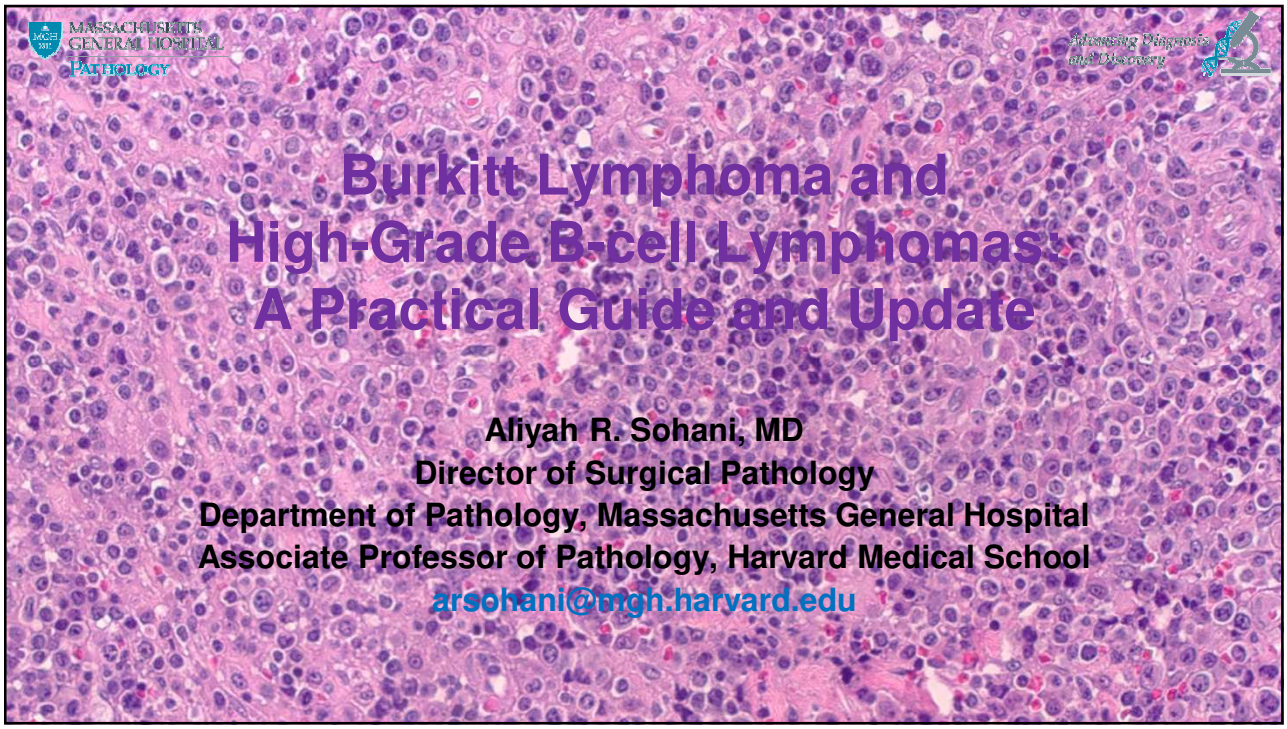
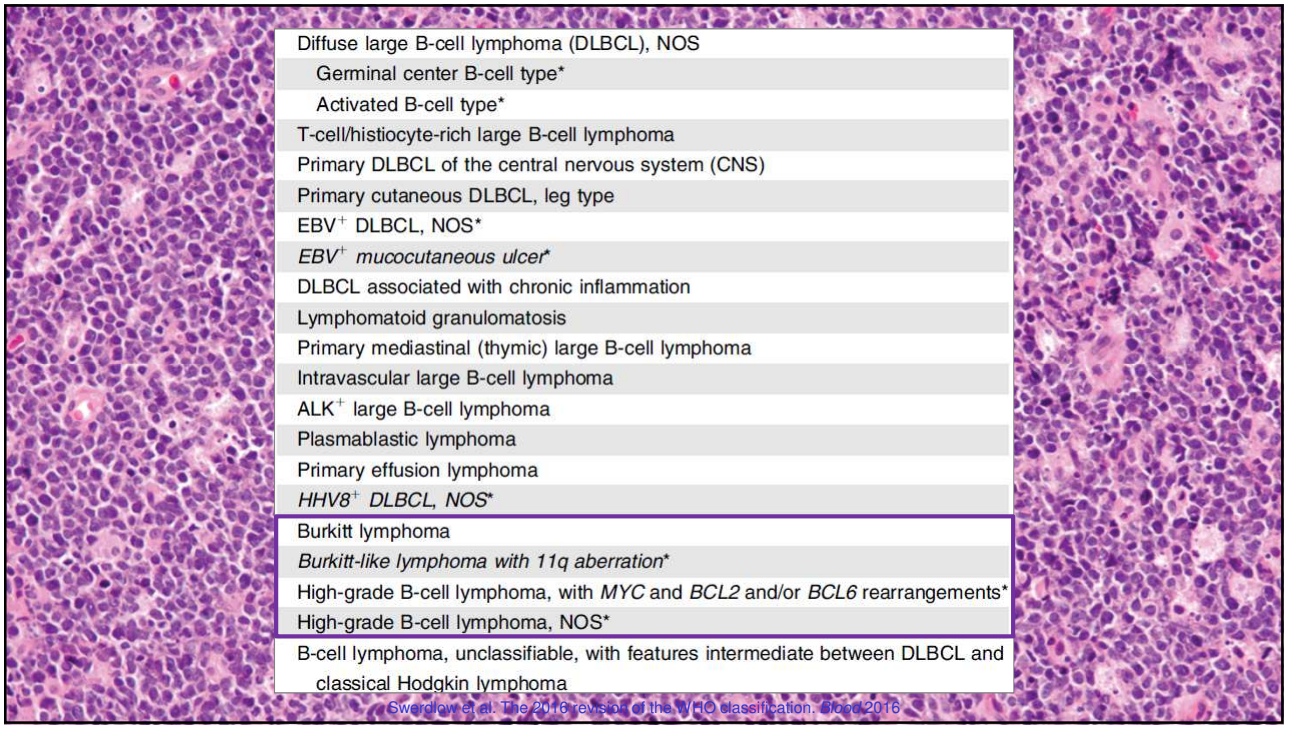


Disclosures

- Consulting: AbbVie Inc., Levin Papantonio PA, Mersana Therapeutics Inc.
- Scientific Advisory Board: AbbVie Inc.

Outline: Aggressive B-cell Lymphomas

- Burkitt lymphoma (BL)
  - Classic Burkitt lymphoma
  - Burkitt-like lymphoma with 11q aberration
  - Differential diagnosis of Burkitt lymphoma
- High-grade B-cell lymphoma (HGBL)
  - with *MYC* and *BCL2* and/or *BCL6* rearrangements
  - not otherwise specified
  - …transformed from follicular lymphoma
  - …with TdT expression

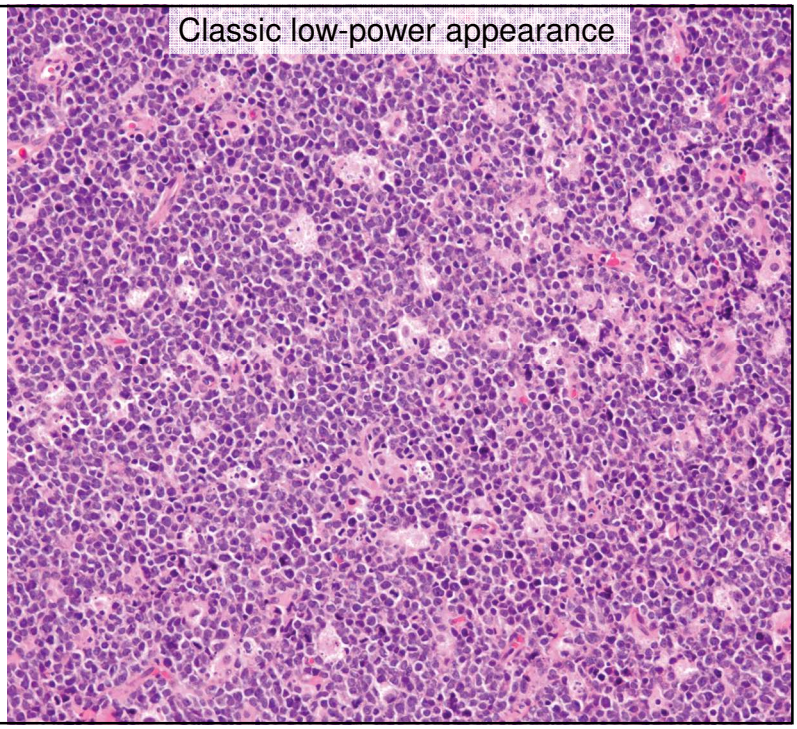


Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
*EBV$^+$ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK$^+$ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8$^+$ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Swerdlow et al. The 2016 revision of the WHO classification. Blood 2016

Burkitt Lymphoma

- Highly aggressive B-cell lymphoma with extremely short doubling time
- May present at extranodal or nodal sites or as an acute leukemia
- “Starry-sky” appearance at low power
- Uniform, round, medium-sized cells
- Nucleoli usually small and multiple
- Cytoplasmic “molding” to adjacent cells



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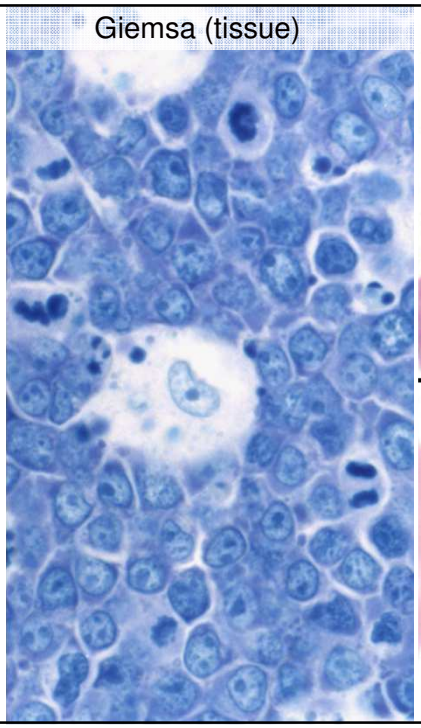
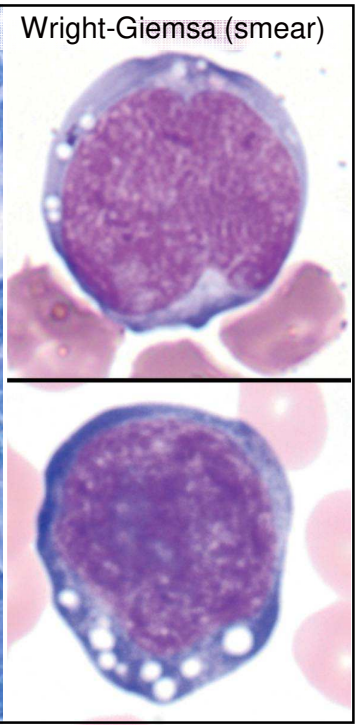
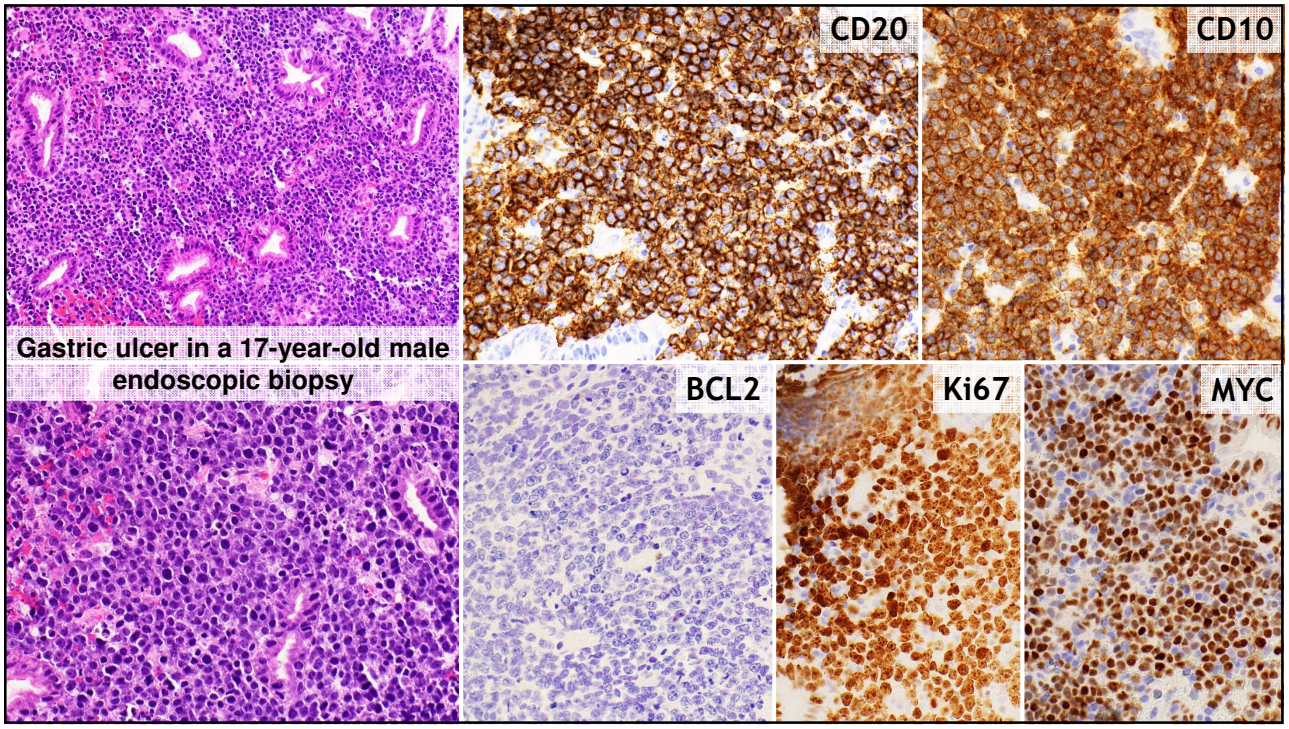


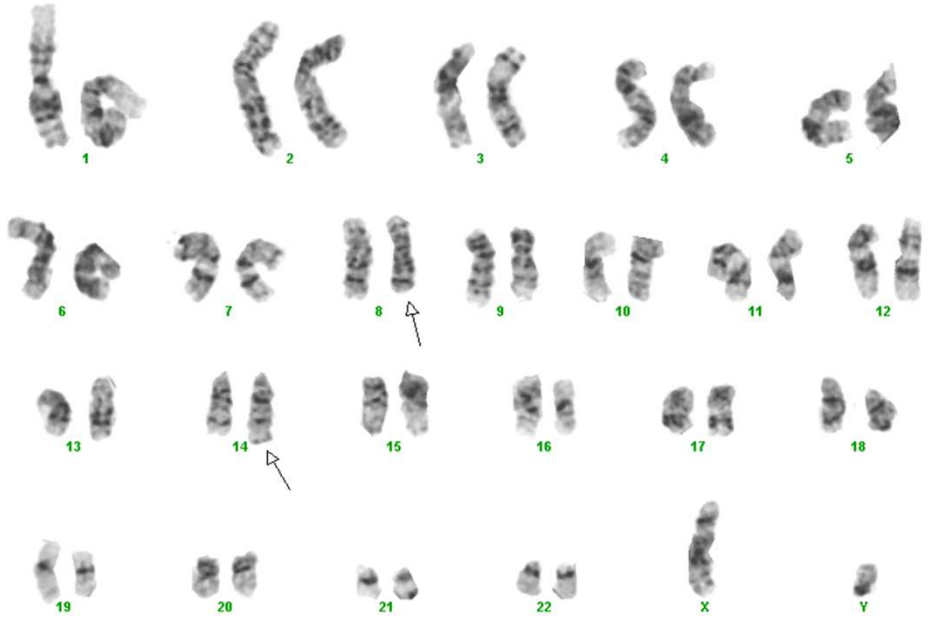


Immunophenotype and Genetics

- Immunophenotype
  - B-cell antigens: CD19+, CD20+, PAX5+, moderate/strong surf IgM + LC
  - Germinal center derivation: strong CD10+, BCL6+, CD38+(bright)
  - LMO2 *negative* (LMO2 negativity correlates with *MYC*-R*)
  - Unlike many other BCLs, BCL2 is *negative* or only weakly positive
  - Relatively few non-neoplastic background T cells
  - Ki67 proliferation index (PI) nearly 100%
  - Variable proportion of cases EBV-driven (EBER+)
- Genetics
  - Translocation involving *MYC* proto-oncogene highly characteristic, but <u>not</u> specific to Burkitt lymphoma

* Colomo et al. *Am J Surg Pathol* 2017
* Liu et al. Diagn Pathol 2019



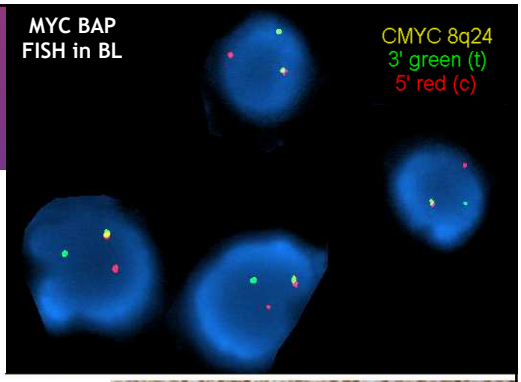


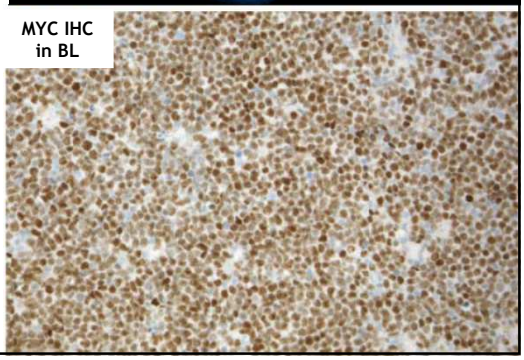
46,XY,t(8;14)(q24;q32)

MYC FISH and Immunohistochemistry

- *IG-MYC* rearrangement in BL
  - *MYC* overexpression drives cell cycle and activates target genes involved in apoptosis
  - Most common partner: *IGH* t(8;14)
  - Less common: *IGL* t(8;22) or *IGK* t(2;8)
  - Sole abnormality or simple background karyotype
    - No *BCL2* or *BCL6* rearrangements
- MYC IHC in BL (clone Y79)
  - 90-100% tumor cell staining of high intensity
  - A relatively high cutoff (≥70% tumor cell staining) 100% sensitive and 93% specific for presence of a *MYC* rearrangement

Green et al. *Am J Surg Pathol* 2012





Burkitt Lymphoma: Clinical Variants

Endemic BL	Sporadic BL	Immunodeficiency-associated BL
Mainly children, peak at 4-7 years	Children and young adults, median 30 years	HIV+ adults, usually preserved CD4+ T-cell count
Equatorial Africa and New Guinea	Worldwide	Worldwide
Extranodal (often jaw and face)	Often extranodal (intrabdominal)	Nodal and/or bone marrow
EBV+ >90%	EBV+ ~30%	EBV+ 25-40%
Uniform nuclei	Classic and “atypical” variant	Plasmacytoid or other “atypical” features

Burkitt Lymphoma: Morphologic Variants

- No longer formally recognized in 2008/2017 WHO Classifications
- Adult cases: greater nuclear irregularity and mild pleomorphism
- Immunodeficiency-associated cases: plasmacytoid +/- nucleolar prominence

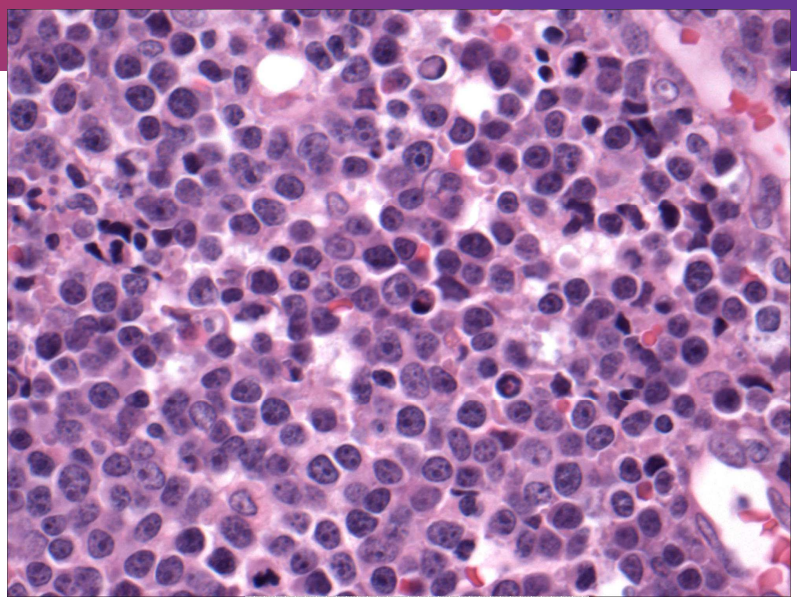


Photo courtesy of Dr Robert Hasserjian, MGH

Differential Diagnosis of Burkitt Lymphoma

- Benign reactive process
  - Floridly reactive follicular hyperplasia
- Lymphoblastic lymphoma
  - TdT+, surface Ig-, no *MYC* rearrangement
- Mature B-cell lymphomas
  - Blastoid variant of mantle cell lymphoma
  - Burkitt-like lymphoma (high-grade B-cell lymphoma) with 11q aberrations
  - High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements (double/triple-hit lymphoma)
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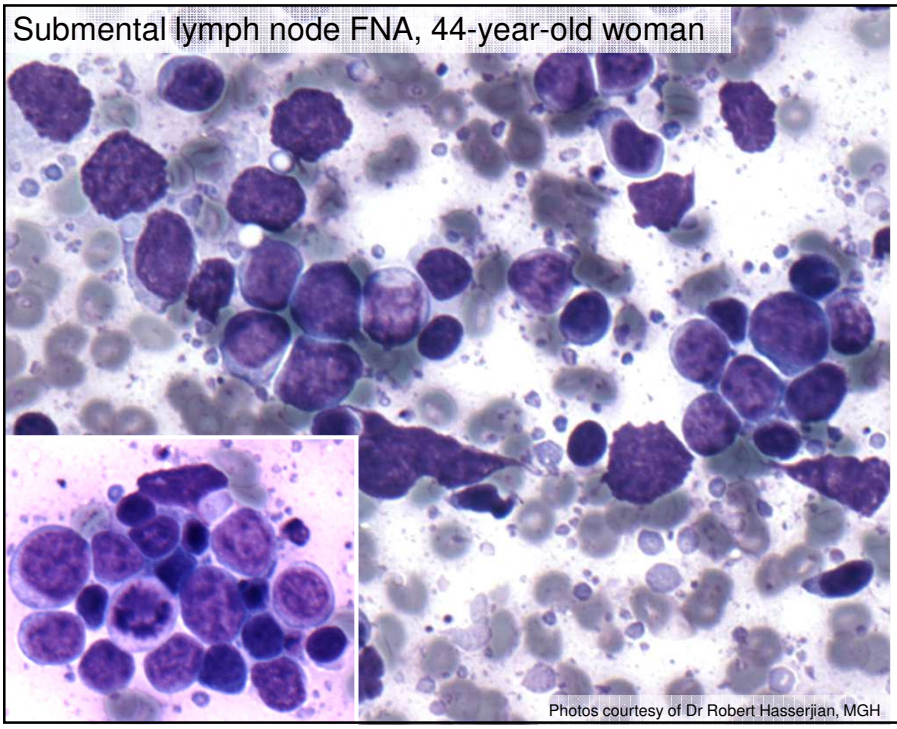
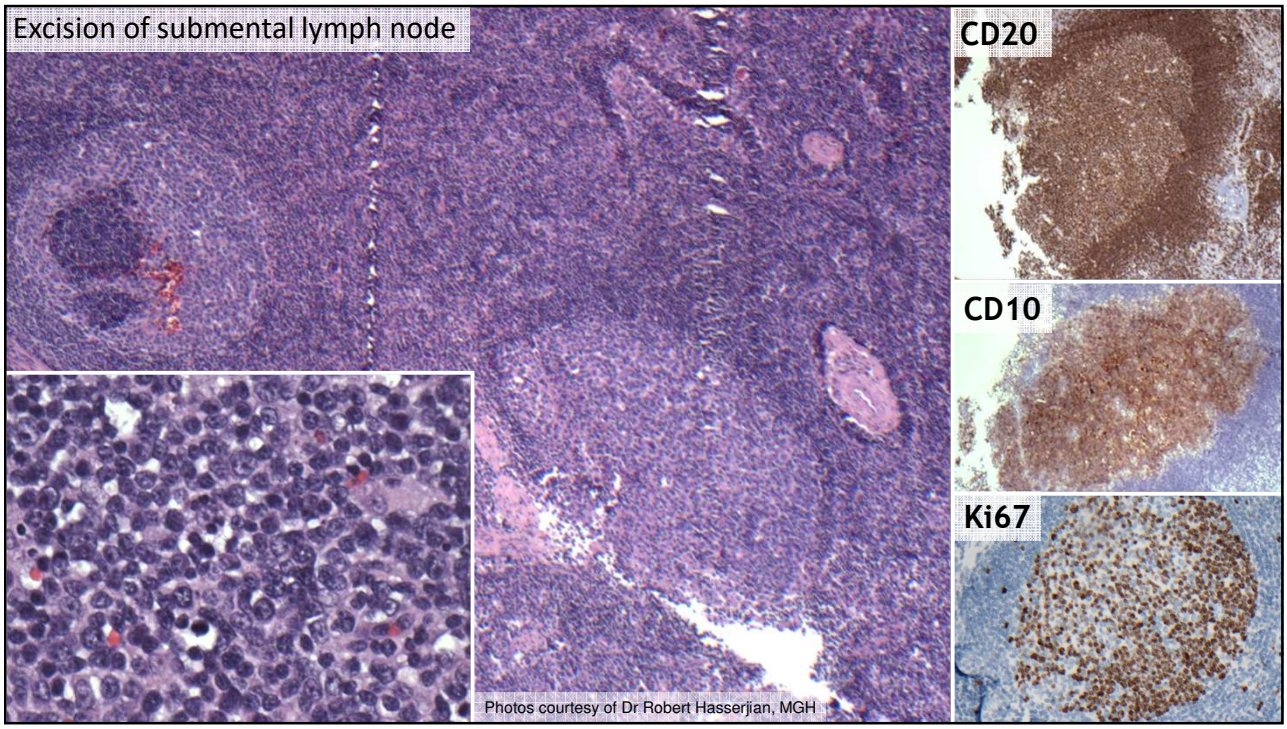
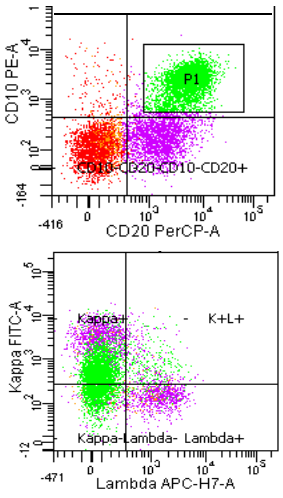


- Predominance of medium-sized cells
- Mitotic figures and apoptotic debris evident
- No FNA material available for flow cytometry or FISH
- No core biopsy submitted for histology or IHC
- Signed out as “atypical, cannot rule out lymphoma”
- Excisional biopsy recommended



Burkitt lymphoma vs. Reactive follicular hyperplasia

- Enlarged germinal centers may resemble BL
  - Predominance of centroblasts and starry-sky
  - Similar IHC: CD10+, BCL6+, BCL2-, high Ki67 (though MYC+ only in scattered cells)
- Diagnosis of malignancy requires proof of clonality on well-sampled specimen
  - Flow cytometry (but CD10+ clonal B cells may be seen in floridly reactive germinal centers*)
  - FISH for *MYC* rearrangement
- Grade 3 follicular lymphoma may also be mistaken for Burkitt lymphoma



* Kussick SJ et al. *Am J Clin Pathol* 2004

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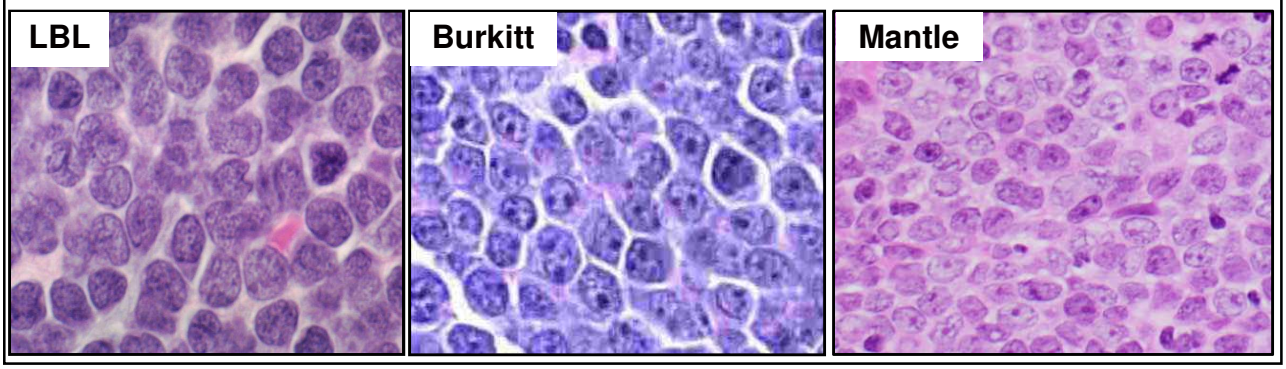
Differential Diagnosis of Burkitt Lymphoma

- **Lymphoblastic lymphoma**: TdT+, surface Ig-, very rare cases *MYC*-R
- **Blastoid variant of mantle cell lymphoma**: Cyclin D1+, SOX11+, CD5+, usually CD10- and no *MYC* rearrangement



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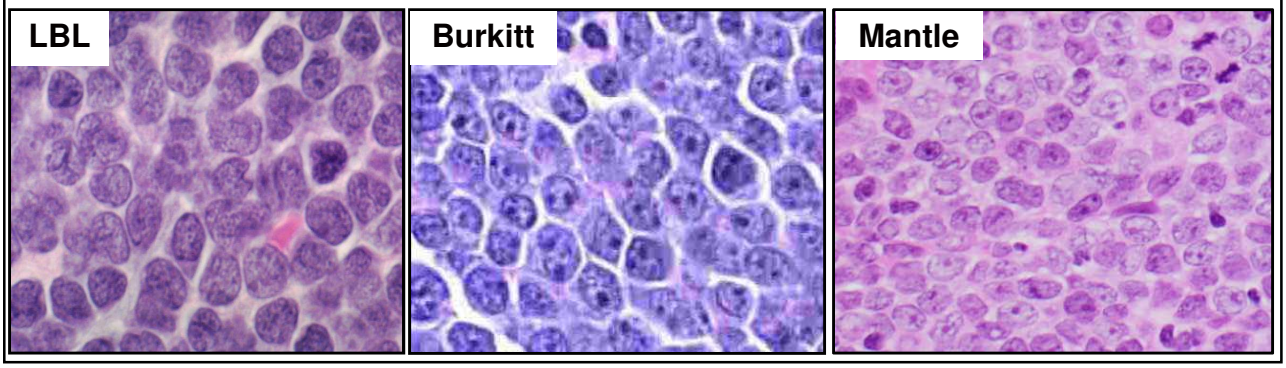

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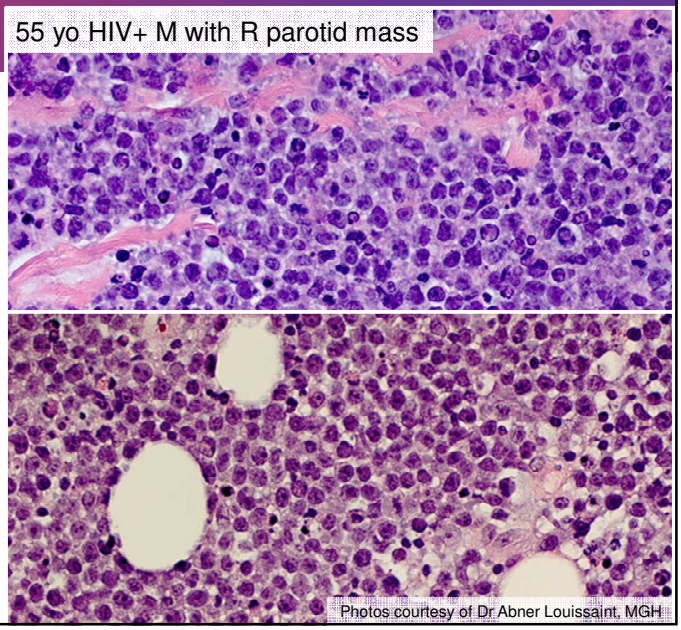
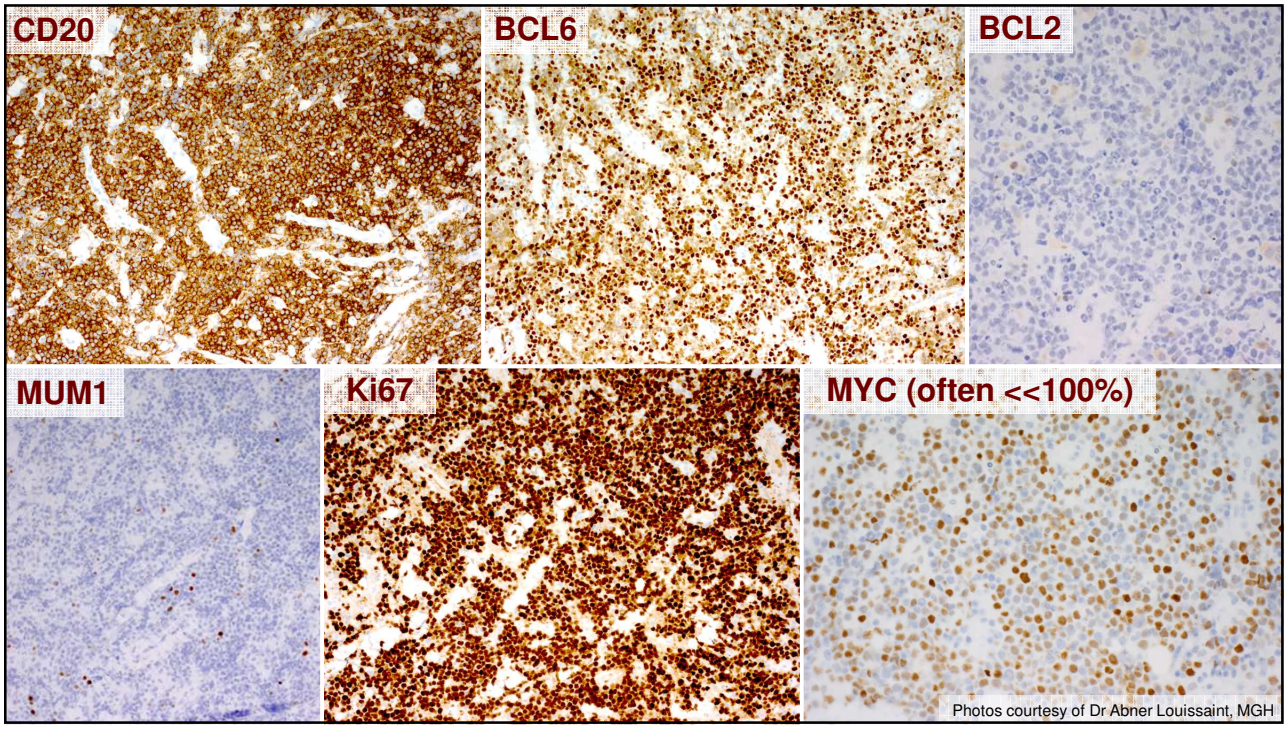
Burkitt-like lymphoma with 11q aberrations

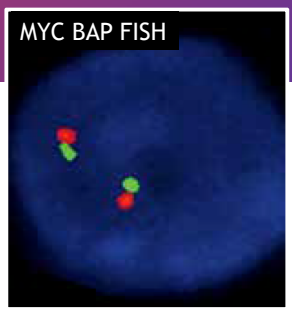
- Localized nodal/extranodal presentation, head & neck or abdomen
- Resembles BL by morph/IHC
  - More variation in nuclear shape and nucleolar prominence
  - Starry-sky macrophages with coarse apoptotic debris
  - Variable MYC expression by IHC
  - LMO2+, CD38dim/-, expression of NK-cell markers: CD56, CD16, CD8
- WHO 5th edition: High-grade B-cell lymphoma with 11q aberrations

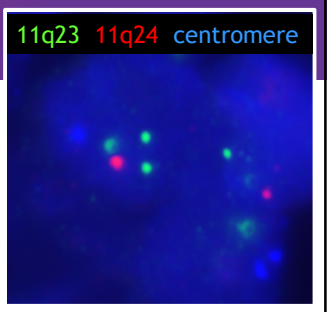


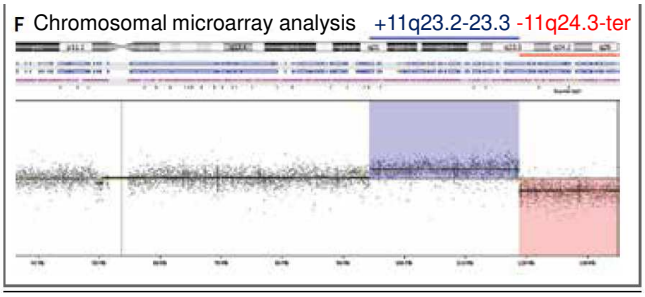


High-grade B-cell lymphoma with 11q aberrations

- Distinct mutational landscape from BL, complex karyotype, and chromosome 11q alteration
  - 11q23 gains, 11q24-ter losses
  - Array CGH or 11q23-24 FISH
  - MYC-R excluded using *MYC* BAP and *IGH::MYC, IGL::MYC, IGK::MYC* FISH probes
- Similar clinical course to BL based on few cases reported
  - Some in immunocompromised setting: HIV or post-transplant







Ard KL et al. Case Records of the MGH. *New Engl J Med* 2018

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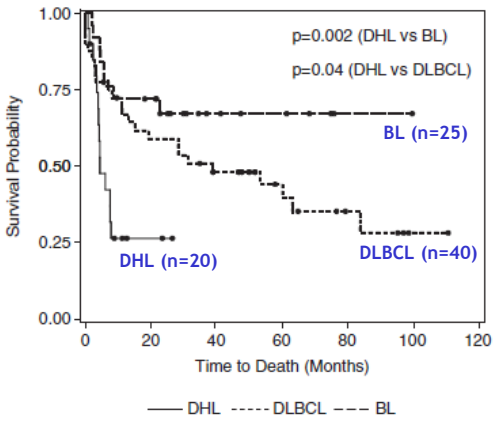
  - High-grade B-cell lymphoma (HGBL), NOS
    - Cases with Burkitt-like morphology lacking detectable *MYC*, *BCL2* or *BCL6* rearrangements

Double/Triple-Hit Lymphoma (DHL/THL): Clinical Features

- Middle-aged or older adults
  - Not typically associated with immune suppression; if present in rare cases, most likely coincidental
- Some patients have history of follicular lymphoma
  - *MYC* rearrangement represents transformation event in pre-existing low-grade FL with t(14;18)/*IGH::BCL2*
- Widespread disease: bone marrow, CNS or other extranodal sites
- Markedly elevated serum LDH, often >3x upper limit of normal
- Therapeutic approach not well established
  - Large multicenter retrospective studies suggest some benefit of intensive therapies (R-EPOCH, R-Hyper-CVAD) over R-CHOP
  - High rates of early treatment failure and death
  - Potential (but limited) role for auto-SCT in relapsed/refractory disease

Petrich et al. *Blood* 2014
Herrera et al. *J Clin Oncol* 2017

Overall Survival of Double-Hit Lymphoma



Snuderl et al. *Am J Surg Pathol* 2010

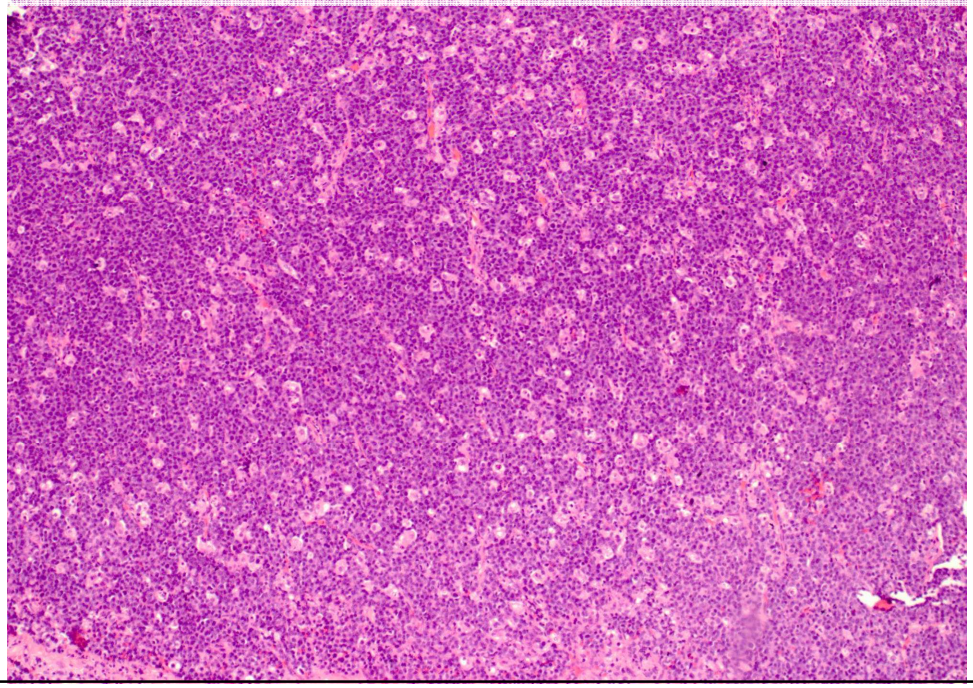
- Retrospective case-control study of 20 DHL patients (*MYC/BCL2*) compared to BL and IPI-matched DLBCL patients
- Median OS DHL: 4.5 months
  - All observed deaths occurred within 8 months of diagnosis
- Median OS DLBCL: 39 months
- Median OS BL: not reached (median follow-up: 32 months)
- Greater variability in outcome with routine FISH assessment of all DLBCL/HGBL

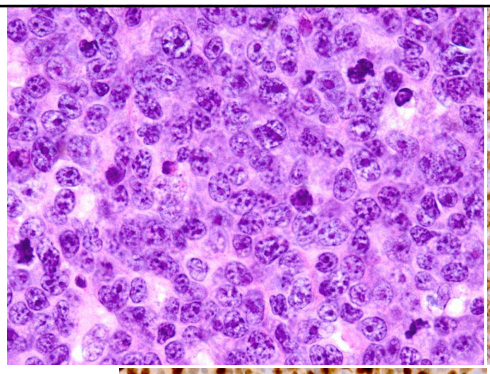
Double/Triple-Hit Lymphoma (DHL/THL)

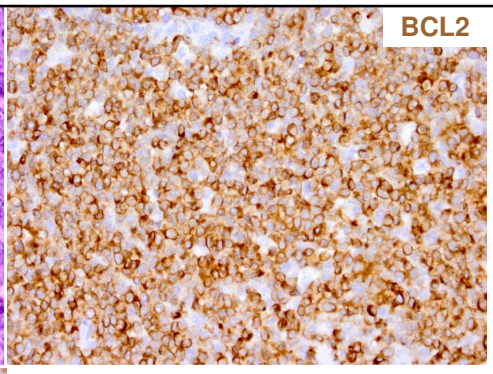
- Pathologic spectrum
  - Intermediate BL/DLBCL (monomorphous cell population with greater nuclear variability than BL, starry-sky pattern may be conspicuous) or blastoid morphology
  - Most cases indistinguishable from DLBCL-NOS
- Immunophenotype
  - **GCB cell-of-origin:** CD10 and BCL6 typically positive, LMO2 usually negative
  - **MYC and BCL2 usually positive** by IHC (particularly in *MYC/BCL2* DHL)
  - **Ki-67:** Proliferation index (PI) typically high, but may be <95%
- Genetics
  - Complex karyotype with many numerical and structural aberrations (median = 10)
  - *MYC* partner: may be *IG* gene, but non-*IG* gene more likely than in Burkitt lymphoma
  - EBV negative by RNA in situ hybridization (EBER)
  - Amplifications or increased copy number of *MYC*, *BCL2* and *BCL6* excluded

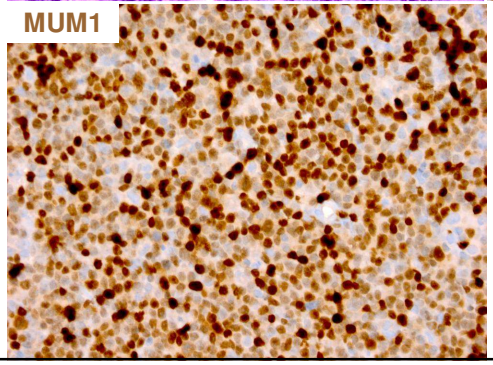
Snuderl et al. *Am J Surg Pathol* 2010
Colomo et al. *Am J Surg Pathol* 2017

68 yo M, axillary LN: Burkitt lymphoma-like morphology



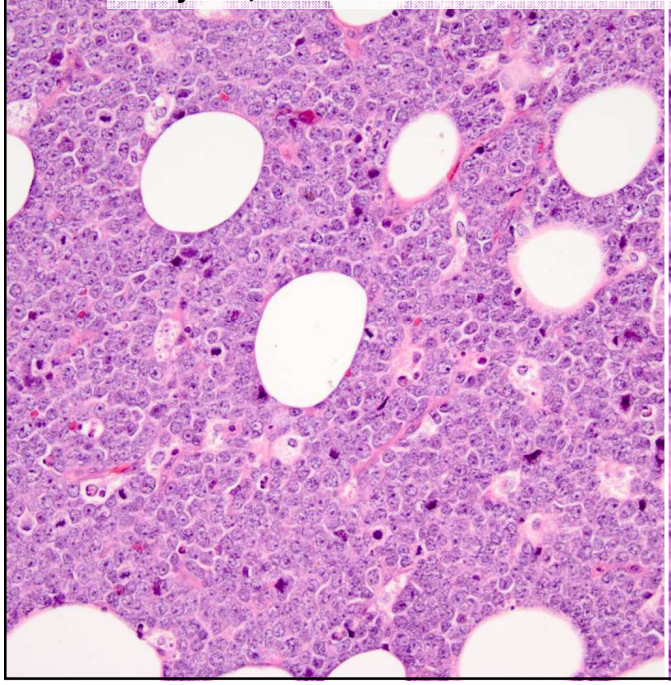


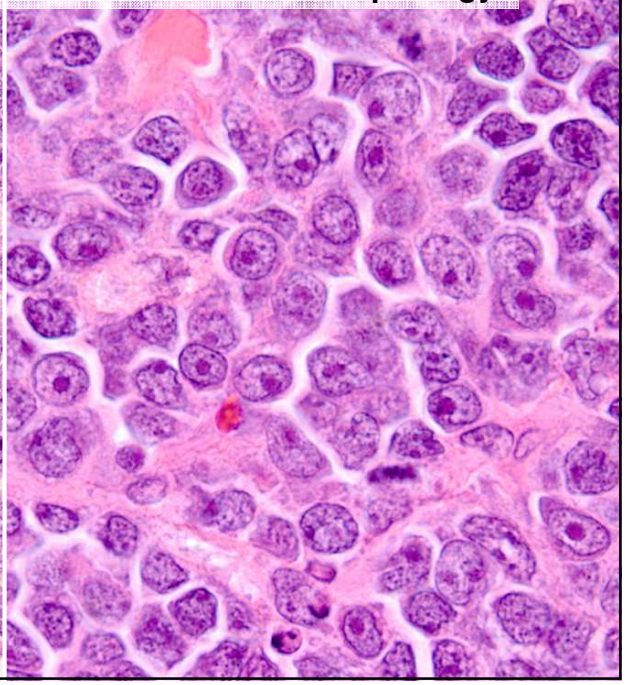


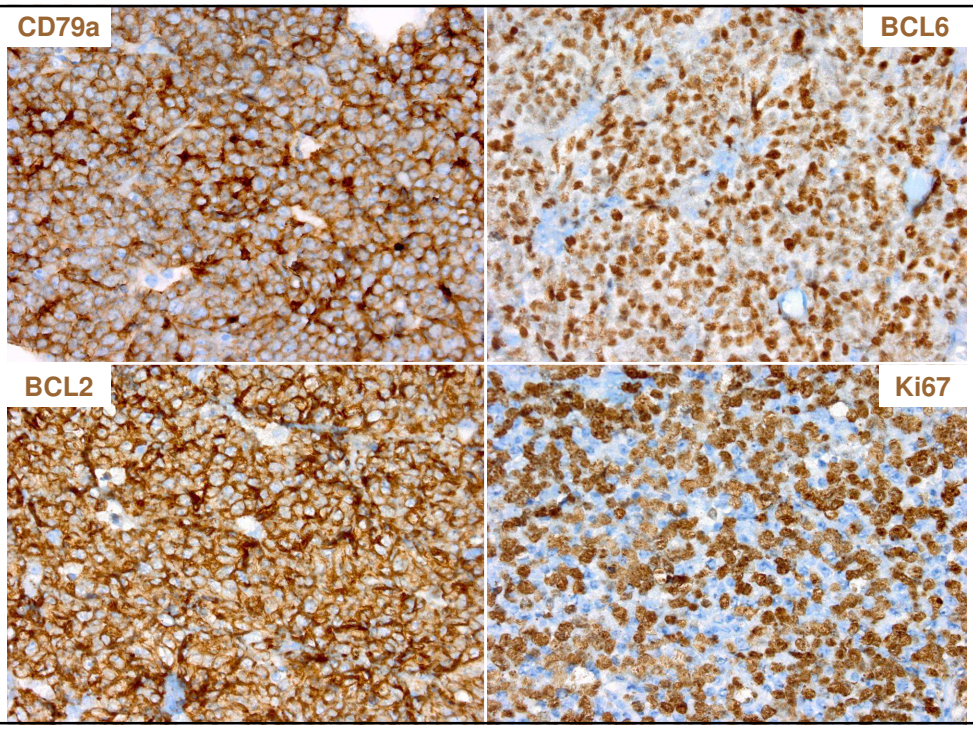


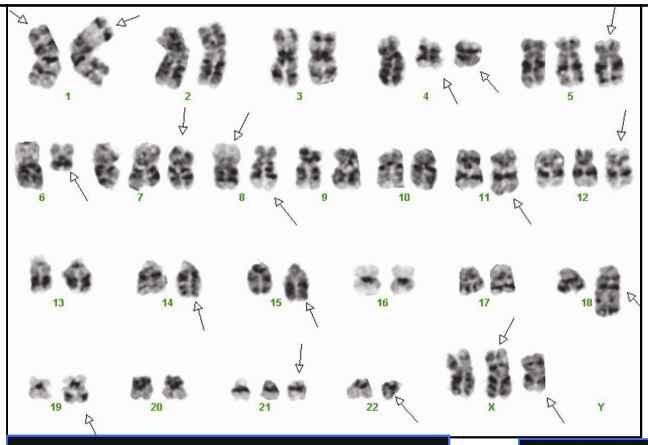
51,XY,t(2;9)(q21;p24),+3,add(5)(p15.3),add(7)(q32),**add(8)(q24),**
+11,+12,**t(14;18)(q32;q21)**,+15,+18,add(19)(q13.1),-22,+2 mar

64 yo M, abdominal wall mass: intermediate BL/DLBCL morphology





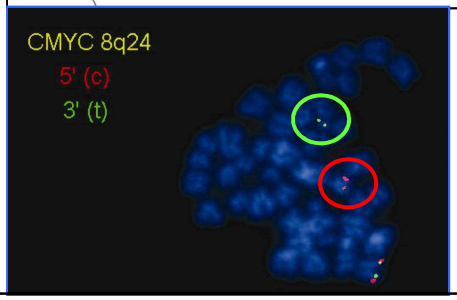


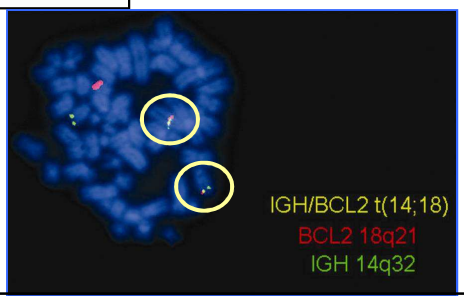


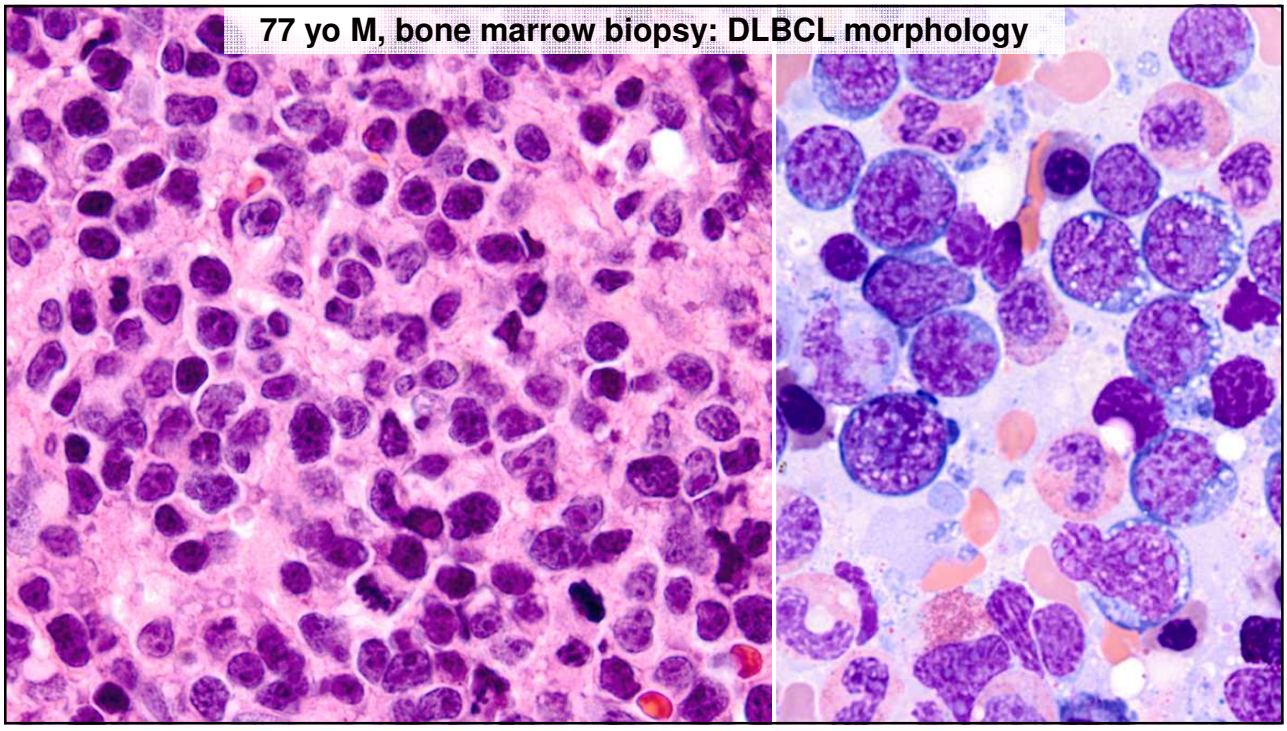
Cytogenetics

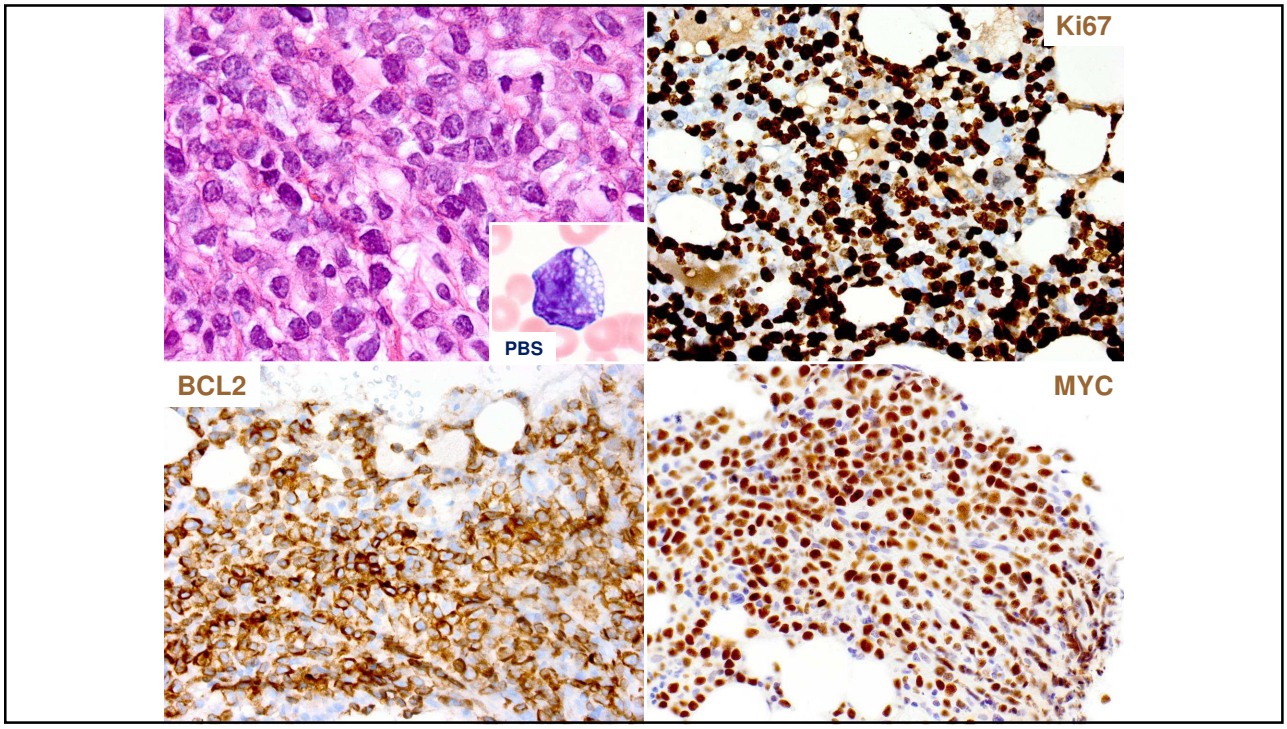
Complex karyotype with multiple abnormalities

52,add(X)(p22.1),del(X)(q22),+X,add(1)(p3?4),add(1)(p3?6),add(4)(q21),+add(4)(q21),+5,del(6)(q13q23),+7,der(8)t(1;8)(p3?4;p2?3),**t(8;22)(q24;q11),**add(11)(q2?3),+12,**der(14)t(14;18)(q32;q21),**add(15)(q22),add(18)(q21),add(19)(q13),+21

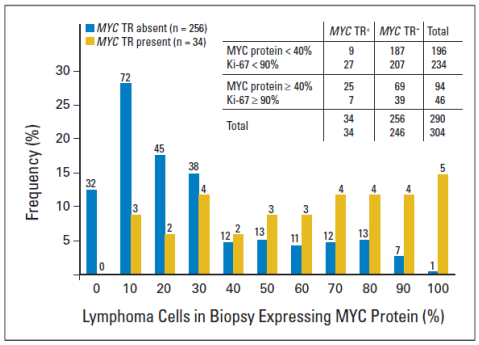





77 yo M, bone marrow biopsy: DLBCL morphology


PBS
Ki67
BCL2
MYC

MYC Expression by IHC in DLBCL and HGBL



	MYC TR+	MYC TR-	Total
MYC protein < 40% Ki-67 < 90%	9 27	187 207	196 234
MYC protein ≥ 40% Ki-67 ≥ 90%	25 7	69 39	94 46
Total	34 34	256 246	290 304

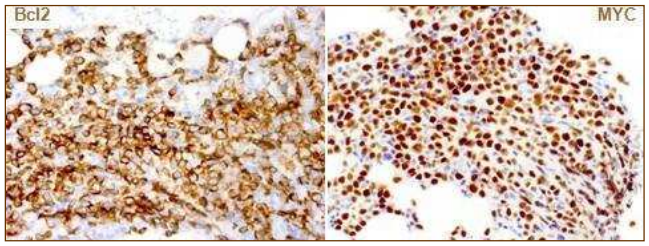
FISH	IHC range	IHC mean*
***MYC* TR +**	**10-100%**	**61%**
***MYC* TR-**	**0-100%**	**29%**

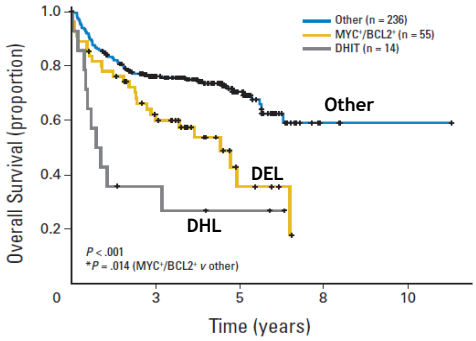
***p<0.001**

Johnson et al. *J Clin Oncol* 2012

- Wide distribution of MYC (clone Y69) expression
- Varying cutoffs for % MYC staining
  - ≥40%: overexpression by IHC seen in ~33% of DLBCL, but only ~1/4 of these have *MYC* rearrangement
- FISH remains gold standard for detection of *MYC* translocation in Burkitt lymphoma, DLBCL and HGBL (DHL/THL)

Double-Expressor Lymphoma (DEL)



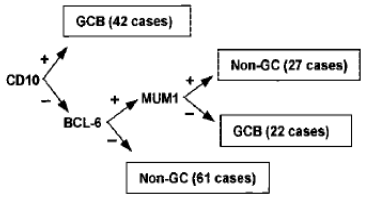


- Utility of MYC immunohistochemistry
  - Prognostic marker with BCL2 IHC
- DLBCL with concurrent high expression of MYC (≥40%) and BCL2 (≥50%)
  - 20-30% of DLBCL (vs. DHL: 5-10% of DLBCL)
- Worse prognosis than non-DEL, though better than double-hit lymphoma
- No distinguishing morphology
- Most are ABC/non-GCB subtype by immunohistochemistry

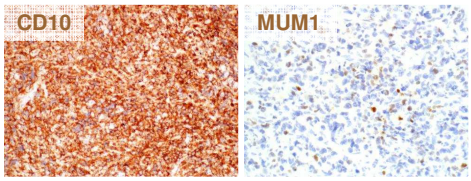
Johnson et al. *J Clin Oncol* 2012

Comprehensive Work-up of BL/HGBL

- Establish cell-of-origin (COO)
  - GCB vs. non-GCB/ABC subtype and IHC algorithm used
- Assess for DEL: MYC and BCL2 IHC
- Assess for DHL: FISH for *MYC*
  - *BCL2* and *BCL6* FISH if *MYC* rearrangement present
  - If DHL, dual color/dual fusion *IG* FISH for *MYC* partner
- Other assessments
  - Ki67 PI, LMO2 (negativity correlates with *MYC*-R)
  - EBV association: EBV-encoded RNA (EBER) stain
  - Blastoid morphology: TdT and cyclin D1/SOX11/CD5 IHC
  - Resembles BL without detectable *MYC* rearrangement
    - Chromosomal microarray or 11q23-24 FISH
    - Check flow cytometry: CD38 / CD56 / CD16 / CD8



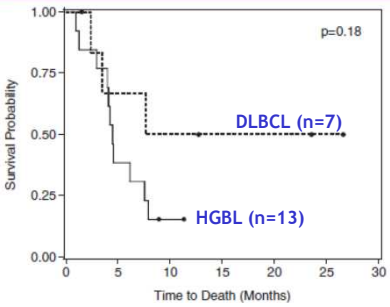
Hans algorithm using CD10, BCL6, MUM1 ıtoffs of >30% tumor cells staining) for GCB vs. non-GCB determination

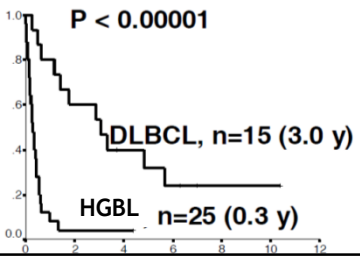


Hans et al. *Blood* 2004

Reporting and Work-up of DHL/THL

- Suggested diagnostic lines
  - High-grade B-cell lymphoma; genetic studies pending.
  - Large B-cell lymphoma; genetic studies pending.
  - Final WHO diagnosis pending genetic studies.
- Report content
  - Describe morphology: high-grade BCL vs. DLBCL
  - Results of IHC, including COO, DEL phenotype
  - Indicate what features raise concern for DHL/THL
  - Await cytogenetics/FISH for *MYC*, *BCL2* and *BCL6*



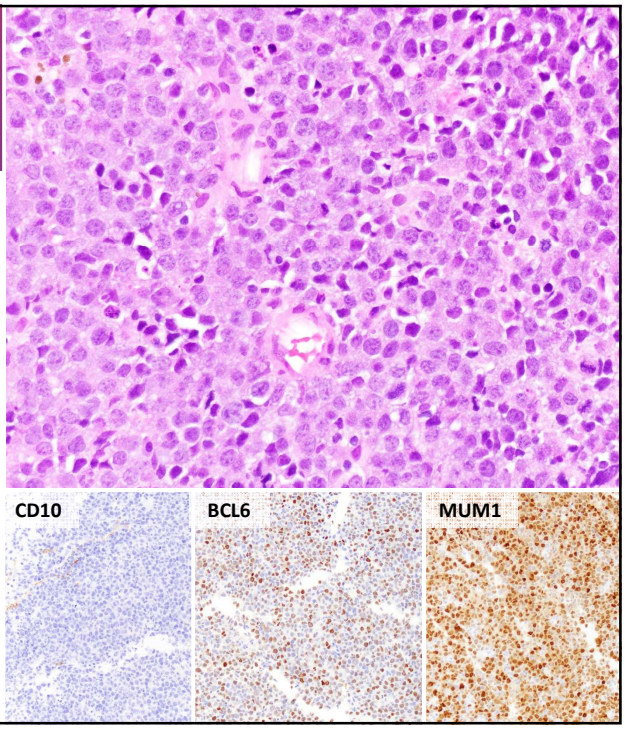


Snuderl et al. *Am J Surg Pathol* 2010 / Johnson et al. *Blood* 2010

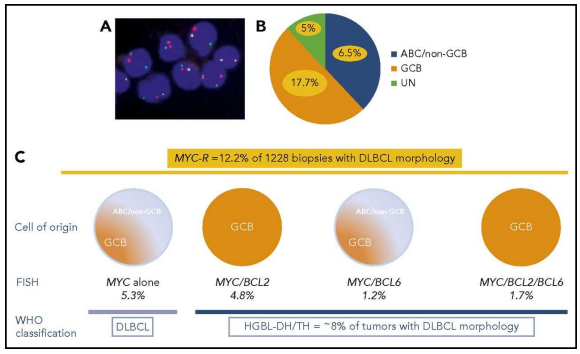
HGBL with *MYC* and *BCL6* rearrangements

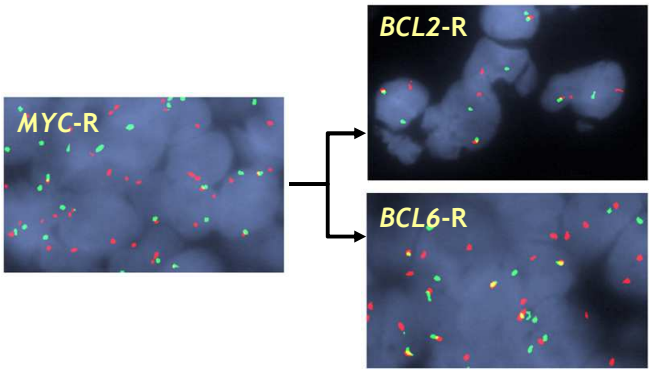
- Comprises only ~5% of DHL/THLs (less common that *MYC/BCL2* DHL and *MYC/BCL2/BCL6* THL)
- Some studies suggest better prognosis
  - Distinct biology from *MYC/BCL2* DHL with fewer GCB COO cases and more heterogeneous mutation profile
  - *MYC* partner more often non-*IG*
    - *IG* partner associated with worse prognosis
    - Up to 40% are "pseudo-DHL" with *MYC::BCL6* → enhancer swap does not result in same level of *MYC* upregulation as *IG::MYC*
- WHO/ICC likely to exclude from HGBL

Ryan et al. *Cancer Discovery* 2015



Targeted Screening Approach for DHL/THL?



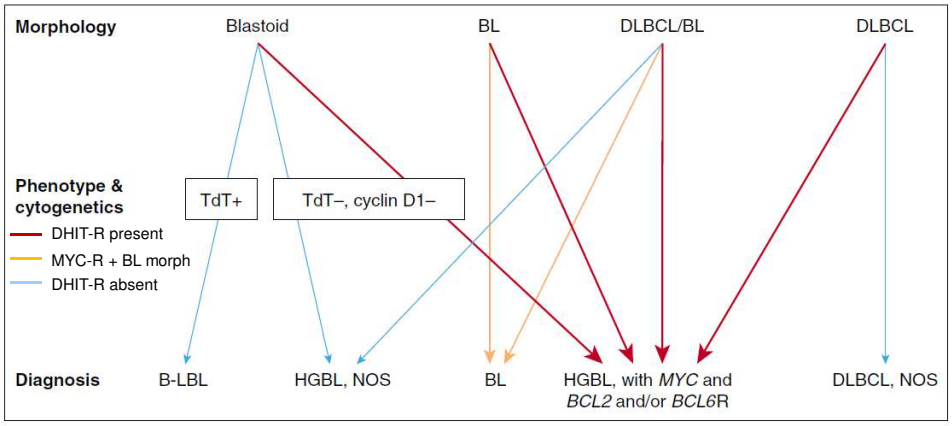


Photos courtesy of Martina Zoi and Dr Joe Lennerz, MGH CID

- Targeted, stepwise FISH screening
  - Restrict FISH to **GCB DLBCL** → would reduce FISH testing by half and still detect >99% of *MYC/BCL2* DHL and *MYC/BCL2/BCL6* THL
  - Most missed cases would have *MYC*-R alone or be *MYC/BCL6* DHL

Copie-Bergman et al. *Blood* 2018

Diagnostic Paradigm for HGBLs



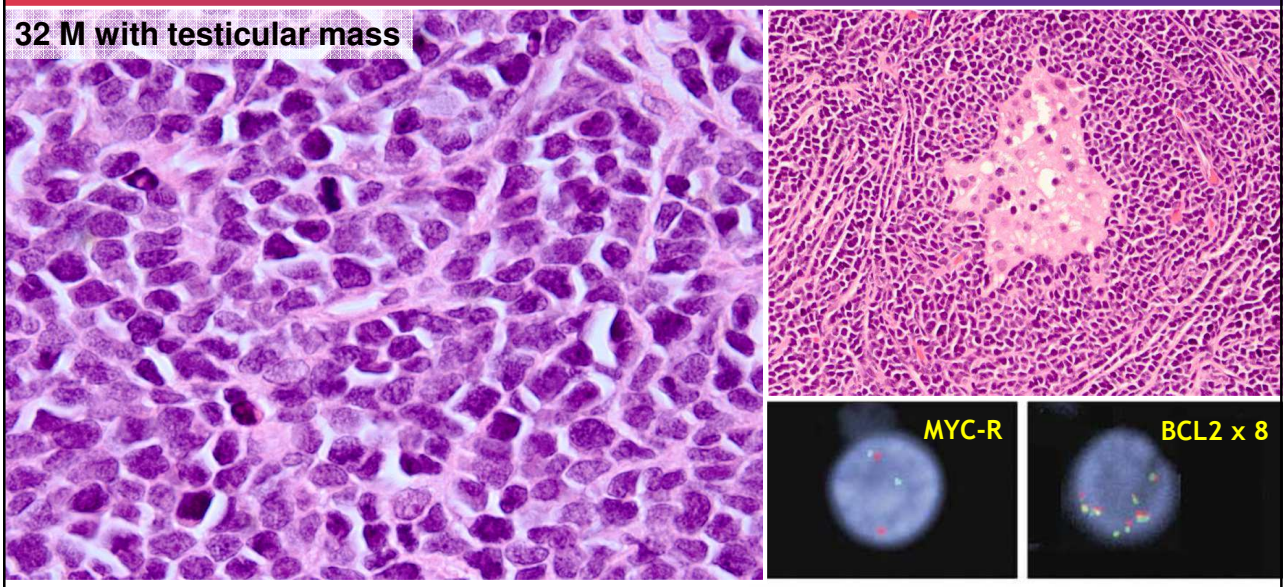
- Issue updated/amended report when cytogenetics/FISH results return
  - If double-hit rearrangement present: "High-grade B-cell lymphoma with rearrangements involving MYC and XXX"
  - If not: "DLBCL" or "high-grade B-cell lymphoma, NOS"

Swerdlow et al. The 2016 revision of the WHO classification. *Blood* 2016

When to diagnose HGBL, NOS?

- **YES**: Morphology on H&E closely resembles BL, but
  - Immunophenotype excludes BL: CD10-, BCL6-, strong BCL2+, or Ki67 <90%, and
  - Lacks double/triple-hit rearrangement, excluding DHL/THL, +/- complex karyotype
- If immunophenotype very good for BL, but no detectable *MYC*-R:
  - Consider Burkitt-like/high-grade B-cell lymphoma with 11q aberrations
  - 10% of BL are *MYC* negative: similar gene expression profile to cases with *MYC*-R
- **YES**: Blastoid morphology on H&E, but
  - Negative for TdT and cyclin D1/SOX11/CD5, and
  - Lacks double/triple-hit rearrangement, excluding DHL/THL
- **NO**: DLBCL morphology with Ki67 >90%, starry-sky pattern, or *MYC*-R

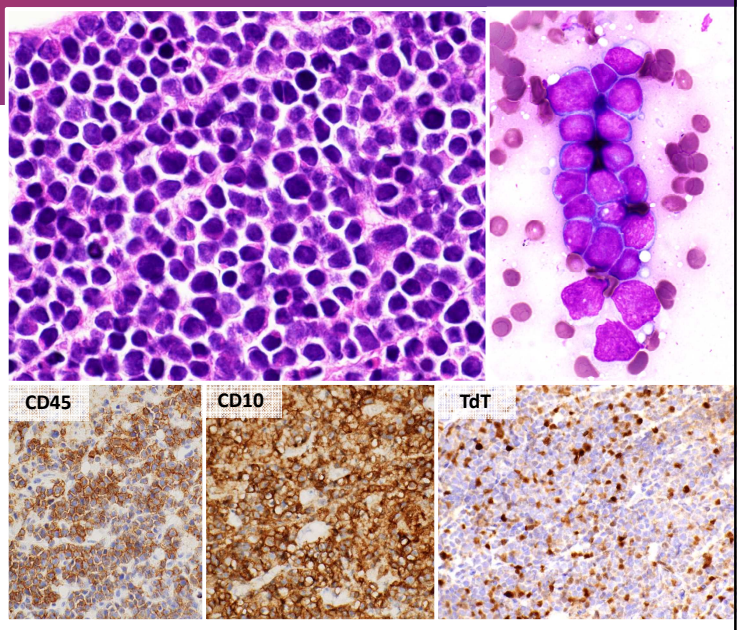
High-grade B-cell lymphoma, NOS



DLBCL/BL overlap or blastoid morphology, no double/triple-hit genetics, TdT-negative

DHL (*MYC/BCL2*) with TdT expression

- TdT+ B cells in ~2% of cases
  - TdT+ cells range from rare to most
  - Blastoid with diffuse architecture
  - CD20-/weak but express sLC
  - GCB COO: CD10+, BCL6-
- History of low-grade follicular lymphoma in some cases
  - Pre-existing *BCL2*-R, *a*cquire *MYC*-R
- DHL vs lymphoblastic lymphoma?
  - Area of controversy
  - If prior FL, best considered HGBL txn rather than de novo B-LBL/ALL
  - *De novo* cases: clinical, flow, and molecular features may be helpful



Ok et al. *Mod Pathol* 2019 / Khanlari et al. *Mod Pathol* 2022

Follicular lymphoma with *MYC* rearrangement

- Not considered HGBL by WHO if low-grade histology (even when *BCL2* or *BCL6* rearranged)
- Rare (~2% of FL), grade 1-2 or 3A, most cases CD10+/BCL6+/BCL2+
- Older patients, higher FLIPI, more aggressive clinical course
  - Shorter PFS
  - Increased risk of lymphoma-related death
  - Greater risk of high-grade transformation

Bussot et al. *Br J Haemtol* 2021
Chaudhary et al. *Hum Pathol* 2021