Gray Zones and Double Hits
The Problem of High-Grade Burkitt-Like/Atypical Burkitt Lymphoma

Burkitt Lymphoma
Burkitt-Like Lymphoma
DLBCL

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Pathology of Typical Burkitt Lymphoma and Diffuse Large B-cell Lymphoma

BL

BL
Burkitt Lymphoma

- Typical immunophenotype:
  - CD20 +
  - CD10 +
  - BCL6 +
  - BCL2 -
  - CD43 +
  - CD5 -
  - TdT -
  - sIg +

BL: BL
LBLL
DLBCL
BL/DLBCL

5/26/2011
Burkitt Lymphoma

- Karyotype:
  Most have translocations linking MYC to Ig genes:
  - t(8;14) → MYC-IGH
  - t(2;8) → MYC-IGL-κ
  - t(8;22) → MYC-IGL-λ

Burkitt Lymphoma with Atypical Morphology:
The Evolution of High-Grade Burkitt-Like/Atypical Burkitt Lymphoma

Burkitt-Like Lymphoma with Atypical Morphology

- Recognition in early lymphoma classifications:
  - Undifferentiated non-Burkitt’s type (revised Rappaport 1974)
  - Small non-cleaved non-Burkitt’s type (Lukes-Collins 1974)
  - Small non-cleaved non-Burkitt’s type (Working Formulation 1982)
- Resemble BL morphologically but have either:
  - More cellular pleomorphism than typical BL (irregular nuclear contours, prominent nucleoli)
  - More large cells than typical BL
High-Grade Burkitt-Like Lymphoma

REAL Classification (1994):

- “...morphologic features intermediate between large cell lymphoma ... and typical Burkitt's lymphoma.”
- CD20+, usually CD10-?
- MYC rearrangement “uncommon”

<table>
<thead>
<tr>
<th></th>
<th>Burkitt</th>
<th>Non-Burkitt</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
<td>17/18</td>
<td>0/10</td>
</tr>
<tr>
<td>BCL2</td>
<td>0/18</td>
<td>3/10</td>
</tr>
</tbody>
</table>

High-Grade Burkitt-Like Lymphoma

REAL Classification (1994):
“We believe this is not a reproducible category..., but it appears to be necessary for cases that are borderline between large B-cell lymphoma and Burkitt’s lymphoma.”

High-Grade Burkitt-Like Lymphoma (a.k.a. Atypical Burkitt Lymphoma)

**Oncologists hated it!**
- Different therapies used DLBCL vs. BL
- Biologically heterogenous category?

**Pathologists hated it!**
- Accused of hedging by oncologists
- Other pathologists could easily disagree
Approach to High-Grade Burkitt-Like Lymphoma in the 2001 WHO Classification?

GET RID OF IT!!

Burkitt Lymphoma

Diagnosis (WHO, 1999):
- Consistent morphology (BL or BLL)
- CD20+ CD10+ BCL-6+
- Evidence of translocation MYC to IGH or IGL: t(8;14), t(2;8), or t(8;22)
  - Classical cytogenetics
  - c-myc rearrangement by Southern blot
  - FISH
  - Proliferation fraction by Ki-67 or MIB-1

“If cytogenetic or Southern blot analysis is not available, it seems likely that the most reasonable surrogate for c-myc rearrangement is ... a proliferation fraction of >99%” (Harris et al. Ann Oncol 10: 1419-1432; 1999)

Burkitt Lymphoma

Diagnosis (WHO, 2001):
- Consistent morphology (BL or BLL)
- CD20+ CD10+ BCL-6+
- “All cases have” a translocation of MYC to either IgH or IgL: t(8;14), t(2;8), or t(8;22)
- Burkitt-like variant (a.k.a. atypical Burkitt lymphoma) declared to be simply variant of Burkitt lymphoma, having greater pleomorphism in nuclear size and shape, but also having:
  - growth fraction by Ki-67/MIB-1 of “nearly 100%”
  - “proven or strong presumptive evidence of MYC translocation”
Problems with the 2001 WHO Approach to Burkitt-Like Lymphoma
Normal Green=Ig Red=MYC

Single balanced Ig/MYC

Multiple Ig/MYC

MYC amplification

Ig/MYC & MYC amplification

Chromosomal analysis of monoblastic cells revealed an abnormal clone characterized by a translocation between the 17 and 21, and trisomy 21 in two cells. Five cells showed loss of the Y chromosome. These two clones shared different abnormalities, as described in the hypotype above. Fifteen cells have an apparently normal chromosome complement, with no evidence of clinically significant numerical or structural chromosome abnormalities.

Although the W1272 is not consistent with follicular lymphoma, it does suggest the presence of a neoplastic clone. Trisomy 21 is a recurrent, but uncommon, finding in hematologic malignancies.
TABLE 2. Summary of FISH Results

<table>
<thead>
<tr>
<th>General Pattern</th>
<th>Childhood BL (n = 9)</th>
<th>Adult BL/LLB (n = 27)</th>
<th>DLBL (n = 76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IG/MYC positive</td>
<td>9/9 (100%)</td>
<td>8/27 (30%)</td>
<td>1/76 (3%)</td>
</tr>
<tr>
<td>IGH/BCL2 negative</td>
<td>—</td>
<td>3/27 (11%)</td>
<td>13/76 (17%)</td>
</tr>
<tr>
<td>Only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGH/BCL2 positive</td>
<td>—</td>
<td>4/27 (15%)</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both (&quot;Double hit&quot;)</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BL, Burkitt lymphoma; BL/LLB, Burkitt-like lymphoma; DLBL, diffuse large B-cell lymphoma.

McClure et al. AJSP 29: 1652; 2005

Cytogenetics and Survival in Burkitt-Like Lymphoma (Adults & Children)

Macpherson et al. J Clin Oncol 17:1558; 1999

- Younger
- Low-stage
- Simpler karyotypes
- Older
- High-stage
- Complex karyotypes

Snuderl et al. AJSP 34: 327; 2010

p=0.002 (DHL vs BL)

p=0.04 (DHL vs DLBCL)
Double Hit Lymphoma

- DHL may explain difference in long-term survival between pediatric & adult BL/BLL
  - Pediatric: 70-80%
  - Adult: 15-25%
- DHL tends to:
  - Occur in older patients (30%)
  - Show very complex karyotypes
  - Show strong staining for BCL2
- But some adults with BL/BLL will have genetically simple single-hit disease – important for us as pathologists identify these different populations!

Double Hit Lymphoma

Not restricted to cases of BL/BLL...

- Described in a number of B-cell lymphomas:
  - Burkitt or Burkitt-like lymphoma (most common)
  - Diffuse large B-cell lymphoma (occ.)
  - TdT+ B-cell lymphoblastic leukemia/lymphoma (occ.)
  - Plasmablastic lymphoma (rare)
  - Low-grade follicular lymphoma (rare)
- So it is a genotype associated with a very poor prognosis in various B-cell neoplasms (but most common in BL/BLL)

Double Hit Lymphoma

Is MYC the second hit?

- Several case reports of DHL arising in patients with low-grade follicular lymphoma with known BCL2 translocation
- Even more case reports of DHL in patients with history of follicular lymphoma
- But most DHL cases clinically de novo

Triple Hit Lymphoma?

- Some authors expand definition of DHL to include cases with:
  - any MYC translocation, plus
  - any translocation of BCL2, BCL6, or CCND1
- 2008 WHO accepts as DHL:
  - any MYC plus any translocation of BCL2 or BCL6 (MYC plus translocations common in FL or DLBCL)
  - calls cases with MYC + BCL2 + BCL6 “triple hit” lymphoma
Lessons Learned from Gene Expression Profiling Studies of Burkitt Lymphoma

Molecular Diagnosis of Burkitt Lymphoma
(Hummel et al. NEJM 354: 2419; 2006)

Pathologic Features of molecular BL Cases

<table>
<thead>
<tr>
<th>CD10+</th>
<th>100%</th>
</tr>
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<tbody>
<tr>
<td>BCL6+</td>
<td>100%</td>
</tr>
<tr>
<td>BCL2+</td>
<td>19%</td>
</tr>
<tr>
<td>Any MYC trans.</td>
<td>91%</td>
</tr>
</tbody>
</table>

Bl can be BCL2+?
9% will lack MYC!

Ki-67 ≥ 95% | 66% |
34% Ki-67 < 95%!

Fate of High-Grade Burkitt-Like Lymphoma in 2008 WHO Classification?

IT’S BA-ACK

And this time, it’s even wordier...
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
### Burkitt Lymphoma vs. “DLBCL/BL”

#### Acceptable for Classical Burkitt?

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Immunophenotype &amp; Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>✓</td>
<td>X</td>
</tr>
</tbody>
</table>

*BCL2+ can be redeemed by FISH MYC+ BCL- BCL6-
**If FISH MYC-, morphology & IP must be perfect for BL dx

### Burkitt Lymphoma WHO 2008

**Diagnostic Criteria**

- **Morphology:**
  - Uniform population of intermediate size cells with scant cytoplasm, round nuclei, clumped chromatin, nucleoli
  - Minimal deviations from classic cytology permitted (increased nuclear irregularity, single prominent nucleoli) permitted, but not significant pleomorphism with many admixed large cells

- **Immunophenotype:**
  - CD20+ CD10+ BCL6+ BCL2- CD43+ TdT- (20% weak BCL2+)
  - If strongly BCL2+, must have translocation of MYC but no translocations of BCL2 or BCL6 genes (not “double hit”)

- **Genotype:**
  - MYC translocations in 90%
  - If no MYC translocation even by FISH, all other features must be completely typical

### B-cell lymphoma, unclassifiable, with features intermediate between DLBCL & BL

**DLBCL/BL Diagnostic Criteria**

- **Morphology:**
  - Some resemble classical BL (but not immunophenotype)
  - Most have mix of BL-like and DLBCL-like cells
  - Cannot have pure large cell morphology (MYC+ DLBCL)

- **Immunophenotype:**
  - Some have classical BL phenotype (but not morphology)
  - Many have strong BCL2 (suggests “double hit”)
  - MIB-1 staining usually high, but can be 50-100%

- **Genotype:**
  - Often complex with multiple abnormalities
  - May lack MYC translocations or be “double hit”

### Negotiating the Gray Zone: Making a Diagnosis of Burkitt Lymphoma or “DLBCL/BL”

1. Morphology c/w BL or BLL
Diagnosing BL vs. DLBCL/BL

First ask: Is morphology really acceptable?

- Could the cells be lymphoblasts?:
  - If yes (often is), exclude lymphoblastic leukemia/lymphoma with negative stains for TdT & CD34
- Are all cells (or almost all) large?
  - If yes, BL & DLBCL/BL is excluded; diagnosis is DLBCL, or pleomorphic mantle cell lymphoma if CD5+ and BCL1+
- Could cells be small lymphocytes?:
  - If yes, look for mitoses, apoptosis, MIB-1 >50%
    • If these are absent, argues against high-grade NHL
Negotiating the Gray Zone: Making a Diagnosis of Burkitt Lymphoma or “DLBCL/BL”

1. Morphology c/w BL or BLL
2. Let immunophenotype & guide you to the diagnosis following WHO guidelines
Molecular Diagnosis of Burkitt Lymphoma
(Hummel et al. NEJM 354: 2419; 2006)

**Molecular Signature**

<table>
<thead>
<tr>
<th>Pathologic Dx</th>
<th>mBL</th>
<th>Intermediate</th>
<th>Non-mBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Atypical BL*</td>
<td>75%</td>
<td>14%</td>
<td>5%</td>
</tr>
<tr>
<td>DLBCL</td>
<td>7%</td>
<td>24%</td>
<td>70%</td>
</tr>
</tbody>
</table>

*Defined as:
- Burkitt-like morphology, OR
- Deviant immunophenotype (not CD20+ CD10+ BCL6+ BCL2- CD5- Ki-67 ≥95%)

**Pathological Diagnosis**

- **Burkitt's Lymphoma**
  - Pathological Diagnosis: Burkitt (N=25)
  - Original Diagnosis: Burkitt's Lymphoma or Burkitt-like Lymphoma (N=41)
- **Atypical Burkitt's Lymphoma**
  - Pathological Diagnosis: Atypical Burkitt's Lymphoma (N=20)
  - Original Diagnosis: Burkitt-like Lymphoma (N=223)
- **ABC**
  - Pathological Diagnosis: ABC (N=78)
  - Original Diagnosis: Burkitt's Lymphoma or Burkitt-like Lymphoma (N=41)
- **GCB**
  - Pathological Diagnosis: GCB (N=82)
  - Original Diagnosis: Burkitt's Lymphoma or Burkitt-like Lymphoma (N=41)
- **PMBL**
  - Pathological Diagnosis: PMBL (N=33)
  - Original Diagnosis: Burkitt's Lymphoma or Burkitt-like Lymphoma (N=41)
- **Unclassified**
  - Pathological Diagnosis: Unclassified (N=10)
  - Original Diagnosis: Burkitt's Lymphoma or Burkitt-like Lymphoma (N=41)