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

<table>
<thead>
<tr>
<th>Clinical application</th>
<th>Parameters measured by flow cytometry</th>
</tr>
</thead>
</table>
| 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 |

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

## Plasma Cell Neoplasm

### A-7-color Panel

<table>
<thead>
<tr>
<th>FITC</th>
<th>PE</th>
<th>PerCP-Cy5-5</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>V450</th>
<th>V500</th>
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<tbody>
<tr>
<td>CD38</td>
<td>CD28</td>
<td>CD19</td>
<td>CD117</td>
<td>CD138</td>
<td>CD56</td>
<td>CD45</td>
</tr>
<tr>
<td>Kappa cyto</td>
<td>Lambda cyto</td>
<td>CD38</td>
<td>-</td>
<td>CD138</td>
<td>CD20</td>
<td>CD45</td>
</tr>
</tbody>
</table>
Plasma cell Neoplasm
Flow Cytometry Analysis
Plasma cell Neoplasm
Aberrant immunophenotype

CD45 V500-A vs CD138 APC-A
- 0.09% CD19 PerCP-Cy5.5-A vs CD56 V450-A
- 0.23% CD28 PE-A vs CD56 V450-A
- 0.26% CD117 PE-Cy7-A vs CD56 V450-A
- 0.03% CD138 APC-A vs CD20 V450-A
- 0.01% cyto Lambda PE-A vs cyto Kappa FITC-A
Plasma cell Neoplasm

B-cell clonality

[Graph and images showing cell counts and percentages for various markers like CD20, CD138, CD38, cyto Kappa, cyto Lambda, and other markers with their respective percentages and scatter plots.]

67.53% cyto Kappa FITC 28.86%
97.18% cyto Lambda PE 0.35%
85.85% CD20 V450-A 13.31%
39.43% CD38 PerCP-Cy5-5-A 2.33%
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

CD45 V500-A vs CD138 APC-A

CD38 FITC-A vs CD28 PE-A

CD19 PerCP-Cy5-5-A vs CD56 V450-A

CD138 APC-A vs CD20 V450-A

Cyto Kappa FITC-A vs Cyto Lambda PE-A
Case Cluster I-Case #2

B-cell clonality

- **CD20 V450-A**
  - 3 × 10^4
  - 4 × 10^4
  - 5 × 10^4

- **FSH/SSH**
  - S S C-A
  - 131072
  - 196608
  - 262144

- **cyto Lambda PE-A**
  - CD20 V450-A
  - -10^2
  - 2 × 10^2
  - 10^3
  - 10^4
  - 10^5

- **cyto Kappa FITC-A**
  - CD20 V450-A
  - -10^2
  - 2 × 10^2
  - 10^3
  - 10^4
  - 10^5

- **CD45 V500-A**
  - 0
  - 65536
  - 131072
  - 262144

- **Percentage**
  - **92.53%**
  - cyto Kappa FITC-A
  - **2.29%**
  - **77.22%**
  - cyto Lambda PE-A
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

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD45</th>
<th>CD19</th>
<th>CD20</th>
<th>CD56</th>
<th>CD38</th>
<th>CD138</th>
<th>CD117</th>
<th>Cytogenetics</th>
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<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>46,XY[cp20]</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>46,XY,t(6;10)(p12;p13)[20]</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XX[20], t(11;14)(q13;q32) (positive by FISH)</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>46,XY,t(11;14)(q12~13.1;q32), del(13)(q14q22),del(17)(p12) [cp2]/46,XY[18]</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>45,XY,t(11;14)(q12~13.1;q32), –13[1]/46,XY[cp19]</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>46,XY,der(14)t(11;14)(q13;q32) [4]/46,XY[8]</td>
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<tr>
<td>10</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>46,XY[20]</td>
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<tr>
<td>11</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>46,XX[20],t(11;14)(q13;q32) (positive by FISH)</td>
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<td>12</td>
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<td>ND</td>
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<td>ND</td>
<td>46,XY[20]</td>
</tr>
</tbody>
</table>

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

- CD38 FITC-A
- CD138 APC-A
- CD138 AP C-A
- CD38 FITC-A
- CD45 V500-A
- Clean CD38/CD138
- FSC-H
- SSC-A
- SSC-A
Case Cluster I-Case #3
FCI Analysis of Plasma Cells

CD45 V500-A
CD19 PerCP-Cy5-5-A
CD138 APC-A
CD117 PE-Cy7-A
CD20 V450-A
CD117 PE-Cy7-0.14%
CD138 APC-A
CD19 PerCP-Cy5-5-A
CD20 V450-A
0.00%
cyto Lambda PE-A
23.84%
cyto Kappa FITC-A
6.43%
Case Cluster I-Case #3
FCI Analysis of Plasma Cells
Case Cluster I-Case #3
Bone Marrow Biopsy
Case Cluster I-Case #3

Immunohistochemistry

PAX5

CD138
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

<table>
<thead>
<tr>
<th>Antigen/Disease</th>
<th>No. (%) Positive</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma (n = 41)</td>
<td>39 (95)</td>
<td>&lt;&lt;.001</td>
</tr>
<tr>
<td>Myeloma (n = 41)</td>
<td>4 (10)</td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma (n = 41)</td>
<td>13 (32)</td>
<td>.81</td>
</tr>
<tr>
<td>Myeloma (n = 41)</td>
<td>11 (27)</td>
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</tr>
<tr>
<td>CD45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma (n = 33)</td>
<td>30 (91)</td>
<td>&lt;&lt;.001</td>
</tr>
<tr>
<td>Myeloma (n = 41)</td>
<td>17 (41)</td>
<td></td>
</tr>
<tr>
<td>CD56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma (n = 16)</td>
<td>3 (33)</td>
<td>.01</td>
</tr>
<tr>
<td>Myeloma (n = 41)</td>
<td>29 (71)</td>
<td></td>
</tr>
<tr>
<td>Surface immunoglobulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma (n = 41)</td>
<td>31 (76)</td>
<td>.006</td>
</tr>
<tr>
<td>Myeloma (n = 41)</td>
<td>13 (44)</td>
<td></td>
</tr>
</tbody>
</table>

PC, plasma cell.

*By the Fisher exact test.

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

CD45 V500-A
CD56 V450-A

CD45 V500-A
CD28 PE-A

CD45 V500-A
CD117 PE-Cy7-A

CD38 FITC-A

22.88% 77.12%
0.00% 0.00%
0.00% CD56 V450-A
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) %

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

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

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

Paiva B et al. Blood 2008;112:4017-4023
FCI in Detection of Minimal Residual Disease
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**

<table>
<thead>
<tr>
<th>FITC</th>
<th>PE</th>
<th>PerCP-Cy5-5</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>V450</th>
<th>V500</th>
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<tbody>
<tr>
<td>CD7</td>
<td>CD26</td>
<td>CD8</td>
<td>CD3</td>
<td>CD4</td>
<td>CD14</td>
<td>CD45</td>
</tr>
<tr>
<td>CD57</td>
<td>CD94</td>
<td>CD16</td>
<td>CD3</td>
<td>CD56</td>
<td>CD8</td>
<td>CD45</td>
</tr>
<tr>
<td>A/B</td>
<td>D/G</td>
<td>CD5</td>
<td>CD3</td>
<td>CD4</td>
<td>CD8</td>
<td>CD45</td>
</tr>
<tr>
<td>CD52</td>
<td>CD2</td>
<td>CD4</td>
<td>CD3</td>
<td>CD10</td>
<td>CD25</td>
<td>CD45</td>
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<tr>
<td>Kappa</td>
<td>Lambda</td>
<td>CD5</td>
<td>CD19</td>
<td>CD10</td>
<td>CD20</td>
<td>CD45</td>
</tr>
</tbody>
</table>
FCI Analysis of T-cells and NK-cells

**Graphs:**
- **CD45 V500-A vs. SSC-A**
  - 35% of cells
- **CD3 PE-Cy7-A vs. SSC-A**
  - 57% of cells

**Legend:**
- CD3
- SSC-A
- CD45 V500-A
FCI Analysis of T-cells and NK-cells

CD3 PE-Cy7-A

- CD4 APC-A
  - 38.5%
  - 17.5%

- CD8 PerCP-Cy5-5-A
  - 36.5%
  - 1.5%

- CD4 APC-A
  - 42.8%
  - 1.2%

- CD8 PerCP-Cy5-5-A
  - 42.5%

- CD3 PE-Cy7-A
  - 41.3%
  - 1.9%

- CD4 APC-A
  - 42.8%
  - 0.6%

- CD8 PerCP-Cy5-5-A
  - 42.5%
  - 21.0%

- CD4 APC-A
  - 38.9%
  - 17.7%

- CD8 PerCP-Cy5-5-A
  - 41.3%
  - 0.4%

- CD4 APC-A
  - 42.8%
  - 1.2%

- CD8 PerCP-Cy5-5-A
  - 42.5%
  - 0.4%

- CD4 APC-A
  - 60.5%
  - 21.2%

- CD8 PerCP-Cy5-5-A
  - 60.1%
  - 21.0%
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

- **CD3 PE-Cy7-A**: 71.9% (71.2%), 25.7% (25.7%), 71.9% (71.9%)
  - CD5 PerCP-Cy5-5-A: 3.2% (3.2%), 0.1% (0.1%), 3.5% (3.5%)
- **CD56 APC-A**: 70.8% (70.8%), 2.9% (2.9%), 27.2% (27.2%)
  - CD5 PerCP-Cy5-5-A: 23.1% (23.1%), 2.7% (2.7%), 17.8% (17.8%)
- **CD57 FITC-A**: 0.9% (0.9%), 78.2% (78.2%), 3.6% (3.6%)
  - TCR D/G PE-A: 10.1% (10.1%), 70.7% (70.7%), 0.4% (0.4%)
- **TCR D/G PE-A**: 17.8% (17.8%), 4.0% (4.0%), 25.7% (25.7%)
  - CD5 PerCP-Cy5-5-A: 23.1% (23.1%), 2.7% (2.7%), 17.8% (17.8%)
- **CD52 FITC-A**: 0.4% (0.4%), 3.6% (3.6%), 78.2% (78.2%)
  - CD5 PerCP-Cy5-5-A: 23.1% (23.1%), 2.7% (2.7%), 17.8% (17.8%)
Case Cluster II-#case 1
Bone Marrow Biopsy
Case Cluster II-#case 1
Bone Marrow Biopsy

CD3
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

- CD3 PE-Cy7-A
  - 5.2% 0.1%
  - 5.2% 0.3%

- CD4 APC-A
  - 89.9%
  - 16.4%

- CD8 PerCP-Cy5-5-A
  - 5.5%
  - 0.0%
  - 78.6%
  - 4.9%

- CD7 FITC-A
  - 38.8%
  - 55.5%

- CD2 PE-A
  - 0.4%
  - 94.3%
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

CD3

Granzyme
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

- CD4 APC-A
  - 4.2% 0.1%
  - 90.1% 5.6%

- CD3 PE-Cy7-A
  - 94.1%
  - 0.1%

- CD8 PerCP-Cy5-5-A
  - 90.1% 5.5%
  - 0.4%

- CD7 FITC-A
  - 2.1% 91.5%

- CD2 PE-A
  - 5.7%

- CD3 PE-Cy7-A
  - 5.3%

- CD5 PerCP-Cy5-5-A
  - 0.1%
  - 93.9%
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 hepatosplenetic T cell lymphoma?
### CD56+ Aggressive Variant LGL

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Absolute WBC ($1 \times 10^9/\text{l}$)</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.8</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XX</td>
</tr>
<tr>
<td>2</td>
<td>29.9</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XY</td>
</tr>
<tr>
<td>3</td>
<td>19.0</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}$</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>17.9</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XY</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XY</td>
</tr>
<tr>
<td>6</td>
<td>18.2</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XY,i(7),i(8:14),+13</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XX,t(2;17),+14</td>
</tr>
<tr>
<td>8</td>
<td>3.0</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XY,i(7),+12</td>
</tr>
<tr>
<td>9</td>
<td>2.6</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>55.0</td>
<td>$\text{CD3}^+\text{CD4}^-\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>i(7q)</td>
</tr>
<tr>
<td>11</td>
<td>9.0</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>NS</td>
</tr>
<tr>
<td>12</td>
<td>106.0</td>
<td>$\text{sCD3}^-\text{eCD3}^+\text{CD8}^+\text{CD56}^-\text{CD57}^-\text{a}$</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{sCD3}^-\text{eCD3}^+\text{CD4}^+\text{CD8}^+\text{CD56}^-\text{CD57}^-\text{b}$</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>53.3</td>
<td>$\text{CD3}^+\text{CD8}^+\text{CD56}^+\text{CD57}^-$</td>
<td>46XY</td>
</tr>
</tbody>
</table>

Summary of LGL leukemia

- **CD4+ variant**
  - CD4+, CD8dimer/neg, CD56+, CD57+
  - often associated with other malignancies
  - do not show cytopenias and autoimmune phenomena

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

Chronic lymphoproliferative disorder of NK-cells (LGL leukemia, NK cell subtype)
## Large Granular Lymphocytic (LGL) Proliferations

<table>
<thead>
<tr>
<th>Type</th>
<th>Clinical features</th>
<th>Associated diseases</th>
<th>Phenotype</th>
<th>TCR</th>
<th>Treatment</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indolent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive lymphocytosis (T or NK)</td>
<td>Transient benign</td>
<td>Viral infection</td>
<td>CD3⁺ CD8⁺, CD57⁺ or CD3⁻ CD16⁺ CD56⁺</td>
<td>Polyclonal</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>T-LGL leukaemia</td>
<td>T-LGLs 2–20 x 10⁹/l &gt; 6 months</td>
<td>RA other AID</td>
<td>CD3⁺ CD8⁺ CD16⁺ CD57⁺</td>
<td>Clonal</td>
<td>Observation MTX, CSA, PAs, MoAbs</td>
<td>&gt;10 years</td>
</tr>
<tr>
<td><strong>CLPD-NK</strong></td>
<td>Often Asymptomatic</td>
<td></td>
<td>KIR (50%)</td>
<td>TIA1, Granymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytopenias, infection, splenomegaly, liver, BM</td>
<td>Malignancy, AID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cells &gt; 2 x 10⁹/l for &gt;6 months</td>
<td>Often Asymptomatic Cytopenias BM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aggressive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-LGL variant</td>
<td>Progressive cytopenias and systemic features</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK-cell leukaemia</td>
<td>Fulminant Blood, BM, Liver, spleen Haemo-phagocytic syndrome</td>
<td>EBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T-cell Clonality Assessment

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)
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

CD45 V500-A

SSC-A

CD45dim gate 68.4%

CD34 PerCP-Cy5.5-A

SSC-A

CD34-PerCP 12.9%
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

CD45 dim gate 82.7%

CD34-PerCP 0.0%
Case Cluster III-case #2

Flow Cytometry Analysis

- **CD34 PerCP-Cy5-5-A**:
  - 0.0% 0.2%
  - 8.9% 90.9%
  - 99.8% 0.0%
  - 0.0% 0.0%

- **CD117 PE-Cy7-A**:
  - 88.5% 0.6%
  - 9.2% 1.7%
  - 95.9% 3.5%
  - 0.0% 0.6%

- **CD33 PE-A**:
  - 69.5% 30.4%
  - 0.0% 0.0%
  - 98.1% 0.0%
  - 3.5% 95.9%

- **CD13 APC-A**:
  - 0.0% 0.0%
  - 69.5% 0.0%
  - 30.4% 0.0%
  - 0.0% 0.6%

- **CD64 PE-Cy7-A**:
  - 0.1% 0.0%
  - 9.2% 1.7%
  - 95.9% 3.5%
  - 0.0% 0.0%

- **CD14 V450-A**:
  - 0.0% 0.0%
  - 0.1% 0.0%
  - 0.0% 0.0%
  - 0.0% 0.0%

- **HLA-DR V450-A**:
  - 0.0% 0.0%
  - 0.0% 0.0%
  - 0.0% 0.0%
  - 0.0% 0.0%

- **CD2 FITC-A**:
  - 0.0% 0.0%
  - 0.0% 0.0%
  - 99.8% 0.0%
  - 99.8% 0.0%
Acute Promyelocytic Leukemia (APL)

Surrogate immunophenotypic profile for M3
HLA-DR\textsubscript{low}, CD11\textsubscript{a}\textsubscript{low}, CD18\textsubscript{low}

Case Cluster III-case #2

Flow Cytometry Analysis
<table>
<thead>
<tr>
<th></th>
<th>APL</th>
<th></th>
<th>non-APL</th>
<th></th>
<th>specificity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(%)</td>
<td>(n)</td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>DR-</td>
<td>35</td>
<td>95%</td>
<td>15</td>
<td>47%</td>
<td>56%</td>
</tr>
<tr>
<td>CD34-</td>
<td>31</td>
<td>84%</td>
<td>18</td>
<td>56%</td>
<td>41%</td>
</tr>
<tr>
<td>CD117+</td>
<td>29</td>
<td>78%</td>
<td>26</td>
<td>81%</td>
<td>21%</td>
</tr>
<tr>
<td>DR-/CD34-/CD117+</td>
<td>23</td>
<td>62%</td>
<td>11</td>
<td>34%</td>
<td>68%</td>
</tr>
<tr>
<td>CD11a-</td>
<td>34</td>
<td>92%</td>
<td>12</td>
<td>38%</td>
<td>62%</td>
</tr>
<tr>
<td>CD18-</td>
<td>34</td>
<td>92%</td>
<td>14</td>
<td>44%</td>
<td>53%</td>
</tr>
<tr>
<td>CD11a - or CD18-</td>
<td>37</td>
<td>100%</td>
<td>20</td>
<td>63%</td>
<td>35%</td>
</tr>
<tr>
<td>CD11a-/CD18-</td>
<td>31</td>
<td>84%</td>
<td>6</td>
<td>19%</td>
<td>79%</td>
</tr>
<tr>
<td>CD11a-/CD18 or CD2+/(CD11a or CD18-)</td>
<td>35</td>
<td>95%</td>
<td>7</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>DR-/(CD11a- /or CD18-)</td>
<td>35</td>
<td>95%</td>
<td>13</td>
<td>41%</td>
<td>62%</td>
</tr>
<tr>
<td>DR-/CD11a-/CD18-</td>
<td>29</td>
<td>78%</td>
<td>4</td>
<td>13%</td>
<td>88%</td>
</tr>
<tr>
<td>DR-/(CD11a-/CD18- or DR- /CD2+/(CD11a- or CD18-)</td>
<td>33</td>
<td>89%</td>
<td>5</td>
<td>16%</td>
<td>85%</td>
</tr>
</tbody>
</table>
Summary (take home message)

FCI is an important tool in hematological Diseases

- Diagnosis
- Classification
- Prognostic markers
- Target therapy
- Monitor 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
CD64, CD15 negative
sCD3, CD5, CD8 negative

CD19, CD20, CD22 negative
Myeloperoxidase?
Immunophenotypic summary

- var CD34
- dim CD45
- CD7
- var CD4
- CD71
- CD33
- CD13
- CD117

AML?
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
Megakaryocytic markers - review

GP IIb/IIIa complex:
- Fibrinogen receptor

CD41
- IIb

CD61
- IIIa

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
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

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
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

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)
Scenario 4:

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

Flow cytometric analysis of peripheral blood
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
Old adage:

*If you hear hoof beats, think horse*

Flow cytometric adage:

*If you hear hoof beats, at least 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
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

- sCD3
- CD5
- CD7
- partial CD2
- br CD45
- CD4
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?
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

Immunophenotypic summary

- sCD3
- CD5
- partial CD2
- CD7
- br CD45
- CD34
- CD1a
- TdT
- CD10
- CD4
Core needle biopsy

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

Identical immunophenotypic profile
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

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Pro-T</td>
<td>+/-</td>
</tr>
<tr>
<td>Pre-T</td>
<td>+/-</td>
</tr>
<tr>
<td>Cortical T</td>
<td>-</td>
</tr>
<tr>
<td>Medullary T</td>
<td>-</td>
</tr>
</tbody>
</table>

**WHO Classification, Lyon 2008**
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 prognosticactor

<table>
<thead>
<tr>
<th>Degree of CD2 expression</th>
<th>6 year EFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;30% positivity)</td>
<td>52.8%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>65.5%</td>
</tr>
<tr>
<td>High (&gt;75% positivity)</td>
<td>71.9%</td>
</tr>
</tbody>
</table>

T-lymphoblastic lymphoma,

medullary T-cell type
• Clinical history is essential

• An old adage …

• Importance of tissue site/specimen
Case 3
Peripheral blood analysis
CD64, CD14 negative
• 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

## Sequence of B-cell maturation in marrow

<table>
<thead>
<tr>
<th>CD</th>
<th>TdT</th>
<th>34</th>
<th>10</th>
<th>19</th>
<th>22</th>
<th>38</th>
<th>20</th>
<th>sIg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>dim</td>
<td>bright</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematogones (stages)</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>dim</td>
<td>bright</td>
<td>dim</td>
<td>-</td>
<td>var</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>dim</td>
<td>bright</td>
<td>+</td>
<td>var</td>
<td></td>
<td></td>
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<tr>
<td>Mature B-cells</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>bright to neg</td>
<td>+</td>
<td>+</td>
<td></td>
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</tbody>
</table>

-McKenna, *Leukemia & Lymphoma*, 2004
Stage 1 hematogones
Stage 2 hematogones
Stage 3 hematogones
Mature B-cells

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

- TdT
- dim CD45
- CD19
- CD22
- CD38
- CD15
- CD34
- CD15
B-lymphoblastic leukemia with t(4;11)(q21;q23)

Immunophenotypic characteristics:

Represents an early precursor (“pro-B”) cell

CD10 negative
- \textit{CALLA} = common acute lymphoblastic leukemia antigen
- Expressed in >90% of childhood and ~75% of adult B-lymphoblastic leukemias

Myeloid coexpression (typically CD15 positive)

\textit{Classic immunophenotypic profile:}

\begin{itemize}
  \item CD45^{\text{dim}}, \text{TdT}^\text{pos}, \text{CD34}^\text{pos}, \text{CD19}^\text{pos}, \text{CD22}^{\text{neg/dim}}, \text{CD20}^{\text{neg}}, \text{CD10}^{\text{neg}}, \text{cyt IgM}^{\text{neg}}, \text{CD15}^{\text{dim}}, \text{mostly CD13}^{\text{neg}}, \text{CD33}^{\text{neg}}, \text{CD9}^{\text{pos}}
\end{itemize}
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]
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
• 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
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
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?

Normal B-cell maturation (healthy adult)

Aberrant B-cell maturation

Residual disease

Ciudad, BJH, 1999
B-cell maturation arrest has clinical significance

<table>
<thead>
<tr>
<th>CD10/CD20/CD19 differentiation</th>
<th>All patients</th>
<th>After induction</th>
<th>During maintenance</th>
<th>After treatment</th>
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<tbody>
<tr>
<td>Normal</td>
<td>n=44</td>
<td>n=25</td>
<td>n=26</td>
<td>n=16</td>
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<tr>
<td>Abnormal</td>
<td>9/31</td>
<td>9/17</td>
<td>1/15</td>
<td>1/14</td>
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<tr>
<td></td>
<td>(29%)</td>
<td>(53%)</td>
<td>(7%)</td>
<td>(7%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>12/13</td>
<td>8/8</td>
<td>11/11</td>
<td>2/2</td>
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<tr>
<td></td>
<td>(92%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
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<tr>
<td>P value</td>
<td>0.0001</td>
<td>0.02</td>
<td>&lt;0.00001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

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)
Relapse: June 2011

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 at least 1 aberrancy
  - 60% of cases demonstrated new aberrancy

- Borowitz, *Cytometry B Clin Cytom*, 2005


- Guglielmi, *Leukemia*, 1997
Final diagnosis

Relapsed/refractory B-lymphoblastic leukemia

with

a phenotypic shift manifest as loss of CD34
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