



CHALLENGING FLOW CYTOMETRIC CASES: BLASTS OR NOT?

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BACKGROUND

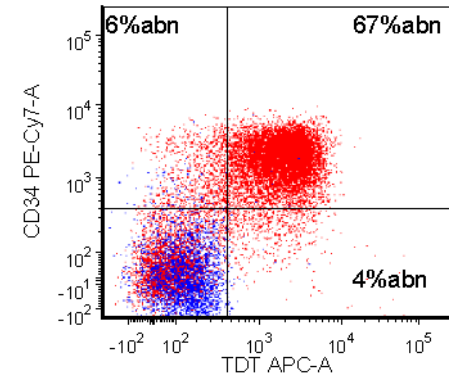
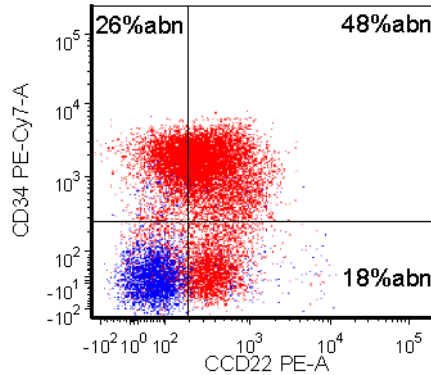
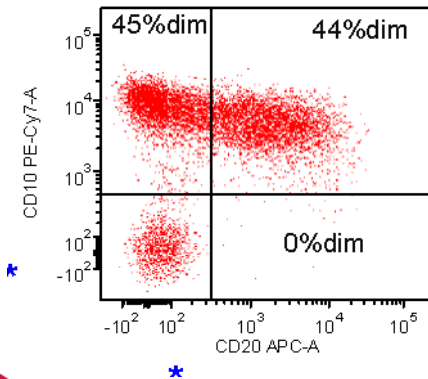
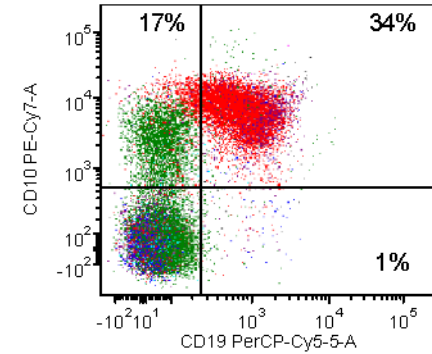
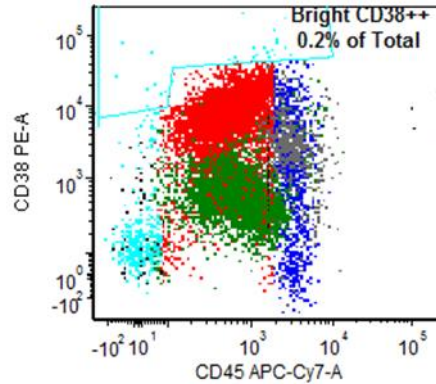
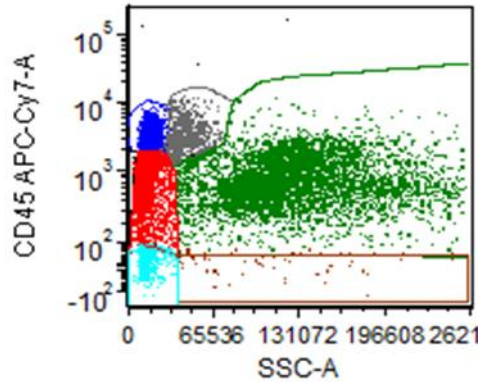
- Flow cytometry is a powerful tool for identifying blasts and determining the lineage of an acute leukemia
- Immunophenotypic features useful in identifying blasts
 - Blasts are typically in the CD45 dim/SSC low region
 - Blasts express immature markers
 - Blasts usually lack terminally differentiated mature markers
- Diagnostic dilemma
 - Blasts could be outside of the typical “blast region”
 - Blasts can aberrantly express mature antigens or lack expression of one or more immature antigens
 - Non-neoplastic blasts may increase in reactive conditions

CASE 1

- 47-year-old female with anemia and leukopenia
- Bone marrow aspirate submitted for flow cytometry
- Accompanying CBC report
 - WBC 1.76 K/uL
 - RBC 3.19 M/uL, Hgb 10.6 g/dL, MCV 100.9 fL
 - Platelets 183 K/uL

CASE 1

Flow Cytometric Analysis



CASE 1

Flow Cytometric Findings

- 38% B lymphoblasts:
 - Positive: CD10, CD19, CD20(partial), CD22, CD34, CD38, CD79a, HLA-DR, and TdT
 - Negative: surface kappa or lambda, T-cell markers, CD13, CD14, CD33, CD41, CD61, CD64, CD71, glycoporphin A, myeloperoxidase
- 1% B cells with normal kappa:lambda ratio (2:1)
- 6% T cells with normal CD4:CD8 ratio (1.9:1)
- 5% Monocytes with normal antigen expression
- 46% Granulocytes with normal maturation pattern

CASE 1

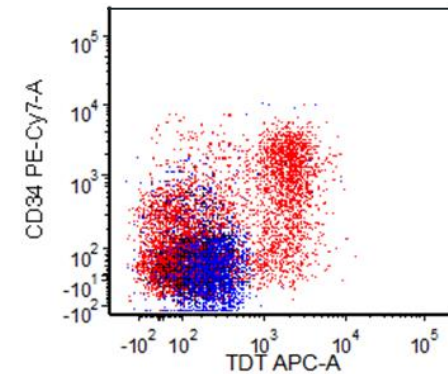
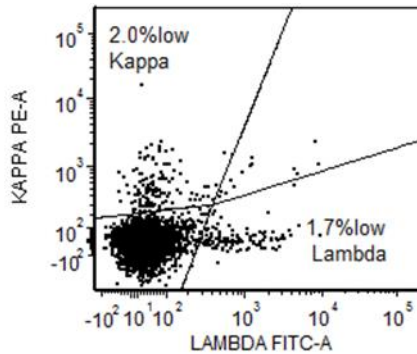
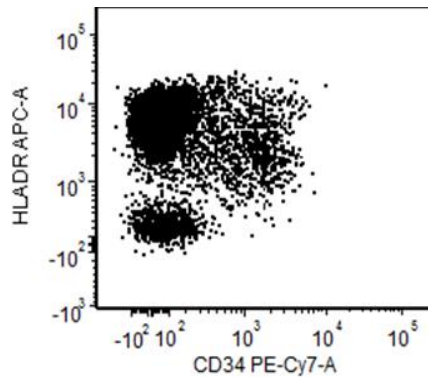
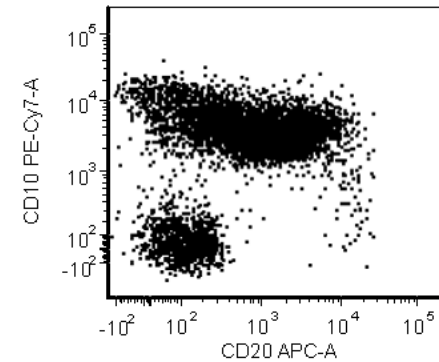
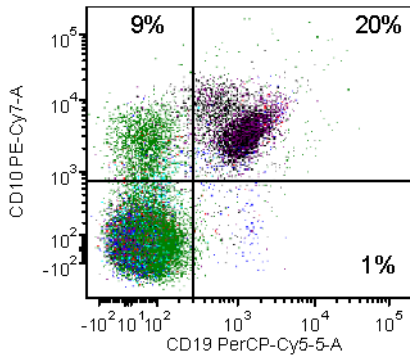
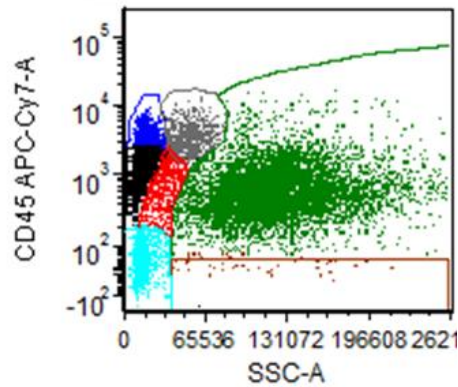
Additional Clinical Information

- The patient had a history of plasma cell myeloma
- Status post treatment with standard induction chemotherapy
- Autologous stem cell transplant in 9/2013 with marginal response
- A 4-cycle chemotherapy completed just one week before the bone marrow biopsy
- Another bone marrow biopsy was scheduled a week later to reassess B lymphoblasts



CASE 1

Flow Cytometric Analysis of the 2nd Marrow



CASE 1

Flow Cytometric Findings of the 2nd Marrow

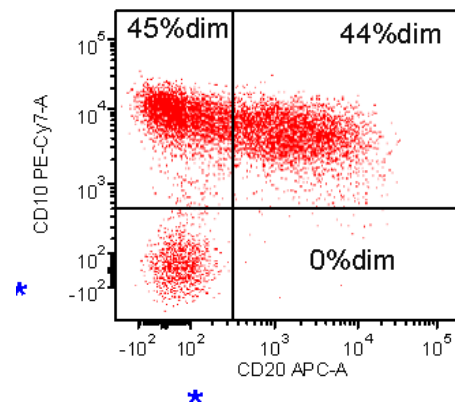
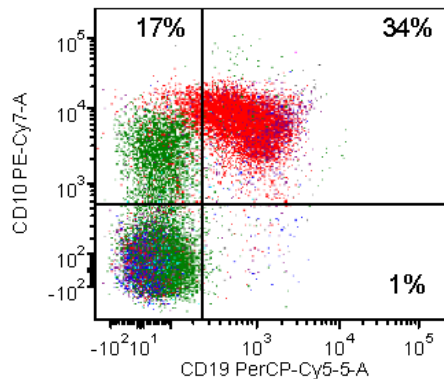
20% Hematogones:

- 4% CD34/TdT positive more immature subset
- 16% maturing hematogones, positive for CD10, CD19, CD20(variable), CD22, CD38(bright), and negative for surface light chains, CD34, or TdT
- <1% more mature hematogones expressing low levels of surface kappa or lambda

CASE 1

Summary

- Hematogones can increase and show a markedly left-shifted maturation in stress-related conditions
- Hematogones show decreased CD10 level when gaining CD20 expression
- Clinical history and correlation are critical for interpretation
- Recommend a repeat marrow biopsy if questions arise

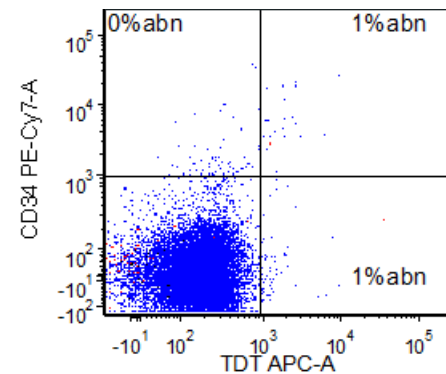
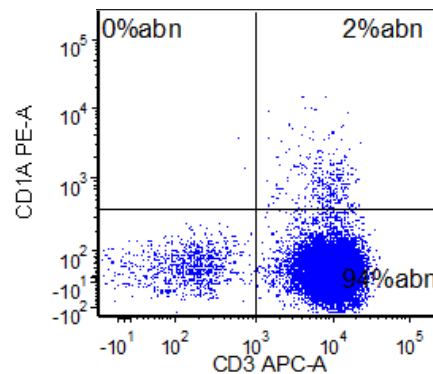
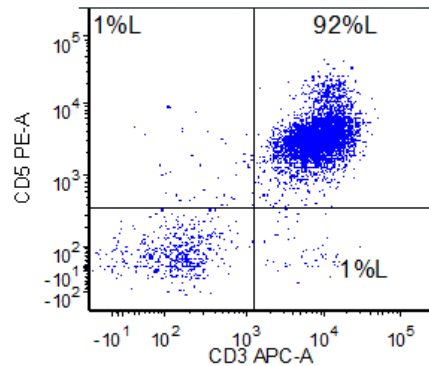
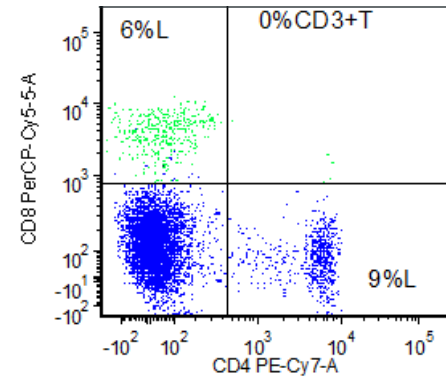
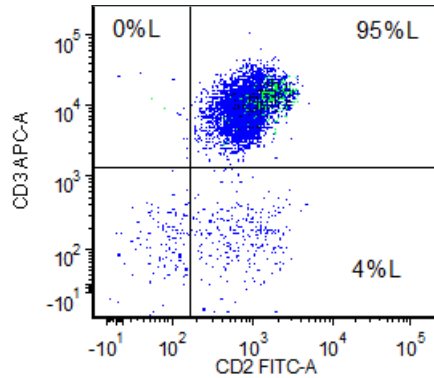
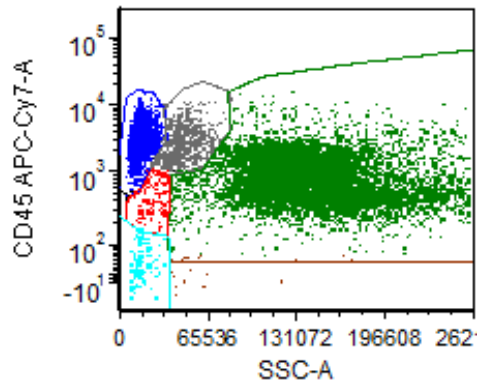


CASE 2

- 29-year-old male
- Bone marrow aspirate submitted for flow cytometric evaluation for acute lymphoblastic leukemia
- No concurrent CBC data

CASE 2

Flow Cytometric Analysis



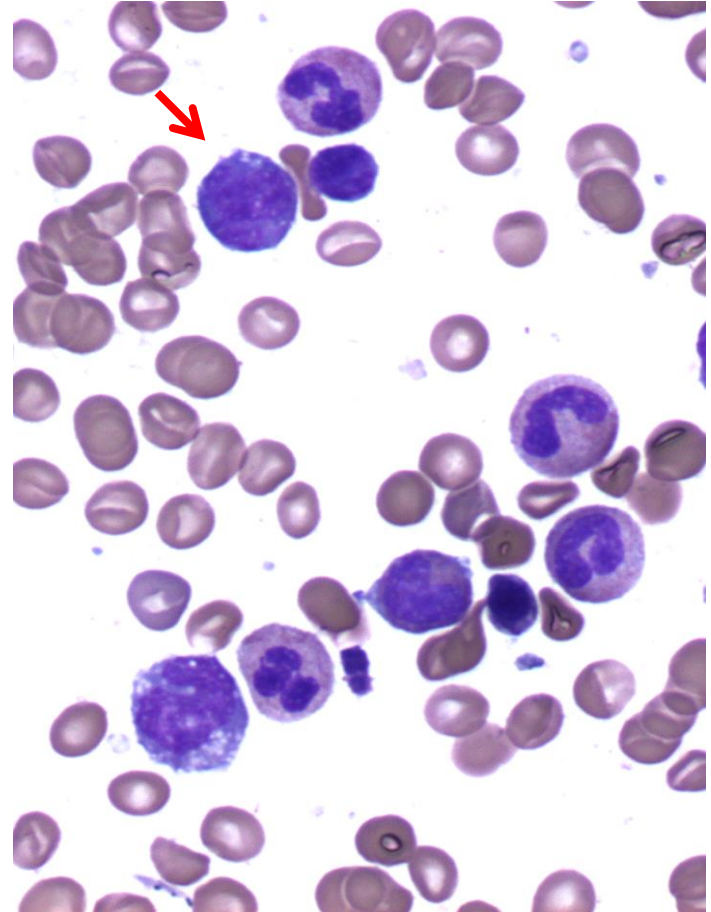
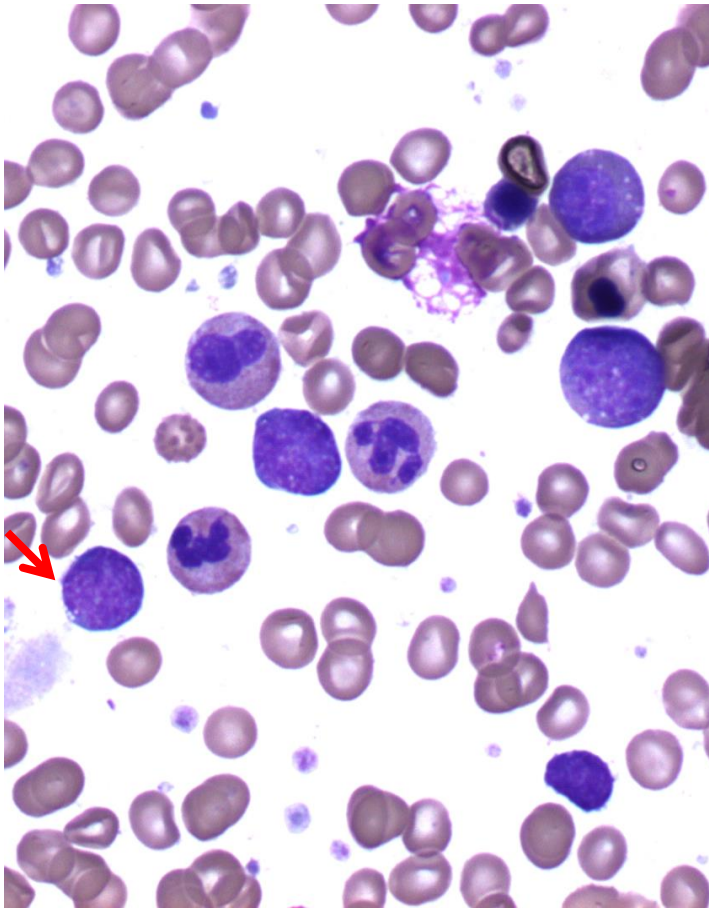
CASE 2

Flow Cytometric Findings

- 19% Aberrant T cells lacking CD4 and CD8 Expression:
 - Positive for: CD2, CD3, CD5(dim), CD7, CD45, TcR-alpha/beta
 - Negative for: CD1a, CD4, CD8, CD16, CD34, CD56, CD57, and TdT
- <1% B cells
- <1% NK cells
- 3% Monocytes with normal antigen expression
- 71% Granulocytes with normal maturation pattern

CASE 2

Marrow Aspirate



CASE 2

T-ALL or Mature T-Cell Neoplasm?

Data supporting an immature process

- Dual CD4-CD8- immunophenotype
 - Most cases of T-ALL (at pro-T or Pre-T stages)
 - Gamma/delta T cells
 - Approximately 10% peripheral T-cell neoplasm
- Blastic morphology in the aspirate smear

Data suggesting a mature process

- Lacking immature markers for T-ALL (CD34, TdT, CD1a)
- Expression of surface CD3
- Expression of a high level of CD45

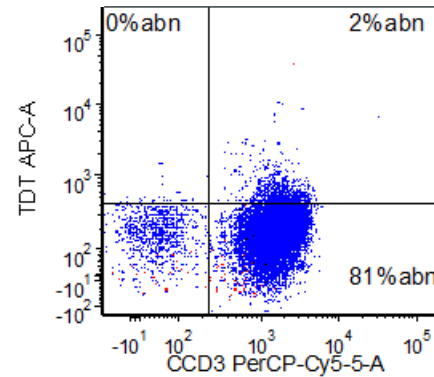
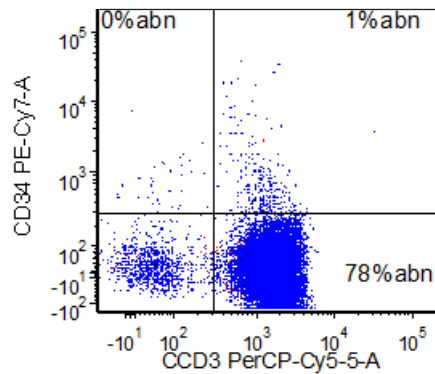
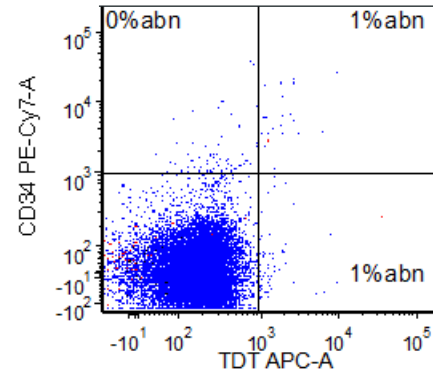
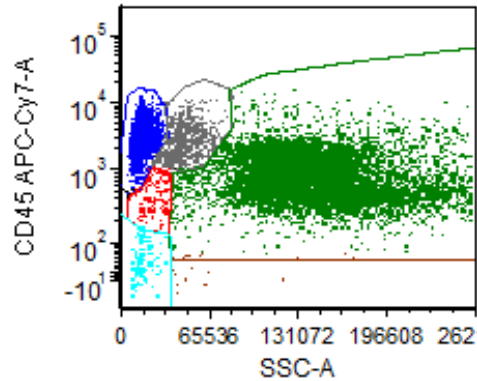
CASE 2

Additional Clinical Information

- The patient had a history of T-ALL, status post chemotherapy
- Flow cytometric report of originally diagnostic marrow at outside institution described a surface CD3-positive, dual CD4/CD8-negative T lymphoblastic population with partial TdT expression

CASE 2

Re-analysis of TdT



CASE 2

Summary

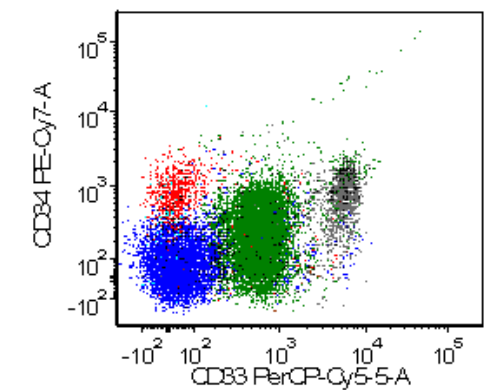
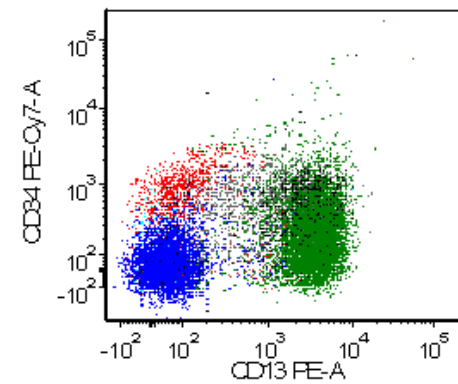
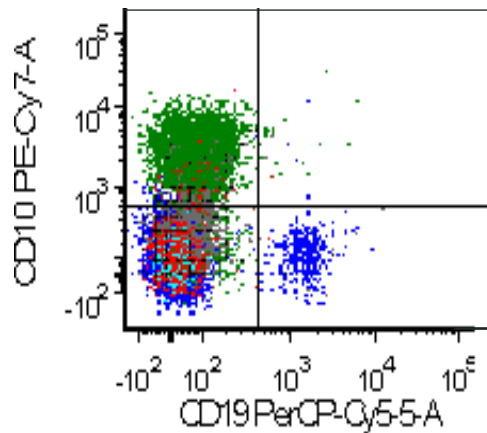
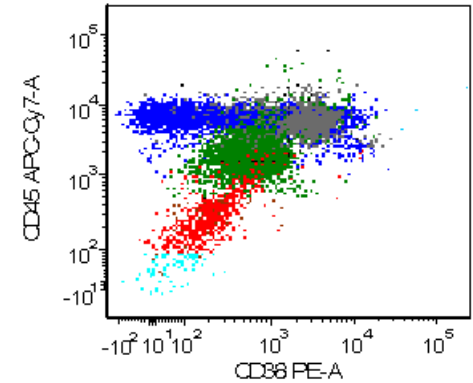
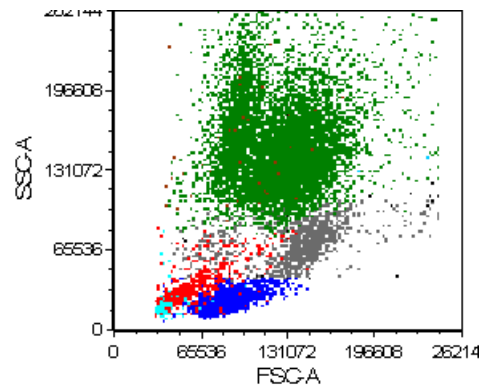
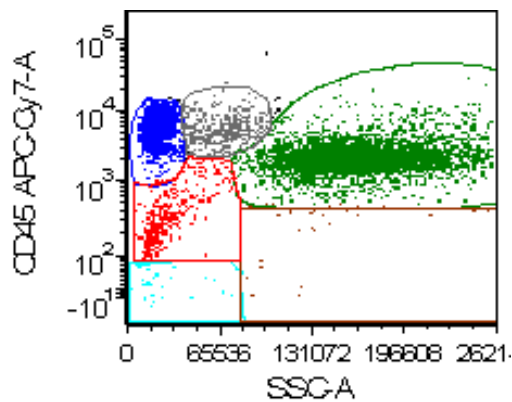
- T lymphoblasts often show stronger CD45 expression compared to B lymphoblasts and may locate in the lymphocyte gate
- Most cases of T-ALL are at pro-T or pre-T stage, lacking CD3, CD4, and CD8 expression
- T-ALL may express surface CD3
- T lymphoblasts may show a change of the intensity of antigen expression or gain and/or loss of an antigen following treatment

CASE 3

- 70-year-old male with a history of anemia
- Blood submitted for flow cytometry
- No concurrent CBC data

CASE 3

Flow Cytometric Analysis



CASE 3

Flow Cytometric Findings

- 5% CD45 dim events:
 - Positive for CD34 and CD38(dim)
 - Negative for CD10, CD13, CD14, CD33, CD56, CD64, CD117, HLA-DR, T- and B-cell markers
- 2% B cells (kappa:lambda=1.5:1)
- 13% T cells (CD4:CD8=2.8:1)
- 9% NK cells
- 7% Monocytes with normal antigen expression
- 56% Granulocytes with mature phenotype

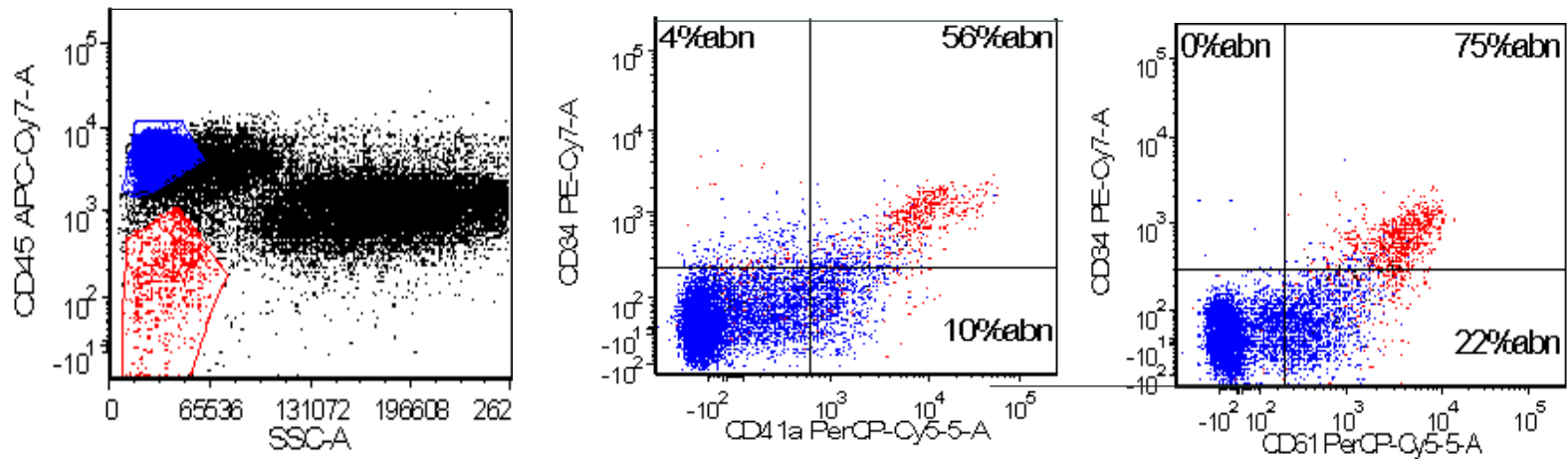
CASE 3

What are the CD34-positive events?

- Aberrant blasts in myelodysplastic syndrome?
- Non-specific CD34 reaction with cellular debris?
- Platelets?

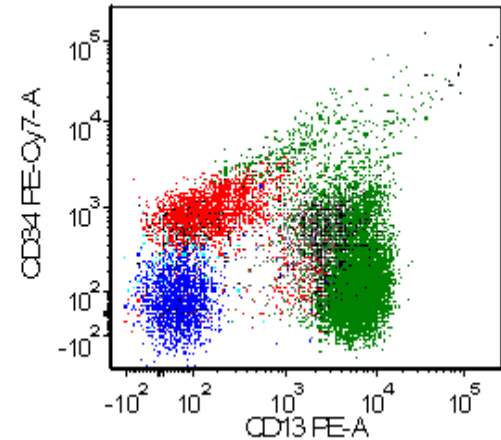
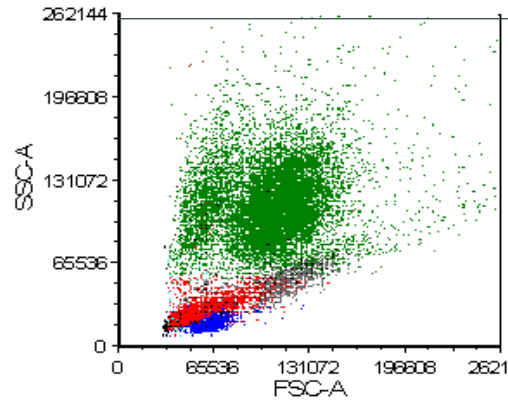
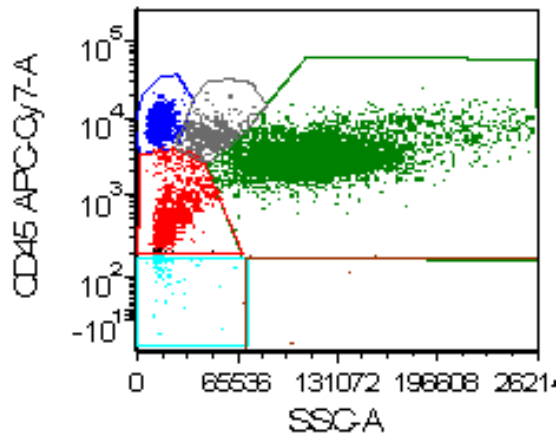
CASE 3

Analysis of Megakaryocytic Markers



CASE 3

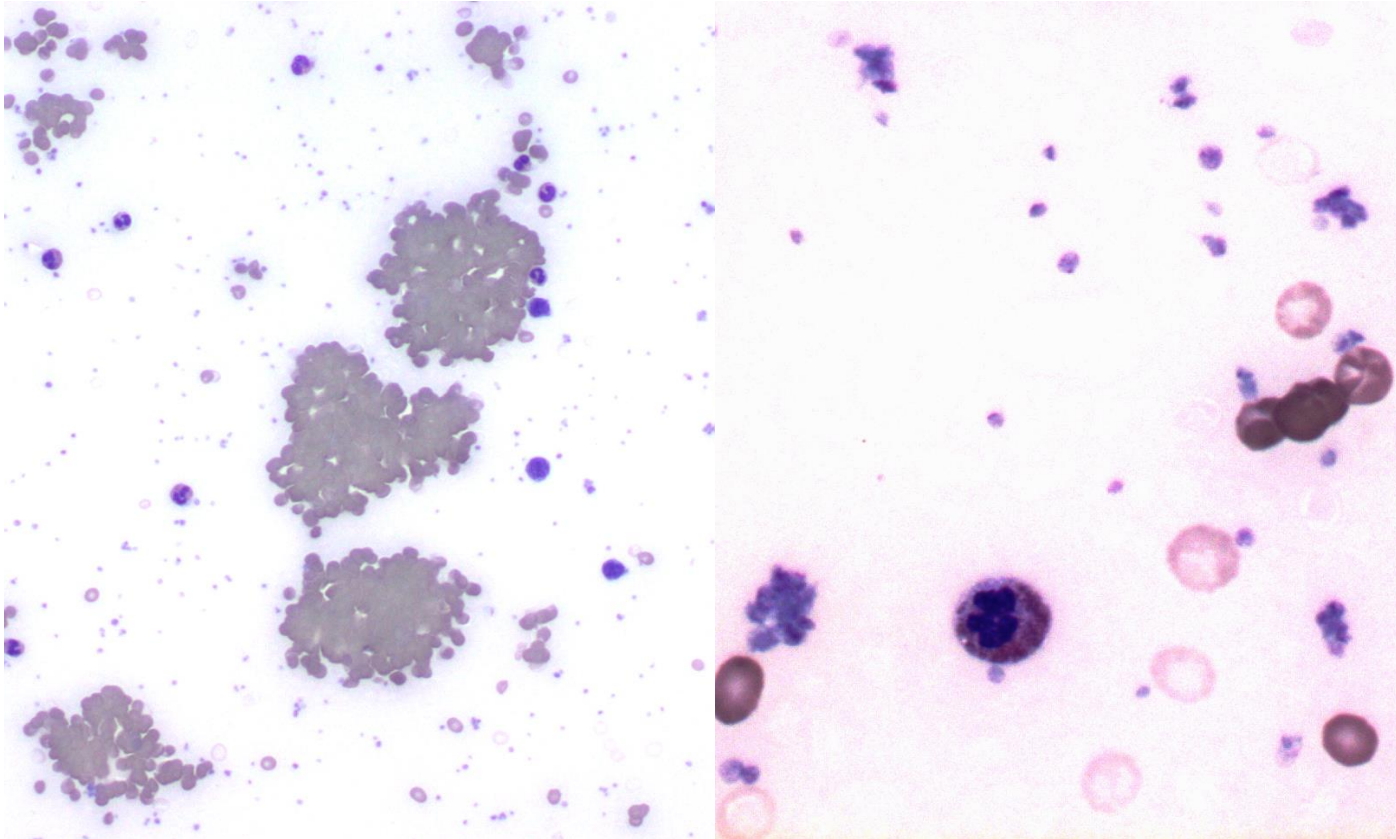
Another Blood Sample 14% CD34-positive/CD45dim Events



84-year-old female with marked normocytic anemia and a history of cold agglutinin

CASE 3

Blood Smear



CASE 3

Summary

- Increased large platelets or platelet clumps appear in the CD45 dim to negative region
- Platelets are positive for CD34 and CD38, and negative for T, B, or myeloid markers
- Platelets are usually smaller than lymphocytes by FSC
- Recognition of this phenomenon helps to avoid a diagnostic pitfall of overestimating blasts



GENOPTIX

QUESTIONS?



A NOVARTIS COMPANY

demand definite answers.