Clone Wars

Matthew Ulrickson

January 25, 2013
Disclosures

• Nothing to disclose have I
Objectives

• Review the biology of lymphocyte development and clonal selection

• Discuss the intersection of malignant and non-malignant lymphocyte clones identified clinically

• Discuss the potential impact of subclones in lymphoproliferative malignancies
Bloody Battles

Hematology

Non-malignant (benign)

Malignant

?
Lymphoma in 1899

• That this conception of a lymphoma was largely theoretical in its deduction is evidenced by the admission of the author that "it is easier to say what a lymphoma is not, than what it is."

• "In no department of surgical pathology do we meet with more confusion than in the difference between benign and malignant tumors and inflammatory swellings of the lymph glands."

An Irritated Description

• “It is indeed probable that this lymphoid tissue gives birth to young cells, and one can see how, *under the influence of a mild irritant*, the production of these cells becomes more abundant.

• It results that tumors occur which have for their common characteristics a reticulated network in which the lymphoid elements are accumulated more or less abundantly. Such tumors or lymphatic growths are designated by the name--lymphoma.”

Lancereaux. Traité d’anatomie pathologique. 1875. I:315
Clones

*Clon,* plural *clons* (pronounced with long o),
NEW HORTICULTURAL AND AGRICULTURAL TERMS.

• "The writer is very much opposed to the wholesale introduction of new terms, as they seldom find use outside of an individual writer's papers. In some cases, however, it is absolutely necessary. "

October 16, 1903

*SCIENCE.*
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- **“Clons, which are groups of plants that are propagated by the use of any form of vegetative parts such as bulbs, tubers, cuttings, grafts, buds, etc., and which are simply parts of the same individual seedling.”**
- “We could then use such expressions as the following: 'The clons of apples, pears, strawberries, etc., are not propagated true to seed, while this is one of the important characters of races of wheat and corn”
We Are Clonal
Figure 12-3 Classical hierarchical map of hematopoietic development

Cantor, A. B. et al. ASH-SAP 2010;2010:331-372
Origin of Hematopoiesis

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Granulocytes</th>
<th>Blood Lymphocytes</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial Pool size</td>
<td>16</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>95% CI</td>
<td>9-23</td>
<td>9-33</td>
<td>3-22</td>
</tr>
<tr>
<td>Variance</td>
<td>0.0156</td>
<td>0.0130</td>
<td>0.0241</td>
</tr>
</tbody>
</table>

Investigated X-inactivation patterns using G6PD heterozygotes

- Statistical modeling inferred number of primordial cells contributing to cell type


Image Courtesy of Jon Fukumoto
The Clone Wars Begin

Overview of Bacterial infections

- Bacterial meningitis
  - Streptococcus pneumoniae
  - Neisseria meningitidis
  - Haemophilus influenzae
  - Streptococcus agalactiae
  - Listeria monocytogenes
- Otitis media
  - Streptococcus pneumoniae
- Pneumonia
  - Community-acquired:
    - Streptococcus pneumoniae
    - Haemophilus influenzae
    - Staphylococcus aureus
  - Atypical:
    - Mycoplasma pneumoniae
    - Chlamydia pneumoniae
    - Legionella pneumophila
- Tuberculosis
  - Mycobacterium tuberculosis
- Skin infections
  - Staphylococcus aureus
  - Streptococcus pyogenes
  - Pseudomonas aeruginosa

Overview of Viral infections

- Encephalitis/meningitis
  - JC virus
  - Measles
  - LCM virus
  - Arbovirus
  - Rabies
- Sinusitis
  - Streptococcus pneumoniae
  - Haemophilus influenzae
- Upper respiratory tract infection
  - Streptococcus pyogenes
  - Haemophilus influenzae
- Gastritis
  - Helicobacter pylori
- Food poisoning
  - Campylobacter jejuni
  - Salmonella
  - Shigella
  - Clostridium
  - Staphylococcus aureus
  - Escherichia coli
- Sexually transmitted diseases
  - Chlamydia trachomatis
  - Neisseria gonorrhoeae
  - Other Enterobacteriaceae
  - Treponema pallidum
  - Ureaplasma urealyticum
  - Haemophilus ducreyi
  - Pseudomonas aeruginosa

- Common cold
  - Rhinoviruses
  - Parainfluenza virus
  - Respiratory syncytial virus
- Pharyngitis
  - Adenovirus
  - Epstein-Barr virus
  - Cytomegalovirus
- Gingivostomatitis
  - Herpes simplex type 1
- Cardiovascular
  - Coxsackie B virus
- Hepatitis
  - Hepatitis virus types A, B, C, D, E
- Skin infections
  - Varicella zoster virus
  - Human herpesvirus 6
  - Smallpox
  - Molluscum contagiosum
  - Human papillomavirus
  - Parovirus B19
  - Rubella
  - Measles
  - Coxsackie A virus
- Sexually transmitted diseases
  - Herpes simplex type 2
  - Human papillomavirus
  - HIV
- Pneumonia
  - Influenza virus, Types A and B
  - Parainfluenza virus
  - Respiratory syncytial virus
  - Adenovirus
  - SARS coronavirus
- Parotitis
  - Mumps virus
- Myelitis
  - Poliovirus
  - HTLV-I
- Gastroenteritis
  - Adenovirus
  - Rotavirus
  - Norovirus
  - Astrovirus
  - Coronavirus
- Pancreatitis
  - Coxsackie B virus
Clonal selection theory

• “Clonal selection theory...allows the antigen no part in impressing pattern on the antibody producing cell.

• The capacity to produce a given antibody is a genetically determined quality of certain clones of mesenchymal cells, the function of the antigen being to stimulate cells of these clones to proliferation and antibody production.”

Burnet. BMJ 1959. 2: 645
Antigen Stimulates B Cell Growth

Wiestner. ASH Education Book 2012. 88-96
Antigen Stimulates B Cell Growth

Wiestner. ASH Education Book 2012. 88-96
The Counterbalance
Intrinsic Pro-Apoptotic Pathway

Mitochondrial outer membrane permeabilization

Bim, Bad, Puma, Noxa

20% MCL biallelic deletion

Mutated/silenced in DLBCL cell lines

Nature Education 2010
p53 inactivation leads to decreased apoptosis
p53 inactivation leads to decreased apoptosis

Anti-Apoptotic

Bcl-xL  Bcl-2

Mcl-1  Bcl-w  A1
Cell Death Pathways

Billard. Leukemia 2012. 26:2032
Clone Hunt

• Our ability to detect a clone and diagnose malignancy depends on the method used
  – SPEP
  – Morphology
  – Flow Cytometry
  – Cytogenetics/FISH
  – PCR
SPEP and Immunofixation

Normal Serum Electrophoresis

Monoclonal Gammopathy

Normal Immunofixation

Monoclonal IgG kappa

McPherson and Pincus, Eds. *Henry's Clinical Diagnosis and Management by Laboratory Methods* 2011
Monoclonal Spike

- **RM**
  - 55yo with CRI
  - monoclonal IgM kappa

- **PW**
  - 70yo with CRI
  - monoclonal IgG kappa
Monoclonal Spike

- RM
  - 55yo with CRI
  - monoclonal IgM kappa
  - Renal biopsy with 3+ IgM and 4+ kappa, revealing MPGN

- PW
  - 70yo with CRI
  - monoclonal IgG kappa

Johnson. NEJM 1993. 328:465
Monoclonal Spike

- **RM**
  - 55yo with CRI
  - monoclonal IgM kappa on SPEP
  - Renal biopsy with 3+ IgM and 4+ kappa, revealing MPGN
  - + Hepatitis C
  - + Cryoglobulins

- **PW**
  - 70yo with CRI
  - monoclonal IgG kappa
  - Hepatitis negative
  - MGUS related MPGN
HCV and Type II (mixed) cryoglobulininemia

- Monoclonal
- Somatic hypermutation present
- Most are IgM kappa
- Antibody directed against the Fc portion of Anti-HCV IgG (RF)

- Antibodies precipitate below 36° C
- May cause vasculitis
  - Urticaria
  - Raynaud’s
  - Glomerulonephritis
  - Peripheral neuropathy
  - Arthritis

Gorevic. Clin Devp Imm 2012 Epub
**HCV-associated MPGN**

**Table 1. Clinical features of patients with MPGN and hepatitis C**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at time of SPEP (years)</th>
<th>Gender</th>
<th>SCr at Time of SPEP (mg/dl)</th>
<th>Proteinuria (g/24 h)</th>
<th>Serum Albumin (g/dl)</th>
<th>Urine Microscopy/hpf</th>
<th>SPEP</th>
<th>SIFE</th>
<th>UPEP</th>
<th>UIFE</th>
<th>RF</th>
<th>Cryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>M</td>
<td>3.8</td>
<td>7.2</td>
<td>3.5</td>
<td>4 to 10 RBCs, &gt;25% DR, 3 to 10 WBCs, + for casts</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Type II</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>F</td>
<td>0.8</td>
<td>0.1</td>
<td>3.4</td>
<td>1 to 20 RBCs, &gt;25% DR, + for casts</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>1370</td>
<td>Type III</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>M</td>
<td>1.3</td>
<td>3.9</td>
<td>3.3</td>
<td>3 to 40 RBCs, &lt;25% DR, later &gt;25% DR, + for casts</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
<td>Type II</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>M</td>
<td>1.5</td>
<td>0.4</td>
<td>3.1</td>
<td>4 to 10 RBCs, &lt;25% DR, + for casts</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>&lt;15</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>M</td>
<td>1.6</td>
<td>6.5</td>
<td>4.6</td>
<td>51 to 100 RBCs, &gt;25% DR, + for casts</td>
<td>Negative</td>
<td>M IgM</td>
<td>Negative</td>
<td>M IgM</td>
<td>272</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>M</td>
<td>1.5</td>
<td>3.0</td>
<td>2.8</td>
<td>4 to 10 RBCs, &gt;25% DR, + for casts</td>
<td>Hypo</td>
<td>M IgM</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>F</td>
<td>2.1</td>
<td>1.7</td>
<td>4.6</td>
<td>11 to 20 RBCs, &gt;25% DR, + for casts</td>
<td>Abnormal band in γ region</td>
<td>Negative</td>
<td>M IgM, P IgG</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>M</td>
<td>1.3</td>
<td>2.7</td>
<td>2.3</td>
<td>41 to 50 RBCs, &lt;25% DR, + for casts</td>
<td>Negative</td>
<td>M IgM, P IgG</td>
<td>ND</td>
<td>ND</td>
<td>70</td>
<td>Type II</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>M</td>
<td>1.7</td>
<td>6.22</td>
<td>2.5</td>
<td>4 to 10 RBCs, &gt;25% DR, + for casts</td>
<td>Polyclonal</td>
<td>M IgM, P IgG</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Type II</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>F</td>
<td>5.4</td>
<td>3.6</td>
<td>4.1</td>
<td>4 to 10 RBCs, &gt;25% DR, + for casts</td>
<td>Polyclonal</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Type II</td>
</tr>
<tr>
<td>11</td>
<td>55</td>
<td>M</td>
<td>1.6</td>
<td>&lt;0.2</td>
<td>2.7</td>
<td>41 to 50 RBCs, &gt;25% DR, + for casts</td>
<td>Polyclonal</td>
<td>M IgM, P IgG</td>
<td>ND</td>
<td>ND</td>
<td>347</td>
<td>Type II</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
<td>M</td>
<td>1.5</td>
<td>3.9</td>
<td>4.1</td>
<td>4 to 10 RBCs, &gt;25% DR, + for casts</td>
<td>Polyclonal</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>1170</td>
<td>Type II</td>
</tr>
<tr>
<td>13</td>
<td>53</td>
<td>M</td>
<td>1.6</td>
<td>0.6</td>
<td>4.1</td>
<td>51 to 100 RBCs, &lt;25% DR, + for casts</td>
<td>Hypo</td>
<td>M IgM, P IgG</td>
<td>Negative</td>
<td>M IgM</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

+ casts, positive for RBC casts; Cryo, cryoglobulins; DR, dysmorphic red cells; Hypo, hypogammaglobulinemia; M, monoclonal; ND, not done; P, polyclonal; RF, rheumatoid factor; SCr, serum creatinine; WBC, white blood cell.

### MGUS-associated MPGN

Table 2. Clinical features of hepatitis-negative patients with MPGN and MGUS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gender</th>
<th>BP (mmHg)</th>
<th>Scr (mg/dl)</th>
<th>CrCl (ml/min)</th>
<th>Proteinuria (g/24 h)</th>
<th>SPEP</th>
<th>SIFE</th>
<th>UPEP</th>
<th>UIFE</th>
<th>Cryo</th>
<th>k FLC, λ FLC, (k/\lambda) Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>F</td>
<td>140/65</td>
<td>2.0</td>
<td>31</td>
<td>2.4</td>
<td>Abnormal fraction</td>
<td>λ light chains</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>1.76, 4.13, 0.43</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>M</td>
<td>143/87</td>
<td>1.6</td>
<td>57</td>
<td>1.5</td>
<td>M spike</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Type I</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>F</td>
<td>179/77</td>
<td>3.1</td>
<td>20</td>
<td>4.3</td>
<td>M spike</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>M</td>
<td>173/94</td>
<td>3.6</td>
<td>22</td>
<td>1.4</td>
<td>M spike</td>
<td>IgG κ</td>
<td>Restricted migration</td>
<td>IgG κ</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>F</td>
<td>165/92</td>
<td>3.1</td>
<td>42</td>
<td>4.6</td>
<td>M spike</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>4.34, 3.39, 1.28</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>F</td>
<td>154/80</td>
<td>5.2</td>
<td>11</td>
<td>1.4</td>
<td>Abnormal fraction</td>
<td>IgG κ</td>
<td>Negative</td>
<td>IgG κ</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>F</td>
<td>142/93</td>
<td>1.4</td>
<td>43</td>
<td>3.5</td>
<td>M spike</td>
<td>IgG λ</td>
<td>M spike</td>
<td>IgG λ</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>8</td>
<td>68</td>
<td>M</td>
<td>150/78</td>
<td>6.4</td>
<td>10</td>
<td>0.83</td>
<td>M spike</td>
<td>IgG λ</td>
<td>M spike</td>
<td>IgG λ</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>9</td>
<td>64</td>
<td>M</td>
<td>150/81</td>
<td>3.7</td>
<td>17</td>
<td>1.5</td>
<td>M spike</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>ND</td>
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<tr>
<td>10</td>
<td>60</td>
<td>M</td>
<td>92/67</td>
<td>3.0</td>
<td>22</td>
<td>3.0</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgG κ</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>54</td>
<td>M</td>
<td>160/80</td>
<td>2.8</td>
<td>25</td>
<td>10.3</td>
<td>Negative</td>
<td>IgG κ</td>
<td>Negative</td>
<td>IgG κ</td>
<td>Negative</td>
<td>2.060, 2.180, 0.945</td>
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<tr>
<td>12</td>
<td>53</td>
<td>F</td>
<td>130/90</td>
<td>1.0</td>
<td>62</td>
<td>0.18</td>
<td>Abnormal fraction</td>
<td>IgG κ</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>0.99, 1.60, 0.62</td>
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<td>13</td>
<td>42</td>
<td>M</td>
<td>153/84</td>
<td>2.4</td>
<td>38</td>
<td>9.8</td>
<td>M spike</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>2.83, 0.97, 2.97</td>
</tr>
<tr>
<td>14</td>
<td>42</td>
<td>M</td>
<td>160/101</td>
<td>1.7</td>
<td>47</td>
<td>1.0</td>
<td>Abnormal fraction</td>
<td>IgG κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>57.50, 2.86, 20.10</td>
</tr>
<tr>
<td>15</td>
<td>56</td>
<td>F</td>
<td>145/84</td>
<td>1.8</td>
<td>29</td>
<td>0.55</td>
<td>Abnormal fraction</td>
<td>IgM κ</td>
<td>Negative</td>
<td>IgM κ</td>
<td>Negative</td>
<td>3.22, 1.88, 1.78</td>
</tr>
<tr>
<td>16</td>
<td>58</td>
<td>M</td>
<td>140/85</td>
<td>1.7</td>
<td>51</td>
<td>2.5</td>
<td>Hypo</td>
<td>IgM κ</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>1.61, 1.45, 1.11</td>
</tr>
</tbody>
</table>

CrCl, creatinine clearance; Cryo, cryoglobulins; FLC, free light chains (reference range \(k\) FLC 0.33 to 1.94 mg/dl, \(\lambda\) FLC 0.57 to 2.63, \(k/\lambda\) ratio 0.26 to 1.65); Hypo, hypogammaglobulinemia; ND, not done; Scr, serum creatinine.
Nonmalignant Monoclonal B cells

- Host or unknown antigen
  - ITP
  - Sjögren syndrome
  - Immunodeficiency
  - Cold agglutinin disease

- Known Foreign Antigen
  - Hepatitis C (some CD5+)
  - EBV infection

Morphology

Reactive Lymph Node

Courtesy of Marshall Kadin
Not always black and white

- PTLD

Early Lesion  Polymorphic  Monomorphonic

### PTLD

<table>
<thead>
<tr>
<th>early lesion</th>
<th>polymorphic</th>
<th>monomorphic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waldeyer’s ring</strong></td>
<td>Commonly extranodal (lung, GI, liver)</td>
<td>Involve allograft in 25%</td>
</tr>
<tr>
<td>Mono-like symptoms</td>
<td>Depends on site</td>
<td>May mimic rejection (GVH in SCT pts)</td>
</tr>
<tr>
<td>EBV +</td>
<td>Most EBV + (80%)</td>
<td>Most EBV positive, though fewer than polymorphic</td>
</tr>
<tr>
<td>Early post-transplant</td>
<td></td>
<td>Usually years post-transplant</td>
</tr>
</tbody>
</table>

References:
<table>
<thead>
<tr>
<th>Waldeyer’s ring</th>
<th>Commonly extranodal (lung, GI, liver)</th>
<th>Involve allograft in 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono-like symptoms</td>
<td>Depends on site</td>
<td>May mimic rejection (GVH in SCT pts)</td>
</tr>
<tr>
<td>EBV +</td>
<td>Most EBV + (80%)</td>
<td>Most EBV positive, though fewer than polymorphic</td>
</tr>
<tr>
<td>Early post-transplant</td>
<td></td>
<td>Usually years post-transplant</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>Many are clonal</td>
<td>Most are clonal</td>
</tr>
<tr>
<td>Usually without mutations</td>
<td>Without p53, myc mutations</td>
<td>Frequent mutations, including p53, myc</td>
</tr>
</tbody>
</table>

Flow Cytometry
Early lymphoid proliferations of uncertain biological potential (ELPUBP)

- Monoclonal B-cell Lymphocytosis
  - ALC <5,000 and asymptomatic
    - atypical phenotype (many CD5+, CD23+)
    - atypical light chain restriction (k:λ >3:1 or <0.3:1)
  - Prevalence 12%, increases with age (Shanafelt. Leukemia 2010. 24:512)
  - Some with chromosomal changes (especially those that present clinically) (Rawstron. Best Pr Res Clin Haem 2011. 23:61)
  - May have lymph node involvement (not pathologically enlarged)
  - 1-2% progression to CLL per year

Fend, J HemPath 2012. 5:169
Early lymphoid proliferations of uncertain biological potential (ELPUBP)

- Monoclonal B-cell Lymphocytosis
  - ALC <5,000, atypical phenotype or atypical light chain restriction; asymptomatic
  - Prevalence of 12%, increasing with age (Shanafelt. Leukemia 2010. 24:512)
  - Some with chromosomal changes (especially those that present clinically) (Rawstron. Best Pr Res Clin Haem 2011. 23:61)
  - May have lymph node involvement (not pathologically enlarged)
    - 1-2% progression to CLL per year

- Polyclonal B-cell Lymphocytosis
  - Females, middle-age, usually smokers
  - Normal kappa:lambda ratio
  - Persists >6M
  - May have mild splenomegaly, no LAD
  - Polyclonal increase IgM
  - Usually DR7+ (90%)
  - Subpopulation with characteristic bilobed nuclei
  - CD5 negative, CD23 negative
  - Cytogenetic changes common (77% with iso 3q10, frequent t(14;18)
  - 90% do not develop lymphoma; unclear if ‘premalignant’

Fend, J HemPath 2012. 5:169
Is it all in the genes?

- Cytogenetics
- FISH
- PCR
**Table 18-2 Phenotypic markers and chromosomal translocations in non-Hodgkin lymphomas**

<table>
<thead>
<tr>
<th>NHL</th>
<th>sIg</th>
<th>CD5</th>
<th>CD10</th>
<th>CD20</th>
<th>Other</th>
<th>Cyclin D1</th>
<th>Cytogenetics</th>
<th>Oncogene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL/SLL</td>
<td>Weak</td>
<td>+</td>
<td>–</td>
<td>Weak</td>
<td>CD23&lt;sup&gt;+&lt;/sup&gt;, FMC&lt;sup&gt;–&lt;/sup&gt;</td>
<td>–</td>
<td>No diagnostic abnormalities&lt;sup&gt;*&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Follicular</td>
<td>++</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>t(14;18)</td>
<td>BCL2</td>
<td>–</td>
<td>Antiapoptosis</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>CD23&lt;sup&gt;–&lt;/sup&gt;, FMC&lt;sup&gt;+&lt;/sup&gt;</td>
<td>+</td>
<td>t(11;14)</td>
<td>Cyclin D1</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td>Marginal zone/ extranodal marginal zone lymphoma</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>t(11;18)</td>
<td>API2-MALT</td>
<td>–</td>
<td>Resistance to <em>Helicobacter pylori</em> treatment</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>CD25&lt;sup&gt;+&lt;/sup&gt;, CD38&lt;sup&gt;+&lt;/sup&gt;, CD11c&lt;sup&gt;+&lt;/sup&gt;, CD25&lt;sup&gt;+&lt;/sup&gt;, CD103&lt;sup&gt;+&lt;/sup&gt;</td>
<td>–</td>
<td>t(9;14)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Weak</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Rare</td>
<td>+/–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>t(14;18), t(3;14), t(3;v), t(8;14), t(2;8), t(2;22)</td>
<td>BCL2, BCL6</td>
<td>–</td>
<td>Antiapoptosis Transcription factor</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>TdT&lt;sup&gt;–&lt;/sup&gt;</td>
<td>t(8;14), t(2;8), t(2;22)</td>
<td>CMYC</td>
<td>–</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>ALC, ALK-positive</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>CD2&lt;sup&gt;+&lt;/sup&gt;, CD3&lt;sup&gt;–&lt;/sup&gt;, EMA&lt;sup&gt;+&lt;/sup&gt;</td>
<td>–</td>
<td>t(2;5)</td>
<td>ALK</td>
<td>Tyrosine kinase</td>
</tr>
</tbody>
</table>

<sup>*</sup>See chapter on CLL/SLL (Chapter 19) for prognostic cytogenetic abnormalities.

ALCL = anaplastic large-cell lymphoma; CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B-cell lymphoma; MALT = mucosa-associated lymphoid tissue; sIg = surface immunoglobulin; SLL = small lymphocytic lymphoma; TdT = terminal deoxynucleotidyl transferase.

**Savage, K. J. et al. ASH-SAP 2010;2010:511-554**
Follicular Lymphoma in-situ

• Cells within germinal centers that otherwise show reactive follicular hyperplasia
• Monoclonal and + t(14;18) (Bcl2/IgH)
• Characteristic immunophenotype of FL
• Rare patients have evidence of FL at other body sites
• Seen in 2.5% of reactive lymph nodes removed in one series (Henopp. Histopath 2011. 59:139)
# FL vs FLBUS

<table>
<thead>
<tr>
<th></th>
<th>FL partial involvement</th>
<th>FLBUS (FLIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histopathology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Architectural alteration</td>
<td>Focal</td>
<td>No</td>
</tr>
<tr>
<td>Germinal centres (GC)</td>
<td>Occasionally enlarged, ill-defined borders</td>
<td>Normal size, well defined, sharply demarcated</td>
</tr>
<tr>
<td>Follicle mantles</td>
<td>Focally attenuated and ill defined</td>
<td>Well defined, normal configuration</td>
</tr>
<tr>
<td>Cytology of GC</td>
<td>Monotonous, mostly centrocytes, but centroblasts may be present</td>
<td>Monotonous, mostly centrocytes</td>
</tr>
<tr>
<td>Perifollicular spread</td>
<td>Occasionally</td>
<td>Absent</td>
</tr>
<tr>
<td>Distribution within LN</td>
<td>Affected follicles often clustered</td>
<td>Affected follicles widely scattered, often not contiguous</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCL2</td>
<td>Positive, variable intensity, whole GC</td>
<td>Strongly positive, sometimes only partial GC involvement</td>
</tr>
<tr>
<td>CD10</td>
<td>Positive, occasional interfollicular tumour cells</td>
<td>Strongly positive, restricted to involved GC</td>
</tr>
<tr>
<td>MIB1</td>
<td>Low proliferation</td>
<td>Low proliferation, except for partially involved GC</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manifest FL at other site</td>
<td>Possible</td>
<td>Absent by definition, but FLBUS may involve more than one node</td>
</tr>
</tbody>
</table>

*FLBUS* FL-like B cells of undetermined significance

Fend, *J HemPath* 2012. 5:169
Circulating t(14;18) B Lymphocytes

- PCR of peripheral blood detects t(14;18) in 20-60% of population
- No increased risk of FL
- Most at very low levels
  - $1 \times 10^5$

An even closer look

Subclones in CLL

• Early Mutations
  – Usually clonal (80-100%)

  Early
  MYD88
  Trisomy 12
  del 13q

• Later Mutations
  – Usually subclonal at diagnosis

  Late
  ATM
  TP53
  SF3B1

146/149 CLL samples tested had subclonal mutations identified

Landau, et al. ASH abstract 2012. 5
Clonal Evolution

• Post-treatment
  – Subclonal mutations progressed to clonal in 10/12 patients

• Even without intervening treatment
  – 1/6 had progression of aggressive subclone

• Subclonal driver mutations present at diagnosis led to shorter remissions and time to first treatment
  – Independent of CD38, cytogenetics

Landau, et al. ASH abstract 2012. 5
Clonal Evolution

- Subclonal driver mutations present at *diagnosis* led to shorter remissions and time to first treatment

- Should the finding of a small subclone at diagnosis change our treatment decisions?

Landau, et al. ASH abstract 2012. 5
Molecular history of Richter syndrome: origin from a cell already present at the time of chronic lymphocytic leukemia diagnosis
Subclones in FL

- CD 20 variably expressed
- t(14;18) in >90%
- MLL2 in 50-89%

Green et al. Blood 2013. Epub
Implications for treatment

• Impact of small subclone present at diagnosis?
  – Should it change treatment?
  – How much PCR product is necessary?
Implications for treatment

• Impact of small subclone present at diagnosis?
• Expectations for ‘targeted’ therapy?
  – What percentage of cells are required for clinical efficacy (and use)?
Implications for treatment

• Impact of small subclone present at diagnosis?
• Expectations for ‘targeted’ therapy?
• Measurement of MRD?
  – Could a change in phenotype/genotype limit our ability to detect residual disease?
  – Could ‘founder’ genetic changes misrepresent the persistence of residual disease?
Implications for treatment

• Impact of small subclone present at diagnosis?
• Expectations for ‘targeted’ therapy?
• Measurement of MRD?
• Clinical evaluation is vital to interpret the laboratory data
Implications for Hematology

Non-malignant (benign) → Hematology → Malignant
Thanks

• Dr. Jan Abkowitz
• Dr. Bob Richard

• Fellowship Program and Fred Hutch
• Faculty
• Co-Fellows
• My Family
Questions