5000 International Clinical Cytometry Society: Practical Flow Cytometry in Hematopathology – A Case-Based Approach

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Hartford Hospital
Disclosures

In the past 12 months, I have not had a significant financial interest or other relationship with the manufacturers of the products or providers of the services that will be discussed in my presentation.

This presentation will include discussion of pharmaceuticals or devices that have not been approved by the FDA or unapproved or "off-label" uses of pharmaceuticals or devices.
Case 1

• 37 year-old woman with thrombocytopenia
• Normal hemoglobin and WBC count
• Bone marrow aspirate
Is Flow Cytometry Indicated?

Original Articles

2006 Bethesda International Consensus Recommendations on the Flow Cytometric Immunophenotypic Analysis of Hematolymphoid Neoplasia: Medical Indications

Medical Indications for Flow Cytometry

- Cytopenias
- Leukocytosis
- Atypical cells in PB/BM
- Evaluation of body fluids
- Plasmacytosis or monoclonal gammopathy
- Organomegaly, lymphadenopathy, tissue infiltration
- Disease monitoring in hematolymphoid neoplasia
What should we look for?
How should we look for it?
What should we look for?

<table>
<thead>
<tr>
<th>Medical indication</th>
<th>Lineage to be evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>B, T, M, P</td>
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<tr>
<td>Leukopenia</td>
<td>B, T, M, P</td>
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<tr>
<td>Thrombocytopenia</td>
<td>B, T, M, P</td>
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<tr>
<td>Pancytopenia</td>
<td>B, T, M, P</td>
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<tr>
<td>Neutrophilia</td>
<td>M (limited)</td>
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<tr>
<td>Monocytosis</td>
<td>M</td>
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<tr>
<td>Lymphocytosis</td>
<td>B, T</td>
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<tr>
<td>Eosinophilia</td>
<td>T, M</td>
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<tr>
<td>Erythrocytosis</td>
<td>M (limited)</td>
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<tr>
<td>Thrombocytosis</td>
<td>M (limited)</td>
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<tr>
<td>Blasts in blood or marrow</td>
<td>B, T, M</td>
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<tr>
<td>Lymphadenopathy</td>
<td>B, T</td>
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<tr>
<td>Extramedullary masses</td>
<td>B, T</td>
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<tr>
<td>Splenomegaly</td>
<td>B, T, M (limited)</td>
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<tr>
<td>Transformation of chronic leukemia—</td>
<td>B</td>
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<tr>
<td>B cell</td>
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<tr>
<td>Transformation of chronic leukemia—</td>
<td>T</td>
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<tr>
<td>T or NK cell</td>
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<tr>
<td>Staging for non-Hodgkin lymphoma—</td>
<td>B</td>
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<tr>
<td>B cell</td>
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<tr>
<td>Staging for non-Hodgkin lymphoma—</td>
<td>T</td>
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<tr>
<td>T/NK cell</td>
<td></td>
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<tr>
<td>Skin rash</td>
<td>B, T</td>
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<tr>
<td>Atypical cells in body fluids (CSF,</td>
<td>B, T, M (limited)</td>
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<tr>
<td>serous, ocular, etc.)</td>
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<tr>
<td>Monoclonal gammapathy</td>
<td>B, P</td>
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<tr>
<td>Unexplained Plasmacytosis of bone</td>
<td>B, P</td>
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<tr>
<td>marrow</td>
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<td>Monitoring of Rx response (unknown</td>
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<td>diagnostic immunophenotype)</td>
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<tr>
<td>Mature B cell neoplasm</td>
<td></td>
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<tr>
<td>Mature T or NK cell neoplasm</td>
<td>T</td>
</tr>
<tr>
<td>Acute lymphoid leukemia—B cell</td>
<td>B</td>
</tr>
<tr>
<td>Acute lymphoid leukemia—T cell</td>
<td>T</td>
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<tr>
<td>Acute myeloid leukemia</td>
<td>M</td>
</tr>
<tr>
<td>MDS/MPD/Overlap Syndrome</td>
<td>M</td>
</tr>
<tr>
<td>Plasma cell neoplasm</td>
<td>P</td>
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</table>

B, B cell; T, T cell; M, myeloid; P, plasma cell.
How should we look for it?

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Primary reagents</th>
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<tbody>
<tr>
<td>B cells</td>
<td>CD5, CD10, CD19, CD20, CD45, Kappa, Lambda</td>
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<tr>
<td>T cells and NK cells</td>
<td>CD2, CD3, CD4, CD5, CD7, CD8, CD45, CD56</td>
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<tr>
<td>Myelomonocytic cells</td>
<td>CD7, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD45, CD56, CD117, HLA-DR</td>
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<tr>
<td>Myelomonocytic cells (limited)</td>
<td>CD13, CD33, CD34, CD45</td>
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<tr>
<td>Plasma cells</td>
<td>CD19, CD38, CD45, CD56</td>
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</table>
## Bone Marrow Screening Panel

<table>
<thead>
<tr>
<th>Differential</th>
<th>CD71</th>
<th>CD33</th>
<th>CD123</th>
<th>CD19</th>
<th>CD34</th>
<th>CD38</th>
<th>HLA-DR</th>
<th>CD45</th>
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<tbody>
<tr>
<td>Myelomonocytic</td>
<td>CD15</td>
<td>CD13</td>
<td>CD16</td>
<td>CD11b</td>
<td>CD34</td>
<td>CD38</td>
<td>HLA-DR</td>
<td>CD45</td>
</tr>
<tr>
<td>B cells</td>
<td>Kappa</td>
<td>Lambda</td>
<td>CD20</td>
<td>CD19</td>
<td>CD10</td>
<td>CD38</td>
<td>CD5</td>
<td>CD45</td>
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<tr>
<td>Plasma Cells</td>
<td>Cyto Lambda</td>
<td>Cyto Kappa</td>
<td>CD20</td>
<td>CD56</td>
<td>CD19</td>
<td>CD38</td>
<td>CD45</td>
<td>CD45</td>
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<tr>
<td>T/NK cells</td>
<td>CD16</td>
<td>CD56</td>
<td>CD5</td>
<td>CD3</td>
<td>CD7</td>
<td>CD8</td>
<td>CD4</td>
<td>CD45</td>
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</tbody>
</table>
Case 1

- 37 year-old woman with thrombocytopenia
- Normal hemoglobin and WBC count
- Bone marrow aspirate
CD45 vs. SSC
Progenitors
Case 1:
No Immunophenotypic Abnormalities
Case 2

• 87 year-old man with lymphocytosis (~75,000/uL)
Chronic Lymphocytic Leukemia

• Characteristic phenotype CD5+/CD10- B cells with:
  – Dim CD20+
  – CD23+
  – FMC7+
  – Dim monotypic light chain
Case 3

- 69 year-old woman with normal CBC
- Monoclonal gammopathy
- Peripheral blood submitted for flow cytometry
Monoclonal B-cell Lymphocytosis

- CD5+ B-cell clone represented 0.5% of WBCs (absolute count: 30/uL)
- Prevalence increases with age (>3.5% over 60)
- Low incidence of clinical CLL (~1%/year)
- In the absence of extramedullary tissue involvement, cytopenias, or disease-related symptoms, must have >5,000/uL cells with CLL phenotype to establish diagnosis of CLL
Case 4

- 60 year-old man with abdominal / retroperitoneal lymphadenopathy
- Splenomegaly
- Bone marrow performed – ? lymphoma
Mantle Cell Lymphoma

- Typical phenotype CD5+/CD10- (but may be CD5- and/or CD10+) with:
  - Moderate/bright CD20+
  - CD23-
  - FMC7+
  - Moderate/bright monotypic light chain
- Also consider “atypical CLL,” CLL/PL, B-PL
- Confirm t(11;14), CCND1 rearrangement, and/or cyclin D1 overexpression by IHC
### Distinguishing CD5+/CD10- B-cell Lymphoproliferative Disorders

<table>
<thead>
<tr>
<th></th>
<th>Chronic Lymphocytic Leukemia</th>
<th>Mantle Cell Lymphoma</th>
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<tbody>
<tr>
<td><strong>CD20</strong></td>
<td>Dim+</td>
<td>Moderate/Bright+</td>
</tr>
<tr>
<td><strong>CD23</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>FMC7</strong></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Ig Light Chain Intensity</strong></td>
<td>Dim</td>
<td>Moderate/Bright</td>
</tr>
</tbody>
</table>
Case 5

- 60 year-old woman with a solitary palpable cervical lymph node (1-2 cm)
- Normal appearance on imaging
- FNA of lymph node submitted
$In\ Situ$ Mantle Cell Lymphoma

- Only 2-3\% monoclonal CD5+ B cells with phenotype of mantle cell lymphoma
- Cyclin D1 immunoreactivity in excised lymph node limited to inner zone of mantle cells
- Remainder of staging work-up negative
Case 6

- 19 year-old boy with recurrent pharyngitis and asymmetric enlargement of tonsils
- Tonsillectomy specimens submitted for flow cytometry - ? lymphoma
Follicular Lymphoid Hyperplasia

- Germinal center B cells typically CD10+, CD20(bright)+, CD38+ with dim or undetectable sIg
- Normal mantle zone cells CD5(partial)+
Case 7

- 44 year-old woman with mediastinal lymphadenopathy
- Core biopsy submitted for flow cytometry
Follicular Lymphoma

• Most common phenotype: CD5-, CD10+ (but significant minority is CD10-)
• CD5-/CD10+ phenotype is non-specific:
  – Burkitt lymphoma, DLBCL, B-cell lymphoma unclassifiable, B lymphoblastic lymphoma, (pleomorphic/blastoid) mantle cell lymphoma
Case 8

- 65 year-old man with palpable 1-2-cm cervical lymph node
- Excised lymph node submitted for “lymphoma work-up”
Follicular Lymphoma (sIg-)

- Complete absence of immunoglobulin is an aberrant phenotype for mature B cells.
- Consider cytoplasmic studies (plasmacytic differentiation).
- Recommend further evaluation.
- Not specific for FL, but most common among GC-type B-NHL (somatic hypermutation)
Follicular Hyperplasia

Follicular Lymphoma
Case 9

- 33 year-old man with anemia, thrombocytopenia, monocytopenia, and lymphocytosis
- Splenomegaly on physical exam
Hairy Cell Leukemia

• Characteristic immunophenotype: CD5-/CD10- (but may be CD10+) with:
  – Bright CD11c+
  – CD25+
  – CD103+
  – CD123+
• Annexin A1+ by IHC
• *BRAF* V600E
Case 10

- 80 year-old woman complains of early satiety
- Splenomegaly on exam
- Peripheral blood lymphocytosis
- Lymphocytes with “cytoplasmic projections,” many with nucleoli
Splenic Marginal Zone Lymphoma

- Must be distinguished from HCL (HCL but not SMZL sensitive to purine nucleoside analogs)
- Pronounced lymphocytosis, normal monocyte count, and nucleoli argue against classic HCL
- SMZL may express CD11c or CD25, but usually not CD103 or CD123
- Annexin A1 and/or *BRAF* V600E as appropriate
Case 11

- 48 year-old man with splenomegaly
- Lymphocytosis with “circumferential projections” and prominent nucleoli
- Normal absolute monocyte count
- Peripheral blood submitted
Hairy Cell Leukemia- Variant

- HCL-v one of two types of splenic B-cell lymphoma/leukemia, unclassifiable (WHO, 2008)
  - Splenic diffuse red pulp small B-cell lymphoma (provisional)
- Higher WBC count than HCL, normal monocyte count, prominent nucleoli
- Variant phenotype: usually CD25-, CD123- despite CD11c+/CD103+
- Annexin A1 and BRAF V600E useful
Distinguishing B-cell LPDs with “Hairy” Cells

<table>
<thead>
<tr>
<th></th>
<th>HCL</th>
<th>SMZL</th>
<th>HCL-v</th>
<th>SDRPSBCL</th>
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<tbody>
<tr>
<td>CD11c</td>
<td>Bright</td>
<td>+/-</td>
<td>+</td>
<td>-(?+)</td>
</tr>
<tr>
<td>CD25</td>
<td>+</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD103</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-(?+)</td>
</tr>
<tr>
<td>CD123</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-(?+)</td>
</tr>
<tr>
<td>Annexin A1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>?</td>
</tr>
</tbody>
</table>
Case 12

- 55 year-old man with absolute lymphocytosis (~12,000/uL) discovered incidentally
- Peripheral blood submitted
Biclonal CLL

- Small minority (<5%) of cases
- Depending upon proportions of kappa and lambda clones, may mimic polytypic light chain expression
- Examination for other aberrancies permits correct diagnosis
Case 13

- 71 year-old man admitted to hospital with sepsis
- Normocytic anemia and large M-protein (5.1 g/dL IgG-κ) noted
- Bone marrow performed - ? myeloma
Plasma Cell Neoplasm

- Requires integration with clinical, radiographic, and morphologic findings (CRAB)
- CD38/CD138 and CD45 useful for gating
- CD19 (lost ~95%), CD56 (increased ~75%) most useful aberrancies; cytoplasmic light chain
- Less common (<50% of cases) aberrancies may be useful for MRD detection: CD20, CD27, CD28, CD117
- Plasma cells underrepresented by flow (up to 10X)
Case 14

• 63 year-old woman with lymphocytosis (~9,500/μL) and neutropenia
• PB submitted - ?CLL
T-cell Large Granular Lymphocyte Leukemia

- Persistent (>6 months) increase (>2,000/uL) in PB granular lymphocytes without cause
- Neutropenia (with or without anemia) typical
- Splenomegaly, rheumatoid arthritis common
- Typical phenotype: CD3+/CD8+ T cells with CD16 and CD57; diminished CD5 and/or CD7 common
Case 15

- 45 year-old man from Trinidad with fatigue, weight loss, and splenomegaly
- WBC count ~ 150,000/uL with abnormal lymphs
- Elevated LDH and hypercalcemia
Adult T-cell Leukemia/Lymphoma

- Typical phenotype: CD3+/CD4+ T cells with loss of CD7 and strong CD25
- Not specific for ATL/L (e.g., SS, T-PL)
- Variant phenotypes (e.g., CD8+, CD4+/8+) – see next
- HTLV-1 serology essential
Case 16

- 78 year-old woman with FUO
- Generalized lymphadenopathy
- Cervical lymph node biopsied, portion submitted for flow cytometry
Angioimmunoblastic T-cell Lymphoma

- Typical phenotype: CD4+ T cells with loss of CD3 and/or CD7, and at least partial expression of germinal center markers (CD10, bcl-6, PD-1)
- Normal CD4+/CD10+ TFH retain CD3, CD7
- Majority of reactive T cells not uncommon
- Follicular hyperplasia in early AITL
Case 17

- 67 year-old man with generalized lymphadenopathy
- Lymphocytosis (~120,000/uL)
- Abnormal lymphoid cells with distinct nucleoli and cytoplasmic “blebs”
- PB submitted for flow cytometry
T-cell Prolymphocytic Leukemia

- Usually CD4+ T cells with retention of CD7 (about 25% CD4+/CD8+, 15% CD4-/CD8+)
- 90% of cases with inv(14q) or t(14;14) involving TCRA
- Responsive to alemtuzumab (anti-CD52) – specific diagnosis permits appropriate treatment
Case 18

- 62 year-old woman with Crohn’s disease, treated with infliximab and azathioprine
- Elevated LFTs
- “Blastoid” cells noted in peripheral smear
- Bone marrow submitted for flow cytometry
Hepatosplenic T-cell Lymphoma

• ~20% arise in setting of immunosuppression (solid organ transplants, azathioprine + infliximab in patients with Crohn’s)
• Typical phenotype: CD3+, CD5- with absent or weak CD8, and cytotoxic markers
• Most are TCR-γ/δ+ (but some TCR-α/β+)
• Isochromosome 7q in most
Case 19

- 35 year-old man referred because of lymphocytosis (~5,200/uL) with “atypical lymphs” in routine peripheral smear
- Was told he had “mono” years ago, but did not ever have fever, sore throat or lymphadenopathy
Chronic Lymphoproliferative Disorder of NK Cells

- Persistent (>6 months) increase (>2,000/uL) in NK cells without cause
- Usually asymptomatic; may have anemia/neutropenia
- sCD3-, CD16+, CD56+ (may be weak)
- May have aberrant CD5 and/or loss of CD2, CD7
- Indolent course
Case 20

- 6 year-old boy with bruising
- CBC reveals anemia, thrombocytopenia, and leukocytosis
- Blasts seen in peripheral smear
- Peripheral blood submitted
B Lymphoblastic Leukemia

- CD19+ with variable CD45 (undetectable to dim)
- Most cases CD10+ (>80%; CD10-/CD15+: MLL rearrangement), CD34+ (~75%), TdT+ (>95%)
- CD20 (typically partial/dim), sIg- (<5% sIg+)
- cyCD22 and cyCD79a+
- Myeloid antigens (esp. CD13/CD33) often +, but cyMPO-
- Risk stratification: age, WBC count, response
Case 21

• 70 year-old man with history of B lymphoblastic leukemia with *BCR-ABL1*
• End-of-induction bone marrow submitted
• Re-evaluate marrow status
Minimal Residual Disease (~0.1%)

- Characteristic phenotypic patterns of normal B-cell maturation
- B cells deviating from patterns are abnormal
- Not all antigens “informative” in every case
- Large number of cells must be acquired (>500,000 for 0.01% sensitivity)
- Phenotypic shifts in blasts due to steroids
Case 22

- 45 year-old man with generalized lymphadenopathy
- Mediastinal mass
- Marked leukocytosis (~240,000/uL)
T Lymphoblastic Leukemia

• cyCD3+ (but sCD3 may be dim or absent) and CD7+ in all cases; TdT+ (~90%)
• Other T-cell markers (CD2, CD4, CD5, CD8) variable
• CD1a (~40%), CD10 (<25%), CD34 (~35%), HLA-DR (10-20%)
• Myeloid antigens (CD13, CD15, CD33: ~30%)
• cyCD79a/CD117 (small minority)
“Early T-cell Precursor” (ETP)
Early T-cell Precursor Leukemia

- ~13% of T-ALL
- Distinct GEP and immunophenotype:
  - CD5 weak
  - CD1a, CD8 negative
  - Immature/myeloid marker(s) (CD13, CD33, CD34, CD117, HLA-DR)
- ? Better treated as AML

Case 23

- 17 year-old boy with several weeks of fatigue
- CBC reveals anemia, leukocytosis with “blasts” in peripheral smear
- Peripheral blood submitted
Acute Myeloid Leukemia

- In this example, with immunophenotypic features of at least partial monocytic differentiation: bright CD33, bright CD64, variable CD14 (maturation)
- Other monocytic features: dim CD4, HLA-DR+, CD34 commonly negative
- However, contemporary risk stratification largely driven by genetics, not differentiation
Case 24

- 44 year-old woman presents with pancytopenia and DIC
- Blasts seen in peripheral smear
- Bone marrow submitted
Acute Promyelocytic Leukemia (Hypergranular) [AML with t(15;17)/PML-RARA]

- Essential to recognize possibility of APL:
  - 1. potentially fatal DIC left untreated
  - 2. unique treatment incorporating ATRA
- Characteristic phenotype: HLA-DR-, CD11b-, CD34- with heterogeneous CD13, absent/dim CD15, bright CD33, CD117+, MPO+
- Such phenotypes should trigger prompt notification of clinicians, and expedited FISH/RT-PCR for PML-RARA
Acute Promyelocytic Leukemia (Microgranular) [AML with t(15;17)/PML-RARA]

- Microgranular variant shows lower SSC
- More likely HLA-DR+/CD34+ (usually partial/dim); also associated with CD2 expression
Case 25

- 60 year old woman with anemia and progressive, unexplained thrombocytopenia
- Normal WBC and absolute monocyte counts
- ? Rare blast seen in peripheral smear
- Bone marrow examination
Normal Progenitors

Patient Progenitors
Refractory Cytopenia with multilineage Dysplasia (RCMD)

- Myeloid neoplasms (MDS, MPN, MDS/MPN, AML) commonly display phenotypic aberrations in both progenitors and myelomonocytic cells:
  - Lymphoid antigen coexpression (e.g., CD7, CD56)
  - Abnormal antigen intensity (increased or decreased)
  - Abnormally homogeneous antigen expression
  - Decreased SSC (granulocytes)
  - Abnormal maturation patterns (e.g., CD16 vs. CD13)
Case 26

- 70 year-old woman with unexplained neutropenia, thrombocytopenia
- Absolute monocytosis
- Bone marrow examination performed
Chronic Myelomonocytic Leukemia-1

• Multiple aberrations:
  – decreased SSC among maturing granulocytes
  – abnormal CD16 vs. CD13 among maturing granulocytes
  – aberrant CD56 among monocytes
  – loss of HLA-DR among monocytes