Clinical Flow Cytometry for the perplexed

Part 3: Lymphomas

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Recap from Parts 1 & 2

• What is Flow Cytometry
• Gating Strategy(ies)
• Technical Issues/Artifacts
Goals for Part 3

• Understand the major diagnostic flow cytometric division in B cell lymphomas
• Recall the phenotype of abnormal plasma cells
• Recognize the maturational pathway of immature B and T cells
Phenotypes you should care about...

- CLL vs MCL
- Follicular Lymphoma
- Things that are big
- Plasma Cell Myeloma
- T cells...???
CD5+ B cell populations (CLL/MCL)

**CLL**
- Decreased "B cell" ness
- Decreased CD20, CD22, light chains
- Absence of FMC7
- Expression of CD200 and/or CD23

**MCL**
- Retained B cell phenotype
- Normal to increased CD20 and CD22
- Retained CD19 and FMC7
- No CD200 or CD23
- Aberrant CD43
Nota Bene

Not everything that is CD5+ is CLL or MCL

• CD5+ Splenic Marginal Zone Lymphoma
• CD5+ DLBCL
• CD5+ B-PLL
CD10+ Follicular Lymphoma

- CD10 is frequently lost in high grade FL
- Benign light chain restricted GC populations
- Cytoplasmic bcl-2 by flow cytometry

- Other things that are CD10+
  - CD10+ HCL
  - CD10+ MZLs
Large Cell Lymphomas

- Gating Strategy must include evaluation of high FS events

  Typical phenotype:
  Preserved or increased CD19, CD20, CD22
  Light chain restricted
  Can be CD5+, CD10+, or both
Large Cell Lymphoma

• Mimics
  » Burkitts
  » B-LBL

• High Grade B cell Lymphoma (Double/Triple Hit)
  » Often decreased CD45, decreased CD20
  » CD10+, increased CD38

• Other things to note:
  » ALK+ DLBCL
  » Plasmablastic Lymphoma
CD5-/CD10- Lymphomas

- HCL
- HCL-\(v\)
- MZL (SMZL, NMZL, MALToma)
- LPL
- CD5- CLL/MCL
- CD10- FL
Hairy Cell Leukemia (CD103omas)

- CD103, CD11c, (CD123, CD25)
- Increased CD20, CD22, Light chains
- 10% with CD10

- HCL-\(v\)
  - Similar except without CD25 and/or CD123

- Splenic Marginal Zone
  - Similar to HCL-\(v\)
Utility of flow in CD5-/CD10- lymphomas

- **MZL vs LPL**
  - Can’t be determined by flow alone (perhaps bright IgM?)
  - CD25 expression
  - CD13 expression

- **HCL vs HCL-v**
  - Slam dunk

- **HCL-v vs SMZL**
  - Little utility

- **CD10-negative FL vs MZL**
  - FL typically associated with higher grade
  - FL will lack CD11c
  - FL with generally have lower CD19 (retained or increased CD20)
B cell lymphomas with plasmacytic differentiation

• B cell LPD
  » Can be anything!
  » MCL, FL, CLL, MZL, LPL

• Plasma Cell
  » Looks ‘normal’
  » LC restricted
  » Retained CD19, gain of CD20
  » No aberrancies in CD56 or CD117
  » Occasionally CD13+
Plasma Cell Neoplasm

• Gating strategy: CD38 high events with variable or decreased CD45
• Cytoplasmic Kappa/Lambda-key to diagnosis
• CD138 isn't a great marker by flow due to antigen loss/shedding
• Phenotypes:
  » CD19-negative 80-90%
  » CD56-positive 50-70%
  *Normal polytypic populations of CD19-/CD56+ PC
Other plasma cell markers

- **CD27**
  » Similar issues with CD138

- **CD81**
  » Normally intermediate, can be increased or decreased

- **CD200**
  » Normally negative, but occasional reactive cases are positive and polytypic

- **CD117**
  » Specific for disease, only 20% of cases
T cells

T cell abnormalities are a dime a dozen and PCR and TCR clones are frequent.

Aberrancy:
- CD4:CD8 as a surrogate “clonality” marker – n.b. New Marker T-cell receptor (TCR) β-chain constant regions (TRBC1) published shows high specificity for clonal processes
- Loss/decreased of CD2, CD3, CD5, CD7

Numerous (normal) T cell subsets exist:
- Memory T cells (CD4+/CD5-)
- Naïve T cells (CD5++/CD4+)
- Gamma-delta T cells (decreased CD8, absent CD5, increased CD3)
T cells

• Large Granular Lymphocytes
  » They happen, is it lymphoma?
  » CD3+, CD57+, CD8+, CD16+, CD56+/-
  » Does it matter? Cytopenias?
  » 6 months persistent LGLs
  » STAT3 mutation may help

• NK cells
  » Morphology like LGLs
  » sCD3-negative, cCD3-maybe+,
  » CD8+
  » CD16+, CD56+/-
Lymphoblastic Leukemias

- B >> T
- Maturation Patterns are the key!
  - Arrested or Dysregulated
Normal B cell Maturation

• Hematogones (all CD19+)
  » Stage 1 – high CD10, CD38, low CD45, absent CD20
  » Stage 2 – gaining CD20 and CD45, retained CD10, high CD38
  » Stage 3 – normal CD20, picks up CD5, some light chains, lose CD10 and CD38, near lymphocyte CD45
3D projections
B-LBL

- CD19, CD22, CD79a
- Decreased CD45 (compared to lymphocytes)
- CD34
- CD10 (aberrant loss)
- CD38 +/-

- Aberrant CD13, CD33, CD20 (low to intermediate)
- Aberrant CD15
<table>
<thead>
<tr>
<th>Marker</th>
<th>Prevalence</th>
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<tbody>
<tr>
<td>CD10</td>
<td>89%</td>
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<tr>
<td>CD13</td>
<td>5%</td>
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<tr>
<td>CD19</td>
<td>100%</td>
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<tr>
<td>CD20</td>
<td>24%</td>
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<tr>
<td>CD22</td>
<td>69%</td>
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<tr>
<td>CD33</td>
<td>31%</td>
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<tr>
<td>CD34</td>
<td>76%</td>
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<tr>
<td>CD45 (bright)</td>
<td>2%</td>
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<tr>
<td>CD45 (moderate)</td>
<td>33%</td>
</tr>
<tr>
<td>CD45 (dim)</td>
<td>36%</td>
</tr>
<tr>
<td>CD45 (negative)</td>
<td>29%</td>
</tr>
<tr>
<td>CD56</td>
<td>36%</td>
</tr>
<tr>
<td>CD79a</td>
<td>88%</td>
</tr>
<tr>
<td>CD117</td>
<td>0%</td>
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<tr>
<td>cytoplasmic IgM</td>
<td>22%</td>
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<tr>
<td>HLA Dr</td>
<td>98%</td>
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<tr>
<td>TdT</td>
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Normal T cell Development

• Normally in the thymus
• Can happen in extrathymic sites
• Thymoma vs normal thymus – not possible by flow alone

• Maturational patterns are preserved
  » Several “subpopulations”
T-LBL

• Arrested maturation
• Aberrant expression of myeloid markers
  » CD13, CD33, CD117, CD15
Theranostics (targeted therapy)

• CD20 - rituximab

• CD22 - inotuzumab ozogamicin

• CD19 – blinatumomab (BITE)

• CD30 - brentuximab vedotin

• CD38 - daratumumab
And next time...

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