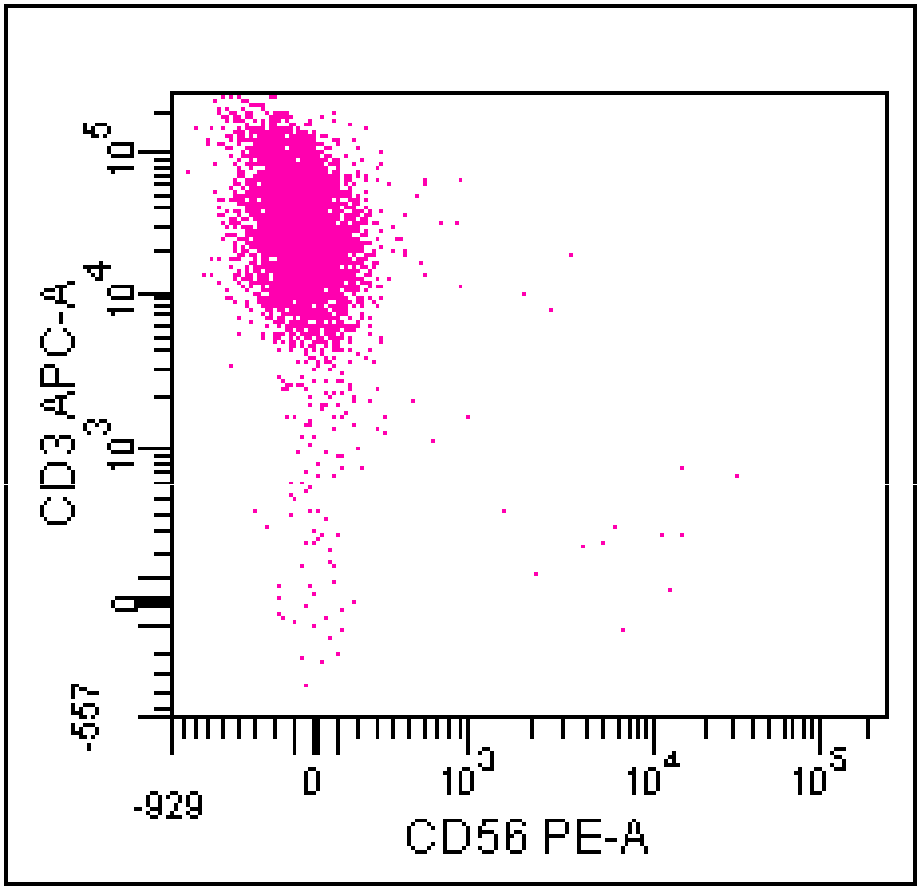
- 8 year old Caucasian female, otherwise healthy
- Parents noted slight facial swelling
- Mediastinal/left chest mass detected radiologically
- Laboratory parameters (only mild anemia)
- No adenopathy, organomegaly, skin lesions

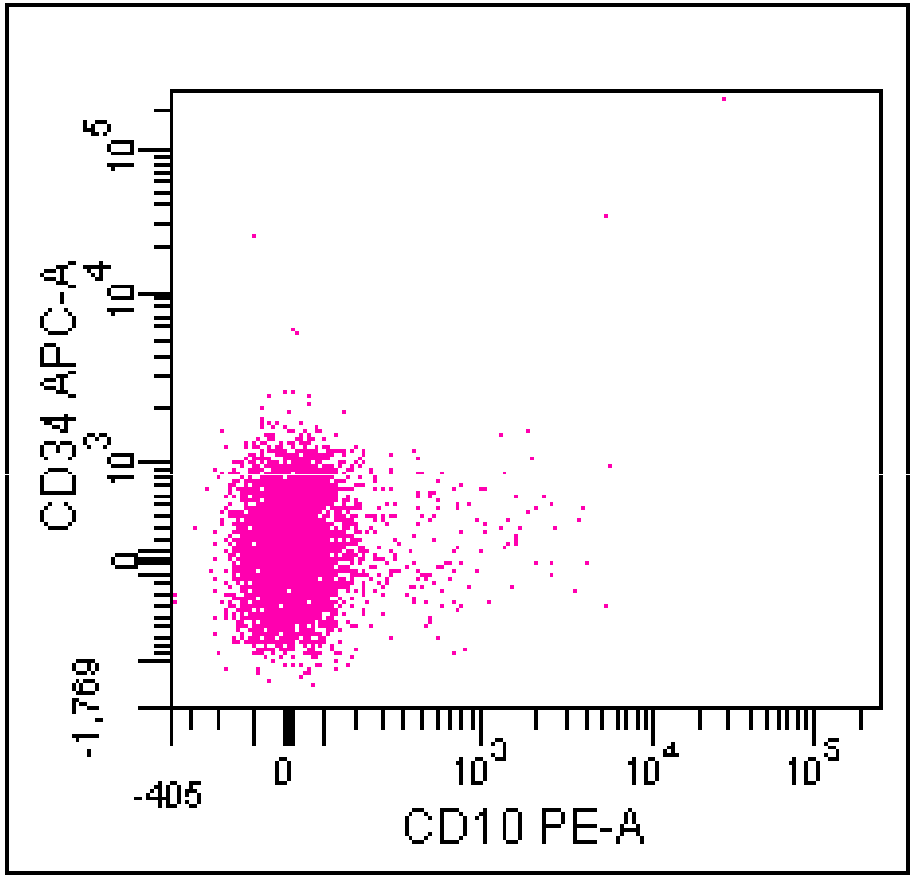
The differential reconsidered

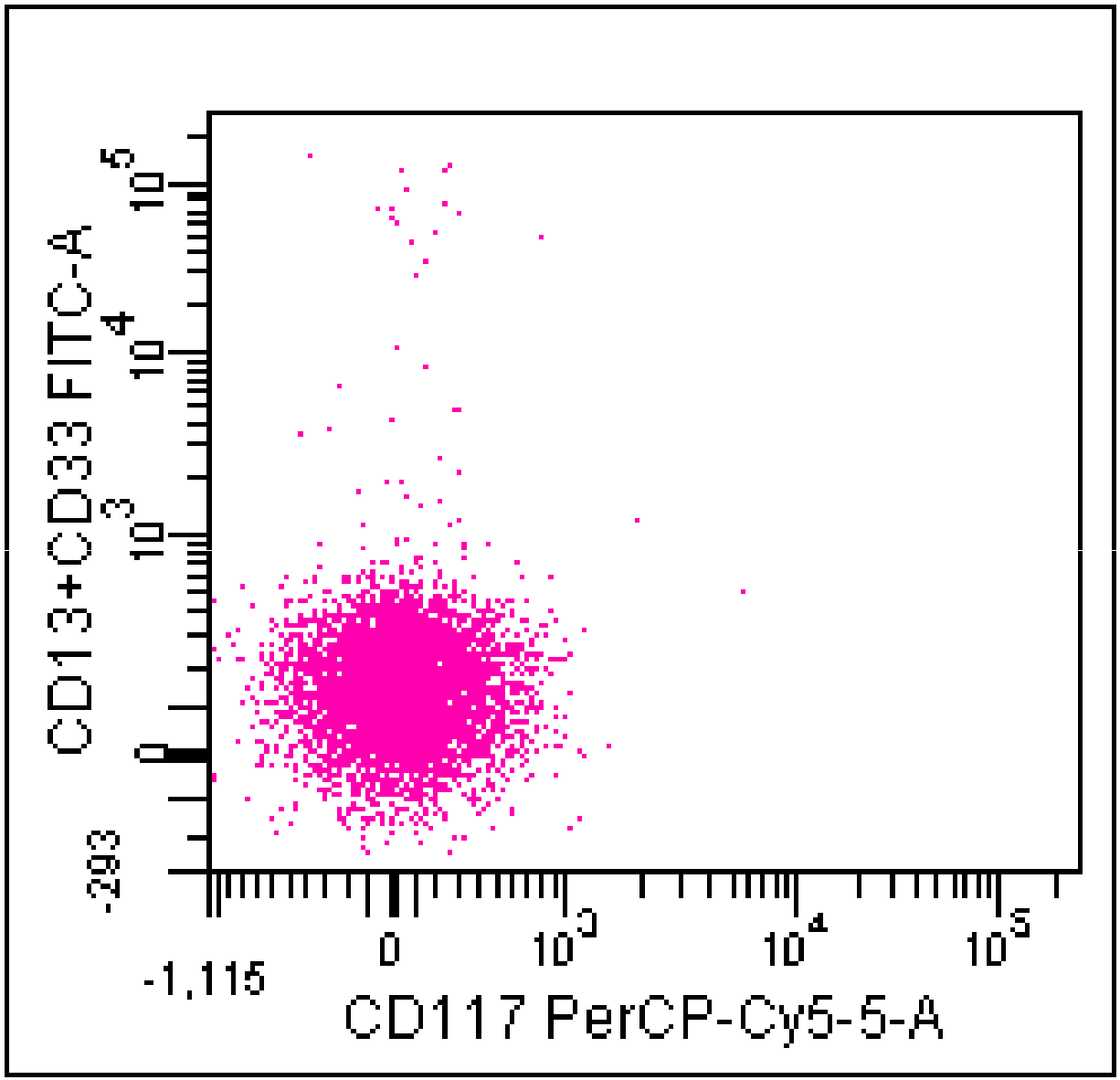
- T-lymphoblastic lymphoma

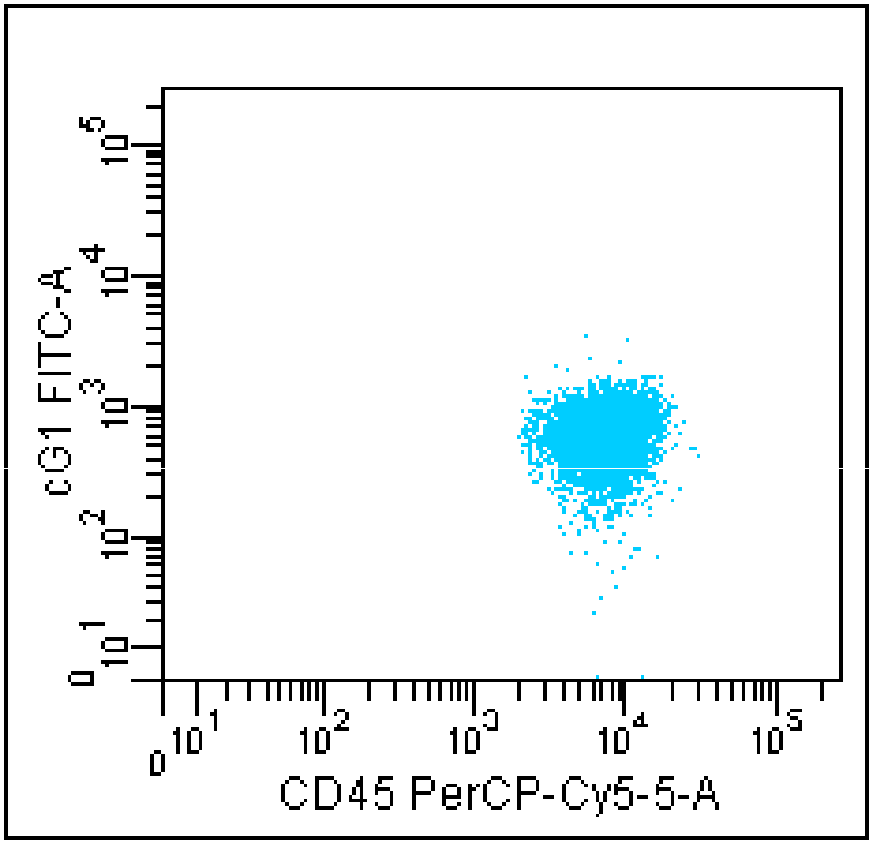
- Anaplastic large cell lymphoma, ALK positive
  - Primarily affects those in first three decades of life
  - Extranodal involvement common
    - Skin, bone, soft tissues, lung, liver
    - Mediastinal disease is less frequent
  - Pleomorphic cytology
  - Immunophenotypic profile (CD30, ALK positive)
    - Surface and cytoplasmic CD3 negative in 75% cases
    - CD2, CD5, CD4 positive in 70% cases
  - t(2;5)(p23;q35) translocation [NPM-ALK]
  - Favorable prognosis

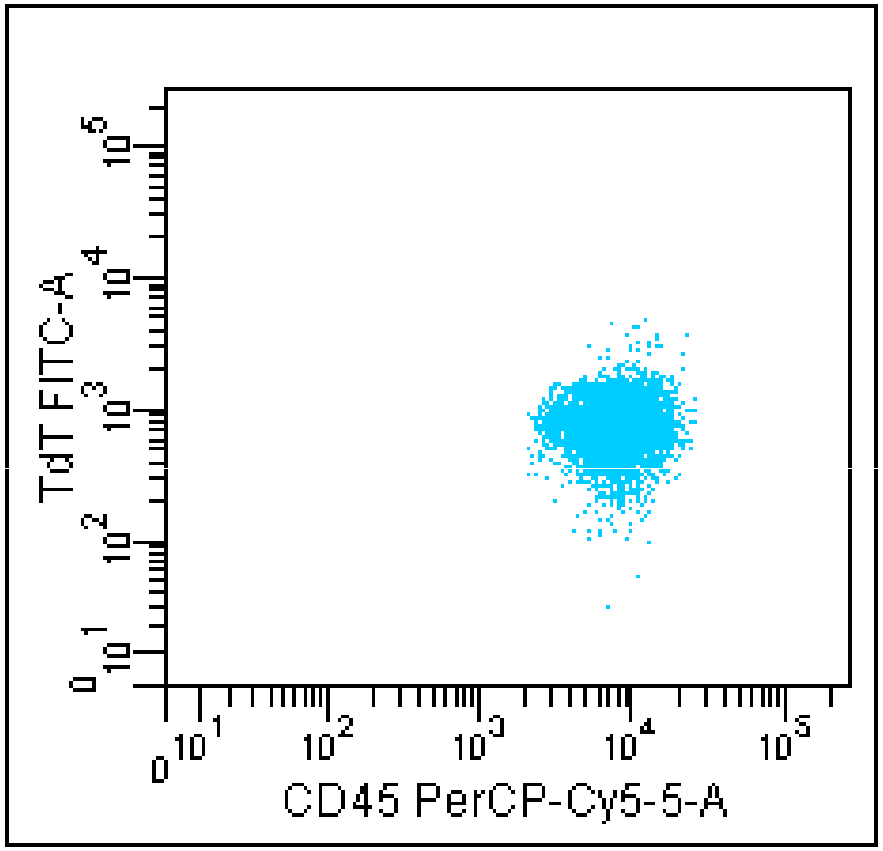
Bonzheim, *Blood*, 2004; Benharroch, *Blood*, 1998


CD3 APC-A
-557
0
10^3
10^4
10^5
-929
0
10^3
10^4
10^5
CD56 PE-A

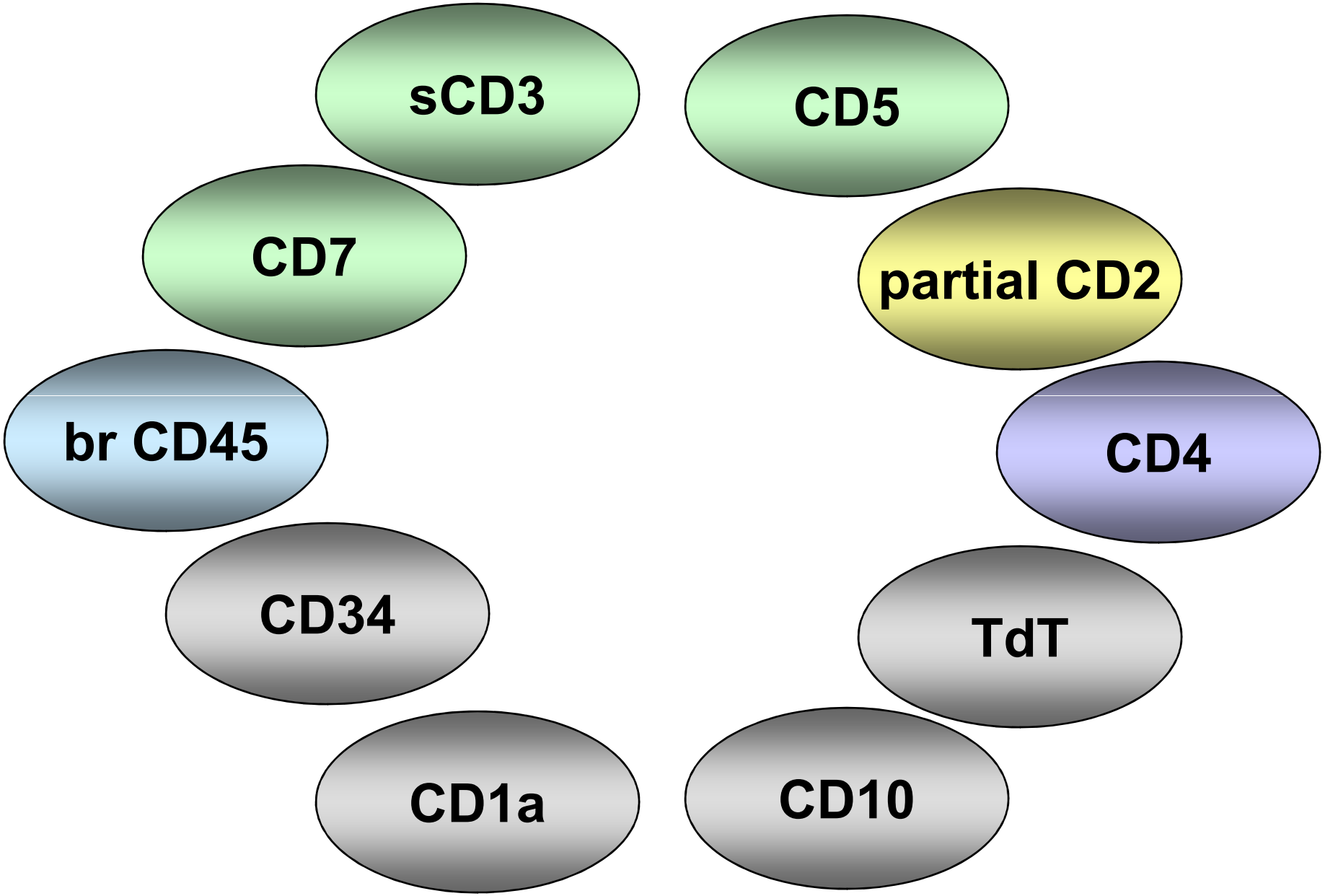

CD34 APC-A
-1,769
0
10^3
10^4
10^5
-405
0
10^3
10^4
10^5
CD10 PE-A


CD13+CD33 FITC-A
-293
0
10^2
10^4
10^5
-1,115
0
10^3
10^4
10^5
CD117 PerCP-Cy5-5-A


cG1 FITC-A
CD45 PerCP-Cy5-5-A
0
10^1
10^2
10^3
10^4
10^5


TdT FITC-A
CD45 PerCP-Cy5-5-A
0
10^1
10^2
10^3
10^4
10^5

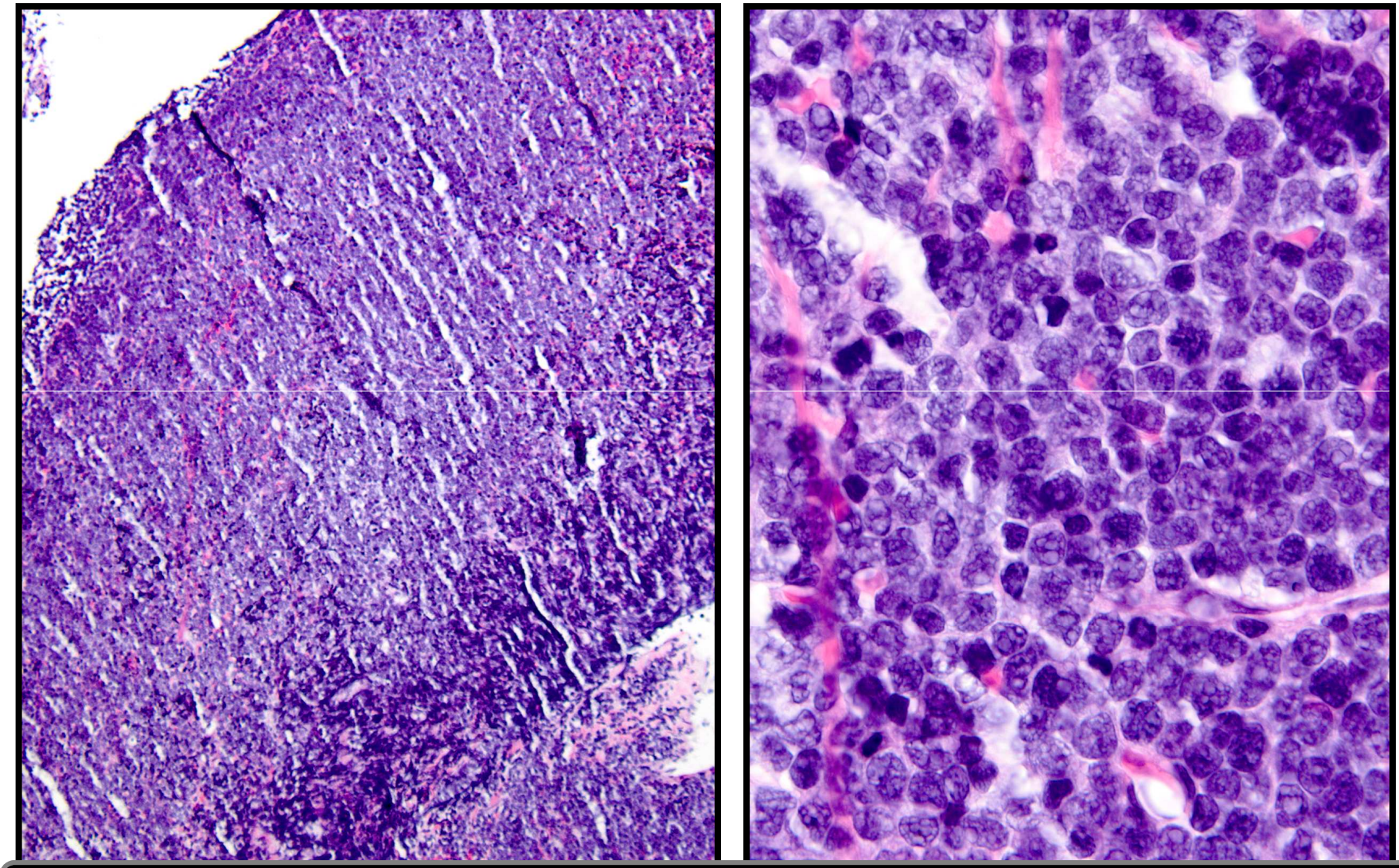
Immunophenotypic summary



Core needle biopsy

- Histology – second assessment
- CD30, ALK
- Artifacts secondary to pleural fluid degeneration?

Morphology



Identical immunophenotypic profile

T-lymphoblastic lymphoma/leukemia

- 15% of pediatric and 25% of adult lymphoblastic leukemias
  - More common in adolescents and in males
- Acute or insidious onset
  - Rapidly growing mediastinal mass with pleural effusions
  - Relative sparing of bone marrow
- <5% TdT negative, rarely TdT *and* CD34 negative
  - 15% express myeloid antigens (CD13 and CD33)
    - not considered biphenotypic (eg. myeloperoxidase positivity)
    - not adverse prognosticator

Han, *AJCP*, 2007

Disease stratification: maturational stages

	CD						
	34	3	7	2	1a	4	8
Pro-T	+/-	cyto	+	-	-	-	-
Pre-T	+/-	cyto	+	+	-	-	-
Cortical T	-	cyto	+	+	+	+	+
Medullary T	-	surf	+	+	-	+ OR -	- OR +

WHO Classification, *Lyon* 2008

Significance of stratification?

- Overall 5 year event-free survival ~75%
- High risk of induction failure, early relapse and isolated CNS relapse
- Medullary (mature) stage of unclear significance
- Strong CD2 expression appears to be favorable prognosticator

Degree of CD2 expression	*6 year EFS*
Low (<30% positivity)	52.8%
Intermediate	65.5%
High (>75% positivity)	71.9%

[Goldman, J Clin Oncol, 2003; Uckun, J Clin Oncol, 1997; Uckun, Blood, 1996; Crist, Blood 1988]

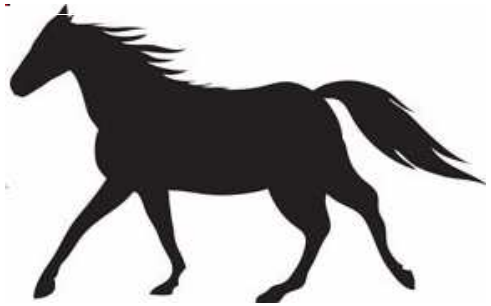
Final diagnosis

T-lymphoblastic lymphoma,

medullary T-cell type

Difficulties

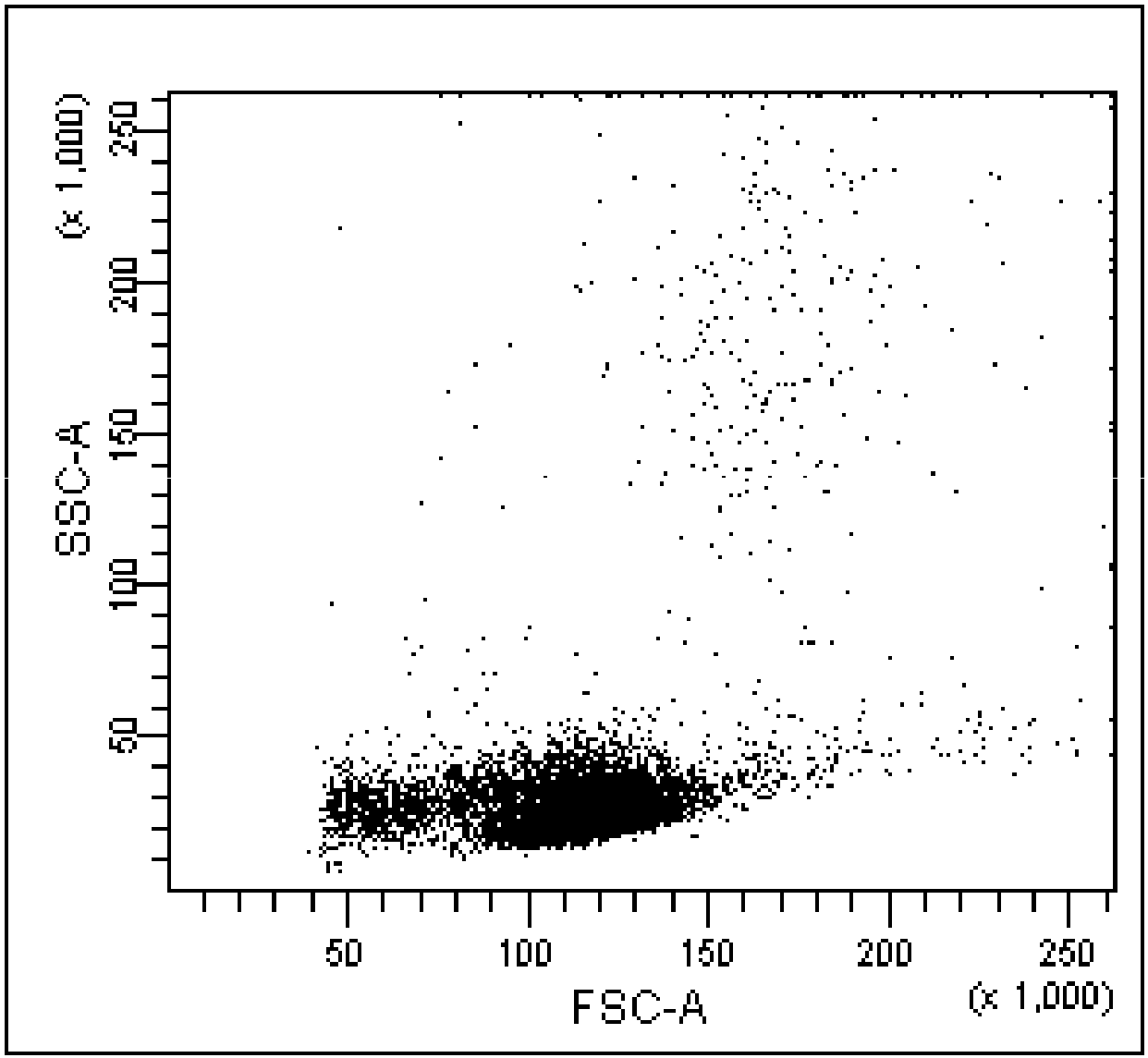
- Clinical history is essential
- An old adage …

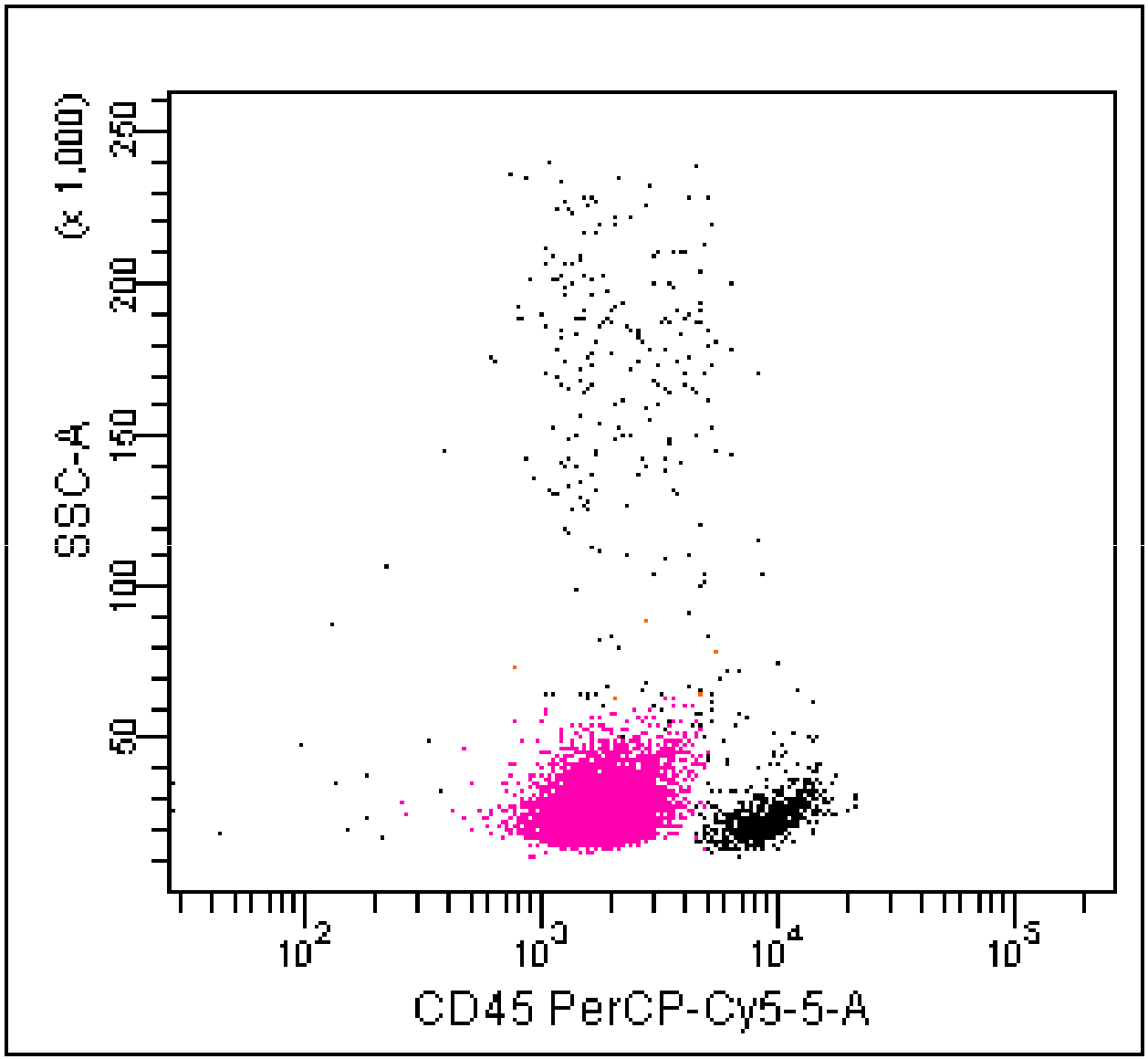


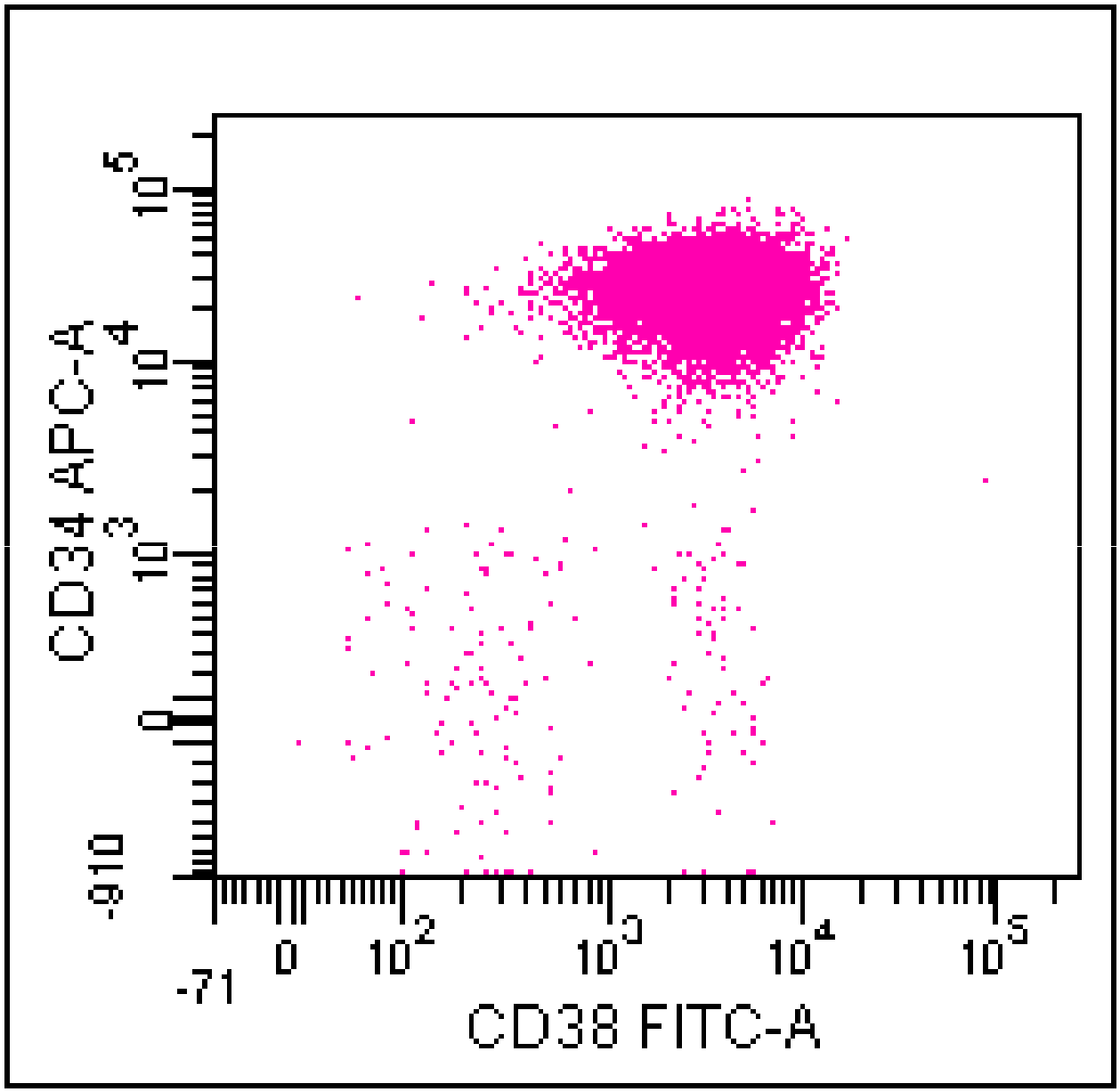
- Importance of tissue site/specimen

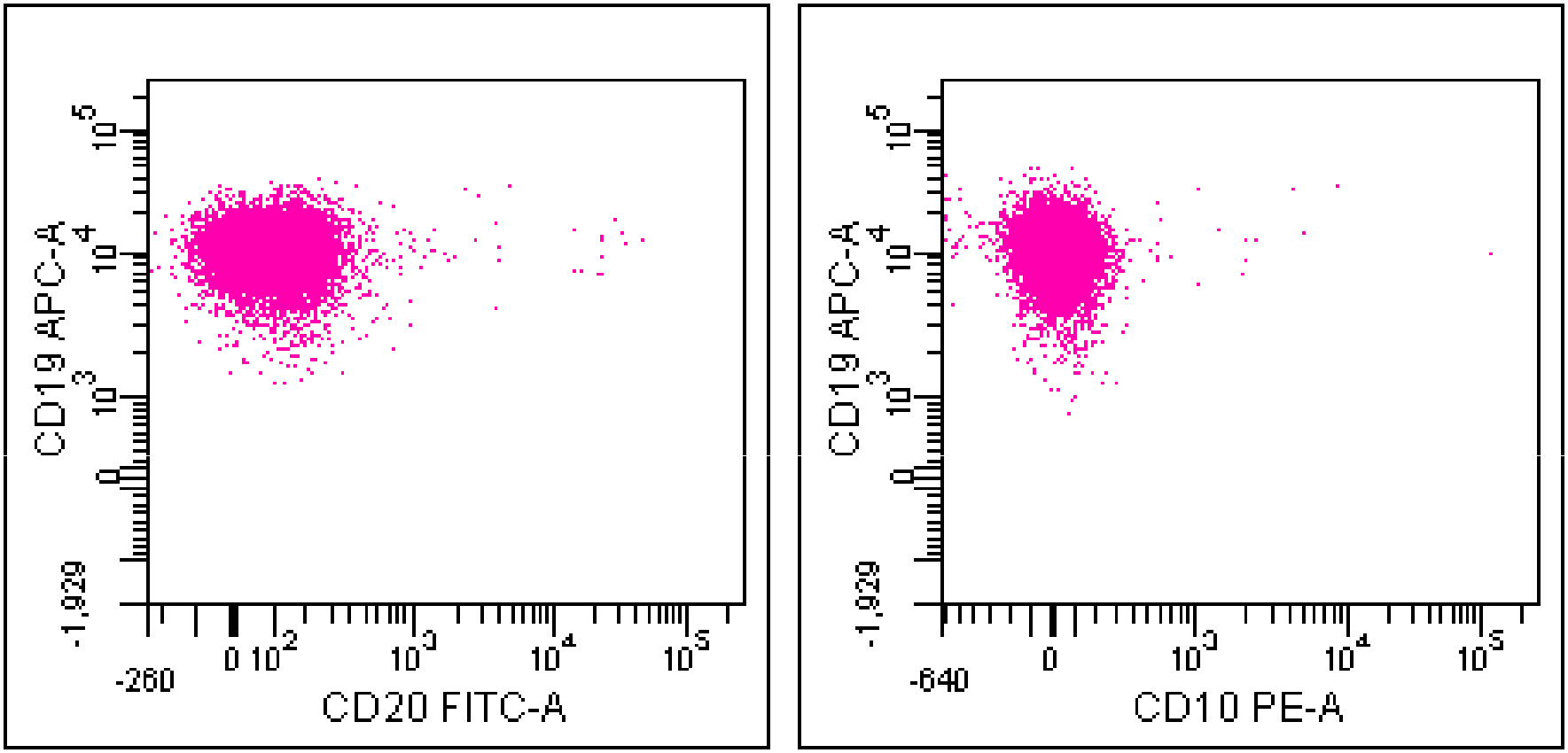
Case 3

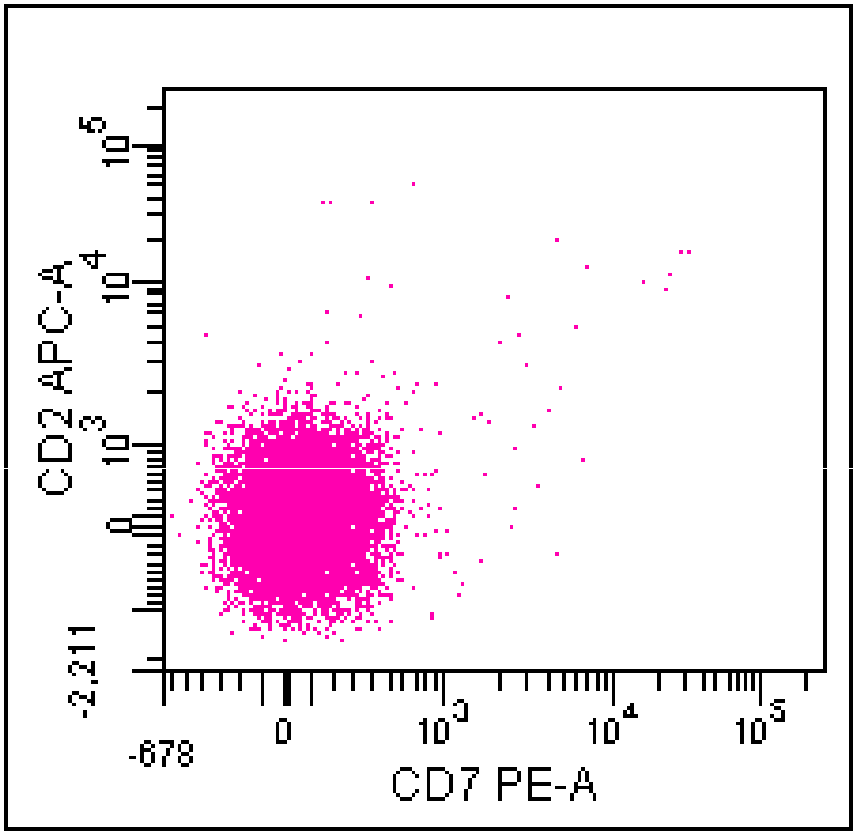
Peripheral blood analysis

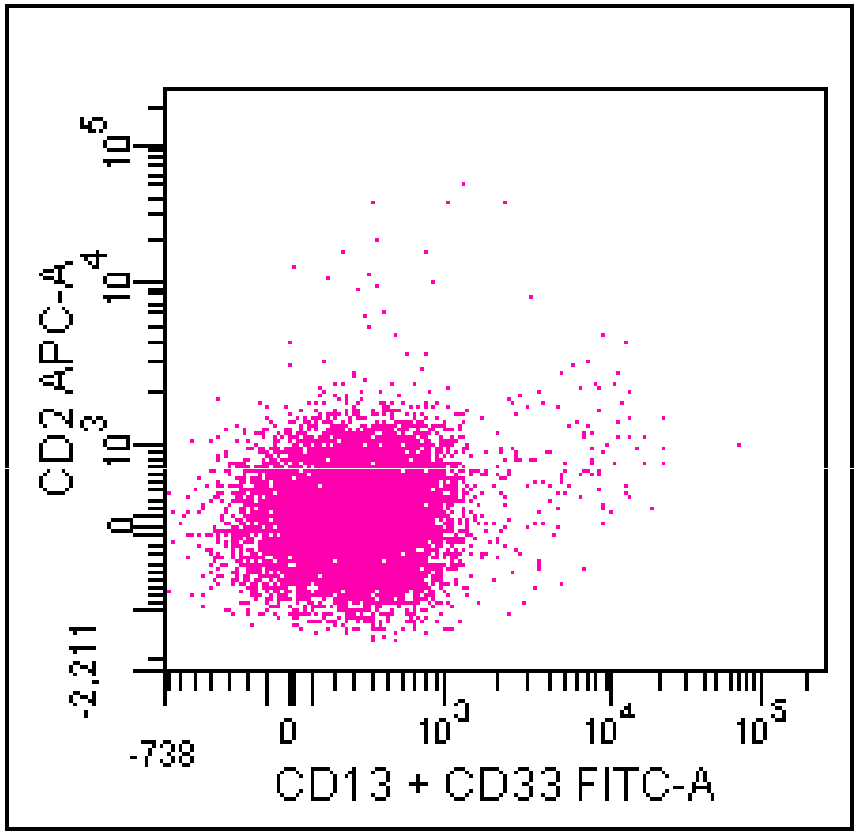


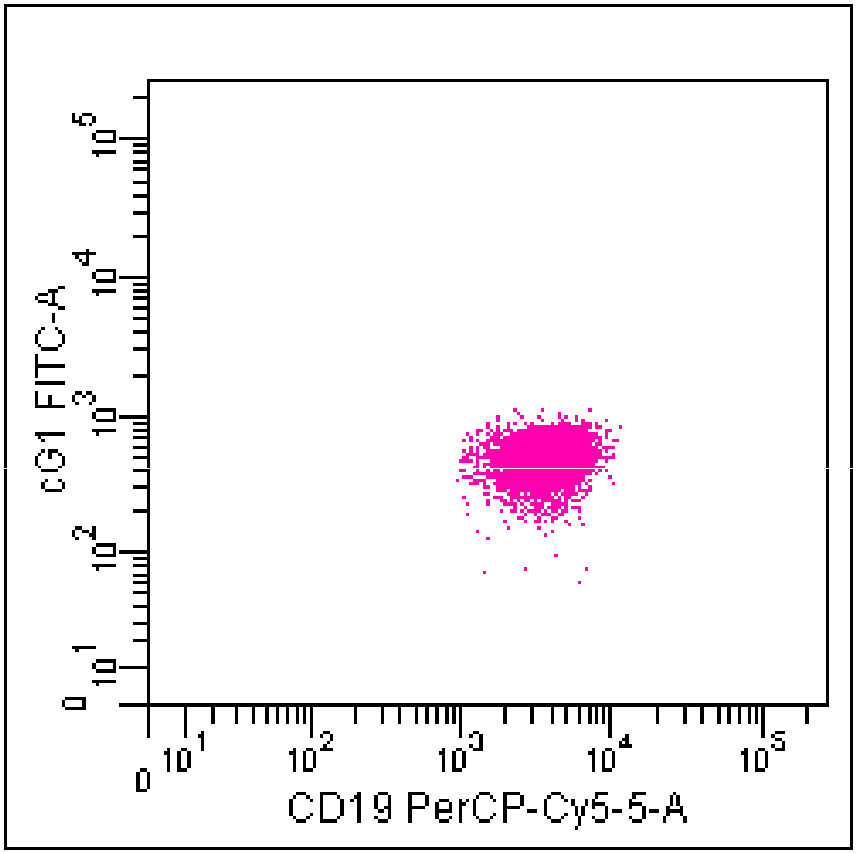

SSC-A (x 1,000)
250
200
150
100
50
10^2
10^3
10^4
10^5
CD45 PerCP-Cy5-5-A

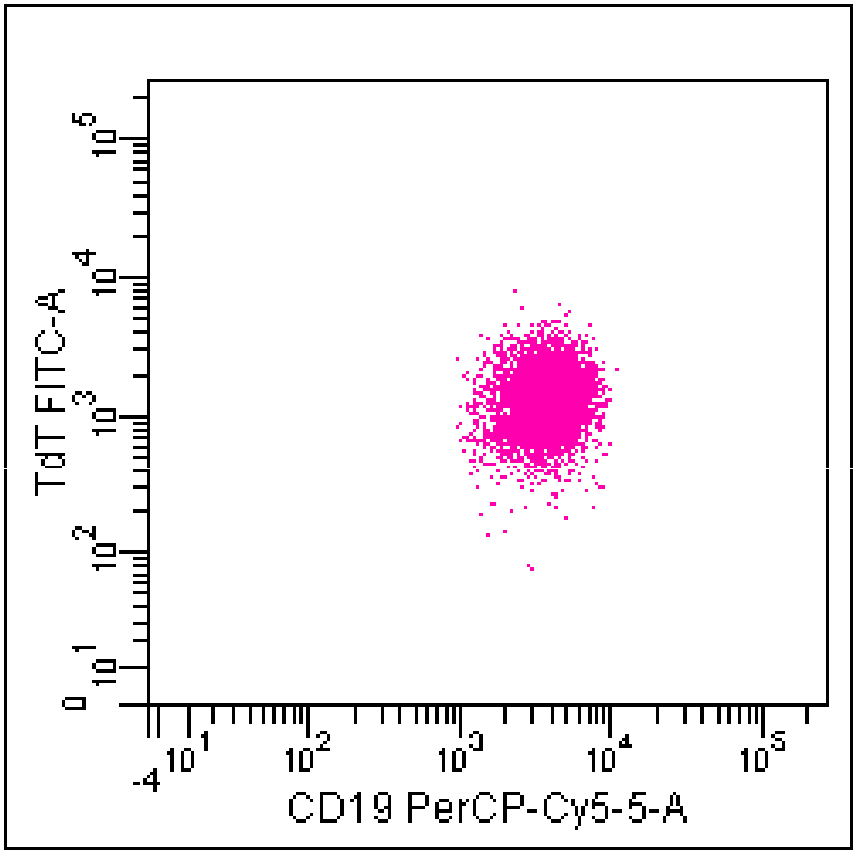

CD34 APC-A
-910
0
10^3
10^4
10^5
-71
0
10^2
10^3
10^4
10^5
CD38 FITC-A

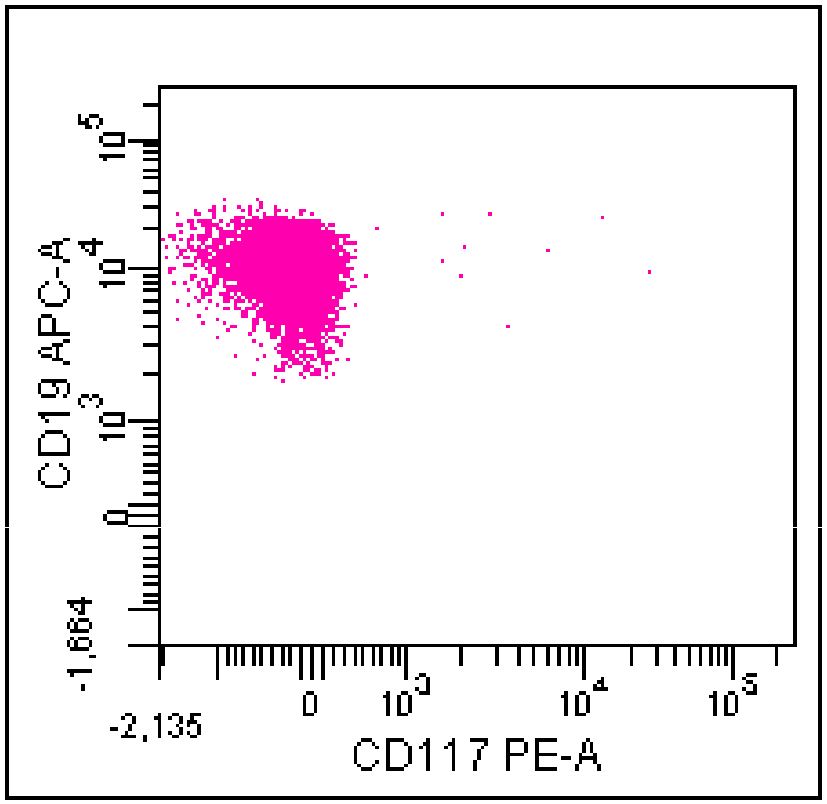

CD19 APC-A
-1,929
0
10^3
10^4
10^5
-260
0
10^2
10^3
10^4
10^5
CD20 FITC-A
CD19 APC-A
-1,929
0
10^3
10^4
10^5
-640
0
10^3
10^4
10^5
CD10 PE-A

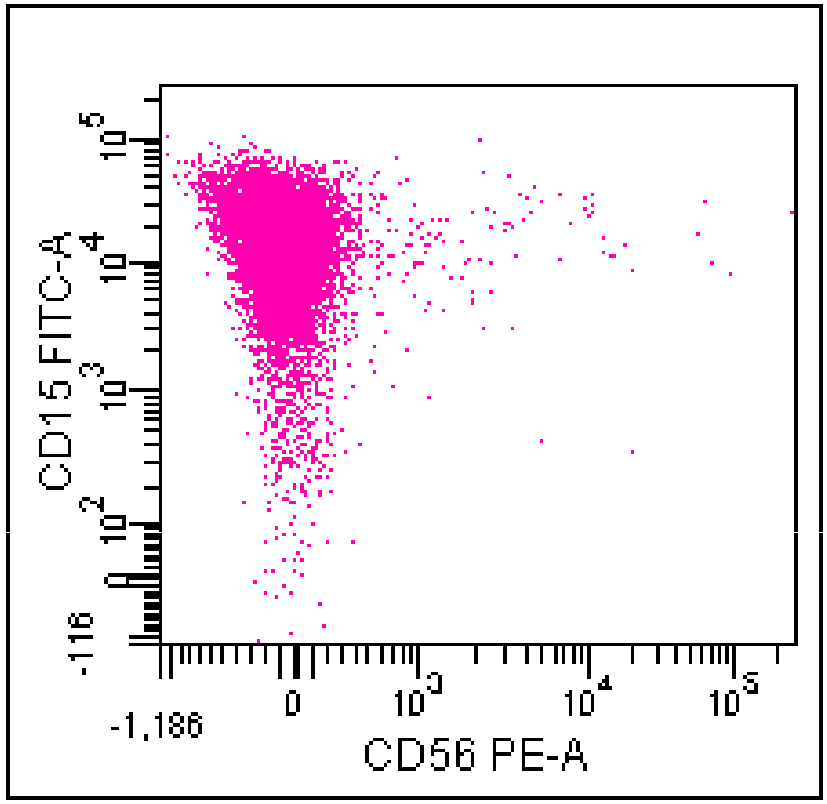

CD2 APC-A
-2,211
0
10^3
10^4
10^5
-678
0
10^3
10^4
10^5
CD7 PE-A


CD2 APC-A
-2,211
0
10^3
10^4
10^5
-738
0
10^3
10^4
10^5
CD13 + CD33 FITC-A


cG1 FITC-A
CD19 PerCP-Cy5-5-A
0
10^1
10^2
10^3
10^4
10^5


TdT FITC-A
CD19 PerCP-Cy5-5-A
0
-4
10^1
10^2
10^3
10^4
10^5



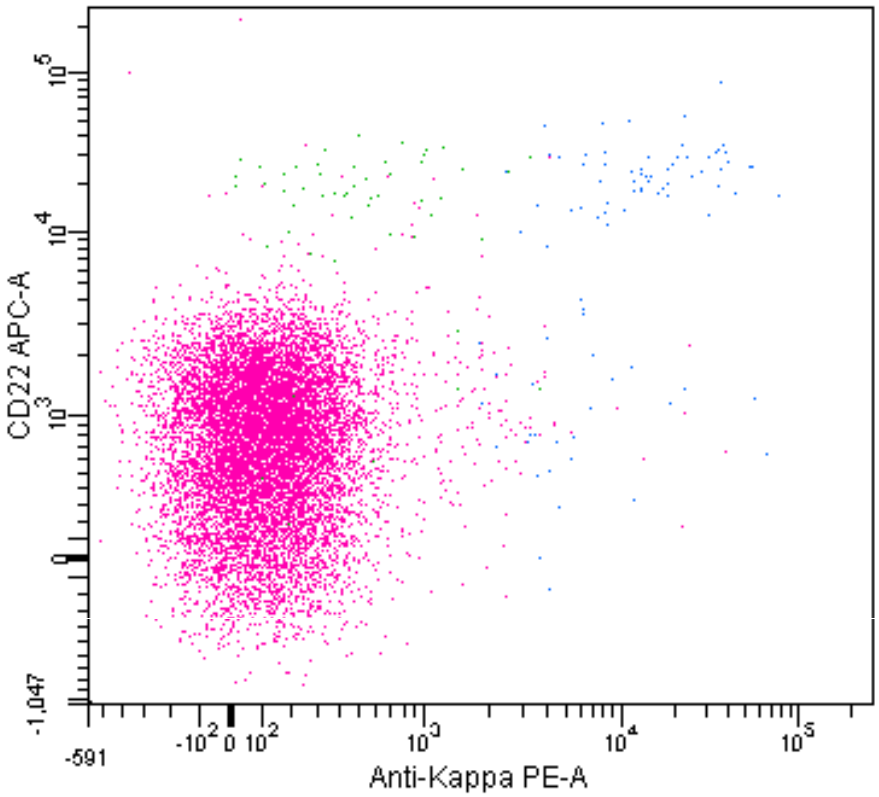


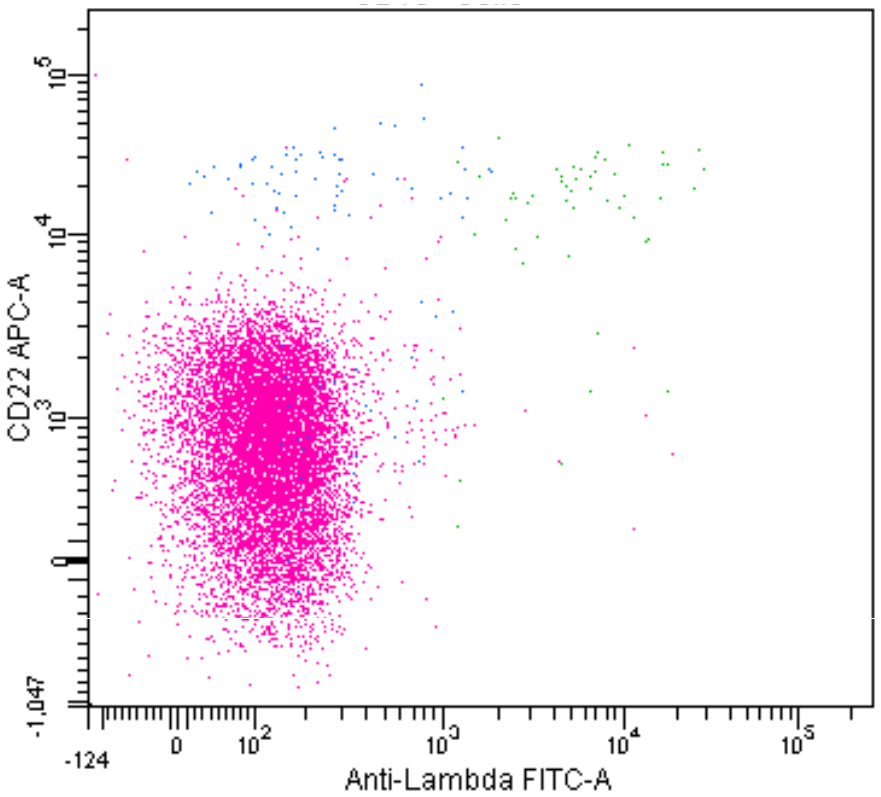
CD64, CD14 negative

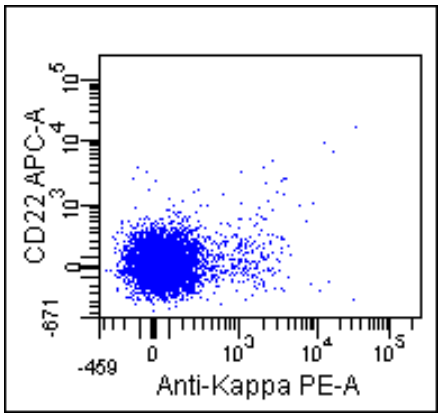
TdT and CD19 in AML

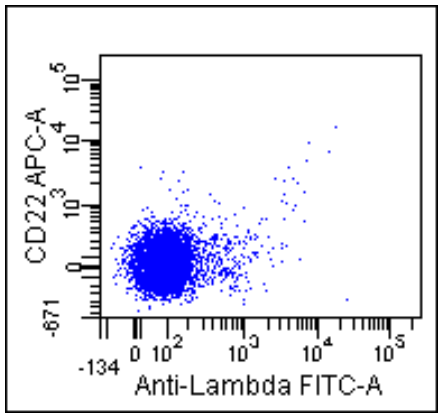
- TdT positivity in 25% of AMLs
- CD19 expressed in <10% pediatric AMLs
  - Increased positivity in association with t(8;21)
- CD3, CD5 and CD10 detected in <5% AMLs

Venditti, *Leukemia*, 1998; Khalidi, *AJCP*, 1998; Drexler, *Leukemia*, 1993


CD22 APC-A
Anti-Kappa PE-A
-1,047
-591
0
10^2
10^3
10^4
10^5


CD22 APC-A
Anti-Lambda FITC-A
-1,047
-124
0
10^2
10^3
10^4
10^5

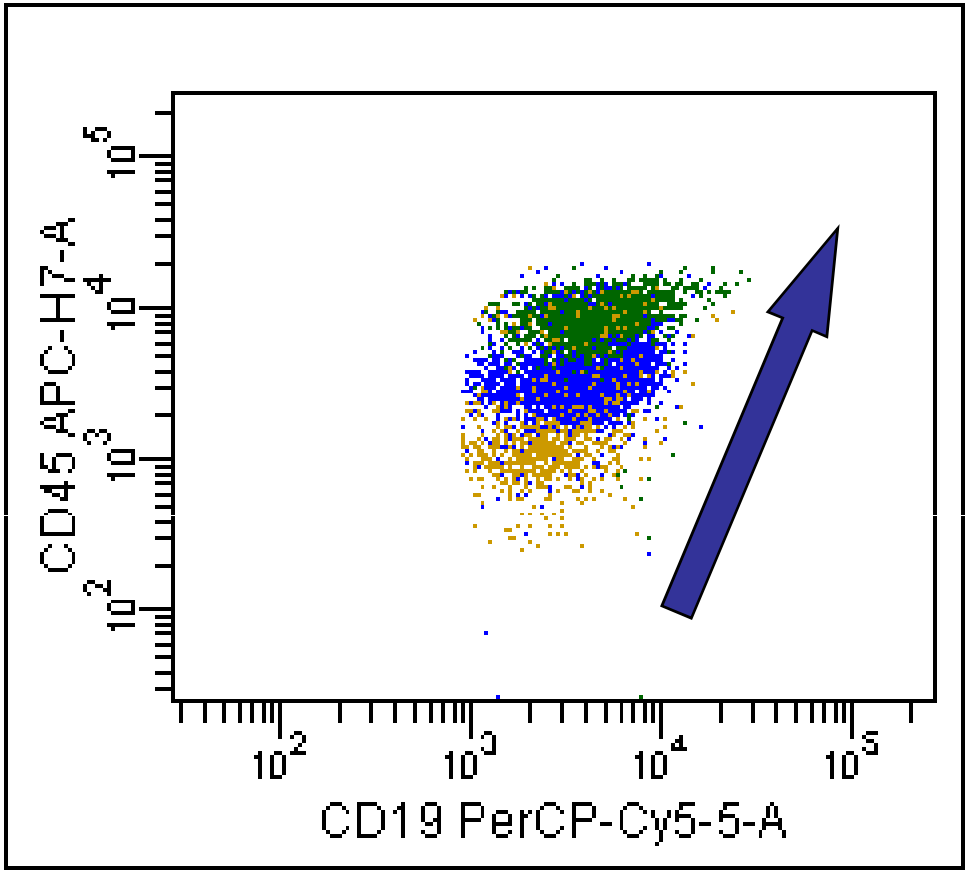

CD22 APC-A
Anti-Kappa PE-A
-671
-459
0
10^3
10^4
10^5


CD22 APC-A
Anti-Lambda FITC-A
-671
-134
0
10^2
10^3
10^4
10^5

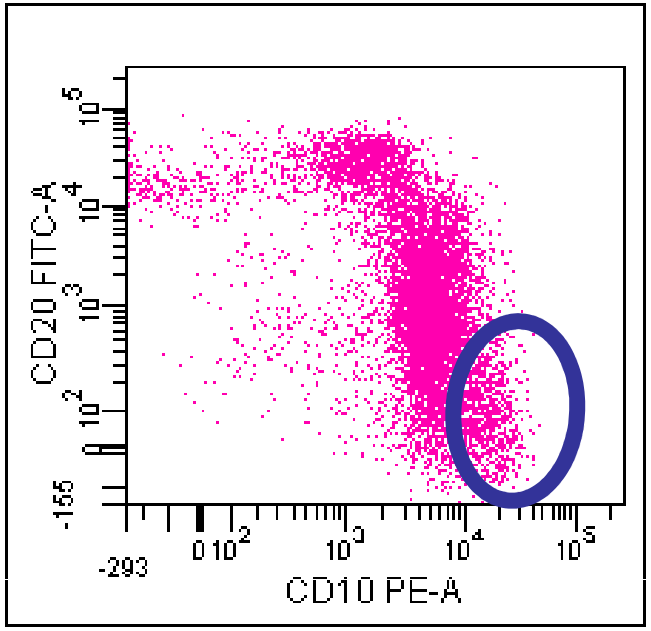
Sequence of B-cell maturation in marrow

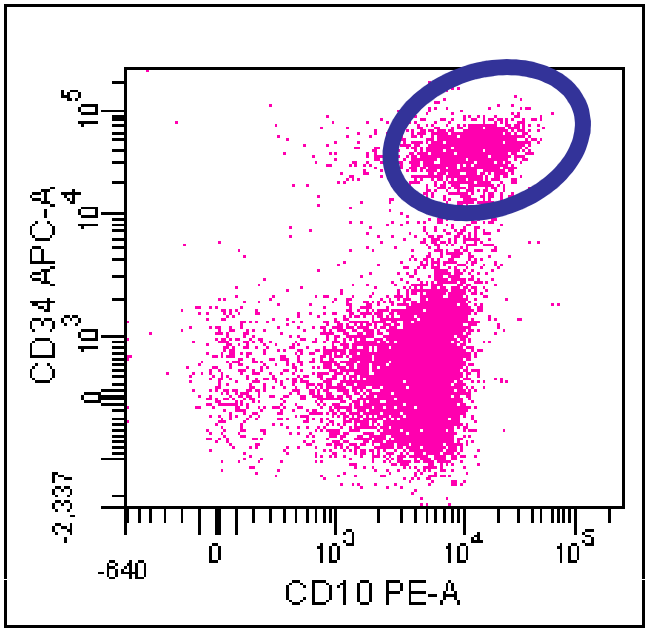
		CD							
		TdT	34	10	19	22	38	20	sIg
Hematogones (stages)	1	+	+	+++	+	dim	bright		
	2			+	+	dim	bright		-
				+	+	dim	bright	dim	var
	3			+	+	dim	bright	+	var
Mature B-cells					+	+	bright to neg	+	+

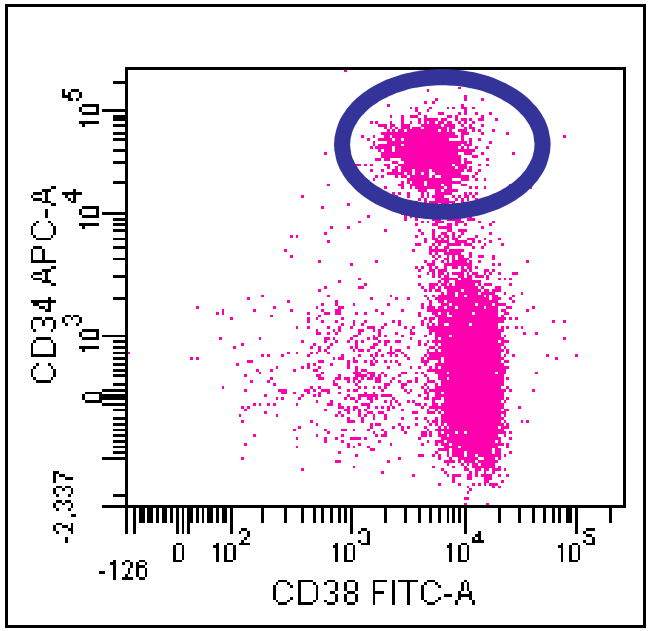
McKenna, *Leukemia & Lymphoma*, 2004

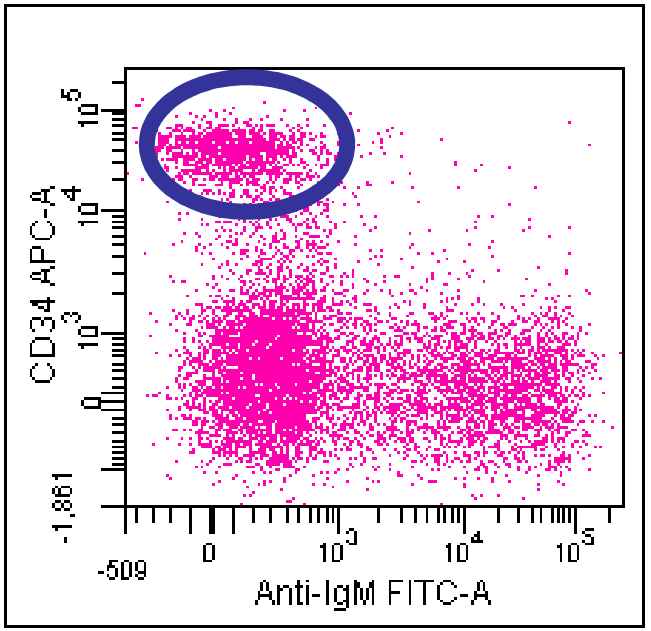

CD45 APC-H7-A
10^2
10^3
10^4
10^5
10^2
10^3
10^4
10^5
CD19 PerCP-Cy5-5-A

Stage 1 hematogones

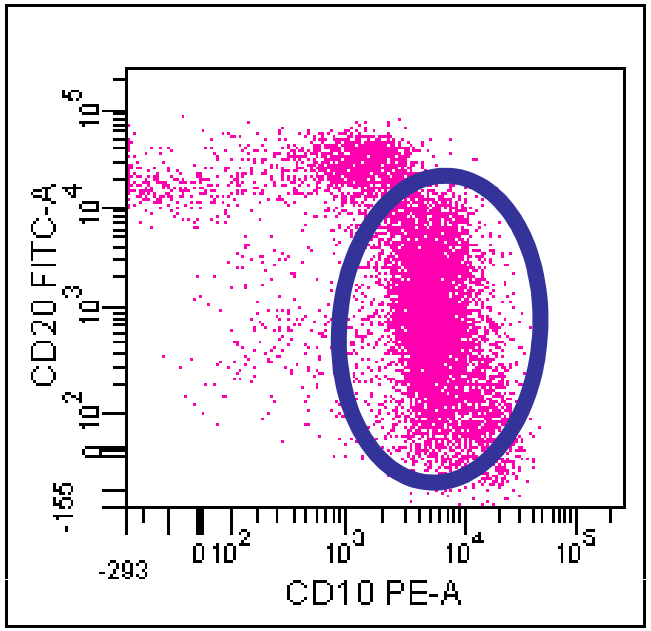


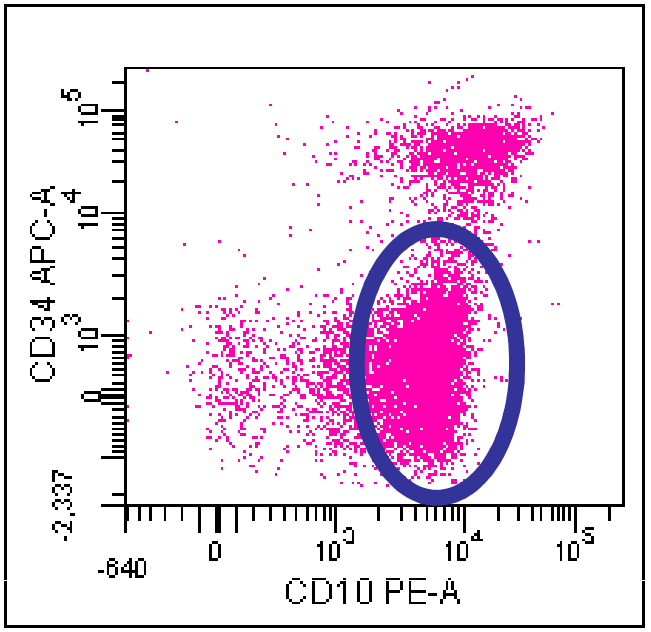


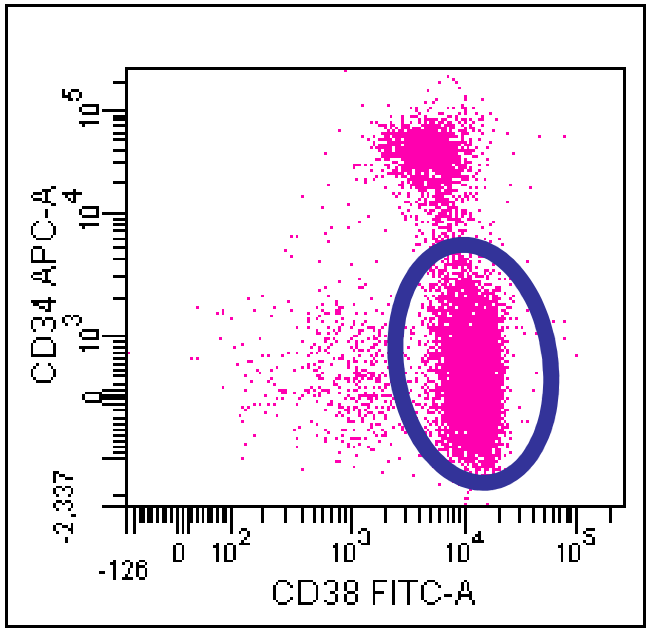


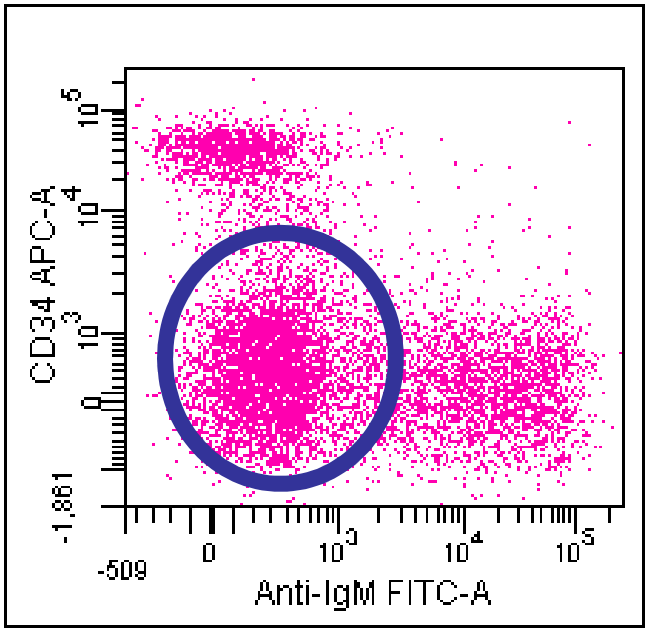


Stage 2 hematogones

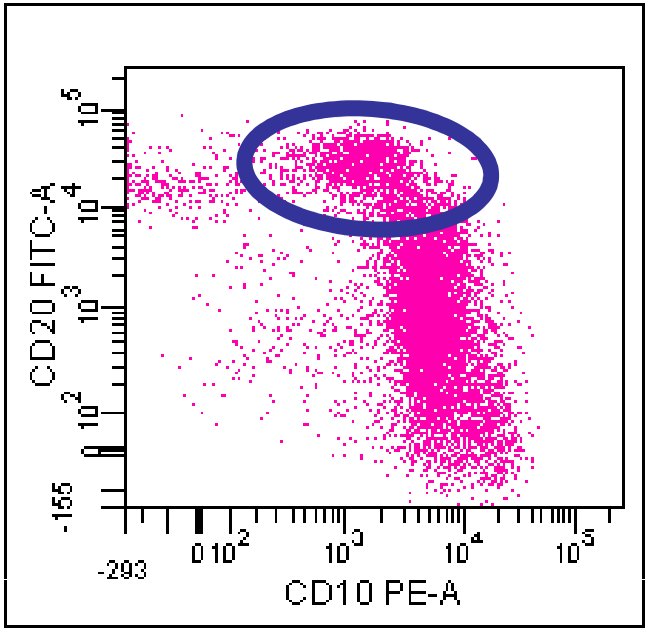


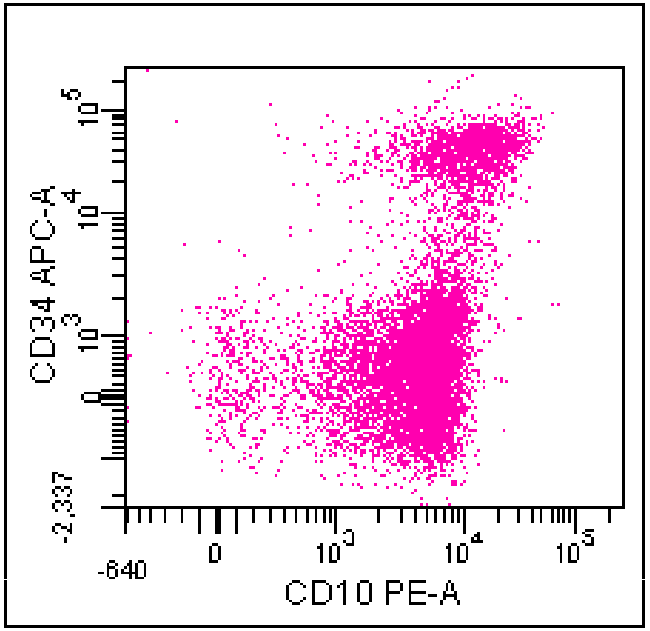


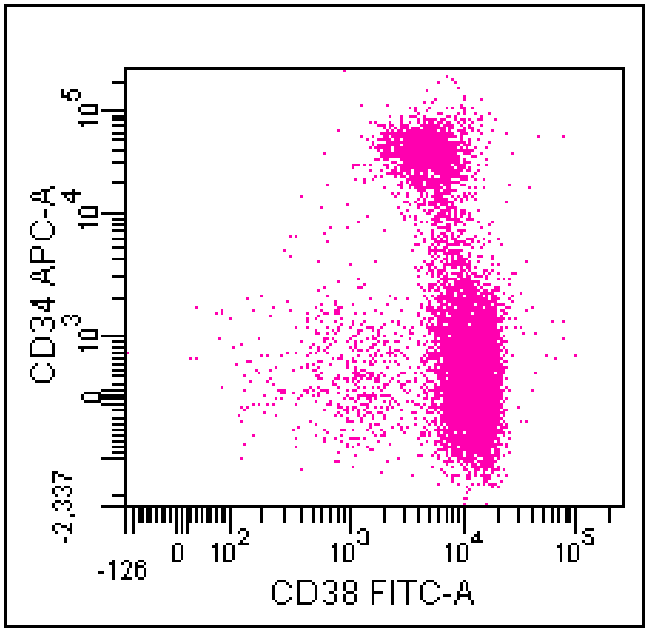


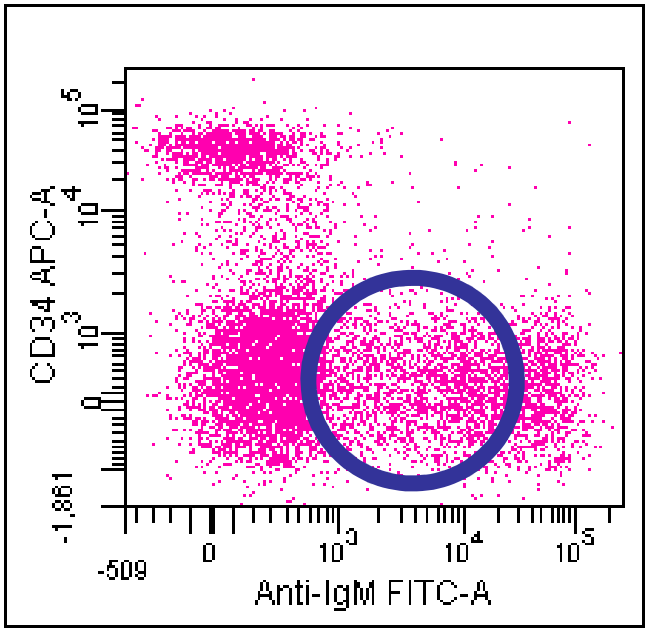


Stage 3 hematogones



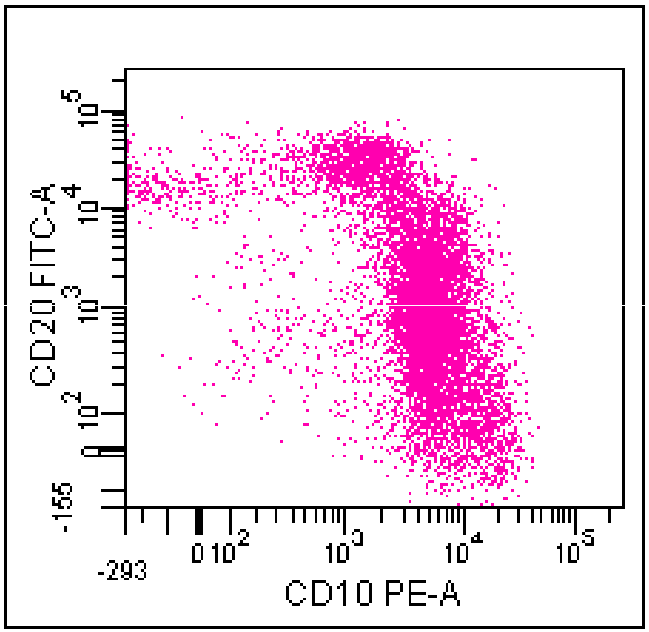




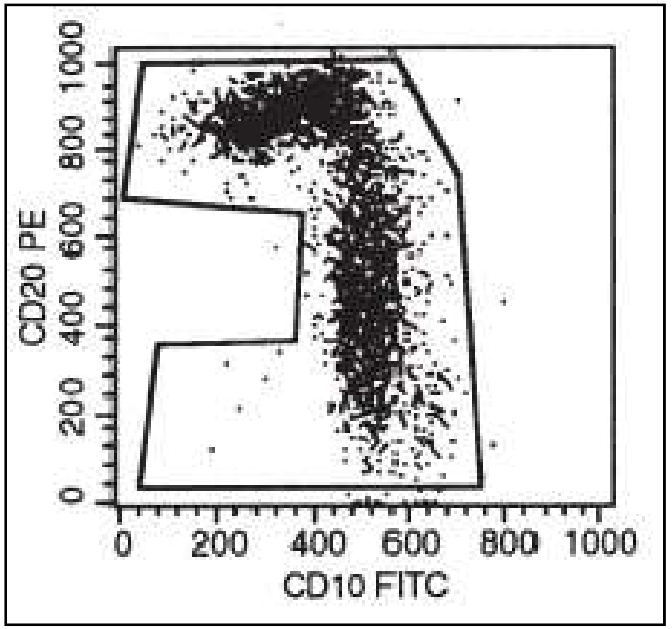


Mature B-cells

Pediatric



Adult



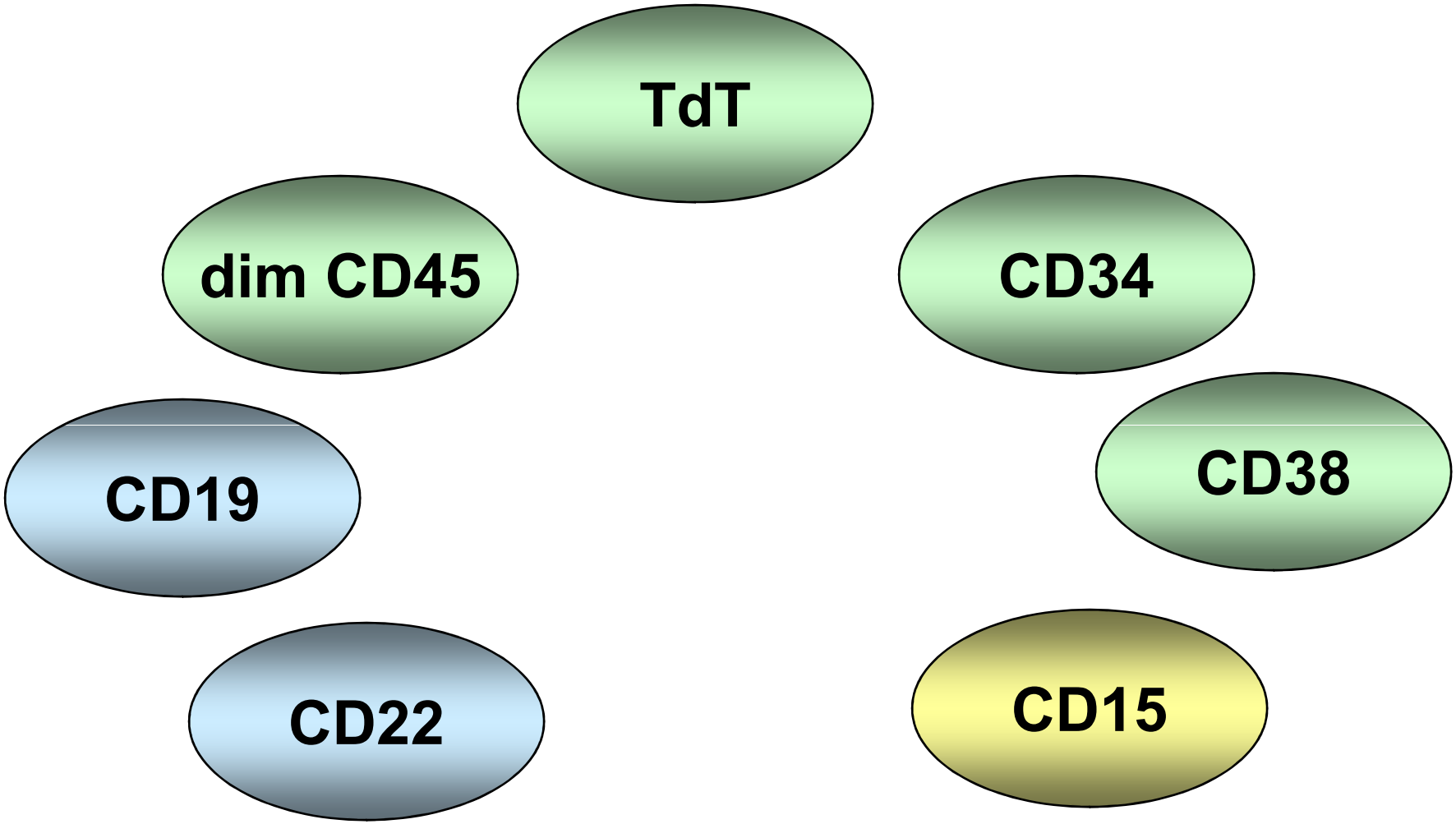
Weir, *Leukemia*, 1999

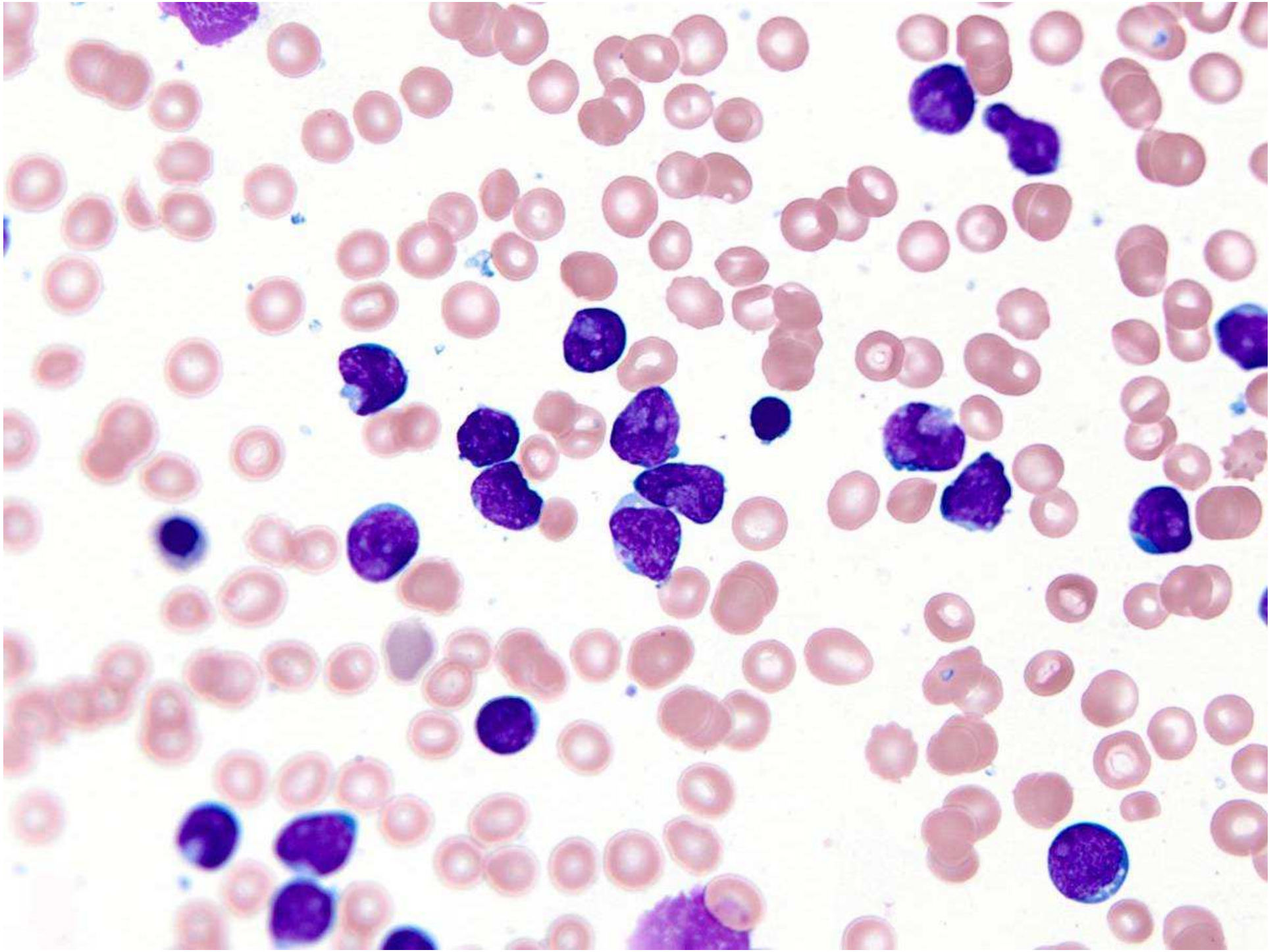
Hematogones

- Sequence and intensity of antigen expression is virtually identical in all individuals
- Most abundant in marrows of infants and young children (10-15%), adults typically <5%
- Exhibit a spectrum of sizes and cytologic features
- Increased in regenerating marrows, autoimmune disorders, solid tumors
- Rarely detected in blood and reactive lymph nodes

McKenna, *Leukemia & Lymphoma*, 2004

Immunophenotypic summary





B-lymphoblastic leukemia with t(4;11)(q21;q23)

Immunophenotypic characteristics:

Represents an early precursor ("pro-B") cell

CD10 negative

- *CALLA* = common acute lymphoblastic leukemia antigen)
- Expressed in >90% of childhood and ~75% of adult B-lymphoblastic leukemias

Myeloid coexpression (typically CD15 positive)

Classic immunophenotypic profile:

$CD45^{dim}$, TdT^{pos}, $CD34^{pos}$, $CD19^{pos}$, $CD22^{neg/dim}$, $CD20^{neg}$, $CD10^{neg}$, cyt IgM^{neg}, $CD15^{dim}$, mostly $CD13^{neg}$, $CD33^{neg}$, $CD9^{pos}$

B-lymphoblastic leukemia with t(4;11)(q21;q23)

Clinical manifestations:

Hyperleukocytosis (median WBC > 150 x10^9/L)

Hepatosplenomegaly

CNS involvement

Pediatric leukemia

- Leukemias are the most common cancers affecting children, representing ~30% of all cancers in those under 15 years of age.

- In the United States, 75% of pediatric leukemias are lymphoblastic leukemia, 15-20% are acute myeloid leukemia (AML), and 5% are chronic myeloid leukemia.

- Infantile ALL (that diagnosed within the first 12 months of life) represents ~2.5-5.0% of pediatric ALL.

Infantile ALL and MLL

- Rearrangements in chromosomal band 11q23, involving the mixed lineage leukemia [MLL] gene, are common in infantile ALL
  - Occurring in ~70% of cases

- Its presence is inversely correlated with age:
  - >90% in those less than 6 months
  - ~50% in those 6 – 12 months
  - ~6-7% in those 12-24 months

- Abnormalities of MLL include deletions, inversions and unbalanced as well as reciprocal translocations
  - Many different translocation partners (greater than 70) have been described

- The most common translocation is t(4;11)(q21;q23), occurring in 30-45% of infants.

Clinical history

- 8 year old female with fever and bruising on extremities

- WBC 120 K/ul, HGB 9.4 g/dl, PLT 86 K/ul

Infantile ALL with t(4;11)(q21;q23) - prognosis

- Very poor
- Long-term rates of event-free survival (EFS) of 28-45%
  - Lower than EFS in older children with ALL, which is ~80%
- Relapses occur very early
  - Typically within the first 2 years of diagnosis
- Therapeutic approaches are also controversial
  - Intensified chemotherapy and hematopoietic stem cell transplantation
- Reasons for poor outcome not well understood

Karyotypic findings

45,XX,t(4;11)(q21;q23)[18] / 46,XY[2]

Additional clinical history

- Diagnosed with MLL-associated ALL at 4 months of age

- In remission for 3 yrs, then relapsed, underwent re-induction, and was subsequently transplanted with unrelated allogeneic marrow from a male [XY] donor

MLL = mixed lineage leukemia gene [11q23]

- Associated with ALL, AML and therapy-related myeloid neoplasms (as its name implies) in both the pediatric and adult populations.

- MLL rearrangements occur in 30-60% of infants with AML
  - t(9;11)(p22;q23) is the most common translocation in this age group
  - t(11;19)(q23;q13.3) is the next most frequent

- MLL-associated AML is most commonly monoblastic and frequently presents with extramedullary infiltrates

Final diagnosis

Relapsed

t(4;11) associated B-lymphoblastic leukemia

occurring after stem cell transplant

in an 8 year old

Difficulties

- Phenotype predicts genotype
  - Helps prognosticate at the outset
  - Marker for recurrent disease

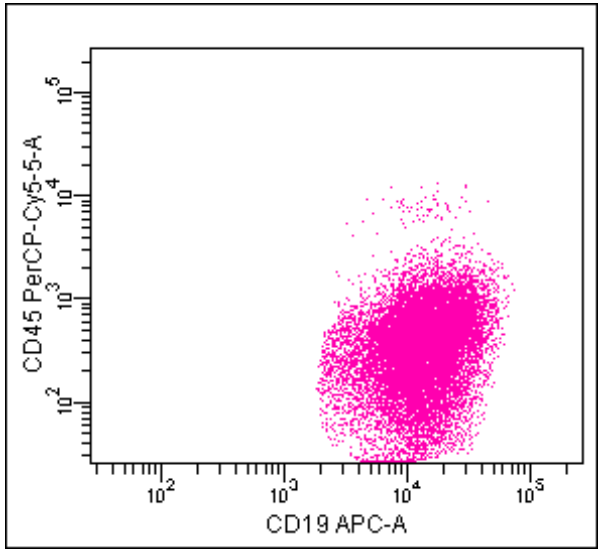
- Caution
  - MLL = mixed lineage leukemia
  - lineage can shift over time or with disease relapse

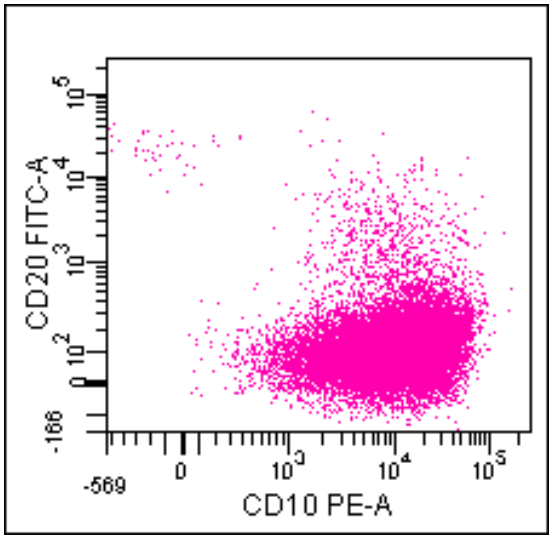
Case 4

Clinical history

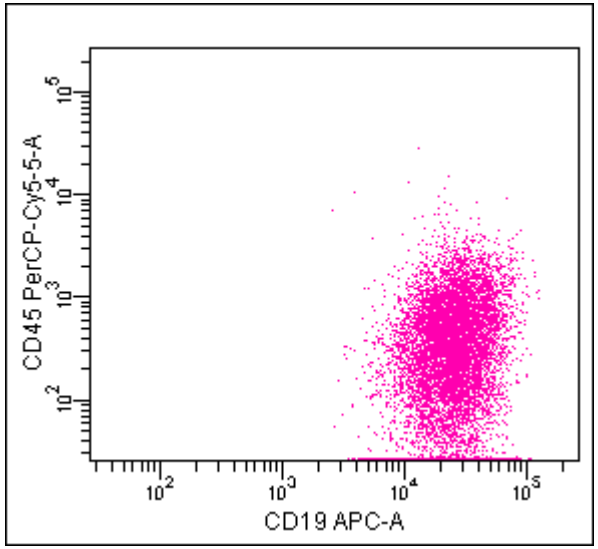
- 6 year old female with history of standard risk B-lymphoblastic leukemia (diagnosed 2 yrs prior)
- Treatment course complicated by pancreatitis
- Presents for end of treatment marrow evaluation
- Clinically stable with appropriate counts

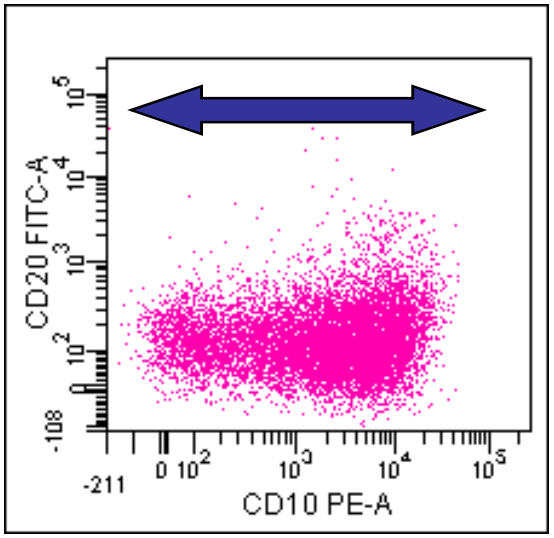
Diagnostic



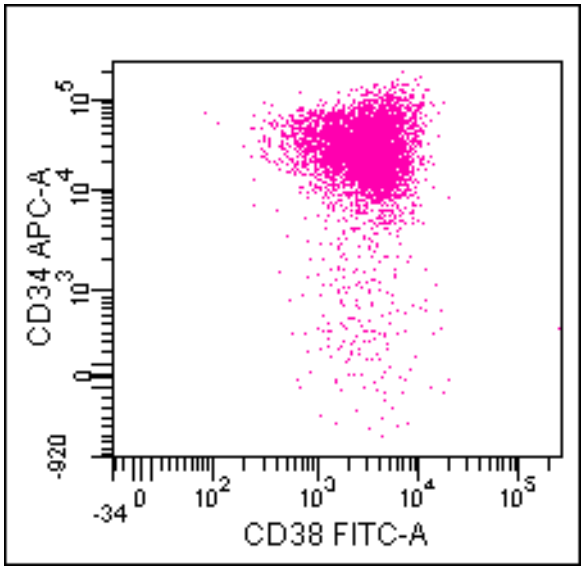


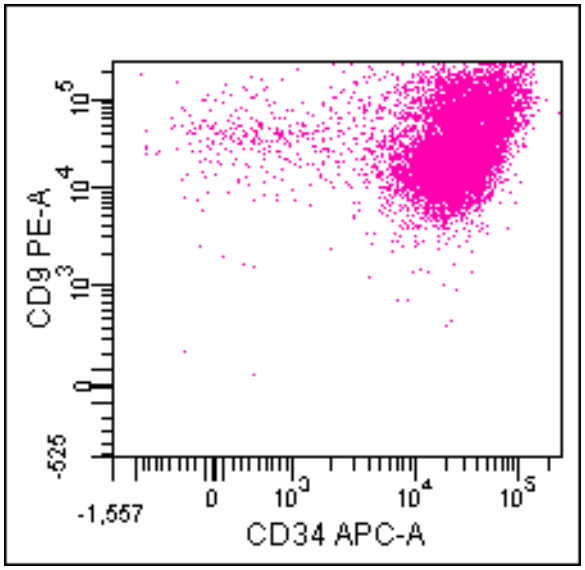
End of treatment



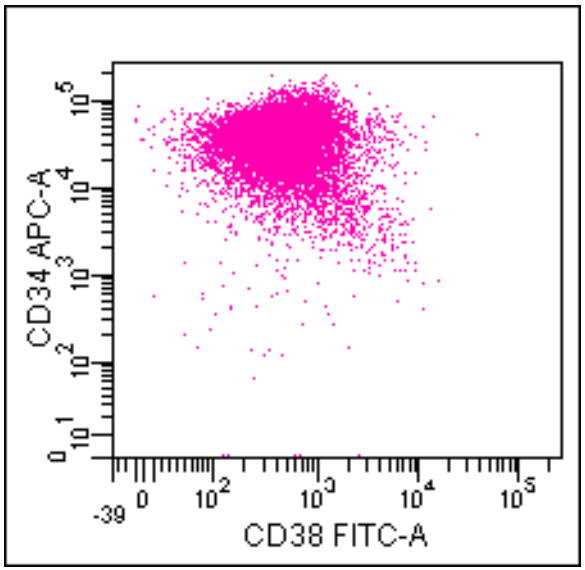


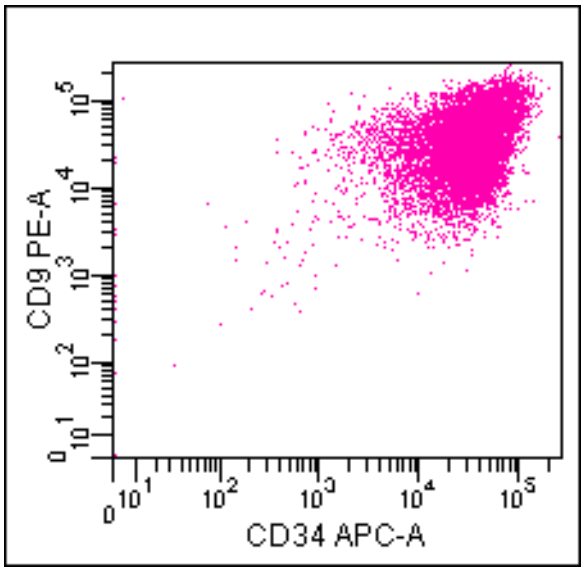
Diagnostic



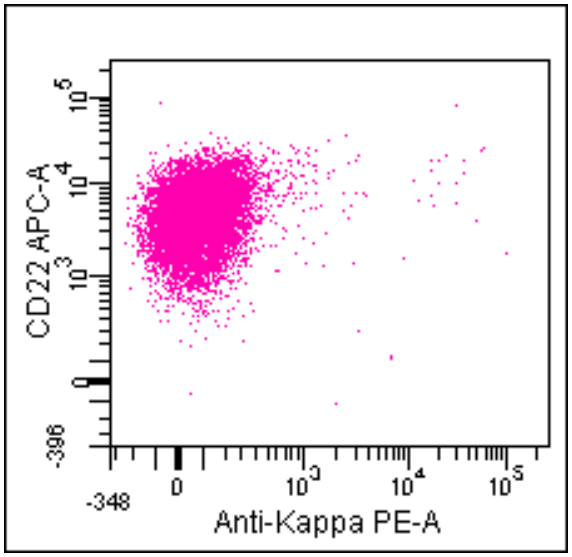


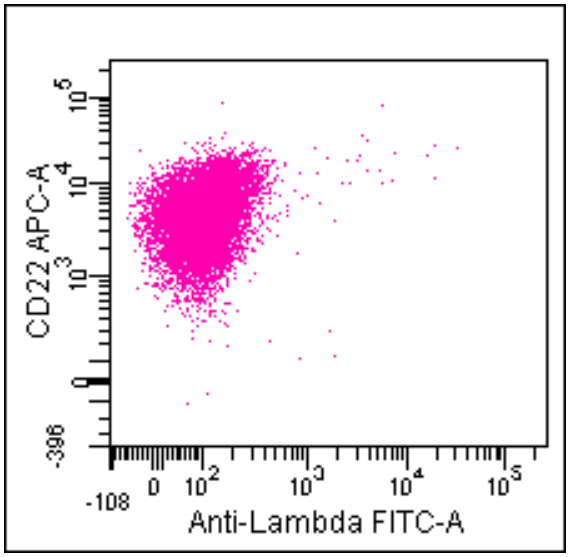
End of treatment

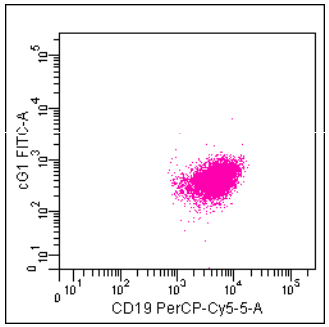


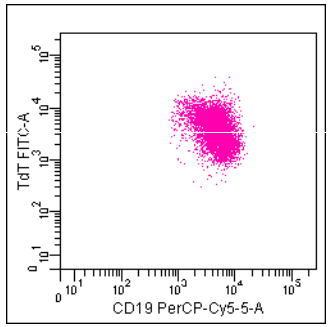


Diagnostic

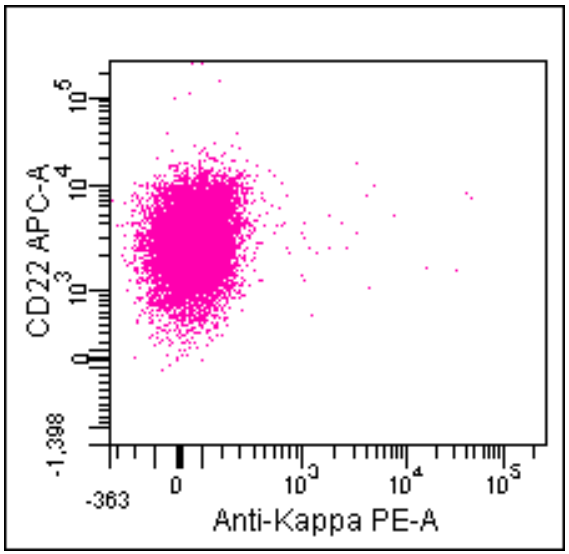


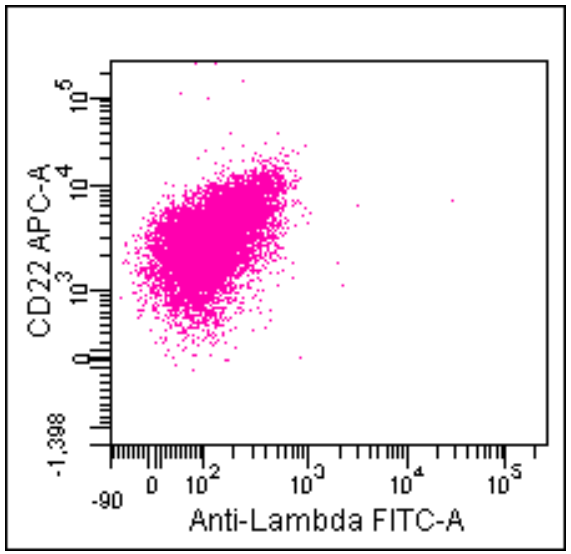




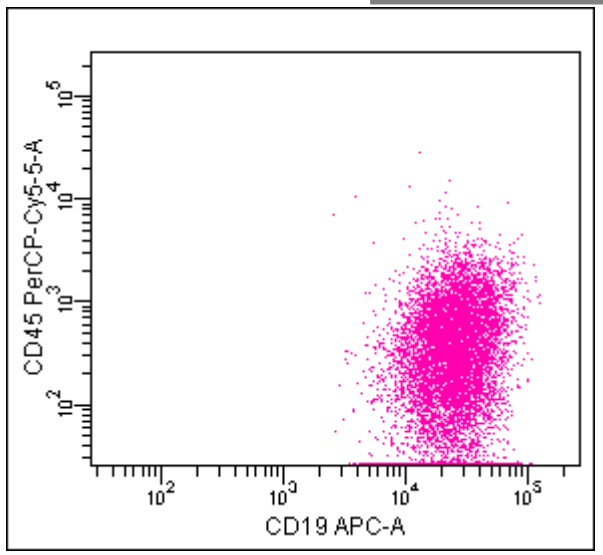


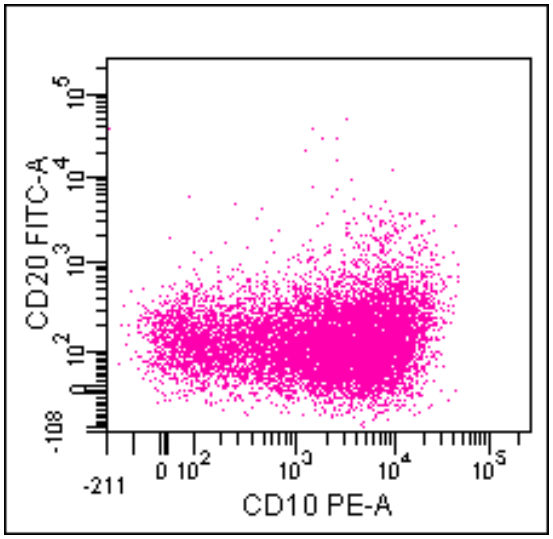
End of treatment

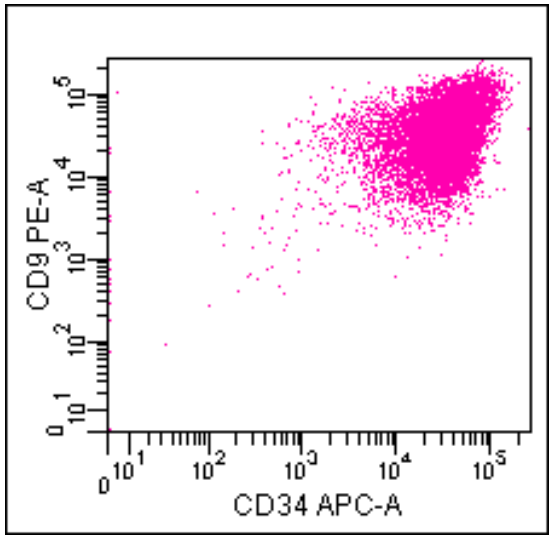




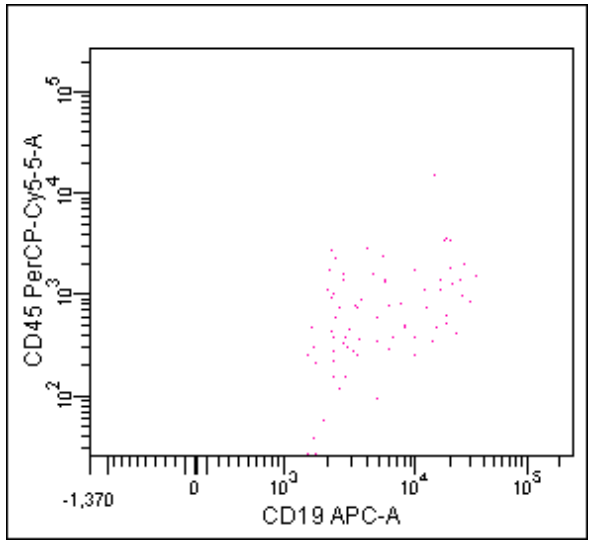
End of treatment/relapse

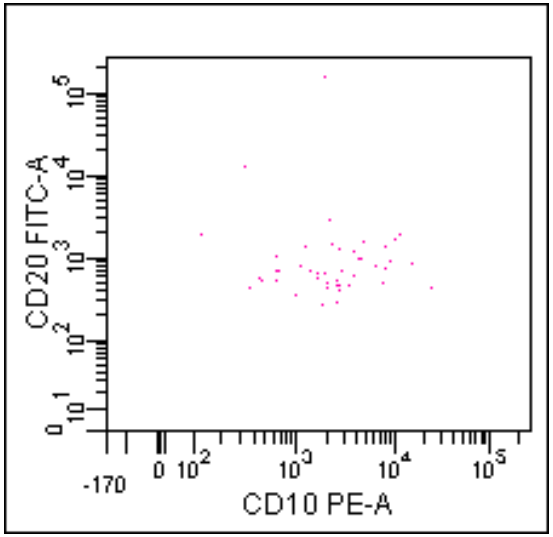


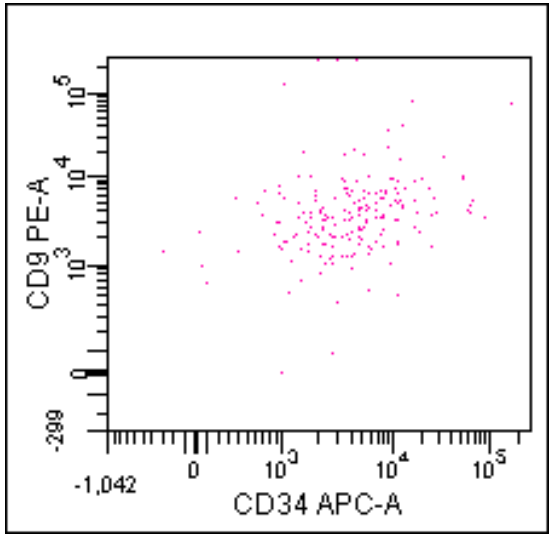




30 day post-reinduction (<1% of total events)







Minimal residual disease – an overview

- Nearly all patients with childhood lymphoblastic leukemia achieve complete remission
  - Histologic remission achieved at less 5% leukemic cells in marrow

- Patients in disease remission can harbor 10^9-10^{10} residual leukemic cells

- Flow cytometric and molecular PCR approaches

- MRD assessment after induction therapy is the most important prognostic factor for outcome in children with ALL

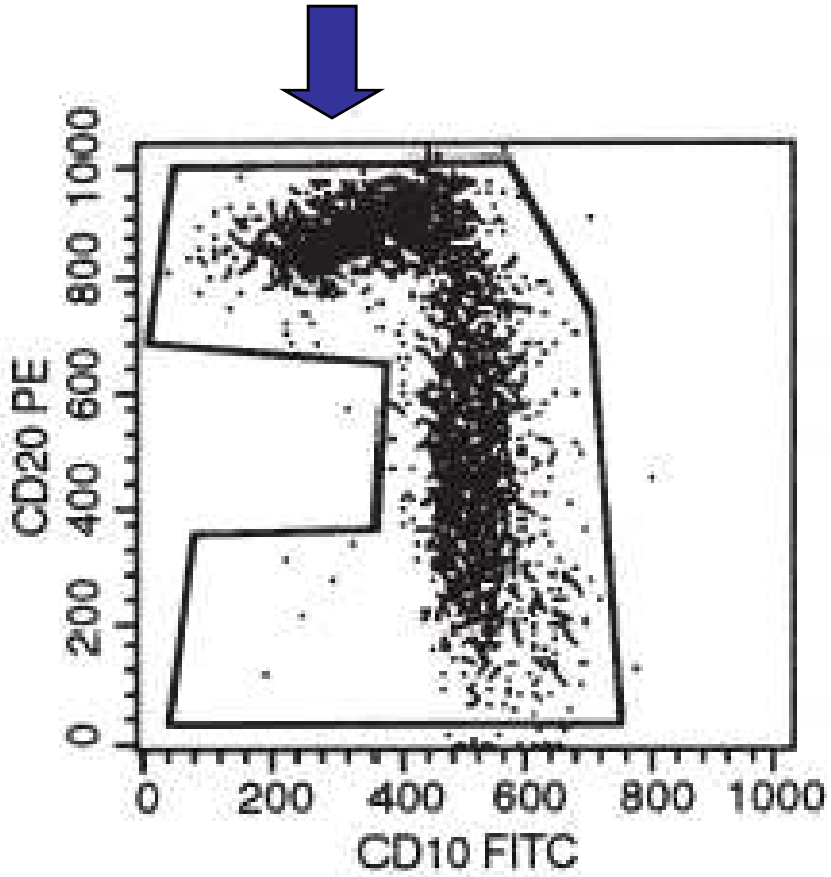
Borowitz, *Blood*, 2008

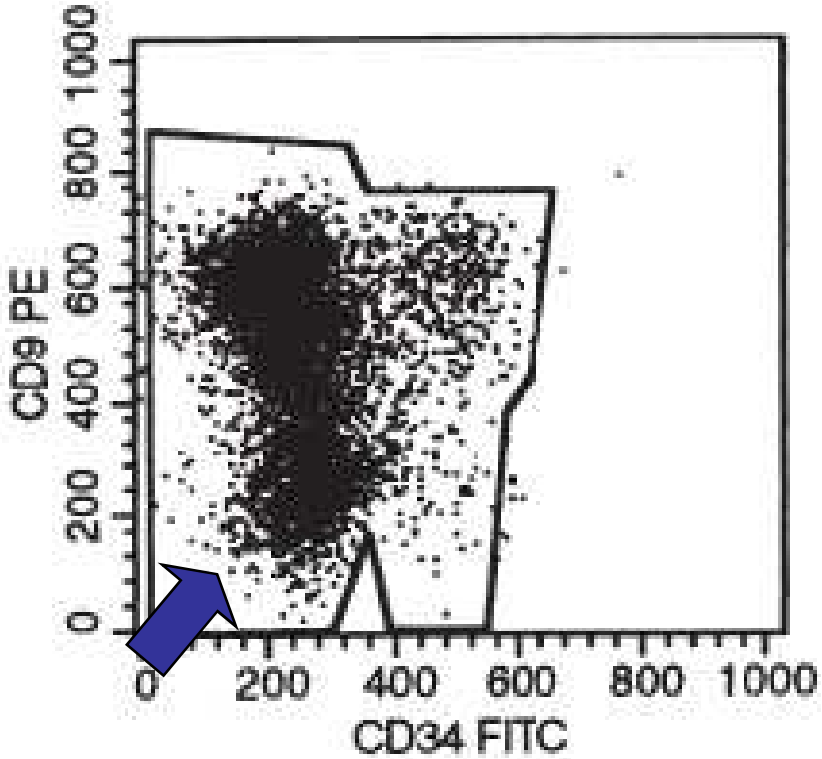
Limited panel for disease detection in B-ALL

- CD19/CD45/CD20/CD10
  - 93% of cases exhibited an aberrant population
- CD19/CD45/CD9/CD34
  - 93% of cases exhibited an aberrant population
- Using both panels in combination
  - 99% of cases demonstrated an abnormality

Weir, *Leukemia*, 1999

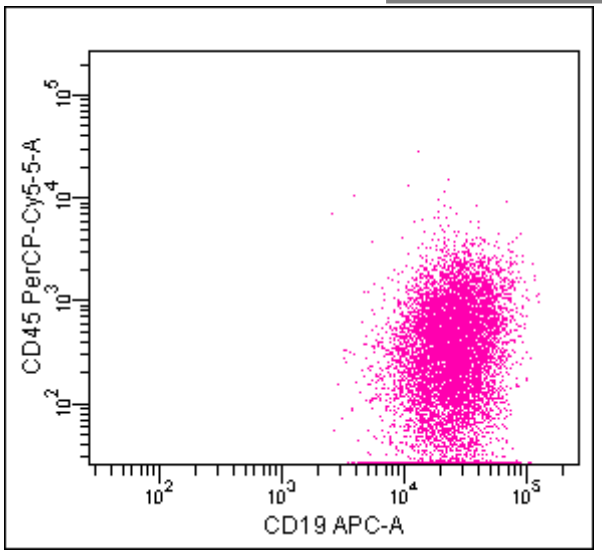
Limited panel for disease detection

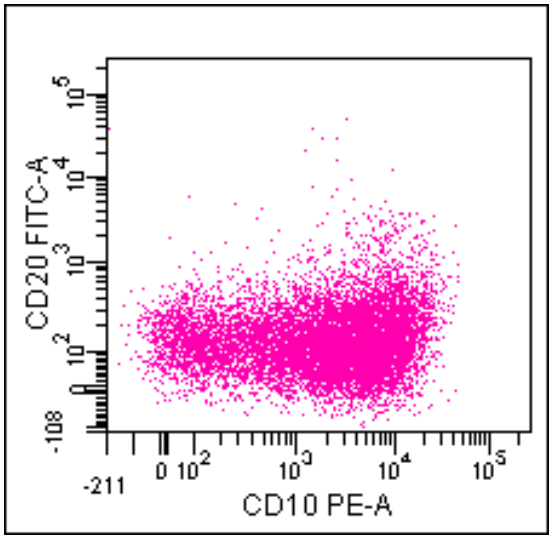


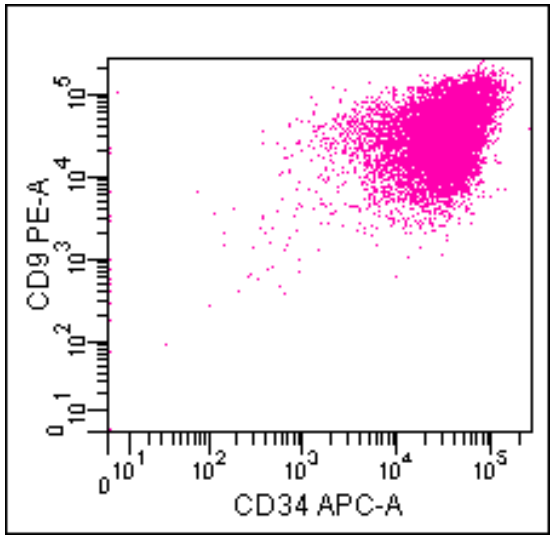


Weir, *Leukemia*, 1999

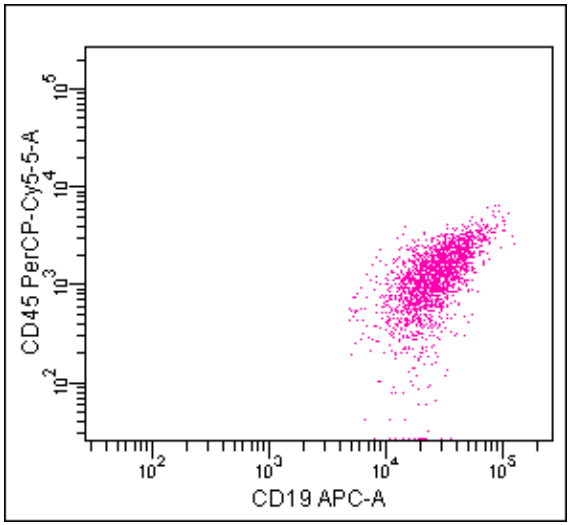
End of treatment/relapse

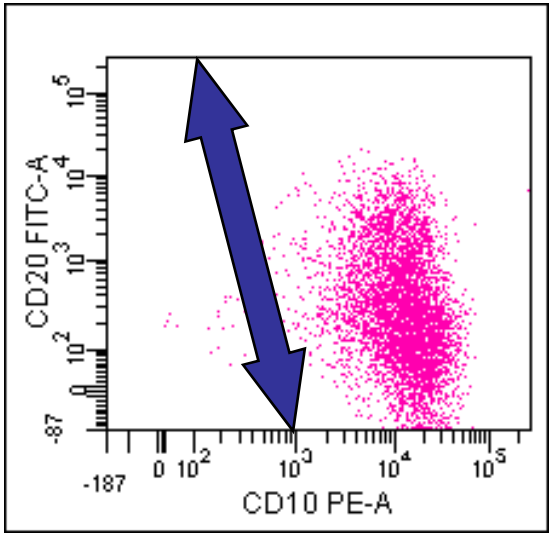


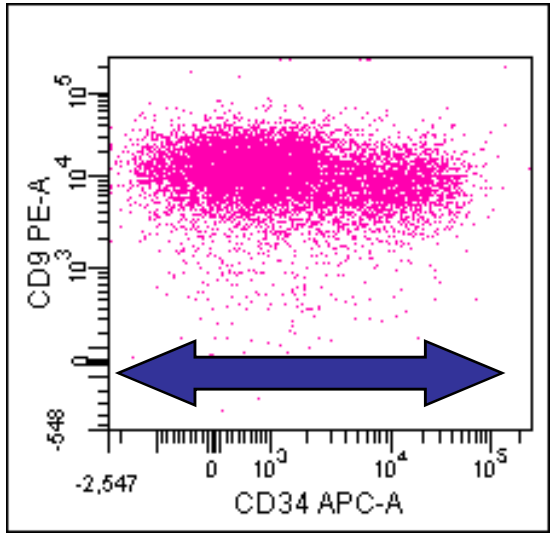




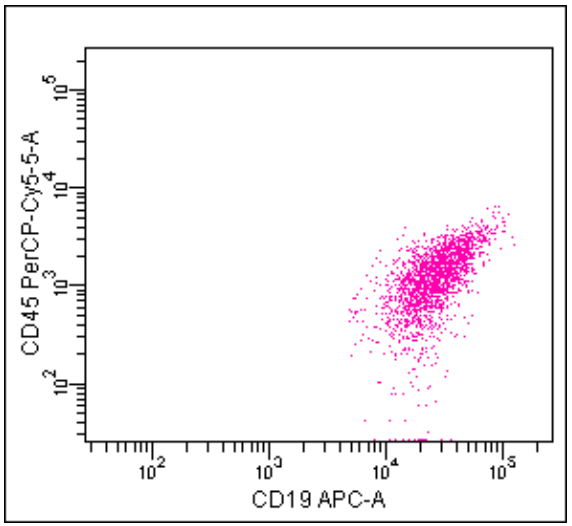
60 day post-reinduction (6% of total events)

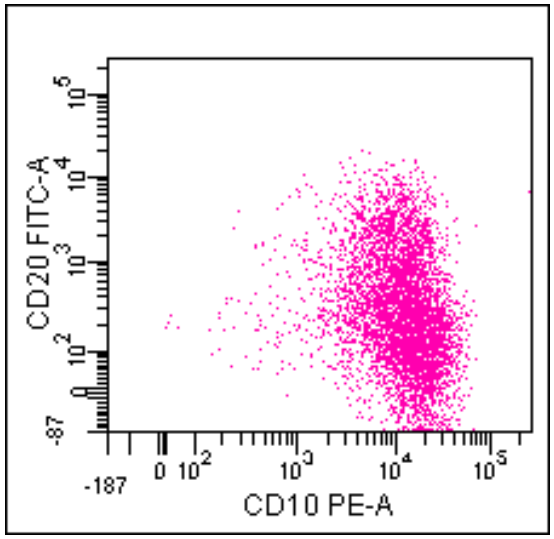


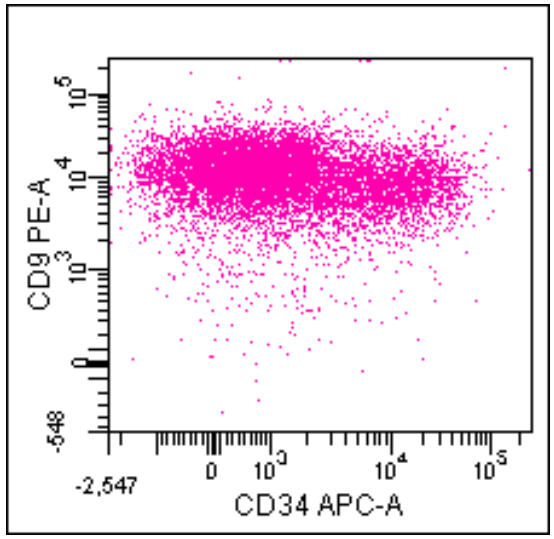




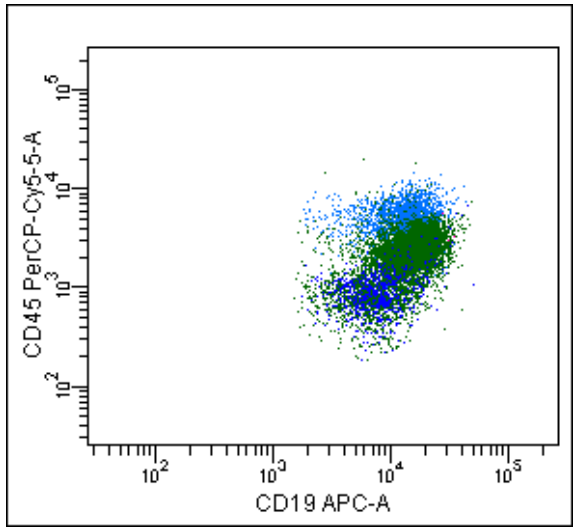
60 day post-reinduction (6% of total events)

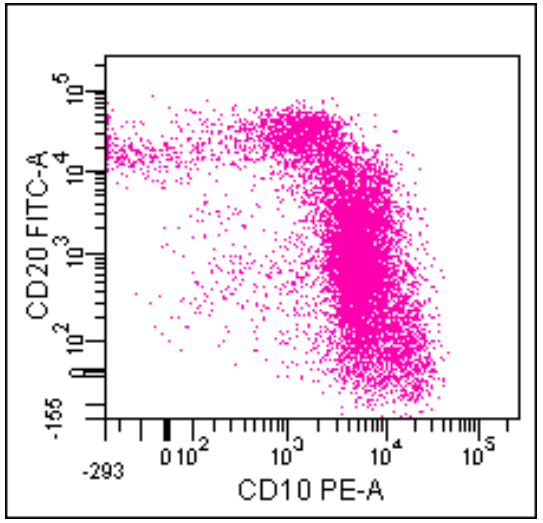


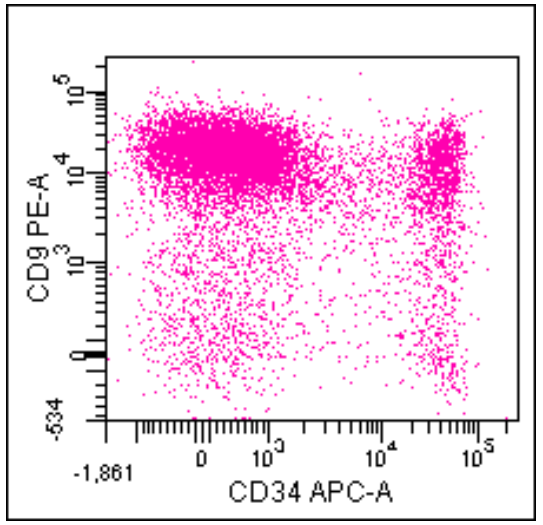




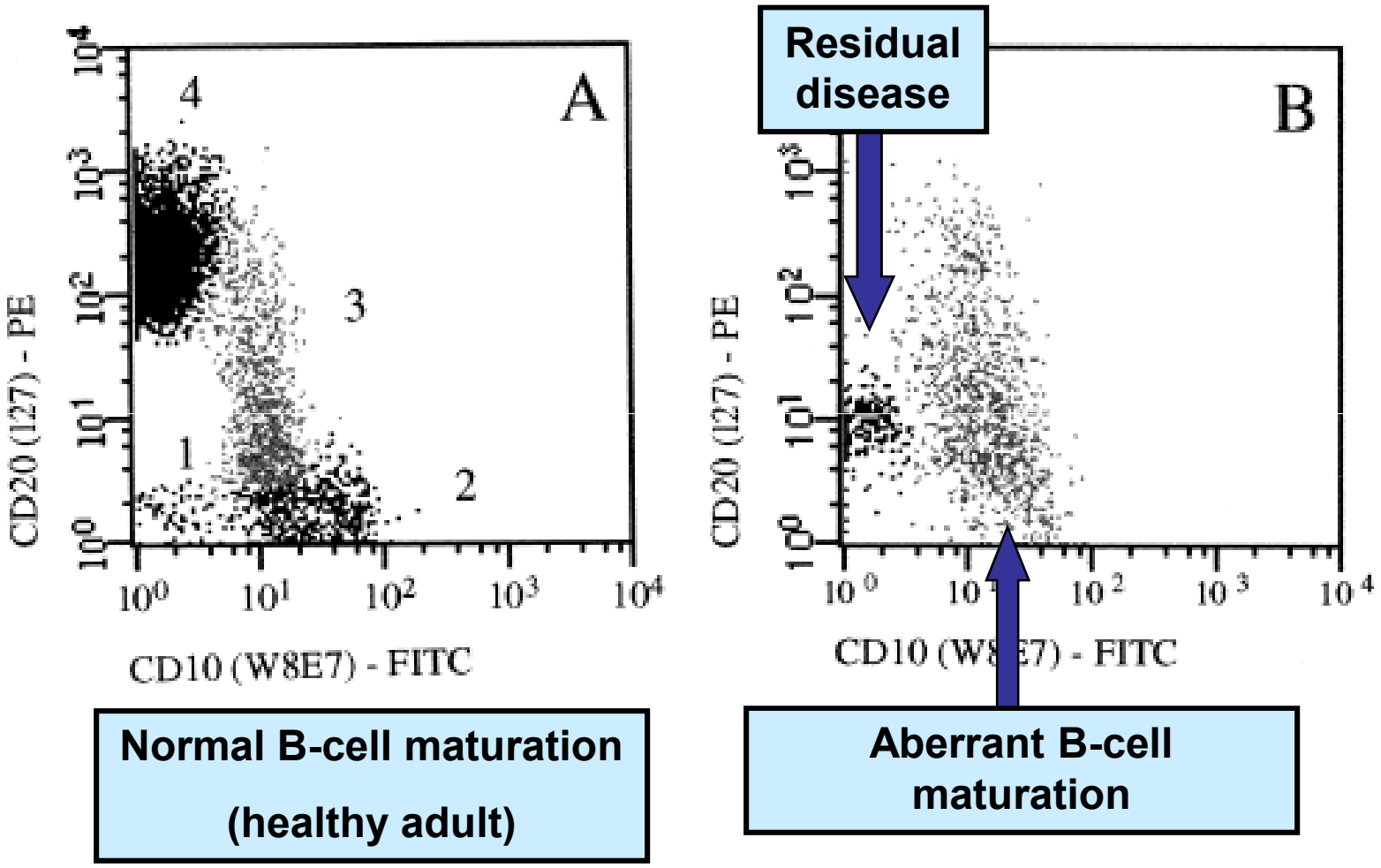
Normal B-cell hematopoiesis







Relevance of arrested B-cell maturation?



Ciudad, *BJH*, 1999

B-cell maturation arrest has clinical significance

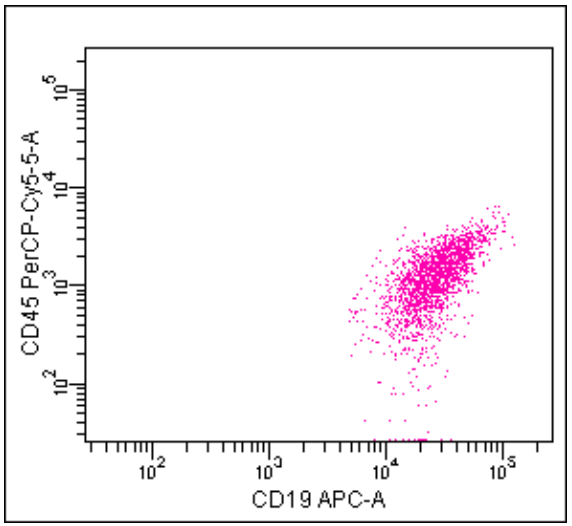
		Relapse rates		
	All patients	After induction	During maintenance	After treatment
CD10/CD20/CD19 differentiation	n=44	n=25	n=26	n=16
Normal	9/31 (29%)	9/17 (53%)	1/15 (7%)	1/14 (7%)
Abnormal	12/13 (92%)	8/8 (100%)	11/11 (100%)	2/2 (100%)
P value	0.0001	0.02	<0.00001	0.002

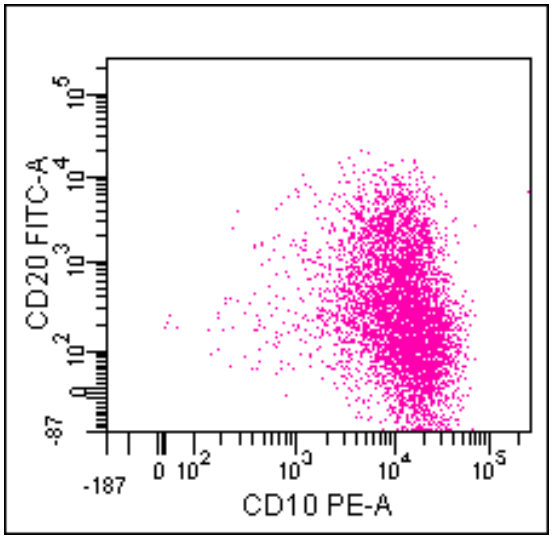
Controls: healthy marrow donors and regenerating marrow from T-ALL patients in cytologic remission

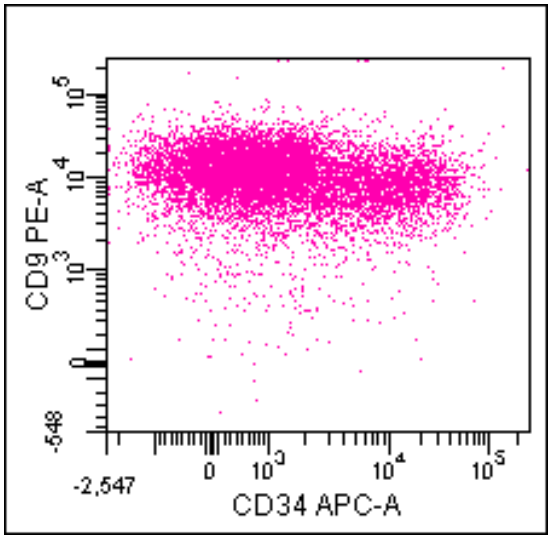
Statistically significant differences present in both children and adults

Ciudad, *BJH*, 1999

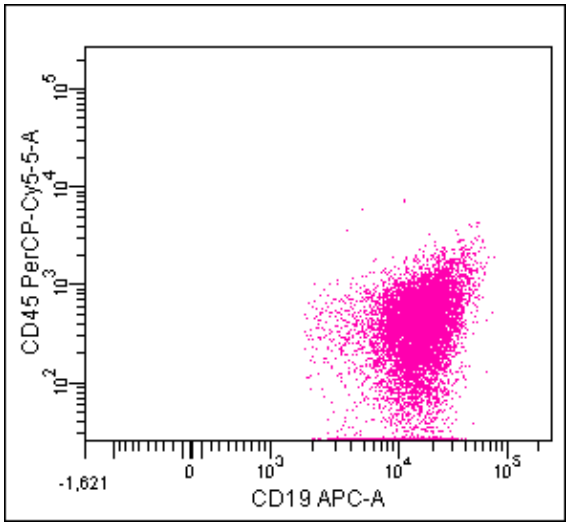
60 day post-reinduction (6% of total events)

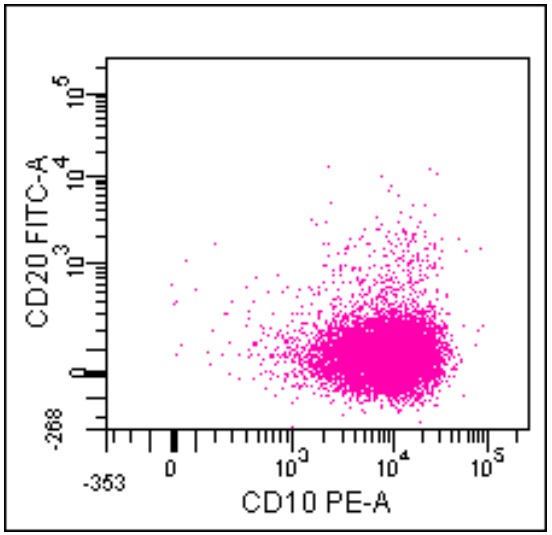


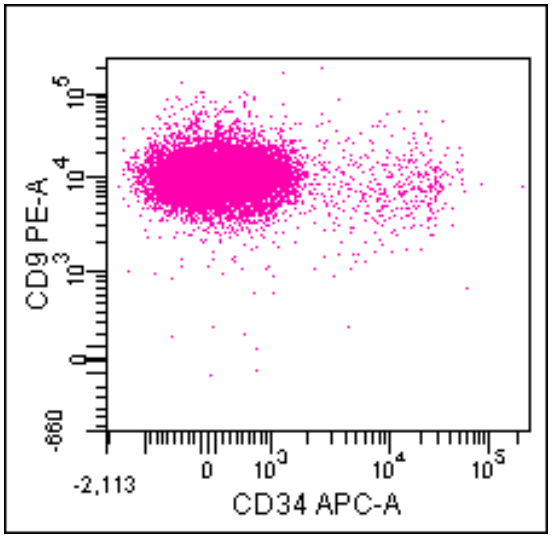




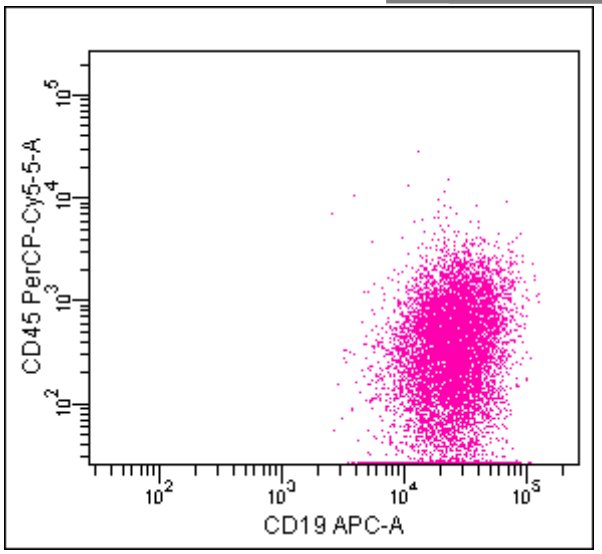
90 day post-reinduction (60% of total events)

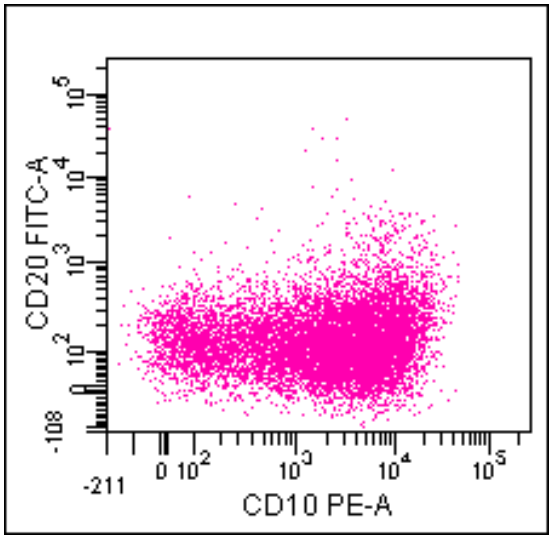


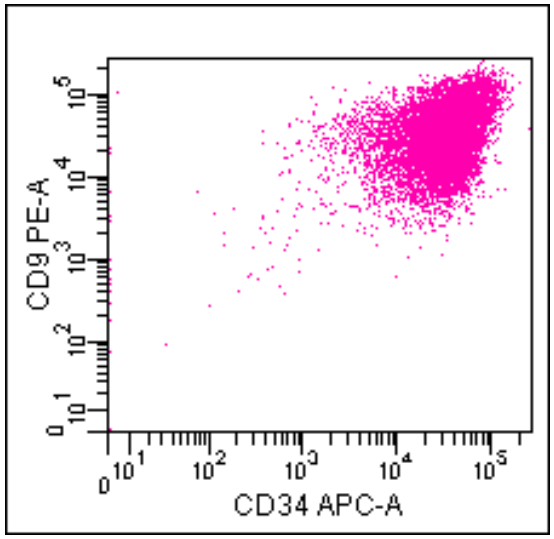




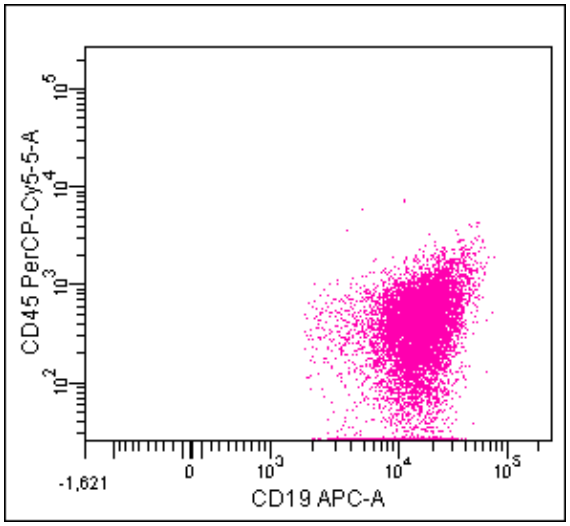
End of treatment/relapse

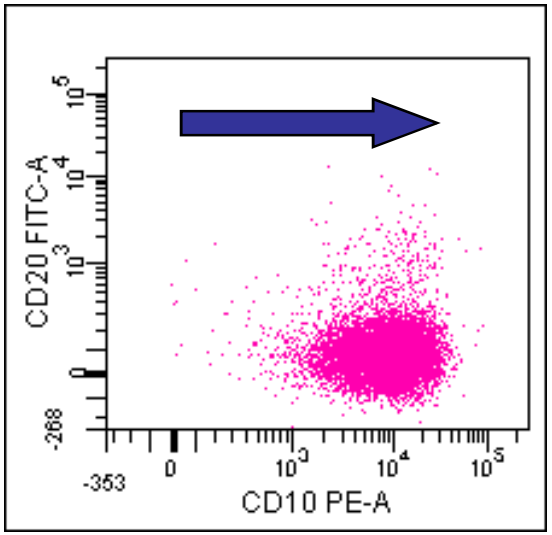


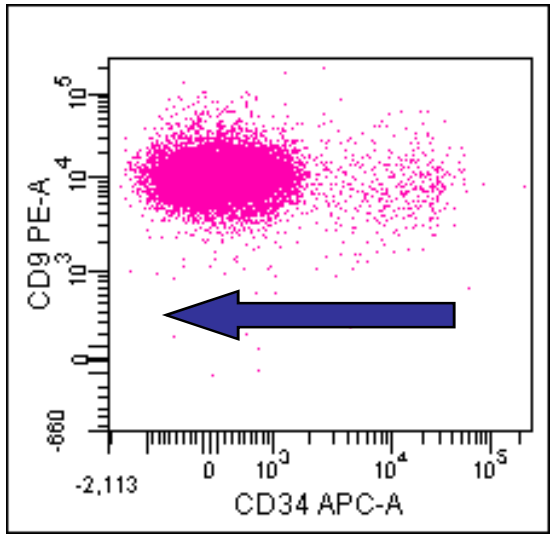




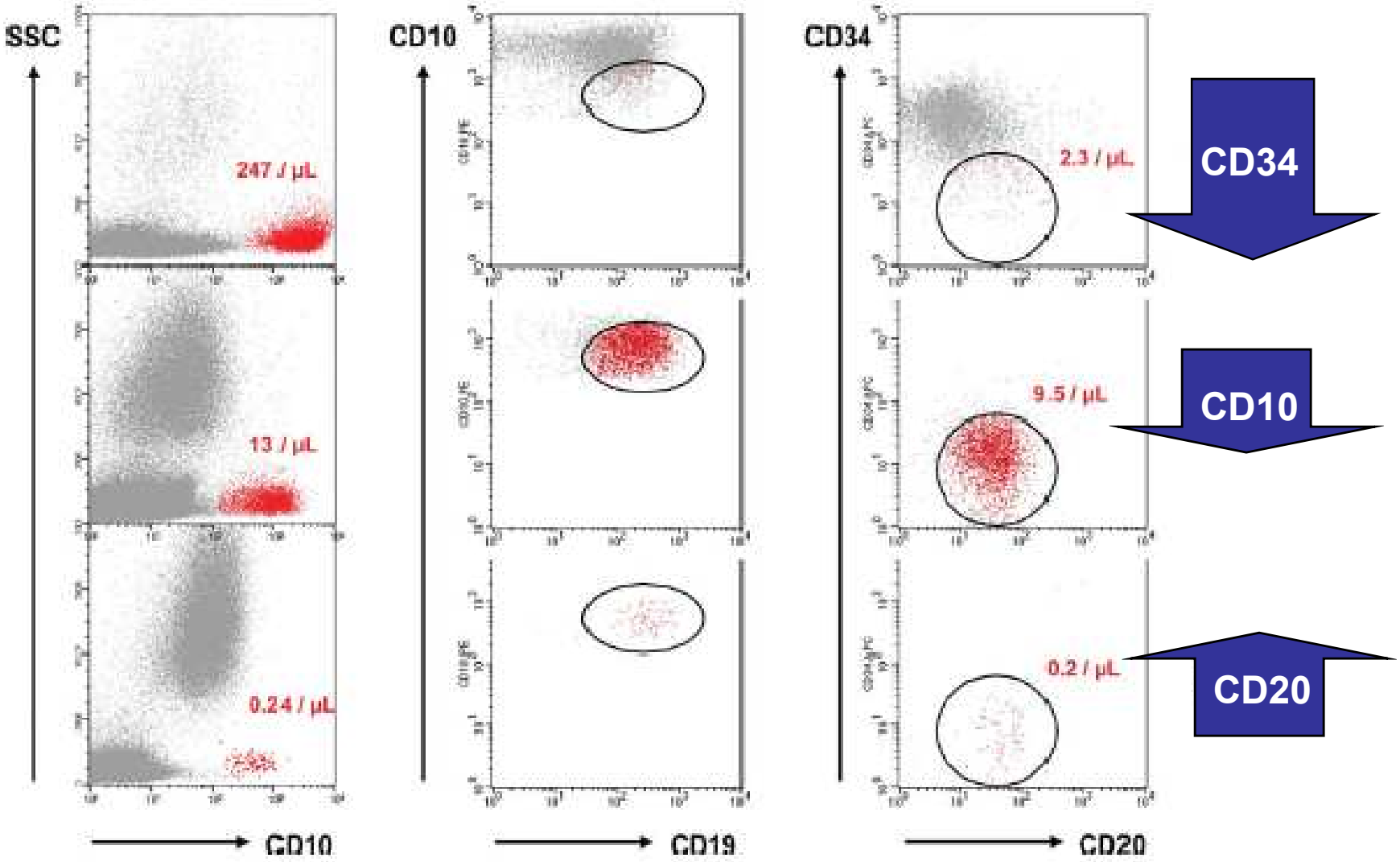
90 day post-reinduction







Drug-induced effects



Dworzak, *Cytometry B Clin Cytom*, 2010

Phenotypic shifts are common in disease relapse

- Chen, *AJCP*, 2007
  - 70% of cases demonstrated loss of atleast 1 aberrancy
  - 60% of cases demonstrated new aberrancy
- Borowitz, *Cytometry B Clin Cytom*, 2005
- van Wering, *Leukemia*, 1998
- Guglielmi, *Leukemia*, 1997

Final diagnosis

Relapsed/refractory B-lymphoblastic leukemia

with

a phenotypic shift manifest as loss of CD34

Difficulties

- Know patterns of normal hematopoiesis
- Aberrancies can indicate disease (or disease risk)
- Phenotypic shifts are not uncommon in relapse, so expect the unexpected

Concluding points

- Context is critical
  - Be specific and complete
- Understand patterns of normal hematopoiesis
- Think horse, but consider zebra
  - Maintain a menagerie of possibilities
- Common is not always classic

Concluding points

- Rare entities can present themselves
- Few markers are truly lineage specific
  - Understand the settings in which “infidelity” occurs
- Phenotype predicts genotype
- Use flow liberally, but judiciously