Gray Zone lymphomas

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Diffuse large B-cell lymphoma: Variants, subgroups and types (WHO 2008)

Diffuse large B-cell lymphoma, unspecified

*Common morphologic variants*
- Centroblastic
- Immunoblastic
- Anaplastic

*Uncommon morphologic variants*

*Molecular subgroups*
- Germinal-center B-cell-like (GCB)
- Activated B-cell-like (ABC)

*Immunohistochemical subgroups*
- CD5-positive DLBCL
- Germinal-center B-cell-like (GCB)
- Non-germinal center B-cell-like (non-GCB)
Diffuse large B-cell lymphoma: Variants, subgroups and types

Primary mediastinal (thymic) large B-cell lymphoma
T cell/histiocyte rich large B-cell lymphoma
  Lymphocytic and histiocytic (L&H)-cell like
  Centroblast-like
  Reed-Sternberg-cell-like
Intravascular large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
ALK positive DLBCL
Plasmablastic lymphoma
Lymphomatoid granulomatosis
EBV positive DLBCL of the elderly
DLBCL associated with chronic inflammation
Primary effusion lymphoma
Lymphoma arising from HHV8-associated multicentric Castleman Disease

Borderline cases
  B-cell lymphoma with features intermediate between DLBCL and BL
  B-cell lymphoma with features intermediate between DLBCL and cHL
PM large B-cell lymphoma

- Tend to affect young adults with female predominance 2:1
- Postulated to arise in thymic B-cells and tend to spread to extranodal organs but usually sparing the BM
- Compartmentalizing fibrosis, tumor cells with abundant pale cytoplasm; cells in some cases may resemble H/RS cells
- Express pan-B markers but no Ig. Variable BCL6+, frequently MUM1+, CD10-, CD30+, MAL+
- Unique pattern of genetic abnormalities
- Extension into surrounding tissues and poor performance status may predict poor outcome
JAK/STAT pathway activation in the pathogenesis of PMBL

Mutation → SOSC1 (45% of cases) → IL4, IL13 and other cytokines

9p24 gain/amplification (60% of cases) → JAK2 → pJAK2

JAK/STAT pathway activation in the pathogenesis of PMBL

JAK2 → STAT5 → pSTAT5 → Target Gene Transcription

PDL1/2 → Modulate host Immune response

Alter the epigenome by dec in H3K27Me3
JAK2 and JMJD2C in chromatin remodeling: Role in the pathobiology of PMBL
B-cell lymphoma, intermediate between DLBCL and CHL

Adapted from Dr. Nancy Harris

• Definition
  – A B lineage lymphoma with overlapping features between CHL and DLBCL, especially PMBL.

• Morphology and IHC:
  – Large cells, including some lacunar, R-S like, in sheets; variable sclerosis, fibrous bands, polymorphic background
  – CD45+, CD30+, Pax5+, CD20+, CD79a+/-, CD15+/-, CD10-, Bcl6-/+

• Clinical:
  – Young person (20-40)
  – mediastinal
  – Poorer outcome than either CHL or PMBL
  – Unclear how it should be managed

Many questions regarding these grey zone cases

• Is this a unique tumor that has overlapping characteristics with PMBL and cHL?
• Are these just cases with atypical morphology and/or aberrant expression of immunophenotypic markers?
• Are these just difficult cases of PMBL or cHL cases, especially with small biopsies?
• Are we lumping cases from all of the above into this grey zone category?
Observations indicating that there are indeed tumors that has overlapping characteristics with PMBL and cHL

• Overlap in GEP of PMBL and HL
• Frequent 9p24, 2p16 and 8q24 gains in both tumors
• Cases of PMBL relapsed with typical NSHL and vice versa
• Recent finding of CIITA fusion transcripts and DNA methylation patterns
• Appear to have worse prognosis whether treated with DLBCL or HL regimens
CIITA in recurrent gene fusions

- CIITA/BX648577 fusion detected in KM-H2 cell line
- Fusion transcript/protein highly expressed and suppresses normal CIITA expression [correlation with low HLA DR expression not confirmed*]
- Frequency of fusion using breakapart probe
  - 8/55 (15%) in HL
  - 29/77 (38%) in PMBL
  - 4/131 (3%) in other DLBCL
  - 8/30 (27%) in gray one lymphoma*
- Multiple fusion partners detected in PMBL samples
- Two of these involves PD-L1 and PD-L2 and induce high expression of the respective proteins

DNA methylation study

- Microdissected tumor cells from cHL, PMBL, MGZL, GCB and ABC-DLBCL
- Comparison of the DNA methylation profiles of selected CpG islands
- Illumina GlodenGate bead assay with ~1500 CpG islands from 800 genes
- PC analysis shows that MGZL has a methylation profile distinct from cHL and PMBL
- Constructed predictors for these entities

Erberle F et al Hematologica 96:558, 2011
DLBCL vs BL: Clinical Advisory Committee 4\textsuperscript{th} ed

- Many cases, especially in adults, cannot be definitively classified as aBL vs DLBCL
- Should not “contaminate” these categories with cases that may be biologically and clinically different
- Provisional category: B-cell lymphoma, unclassifiable, intermediate between BL and DLBCL
  - A heterogeneous category that needs to be further refined; not a distinct entity
  - Allows classification of cases not meeting criteria for classical BL or DLBCL
  - Individualized decisions about treatment

Dr. Nancy Harris
High-grade B-cell lymphoma, unclassifiable, intermediate between BL and DLBCL

Cannot be confidently put into either of the category because of atypia in:

• Morphology:
  – Intermediate between BL and DLBCL (many large or smaller cells; prominent central nucleolus)
• Immunophenotype:
  – CD10+, BCL6+ but also BCL2+
  – Ki67 not close to 100% with adequate staining
• Genetics:
  – *MYC* and *BCL2* translocated (double hit)
  – Non *Ig/MYC*
  – complex karyotypes
Hierarchical Clustering of Burkitt Lymphoma Predictor Genes

Dave SS et al NEJM 2011
Molecular BL (mBL) and intermediate cases

- Discrepancies between pathologic and mBL classification
  - mBL cases called DLBCL/HGU by pathologists (15-30%)
  - Non-mBL cases called aBL by pathologists (5%)
- Some cases had expression profiles borderline between mBL and DLBCL
- Borderline mBL cases had more complex karyotypes +/- MYC-R, MYC & BCL2-R, MYC/non-IG
- Prognosis for non-mBL cases with MYC-R worse than either mBL or DLBCL

Hummel et al, Dave et al, NEJM 2006. Adapted from Dr. N. Harris
Double hit cases

• Usually refer to both MYC and BCL2 translocated cases
• Morphologically heterogeneous: could be DLBCL or gray zone
• Generally BCL2 protein positive
• Poor prognosis in most series
Effect of Treatment on Survival in Burkitt Lymphoma: Adult Patients Only

![Graph showing probability of survival over years for intensive regimens versus CHOP-like regimens. The graph indicates a statistically significant difference (P=0.02).]
Non-coding RNAs

- Ribosomal RNAs
- Transfer RNAs
- microRNAs
- Piwi-associated RNAs
- siRNA
- Various longer non-coding RNAs
  - mRNA splicing
  - Ribosome biogenesis
  - Gene silencing, imprinting
- New members of these classes and new classes may be discovered by high throughput sequencing especially in unique cell types
The RISC complex

- Dicer
- One of the Argonaute (AGO) proteins
- TRBP
- A single strand of a miRNA
miRNA in health and disease

- Central role in the control of development and differentiation
- Dysregulation of miRNA expression (deletions, gains/amplification, abnormal regulations)
- Mutation/SNP in targets: introduce or impair target recognition
- Pathogens manipulate host miRNA
  - Viral mimics: KSHV miR K12-11/miR155
miRNA: in cancer diagnosis

• miRNA profiling in diagnosis
  – Cancer of unknown primary
  – Subclassification of a cancer type: DLBCL/BL
  – Prognostic marker
  – Application on FFPE tissue

• Multiple platforms used
  – Array based
  – Bead based
  – Q-RT-PCR
  – sequencing
MicroRNA in molecular diagnosis:
Correlation of miRNA profiles between paired frozen and FFPE samples

FFPE block in years

Fresh Frozen samples (ΔCt)  FFPE samples (ΔCt)

r = 0.94

r = 0.94

r = 0.95

r = 0.95
Would miRNA profiling help in further defining these gray zone lymphomas
miR-17~92 Cluster within c13orf25
Expression of miR-17~92 in Mature B-cell Lymphoma

> 5-fold in 65% of B-cell Lymphomas

(n=46, 13 DLBCL & 6 FL) (n=47)
miRNA classifier for Burkitt Lymphoma
MiR-17\textasciitilde92 Overexpression Induces the PI3K/AKT Pathway Activation

\begin{itemize}
\item pAKT-S\textasciitilde473
\item Total AKT
\item GSK-3\beta
\item p70S6K
\item Tubulin
\end{itemize}

\begin{figure}
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To move forward

- Perhaps where to put these cases is not that important
- Emphasis should be on the abnormal pathways in each of these tumors