Double Hit Lymphomas

• What are double hit lymphomas?
• Historical perspectives
• Recognizing DHLs among Burkitt and Burkitt-like lymphomas
• Recognizing DHLs among DLBCL
• Beyond Double Hits: Recognizing “double hit biology” by IHC

Double Hit Lymphomas

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MYC

BCL2

BCL6

MYC

BCL2

or

BCL6
Fluorescent in situ hybridization of tumor cells demonstrating (A) presence of t(14;18) and (B) split at MYC locus (red 5′ and green 3′ regions).

Double Hit Lymphoma

Is MYC the second hit?

- Many DHLs arise in patients with prior follicular lymphoma, often with known BCL2 translocations
- BCL2 translocations mediated by recombinase activating gene 1/2 (RAG1/2) in precursor B-cells; MYC translocations mediated by activation induced cytidine deaminase (AICDA) in mature B-cells
- MYC translocations in DHL often to IG light chain genes or non-IG genes

DHL is a genotype associated with a very poor prognosis in various B-cell neoplasms

Lymphomas with double-hit genotype:
- Burkitt or Burkitt-like lymphoma (most common) (many in WHO “gray zone” category B-UNC/DLBCL/BL)
- Diffuse large B-cell lymphoma (less common)
- TdT+ B-cell lymphoblastic leukemia/lymphoma (occ.)
- Low-grade follicular lymphoma (rare)
- Plasmablastic lymphoma (rare)

Historical Perspectives
High-Grade Burkitt-Like Lymphoma

High-Grade Burkitt-like (REAL 1994):

- Generally resembles Burkitt lymphoma but has:
  - “Morphologic features intermediate between large cell lymphoma ... and typical Burkitt's lymphoma”

- Noted to have frequent translocations of BCL2 (unusual in typical Burkitt lymphoma)

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

- MYC only
- MYC + BCL2 dual (double hit)

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

Younger
- Low-stage
- Simpler karyotypes

Older
- High-stage
- Complex karyotypes

Li et al. Mod Pathol 25:145

Normal Green=Ig Red=MYC

Single balanced Ig/MYC

Multiple Ig/MYC

MYC amplification

Ig/MYC & MYC

McClure et al. AJSP 29: 1652; 2005
Both! ("Double hit")

Snuderl et al. AJSP 34: 327; 2010

**Double Hit**

Snuderl et al. AJSP 34: 327; 2010
Recognizing DHLs Among Lymphomas Resembling Burkitt Lymphoma

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!

HGBL vs. Gray Zone
- High-Grade Burkitt-like (REAL 1994):
  Generally resembles BL but has:
  - “Morphologic features intermediate between large cell lymphoma ... and typical Burkitt’s lymphoma”
- B-UNC/DLBCL/BL (WHO 2008):
  Generally resembles BL (most cells Burkitt-like), but has either:
  - significant population of large cells, or
  - abnormal immunophenotype (esp. BCL2+),
Too Many Large Cells

DLBCL

Burkitt Lymphoma

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

“I entirely agree with you (and feel myself supported by many others like Elaine Jaffe) that from a morphologic point of view, we really should be liberal in diagnosing Burkitt lymphoma as long as the tumor cells have a typical phenotype and MYC breakpoint (and lack a BCL2 and BCL6 breakpoint).”

- Philip Kluin, 2011 (personal communication)
**Molecular Diagnosis of Burkitt Lymphoma**

(Hummel et al. NEJM 354: 2419; 2006)

Pathologic Features of molecular BL Cases

- **CD10+** 100%
- **BCL6+** 100%
- **BCL2+** 19% ← BL can be BCL2+?
- Any MYC trans. 91% ← 9% will lack MYC!
- Ki-67 ≥95% 66% ← 34% Ki-67 <95%!

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**Burkitt Lymphoma**

- **Typical 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**
  - **But…**
    - Non-IG partner favors DHL (34%) over BL (2%)
    - 10% BL will lack **MYC** translocations by FISH, 2008 WHO still permits dx of BL provided case is “otherwise completely typical”

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**Guidelines & rules of thumb for diagnosis**

- Be liberal in diagnosing Burkitt lymphoma as long as the tumor cells have a typical phenotype and MYC translocation (and lack BCL2 & BCL6 translocations)
- DLBCL morphology excludes Burkitt lymphoma
- Burkitt lymphoma will lack MYC translocation in 10% of cases; diagnosis still permitted if all other features completely typical
- Non-IG translocations rare in true Burkitt lymphoma
- Complex karyotype by standard cytogenetics argues against Burkitt lymphoma
Recognizing DHLs Among Diffuse Large B-Cell Lymphomas

Finding DHLs Among DLBCLs

The Problem...
- DLBCLs that are DHLs often present with high-stage disease, but so do many other DLBCLs
- No distinctive morphology or immunophenotype
- Most CD10+ BCL6+ MUM1- by IHC, consistent with “good prognosis” DLBCL subgroup per Hans et al., yet have highly lethal with median survival <1 year
- DHLs only ~5% of DLBCLs, so FISH testing of all DLBCL cases would yield many negative results
- Yet DLBCL so much more common than Burkitt lymphoma that DLBCL DHLs nearly as numerous as Burkitt-like DHLs in total number

The Solution?
- New antibody recently developed that correlates well with MYC protein levels in tissue by IHC
- Long known that translocation only one mechanism of MYC activation (amplification, mutation, miRNA dependent mechanisms are others)
- MYC protein expression may be “final common pathway” for all MYC activation mechanisms.
- Three IHC studies recently published to test whether IHC for MYC and BCL2 protein could be as good or better than FISH to detect “double hit biology.”
Detecting DHL in DLBCL by IHC


"Double Hit Score" (DHS):
- MYC ≥40% cells (median): 1 point
- BCL2 ≥70% cells (median): 1 point
- Total point range: 0-2 points

Increased identification of patients with "double hit biology" from 6% to 29%!

Detecting DHL in DLBCL by IHC


IHC Scoring:
- MYC+: ≥40% cells
- BCL2+: ≥50% cells

- Survival difference by IHC score independent of IPI score, COO subtype, & presence of true double-hit by FISH
- High level MYC staining insignificant unless BCL2 overexpressed as well
- 2/3 DHL long-term survivors MYC <40%

Detecting DHL in DLBCL by IHC

Horn et al. Blood 121:2253; 2013

"Sum Score":
- MYC hi (>40% cells): 1 point
- BCL2 hi (>0% cells): 1 point
- BCL6 hi (>25% cells): 1 point
- Sum point range: 0-3 points

Double-hit B-cell Lymphomas with BCL6 and MYC Translocations

Overall Survival (Months)
Diffuse Large B-Cell Lymphoma

Prognostic Markers

- CD10
- BCL6
- MUM1
- MYC
- BCL2

“Cell of origin” per Hans et al.

“Double hit score” per Green et al.