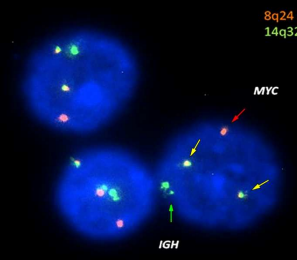
Molecular Diagnostics of B Cell Lymphomas



Scott B. Lovitch, MD, Ph.D.
Associate Pathologist, Brigham and Women's Hospital
Assistant Professor of Pathology, Harvard Medical School

September 15, 2021

Image credit: Atlas of Haematological Cytology (http://www.leukemia-cell.org/atlas)

Disclosure

I have no financial relationship with any commercial entity producing healthcare (or healthcare education) related products and/or services.

Outline

Since I couldn't possibly cover everything about molecular diagnostics of B cell lymphomas in 25 minutes, I will focus on practical applications that are (or will soon be) part of the standard of care for diagnosis and management of BCL:

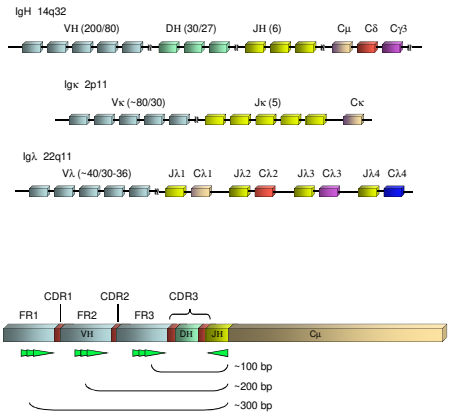
- Molecular methods applied to diagnosis and classification of BCL (brief overview)
- Molecular diagnostics in management of *low-grade* B cell lymphomas
  - CLL/SLL: prognosis, assessment of resistance to targeted therapy
  - LPL: MYD88 and CXCR4 mutation
  - HCL and HCLv: BRAF and MAP2K1 mutation
- Molecular diagnostics in management of *high-grade* B cell lymphomas
  - Cell-of-origin classification of DLBCL
  - Double- and triple-hit lymphomas
  - Identification of unique subtypes of high-grade lymphoma

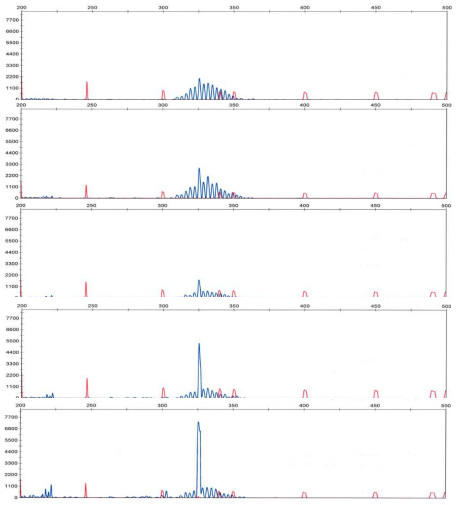
Outline

Since I couldn't possibly cover everything about molecular diagnostics of B cell lymphomas in 25 minutes, I will focus on practical applications that are (or will soon be) part of the standard of care for diagnosis and management of BCL:

- Molecular methods applied to diagnosis and classification of BCL (brief overview)
- Molecular diagnostics in management of *low-grade* B cell lymphomas
  - CLL/SLL: prognosis, assessment of resistance to targeted therapy
  - LPL: MYD88 and CXCR4 mutation
  - HCL and HCLv: BRAF and MAP2K1 mutation
- Molecular diagnostics in management of *high-grade* B cell lymphomas
  - Cell-of-origin classification of DLBCL
  - Double- and triple-hit lymphomas
  - Identification of unique subtypes of high-grade lymphoma

IGH clonality testing for diagnosis and MRD assessment





1% clonal population

3% clonal population

5% clonal population

10% clonal population

25% clonal population

From: Wu, Lovitch, Kim. *Molecular Genetics of Non-Hodgkin Lymphomas*. Wintrobe's Clinical Hematology, 14th ed., chapter 88.

Cytogenetic abnormalities in B-cell NHL

	Translocation	Product	Disease Association
	t(1;14)(p22;q32)	*BCL10* overexpression (*IGH*)	MALT[b]
	t(1;14)(q21;q32)	*BCL9* overexpression (*IGH*)	B-LBL and others
	t(1;14)(q21;q32)	*FCRL4/5* overexpression (*IGH*)	Myeloma (<5%)
	t(1;14)(q22;q32)	*MUC1* overexpression (*IGH*)	DLBCL
	t(1;22)(q23;q11)	*FCGR2B* overexpression (*IGL*)	Transformed FL
	t(2;7)(p12;q21-q22)	*CDK6*	SMZL
	t(2;14)(p16.1;q32)	*BCL11A* overexpression (*IGH*)	CLL, DLBCL
	t(2;17)(p23;q23)	*CLTC-ALK*	ALK-positive LBCL
	t(3;14)(q27;q32)/t(3;v)(q27;v)	*BCL6* overexpression (*IGH* or *IGK/IGL*)	DLBCL (5%-10%) and others
	t(3;14)(p14;q32)	*FOXP1* overexpression (*IGH*)	MALT
	t(4;14)(p16;q32)	*WHSC1 (MMSET)/FGFR3* overexpression (*IGH*)	Myeloma (20%-25%)
	t(5;14)(q23-q31;q32)	*IL3* overexpression (*IGH*)	B-LBL
	t(6;14)(p25-p23;q32)	*IRF4* overexpression (*IGH*)	Myeloma (~20%)
	t(6;14)(p21;q32)	*CCND3* overexpression (*IGH*)	Myeloma (<5%), DLBCL, SMZL, MZL
	t(6;14)(p22;q32)	*ID4* overexpression (*IGH*)	B-LBL (<1%)
	t(8;14)(q24;q32)/t(8;v)(q24;v)	*MYC* overexpression (*IGH* or *IGK/IGL*)	BL (>98%), DLBCL,[a] FL,[a] PLL, myeloma
*	t(8;14)(q11;q32)	*CEBPD* overexpression (*IGH*)	B-LBL (<1%)
	t(9;14)(p13;q32)	*PAX5* overexpression (*IGH*)	LPL (1%-2%), other neoplasms with plasmacytic differentiation, DLBCL
	t(10;14)(q24;q32)	*NFKB2* overexpression (*IGH*)	DLBCL
*	t(11;14)(q13;q32)	*CCND1* overexpression (*IGH*)	MCL (>95%), PLL, SMZL, myeloma[b] (20%-25%)
	t(11;14)(q23;q32)	*PAFAH1B2* overexpression (*IGH*)	PMBCL
	t(11;14)(q23;q32)	*DDX6* overexpression (*IGH*)	DLBCL
*	t(11;18)(q22;q21)	*BIRC3-MALT1* fusion	MALT[b]
	t(12;14)(q23;q32)	*CHST11* overexpression (*IGH*)	DLBCL, CLL (rare)
	t(12;14)(q24;q32)	*BCL7A*	BL, myeloma
	t(12;15)(q32;q11-13)	*NBEAP1*?	DLBCL
	t(12;22)(p13;q11)	*CCND2*	CLL
	t(14;16)(q32;q22-q23)	*MAF* overexpression (*IGH*)	Myeloma (20%-25%)
*	t(14;18)(q32;q21)	*BCL2* overexpression (*IGH*)	FL (~80%), DLBCL (~20%)
	t(14;18)(q32;q21)	*MALT1* overexpression (*IGH*)	MALT
	t(14;19)(q32;q13)	*BCL3* overexpression (*IGH*)	CLL
	t(14;19)(q32;q13)	*CEBPA* overexpression (*IGH*)	B-LBL (<1%)
	t(14;20)(q32;q11-q13)	*MAFB* overexpression (*IGH*)	Myeloma

Rearrangements/ fusions:

- MYC rearrangement: BL, DHL
- BCL6 rearrangement: DLBCL, DHL
- CCND1 fusion: MCL
- BIRC3-MALT1 fusion: MALT
- IGH-BCL2 fusion: FL, DHL

Gains/losses:

- 7q deletion (SMZL)
- Trisomy 12, 11q deletion, 13q deletion (CLL)
- 17p deletion (many – poor prognosis)

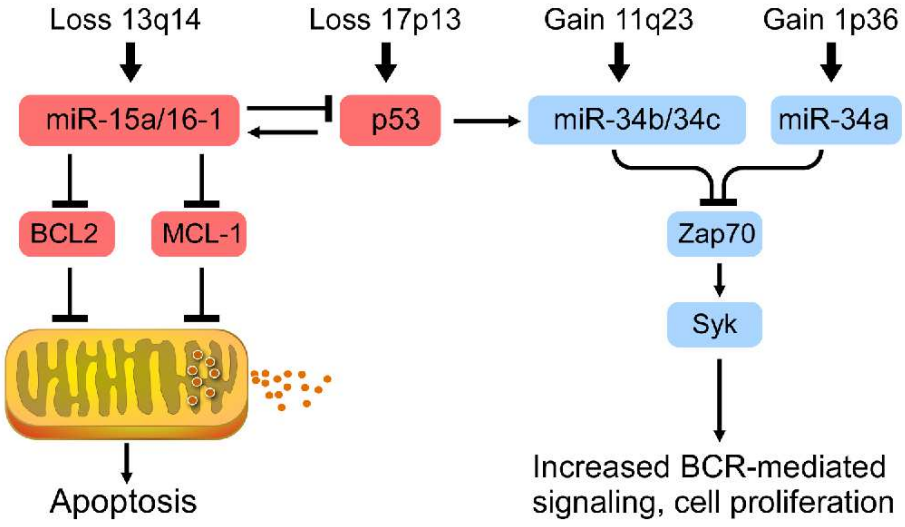
From: Wu, Lovitch, Kim. *Molecular Genetics of Non-Hodgkin Lymphomas*. Wintrobe's Clinical Hematology, 14th ed., chapter 88.

Outline

Since I couldn't possibly cover everything about molecular diagnostics of B cell lymphomas in 25 minutes, I will focus on practical applications that are (or will soon be) part of the standard of care for diagnosis and management of BCL:

- Molecular methods applied to diagnosis and classification of BCL (brief overview)
- Molecular diagnostics in management of *low-grade* B cell lymphomas
  - CLL/SLL: prognosis, assessment of resistance to targeted therapy
  - LPL: MYD88 and CXCR4 mutation
  - HCL and HCLv: BRAF and MAP2K1 mutation
- Molecular diagnostics in management of *high-grade* B cell lymphomas
  - Cell-of-origin classification of DLBCL
  - Double- and triple-hit lymphomas
  - Identification of unique subtypes of high-grade lymphoma

CLL/SLL: cytogenetic aberrations drive disease biology



From: Wu, Lovitch, Kim. *Molecular Genetics of Non-Hodgkin Lymphomas*. Wintrobe's Clinical Hematology, 14th ed., chapter 88.

CLL/SLL: cytogenetic findings and their significance

- **13q deletion *(most common recurrent abnormality; 50-60% of cases)***
  - Can be homozygous or heterozygous; both have favorable prognosis
  - Results in loss of two microRNAs (miR-15a and miR-16-1) that repress anti-apoptotic genes including BCL-2, resulting in overexpression
- **11q22-23 deletion *(17% of cases)***
  - Results in loss of **ATM** – phosphorylates and activates p53
  - Also results in loss of two miRNAs, miR-34b and miR-34c, that suppress ZAP70
  - Associated with poor prognosis; add alkylating agent to chemotherapy
- **Trisomy 12 *(20% of cases)***
  - Associated with atypical morphologic and immunophenotypic features (irregular nuclear contours, open chromatin, increased CD11c)
  - Intermediate risk factor; prognostic impact modified by other abnormalities present
- **17p13 deletion *(8% of cases)***
  - Results in loss of **TP53** (*always* associated with a poor prognosis)

CLL/SLL and somatic hypermutation (SHM)

CLL/SLL is really two different diseases!

- **SHM-positive/IGHV-mutated (50-60%)**
  - Derived from memory B cells that have been through the germinal center reaction
  - Median overall survival 293 months from diagnosis (>22 years!)
- **SHM-negative/IGHV-unmutated (50-60%)**
  - Derived from innate memory B cells that have not been through the germinal center reaction
  - Based on epigenetic studies, more biologically similar to naïve B cells
  - Median overall survival 95 months (~9 years) for low-stage disease – so more aggressive disease than IGHV-mutated CLL/SLL
- ZAP70 expression can be used as a surrogate if IGHV testing is not feasible

CLL/SLL: somatic mutations

- **TP53 mutation** (15% of cases; higher percentage post-chemo)
  - Often associated with 17p deletion (biallelic inactivation)
- **NOTCH1 mutation** (4-12% of cases; often associated with trisomy 12, increase in Richter transformation)
  - Most common mutation is 2bp deletion (delCT) in PEST domain (gain of function)
  - Decreased overall survival
- **SF3B1 mutation** (~5% of cases at diagnosis; higher post-treatment and post-relapse)
  - K700E is most common mutation (same in MDS); others at codon 662, 666
  - Result in aberrant splicing
  - Predominantly found in IGHV-unmutated CLL
- **MYD88 mutation** (~10% of cases; vs. >90% in LPL)
  - Predominantly found in IGHV-mutated CLL
  - Patients tend to be younger and have more advanced disease

CLL/SLL: the new treatment landscape

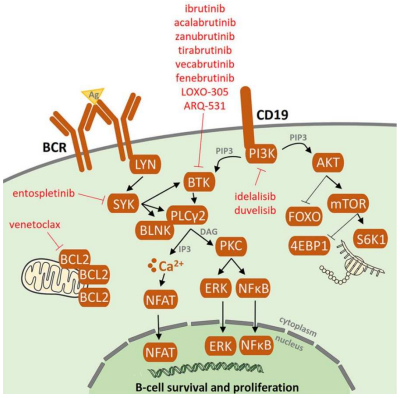
BTK inhibitors (ibrutinib/acalabrutinib)

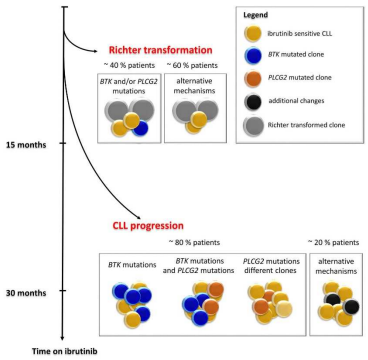
© Abbvie/Pharmacyclics

BCL2 inhibitors (venetoclax)

© Abbvie/Genentech

CLL/SLL: targeted therapy resistance mutations





Sedlarikova *et al.*, *Front Oncol.* (2020)

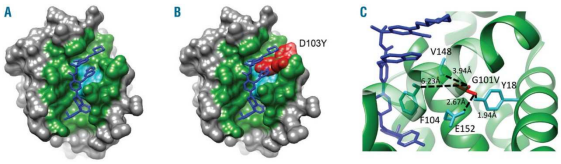
Resistance to BTK inhibitors (ibrutinib, acalabrutinib, etc):

- BTK mutations (**C481S**, rarely C481R/F/Y and others)
- PLGC2 mutations (several hotspots)

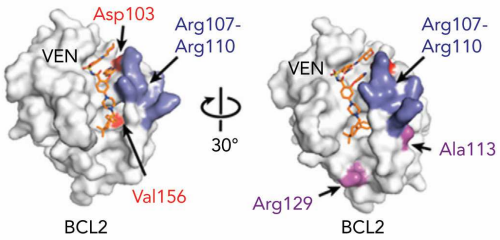
80%

- Remainder likely due to del(8p) and other point mutations

CLL/SLL: targeted therapy resistance mutations



Tausch *et al.*, *Haematologica* (2019)



Blombery *et al.*, *Blood* (2020)

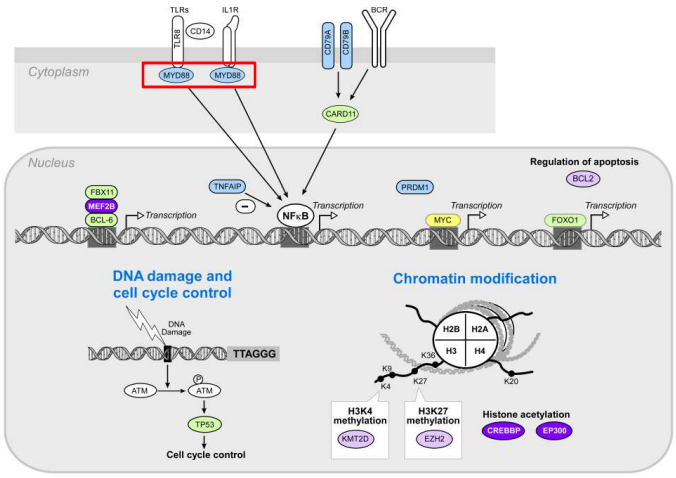
Resistance to BCL2 inhibitors (venetoclax) – vast majority due to BCL2 mutations

- **G101V (~50%)**
- D103Y/E/V
- R107_R110dup
- A113G
- R129L
- V156D
- Rarely others

Most mutations interfere with drug binding

In patients with venetoclax *and* ibrutinib resistance, rare mutations are more likely

Lymphoplasmacytic lymphoma and MYD88 mutation



MYD88 – "master regulator" of innate immunity

- Signals downstream of TLRs, IL-1R, others to activate NFkB pathway
- Constitutively activating mutations (L265P is most common) found in >90% of cases of LPL
- Not entirely specific for LPL – also identified in ~30% of DLBCL and ~10% of CLL/SLL *(BUT absent in myeloma)*
- Presence of mutation predicts response to ibrutinib
- Can be detected by allele-specific PCR or NGS

From: Wu, Lovitch, Kim. *Molecular Genetics of Non-Hodgkin Lymphomas*. Wintrobe's Clinical Hematology, 14th ed., chapter 88.

Lymphoplasmacytic lymphoma and CXCR4 mutation

- **CXCR4 – chemokine receptor** that promotes adhesion to bone marrow stroma
- Mutations found in 27-36% of cases of LPL – in nearly all mutated cases (>98%) MYD88 L265P mutation is also present, so most likely acquired late in disease as a subclonal event
- Nonsense and frameshift mutations that result in loss of C-terminal regulatory domain, resulting in constitutive signaling
- Associated with more aggressive phenotype
  - Higher serum IgM
  - Higher bone marrow disease burden
  - Higher likelihood of requiring therapy
- Associated with resistance to ibrutinib – $MYD88^{mut}CXCR4^{mut}$ cases are less sensitive than $MYD88^{mut}CXCR4^{wt}$, but still more sensitive than $MYD88^{wt}$
- Nonsense mutations *may* be associated with a worse prognosis than frameshift mutations
- Clinical significance in isolation (i.e. without MYD88 mutation) is uncertain
- NGS is preferred method of detection due to heterogeneity of mutation

Hairy cell leukemia and BRAF mutation

- BRAF V600E mutation – found in ~98% of cases of classical HCL
  - Same mutation found in solid tumors (e.g. melanoma, papillary thyroid carcinoma)
  - However, ***not*** found in morphologic mimics of HCL (splenic marginal zone lymphoma, hairy cell leukemia-variant, splenic diffuse red pulp small B-cell lymphoma)
  - Confers resistance to BRAF inhibitors (e.g. vemurafenib; response rate 96-100%)
  - Can be detected by mutation-specific PCR-based assays or by NGS; latter may be preferred as it will also detect functionally equivalent mutations (e.g., C597S, V600K)

Hairy cell leukemia variant and MAP2K1 mutation

- Hairy cell leukemia variant – defined as B cell neoplasm with morphologic features of HCL, but variant clinical presentation and/or immunophenotype
  - Typically present with leukocytosis rather than cytopenias
  - Lack expression of one or more canonical HCL markers (CD11c, CD25, CD103)
  - Poor response to purine analogues (cladribine, pentostatin)
- Universally (so far) negative for BRAF V600E mutation
- Activating mutations in MAP2K1 found in 42% of cases
  - Kinase downstream of BRAF – so alternate route to activation of same pathway
  - MAP2K1 mutations also identified in (rare) cases of $BRAF^{wt}$ classical HCL – may be more predictive of clinical behavior than conventional classification

Outline

Since I couldn't possibly cover everything about molecular diagnostics of B cell lymphomas in 25 minutes, I will focus on practical applications that are (or will soon be) part of the standard of care for diagnosis and management of BCL:

- Molecular methods applied to diagnosis and classification of BCL (brief overview)
- Molecular diagnostics in management of *low-grade* B cell lymphomas
  - CLL/SLL: prognosis, assessment of resistance to targeted therapy
  - LPL: MYD88 and CXCR4 mutation
  - HCL and HCLv: BRAF and MAP2K1 mutation
- Molecular diagnostics in management of *high-grade* B cell lymphomas
  - Cell-of-origin classification of DLBCL
  - Double- and triple-hit lymphomas
  - Identification of unique subtypes of high-grade lymphoma

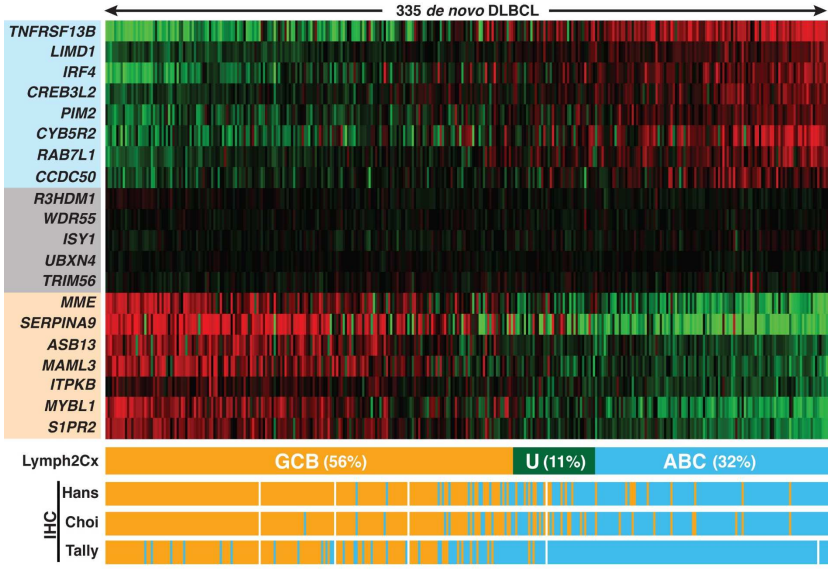
Cell-of-origin classification in DLBCL

Background

- WHO 2008 recognized molecular subgroups of DLBCL based on gene expression profiling (GEP)*:* Germinal center B cell-like (GCB), activated B cell-like (non-GCB/ABC), and unclassifiable
- However, subclassification was considered *optional* because:
  - GEP not routinely available
  - IHC didn't "exactly correlate" with molecular categories
  - Didn't affect therapy
- Better understanding of molecular pathogenesis of GCB and non-GCB subtypes, and emerging impact on selection of treatment, led WHO to *require* cell-of-origin classification (as GCB or non-GCB) in 2016 revision

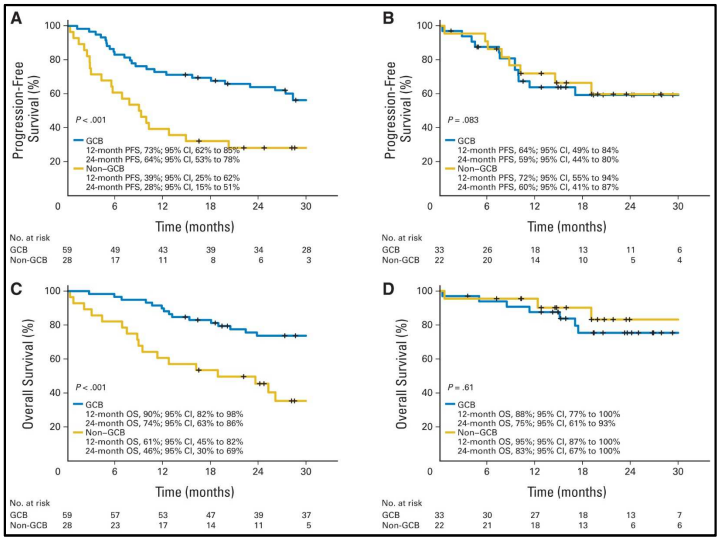
Cell-of-origin classification in DLBCL:

Comparison of IHC vs. GEP



Cell-of-origin classification in DLBCL:

Why it matters



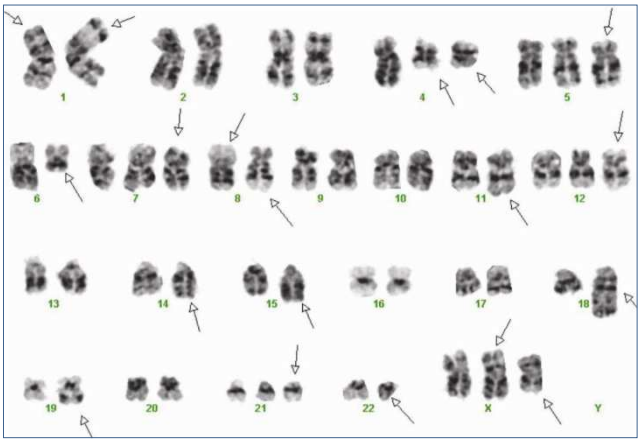
Progression-free and overall survival for patients treated with standard R-CHOP chemotherapy (A and C) and with R-CHOP + lenalidomide (R2CHOP; B and D)

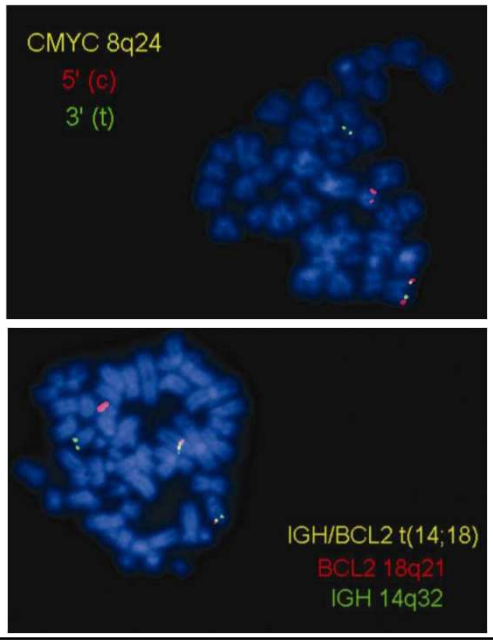
Addition of lenalidomide improves survival in non-GCB but not GCB-type DLBCL

Large B cell lymphomas: double/triple-hit

- Gene rearrangements of BCL2, BCL6, and/or MYC identified in ~65% of large B cell lymphomas
- **Double/triple-hit lymphoma – MYC rearrangement + BCL-2 and/or BCL-6 rearrangement**
  - Patients are generally immunocompetent, middle-aged/older adults
  - Present with widespread disease and markedly elevated serum LDH
  - Very poor prognosis – median OS 4.5 months (vs. 39.8 months for DLBCL, NOS) with nearly all patients dead within 8 months of diagnosis
  - May be benefit to more intensive upfront therapy (R-EPOCH or R-hyper-CVAD) over R-CHOP
  - Potential role for auto-SCT in relapsed/refractory disease
- Standard approach is to FISH for MYC rearrangement → reflex BCL2 and BCL6 FISH if positive

Large B cell lymphomas: double/triple-hit



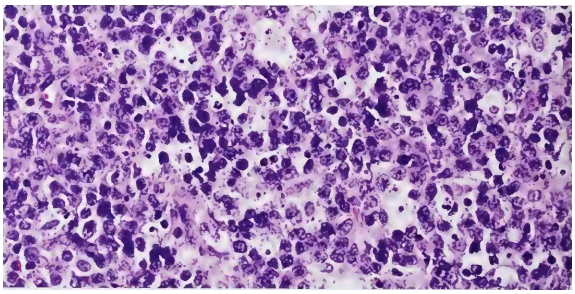


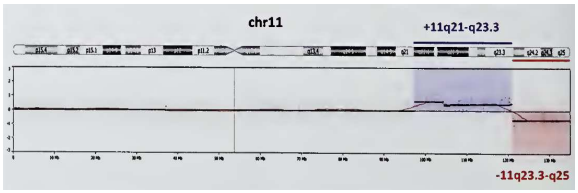
Complex karyotype including t(8;22) and t(14;18); MYC rearrangement and IGH-BCL2 fusion detected by FISH

Large B cell lymphoma with IRF4 rearrangement

- Rare subtype of LBCL associated with ***strong IRF4/MUM-1 expression*** and ***IRF4 gene rearrangement on 6p25***
- Most common in children and young adults; frequently involves Waldeyer's ring and/or cervical LN (less common: GI tract)
- Medium-sized to large neoplastic cells with open chromatin and small nucleoli
- Often "triple-positive" for CD10, BCL-6, and IRF4/MUM-1
  - ➢ This immunophenotype should prompt screening for IRF4 rearrangement
- **Rearrangement is cytogenetically cryptic and often missed on karyotype (requires FISH for detection)**
- Good prognosis following immunochemotherapy +/- radiation
- Distinction from pediatric-type FL is essential (local management is sufficient for the latter)

Burkitt-like lymphoma with 11q aberration





WHO, revised 4th ed. (2017)

- High-grade B cell lymphoma that closely resembles Burkitt lymphoma (morphology, clinical phenotype, gene expression profile) but lacks MYC rearrangement
- Associated with characteristic aberration of 11q, with *proximal gains* and *telomeric losses* (typically need to use aCGH to detect)
- Clinical course similar to BL (relatively few cases reported)
- Important to identify 11q aberration to distinguish from HGBL, NOS


Thank You