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Comprehensive Cancer Center Tübingen

Pathology of aggressive lymphomas

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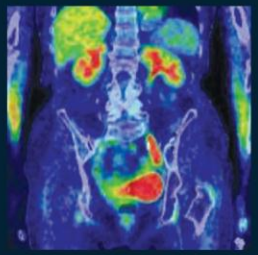
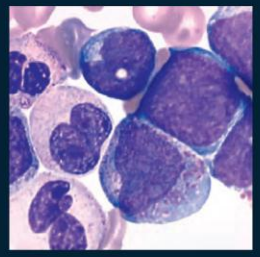
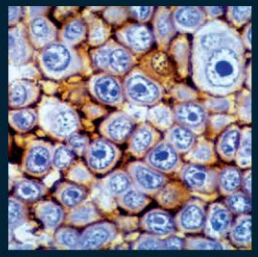
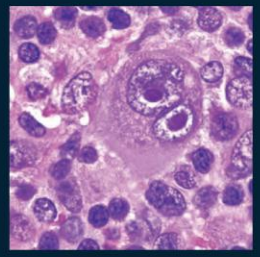
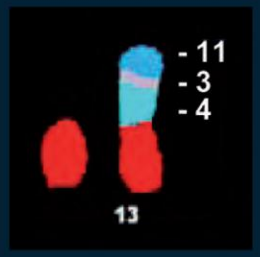
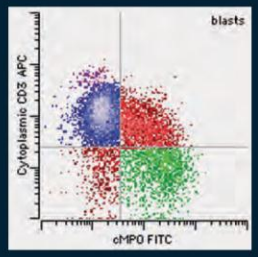
Eberhard Karls
Universität
Tübingen

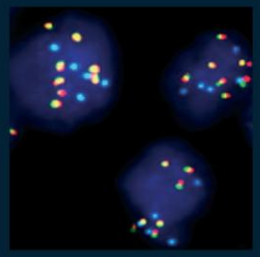
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri,
Harald Stein, Jürgen Thiele, Daniel A. Arber, Robert P. Hasserjian,
Michelle M. Le Beau, Attilio Orazi, Reiner Siebert





WHO

Changes in the new 2016 WHO

- Aggressive B-cell lymphoid neoplasms
  - Major changes that impact how cases should be evaludated and diagnosed (pathologist)
  - Therapeutic implications (hematologist)
  - New biological information

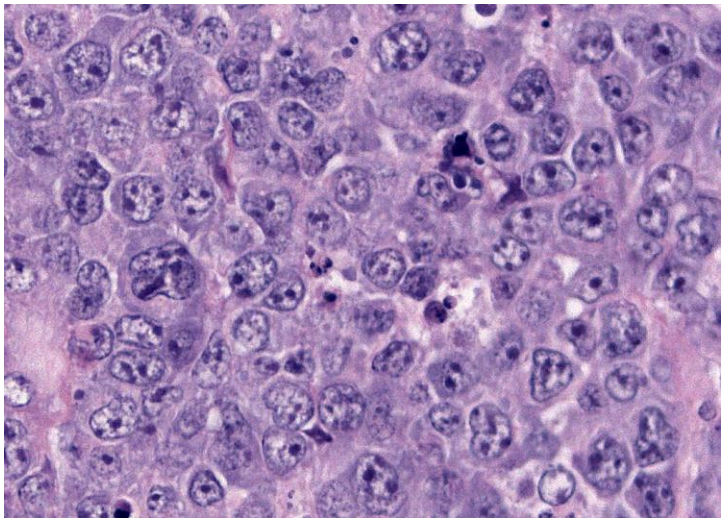
Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

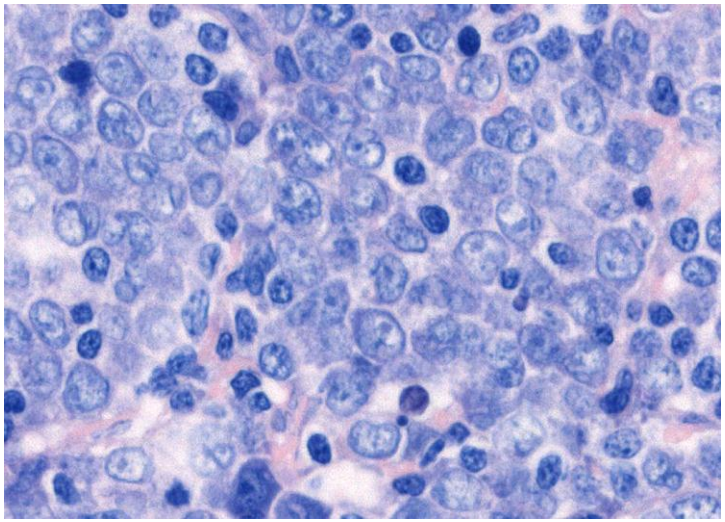
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
*EBV$^+$ mucocutaneous ulcer**
DLBCL associated with chronic 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

Provisional entities are listed in italics.
*Changes from the 2008 classification.

Diffuse large B-cell lymphoma, NOS

Definition: Diffuse large B-cell lymphoma is a neoplasm of large B lymphoid cells more than twice the size of a normal lymphocyte and with diffuse growth pattern.

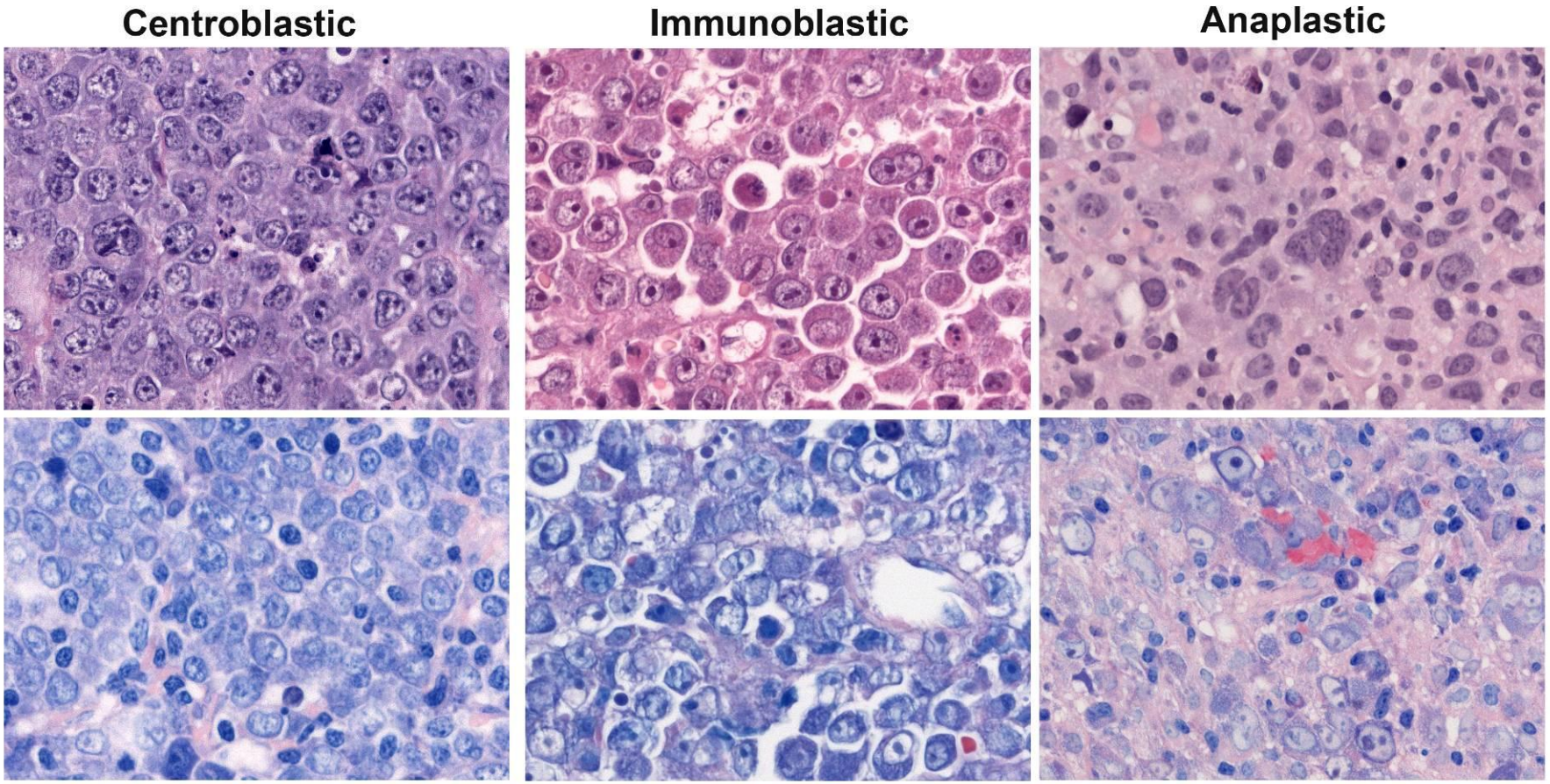




➢DLBCL is clinically, morpholgically and biologically a heterogeneous disease reflected in the highly variable clinical course

WHO 2008

Diffuse large B-cell morphology, NOS



Anaplastic morphology is independent of ALK expression

Diffuse large B-cell lymphomas

Diffuse large B-cell lymphoma (DLBCL), NOS
- Germinal centre B-cell subtype
- Activated B-cell subtype

T-cell/histiocyte-rich large B-cell lymphoma

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

EBV-positive DLBCL, NOS

EBV-positive mucocutaneous ulcer

DLBCL associated with chronic inflammation
- Fibrin-associated diffuse large B-cell lymphoma

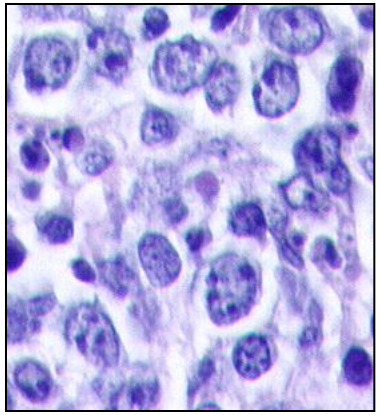
Lymphomatoid granulomatosis, grade 1,2

Lymphomatoid granulomatosis, grade 3

Primary mediatinal (thymic) large B-cell lymphoma

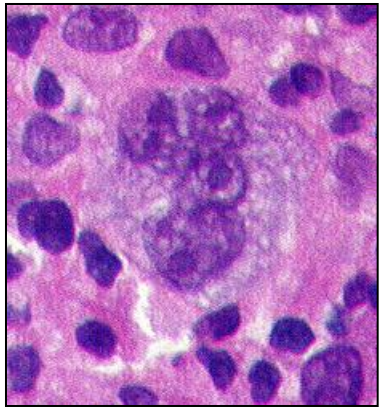
Intravascular large B-cell lymphoma

ALK-positive large B-cell lymphoma

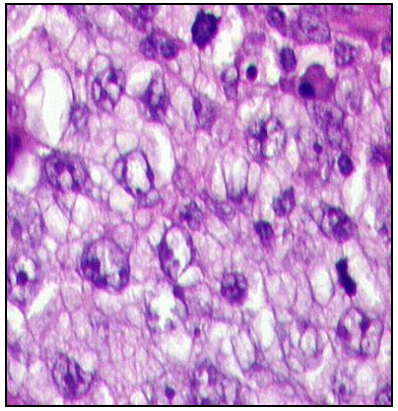


Centroblastic

Immunoblastic



T-cell rich



Mediastinal LBCL

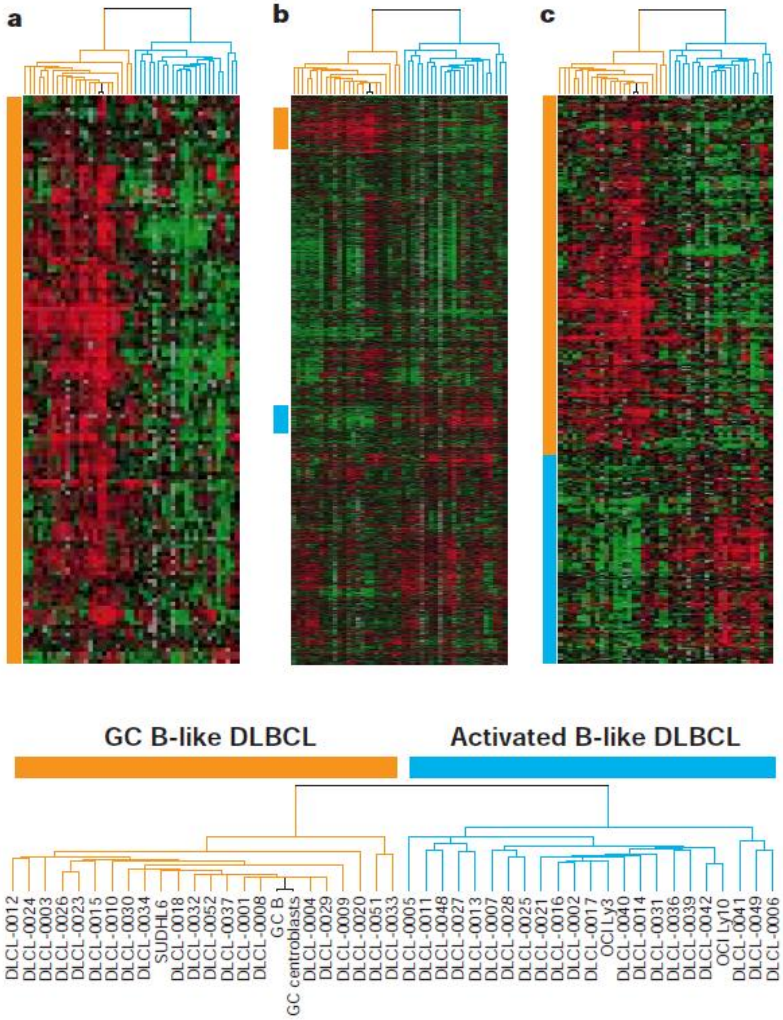
Revised 4th edition WHO classification

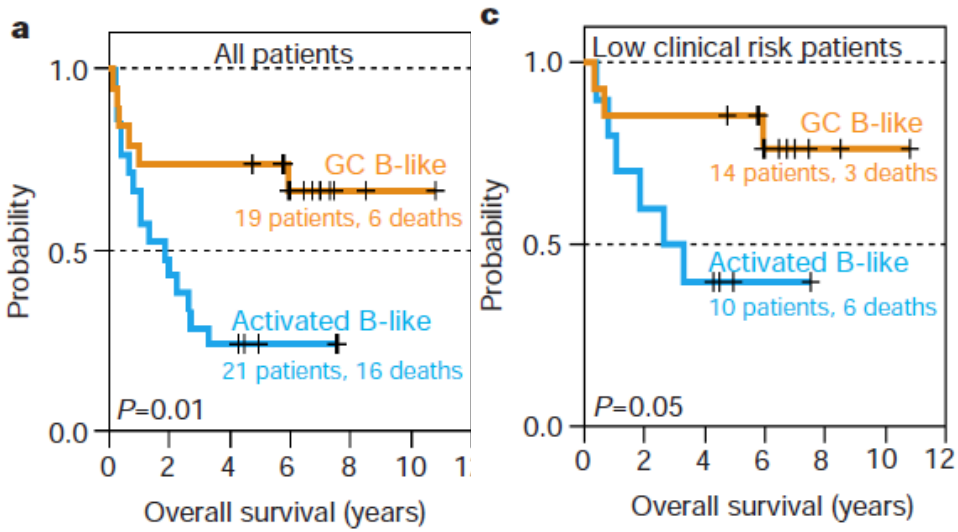
Diffuse large B-cell lymphomas

- R-CHOP – 30% will relapse or do not respond to therapy

- To understand the molecular changes underlying DLBCL
- There is an effort to tailor therapy based on specific types of DLBCL
  - Cell of origin
  - Molecular pathways
- To identify prognostic markers
  - *MYC*
  - *BCL2*

Diffuse large B-cell lymphomas, molecular signature



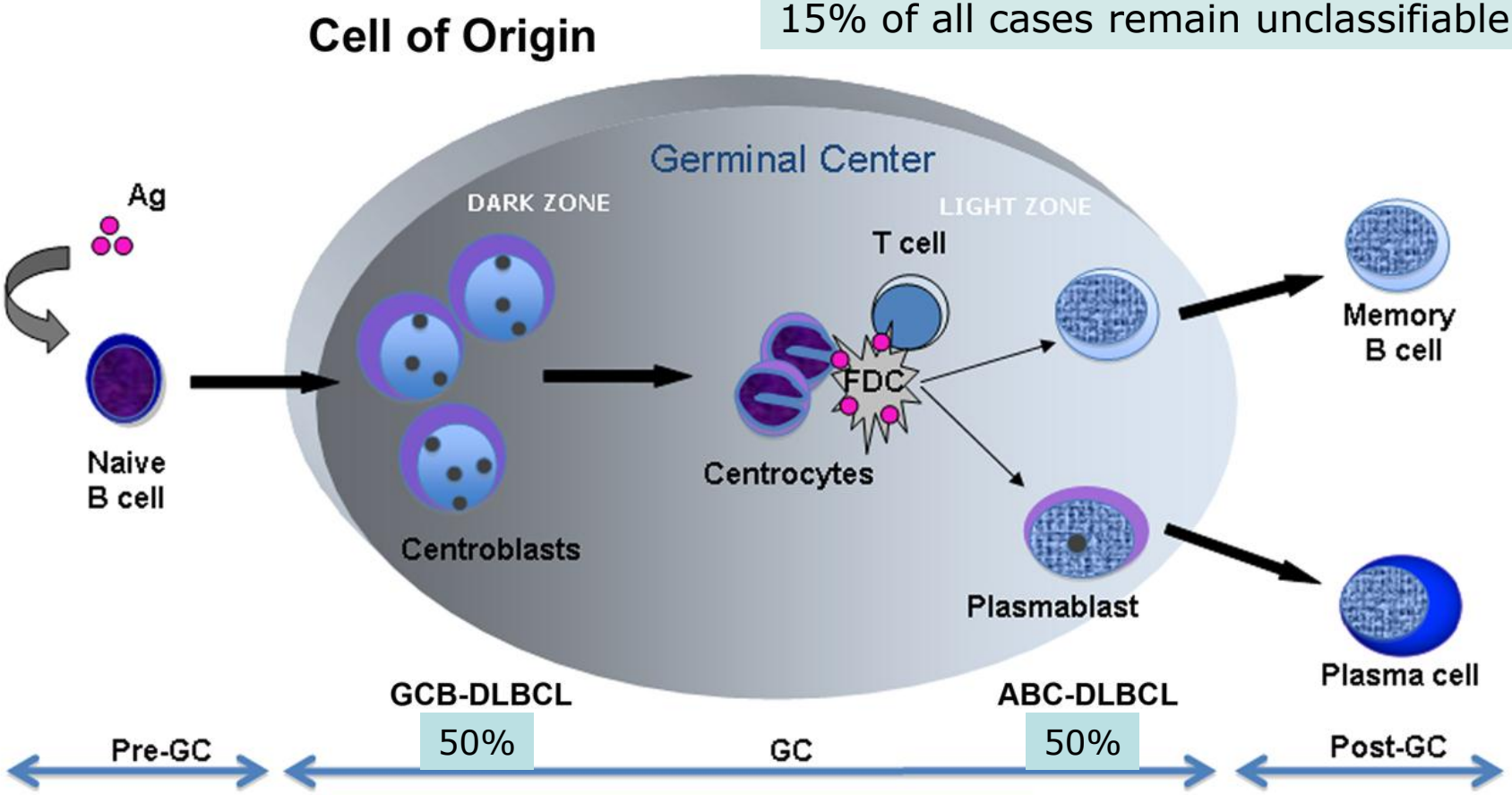


- The most frequent category representing 25-40% of all lymphomas

Alizadeh et al, Nature 2000; 403:503

Diffuse large B-cell lymphomas

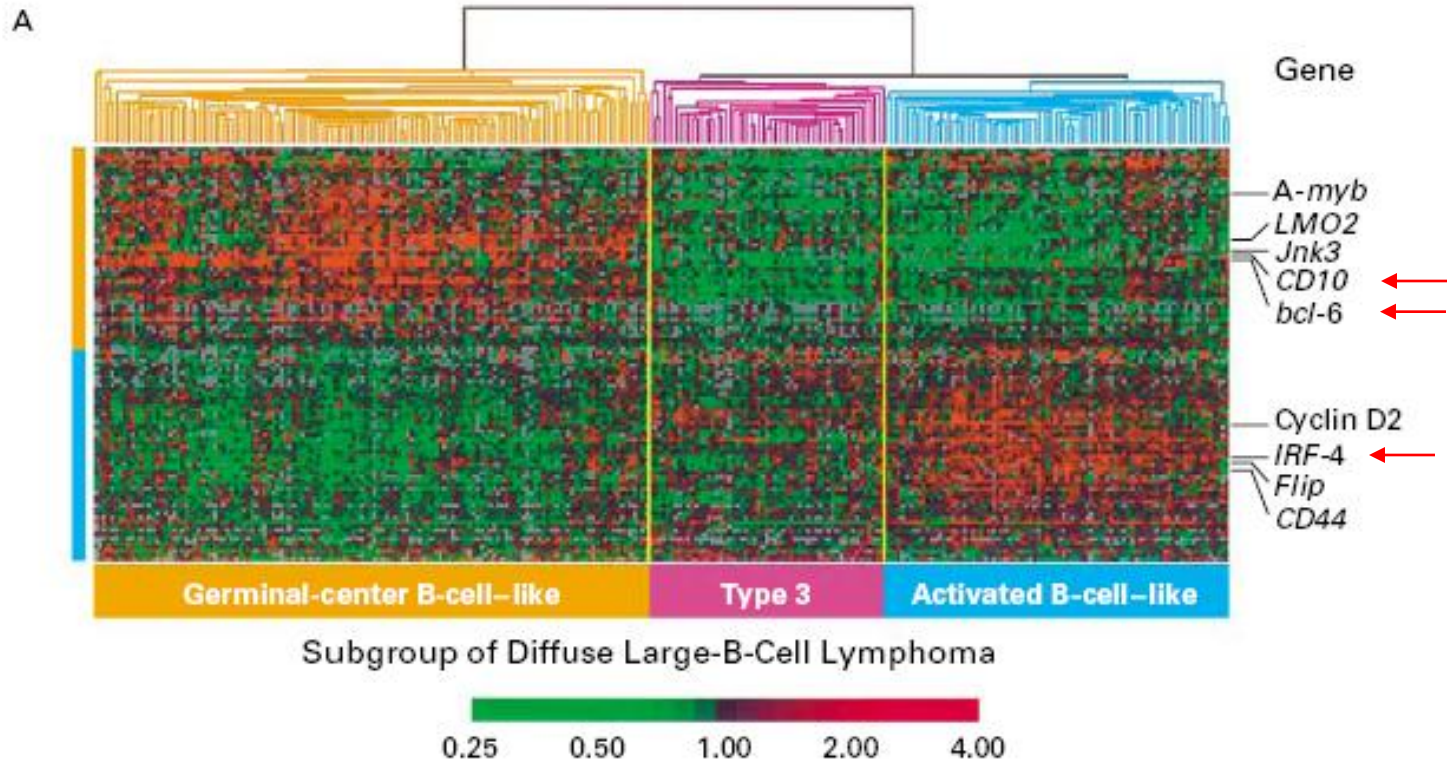
15% of all cases remain unclassifiable



CD10+ BCL6+
Highly mutated IG
Ongoing SHM

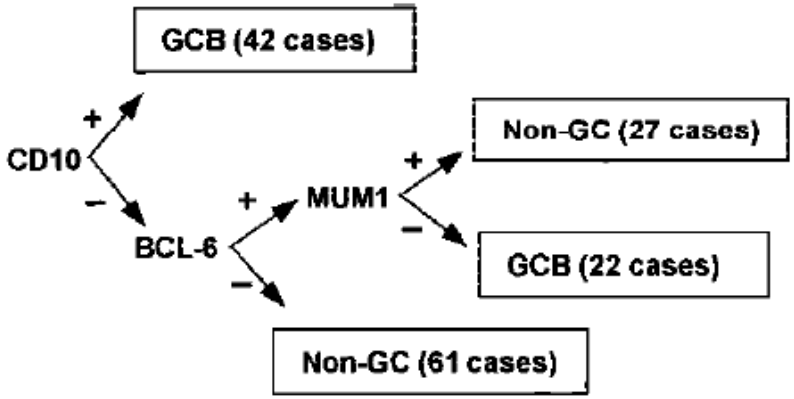
BCR activated B-cells
MUM1+FOXP1+
Activation of the NF-κB pathway

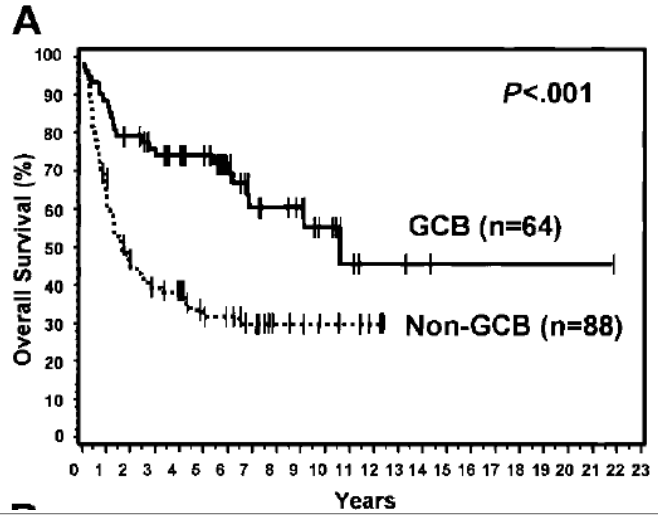
Diffuse large B-cell lymphomas
Determining the cell of origin

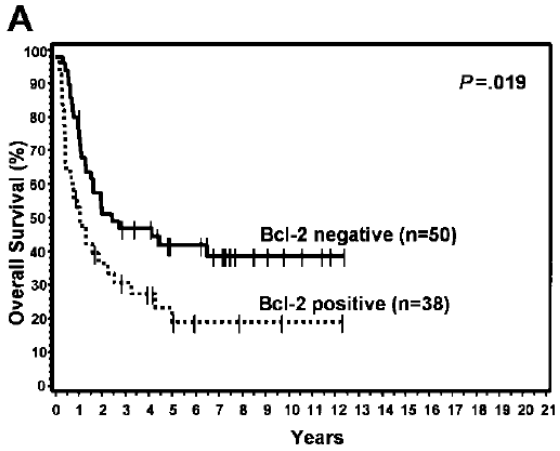


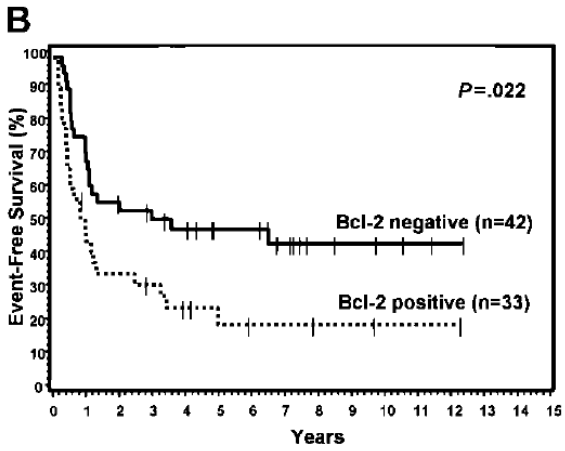
Rosenwald et al, NEJM 2002;346:1938

Hans Algorithm for molecular classification of DLBCL



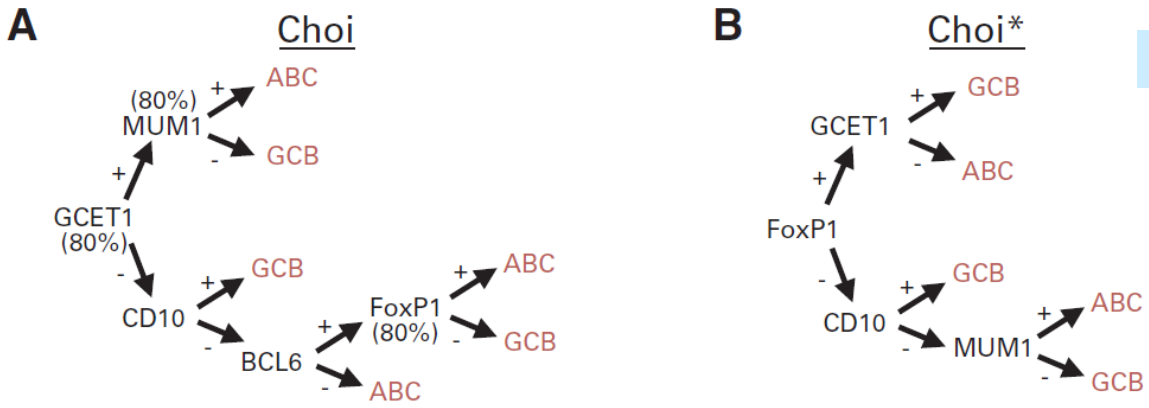






BLOOD, 1 JANUARY 2004 • VOLUME 103, NUMBER 1

Determining the cell of origin



Concordance with GEP of 87%

CHoi et al, Clin Cancer Res, et al 2009; 15:5494

G

Natkunam

LMO2 ≥ 30% → GCB

LMO2 < 30% → ABC

Natkunam et al, JCO 2008, 26: 447

H

Tally

"+" = 1, "-" = 0

GCB	ABC	Score
CD10 (+ or -)	Mum1 (+ or -)	GCB > ABC
GCET1 (+ or -)	FoxP1 (+ or -)	or
Score (0, 1, 2)	Score (0, 1, 2)	ABC > GCB

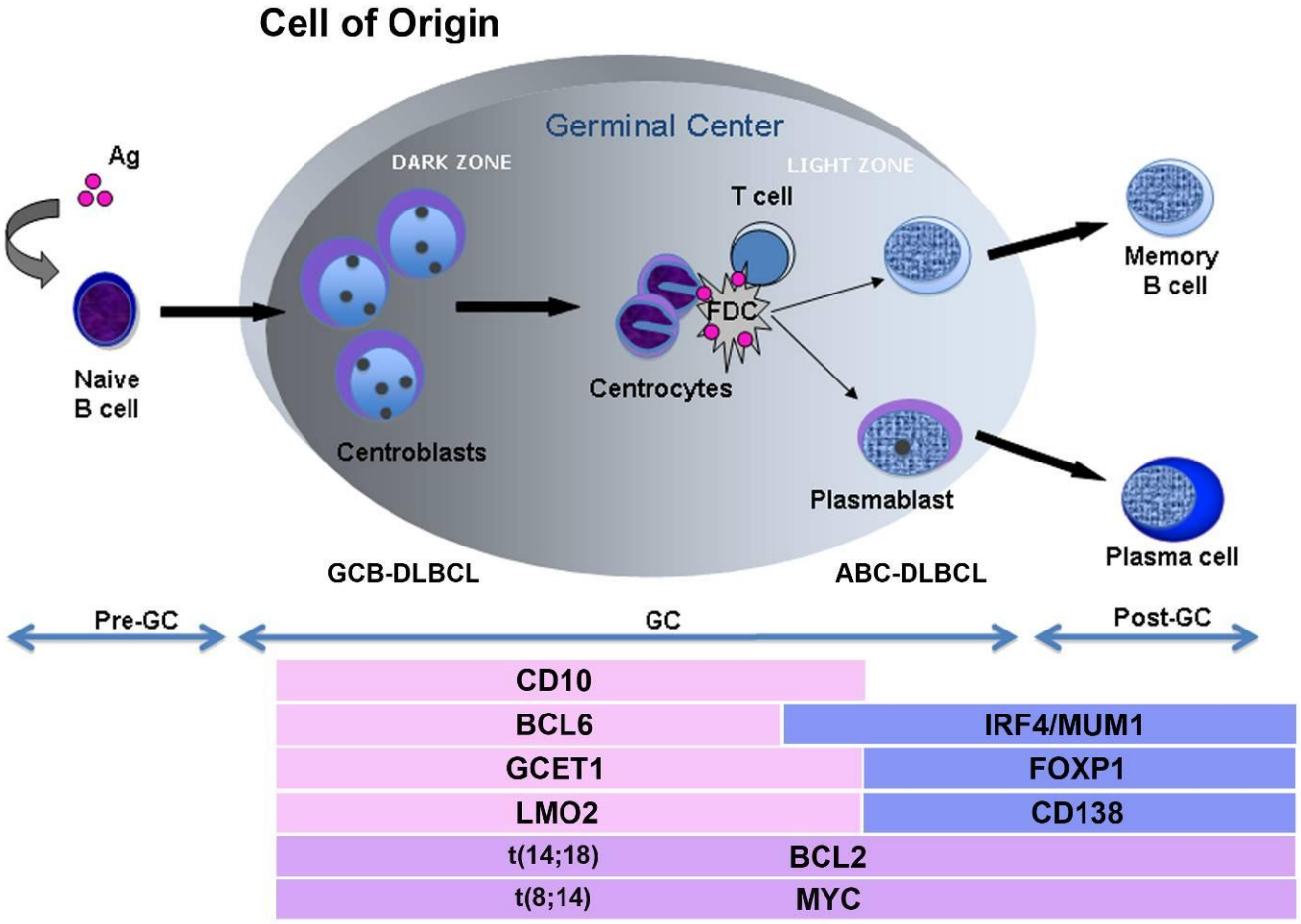
If GCB Score = ABC Score:
LMO2 ≥ 30% → GCB
LMO2 < 30% → ABC

Concordance with GEP of 93%

Meyer et al, JCO 2010; 29:200

WHO 2016 determining the cell of origin

It is acceptable to investigate the cell of origin in DLBCL with IHC algorithms



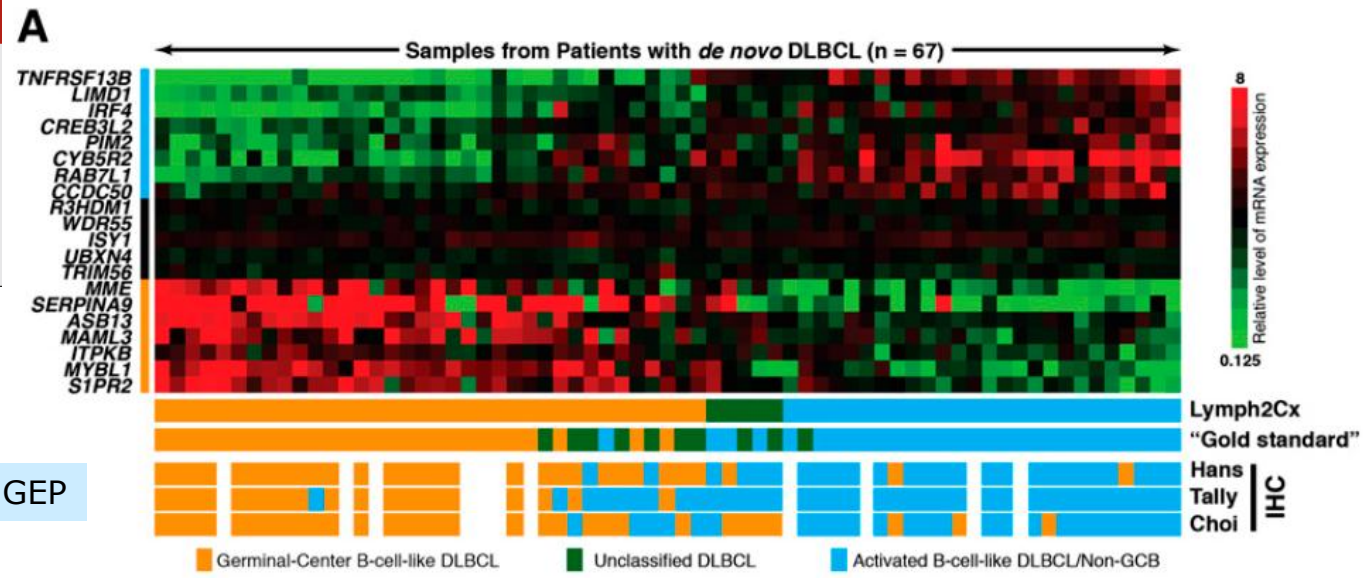
Quintanilla-Martinez, Hematological Oncology 2015

Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue

David W. Scott,[1] George W. Wright,[2] P. Mickey Williams,[3] Chih-Jian Lih,[3] William Walsh,[3] Elaine S. Jaffe,[4] Andreas Rosenwald,[5] Elias Campo,[6] Wing C. Chan,[7] Joseph M. Connors,[1] Erlend B. Smeland,[8] Anja Mottok,[1] Rita M. Braziel,[9] German Ott,[10] Jan Delabie,[11] Raymond R. Tubbs,[12] James R. Cook,[13] Dennis D. Weisenburger,[14] Timothy C. Greiner,[7] Betty J. Glinsmann-Gibson,[15] Kai Fu,[7] Louis M. Staudt,[16] Randy D. Gascoyne,[1,17] and Lisa M. Rimsza[15]

Key Points

- A 20-gene gene expression-based assay accurately and robustly assigns COO subtypes of DLBCL using formalin-fixed paraffin-embedded tissue.

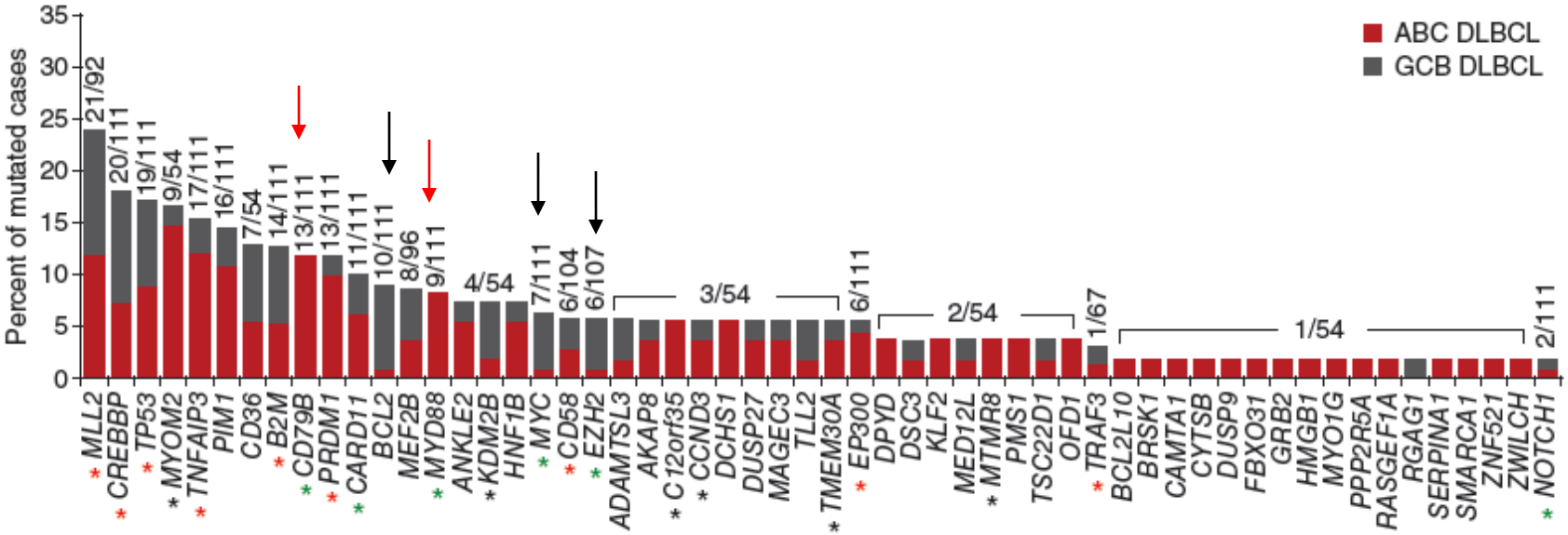


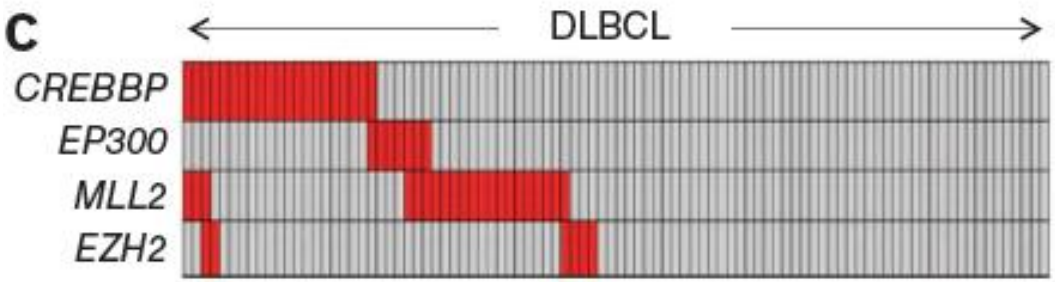
>95% concordance with GEP

200 ng RNA using NanoString technology
Tumor cells >60%
10µm scrolls of FFPET

BLOOD, 20 FEBRUARY 2014 • VOLUME 123, NUMBER 8

Recurrent somatic mutations in DLBCL

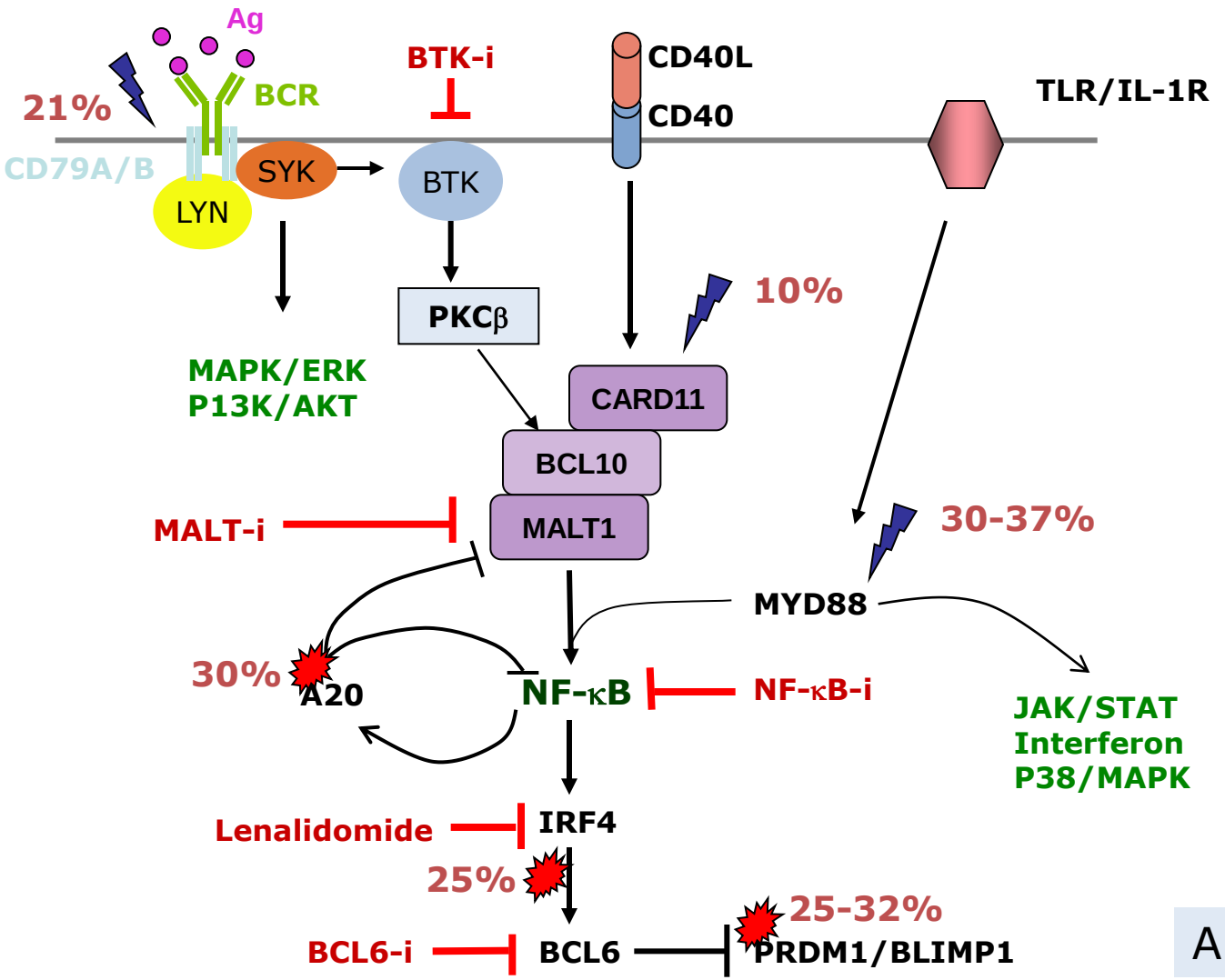




Aberrant histone/ chromatin modification

Pasqualucci L et al. Nat Genet 2011.

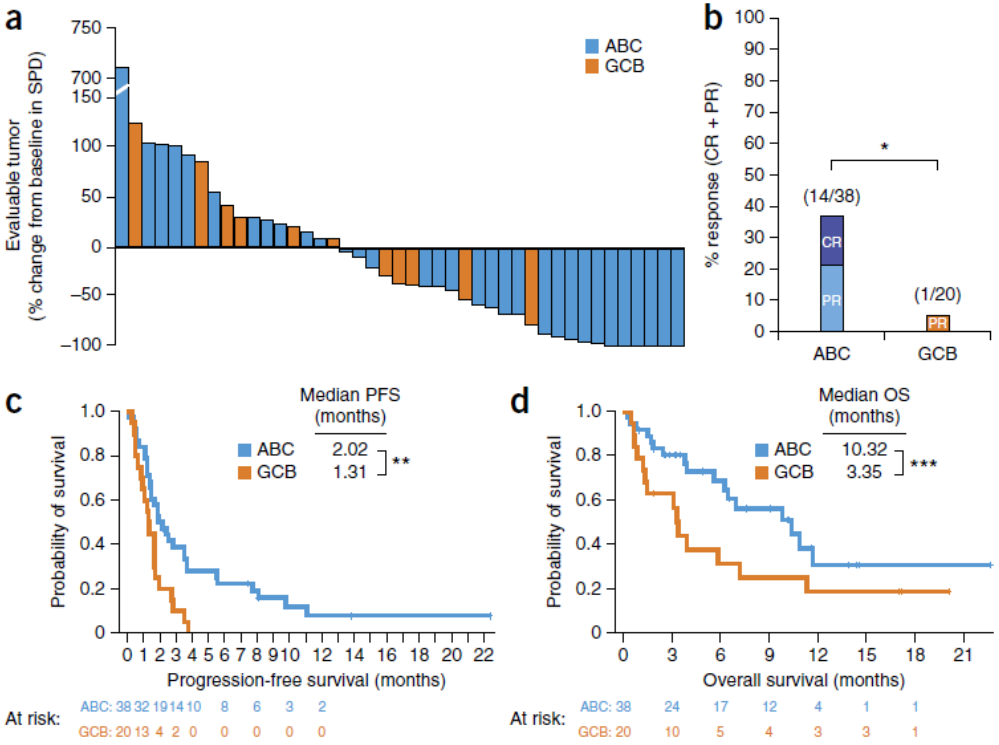
Constitutive BCR and NF-κB signaling

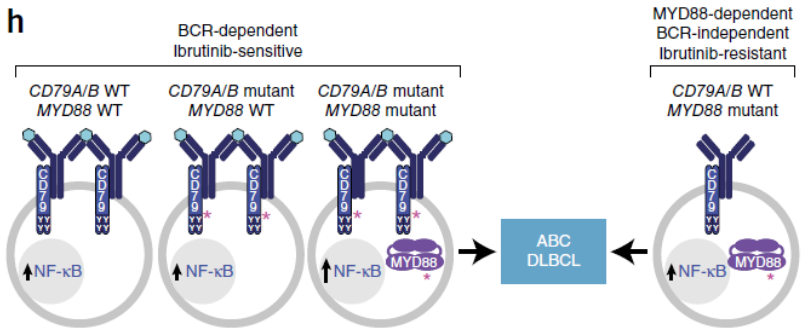


ABC-DLBCL

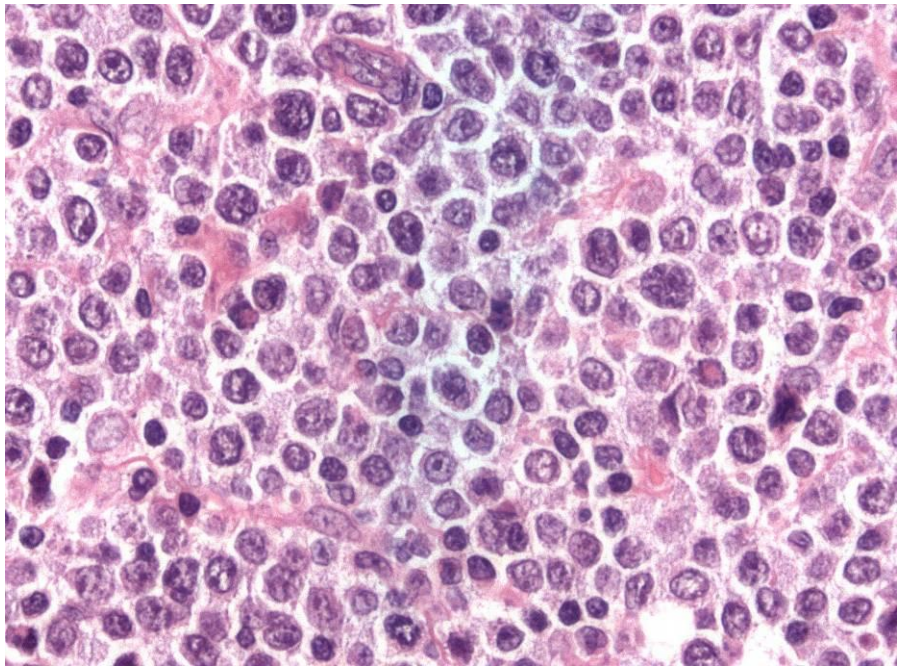
Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma

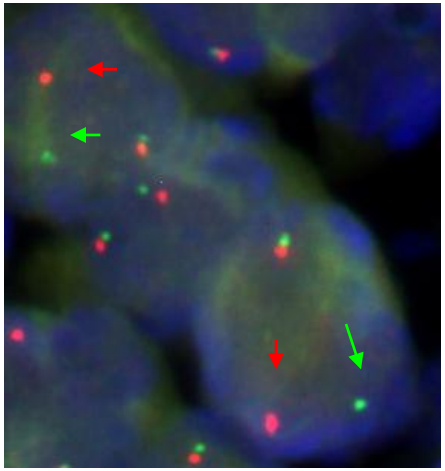
Wyndham H Wilson[1], Ryan M Young[1], Roland Schmitz[1], Yandan Yang[1], Stefania Pittaluga[2], George Wright[3], Chih-Jian Lih[4], P Mickey Williams[4], Arthur L Shaffer[1], John Gerecitano[5,6], Sven de Vos[7], Andre Goy[8], Vaishalee P Kenkre[9], Paul M Barr[10], Kristie A Blum[11], Andrei Shustov[12], Ranjana Advani[13], Nathan H Fowler[14], Julie M Vose[15], Rebecca L Elstrom[16], Thomas M Habermann[17], Jacqueline C Barrientos[18], Jesse McGreivy[19], Maria Fardis[19], Betty Y Chang[19], Fong Clow[19], Brian Munneke[19], Davina Moussa[19], Darrin M Beaupre[19] & Louis M Staudt[1]





Prognostic importance of *MYC* translocation in DLBCL





MYC break-apart probe

Ott et al, Blood 2013;122:3884-91
Karube K & Campo E, Semin Haematol 2015;52:97
Aukema et al., Blood 2011,117:2319
Salaverria et al., JCO 2011

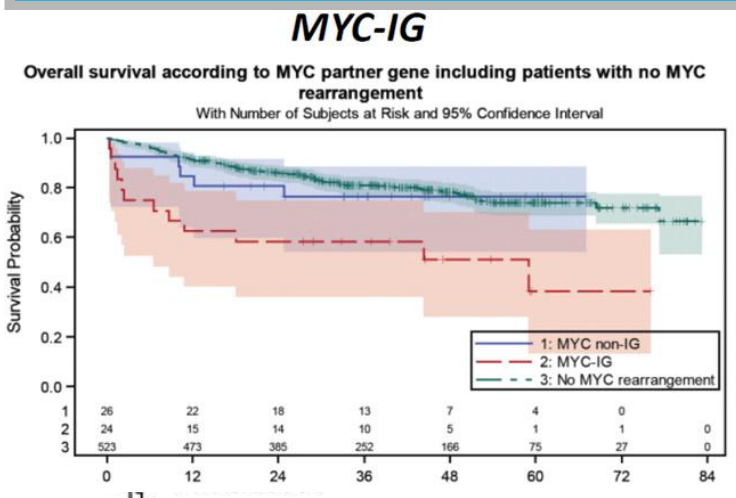
DLBCL morphology with *MYC* translocation

Reference	DH/TH (%)	*MYC*-R (%)	Type of DH	% GCB type
Niitsu 2009	5%	11%	*BCL2*	84%
Johnson 2012	5%	12%	*BCL2*	64%
Green 2012	6%	11%	*BCL2*	91%
Akyurek 2012	3%	6%	*BCL2 & BCL6*	71%
Visco 2013	2%	8%	*BCL2*	88%
Valera 2013	4%	7%	*BCL2 & BCL6*	71%
Hu 2013	3%	N/A	*BCL2*	90%
Tzankov 2014	3-4%	9%	*BCL2 & BCL6*	80%*
Copie-Bergman 2015	6%	8.8%	*BCL2 & BCL6*	N/A

BCL2 R- 60-70%
BCL6 R – 6%
Triple hit: 15-20%

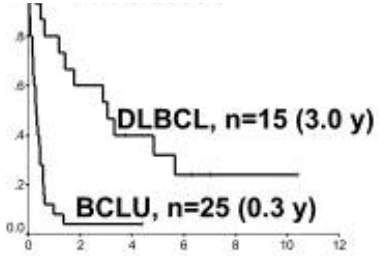
**BCL6* DH only 50% GCB
>90% have a GCB phenotype

Not all *MYC* translocated DLBCL are the same. Modulators of prognosis



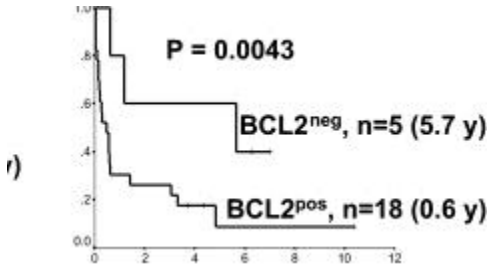
MYC partner matters? IG vs non-IG

Pedersen et al, European J Haematol 2014;92-42-49
Copie-Bergman et al, Blood 2015



Morphology matters? BCLU vs DLBCL

Johnson NA et al Blood 2009;114:2273-2279

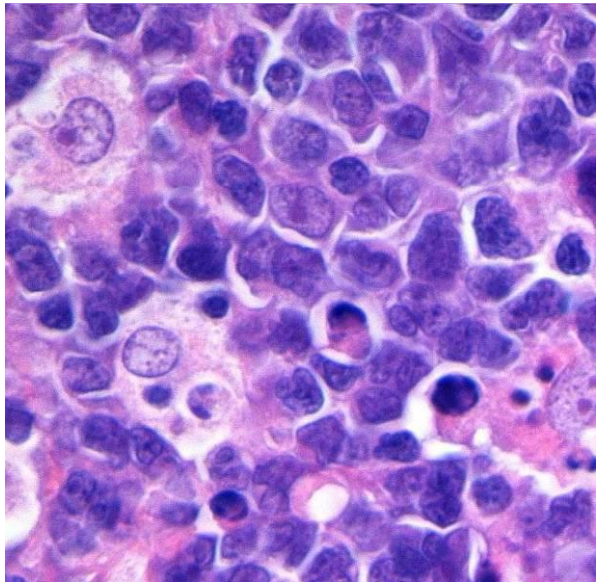


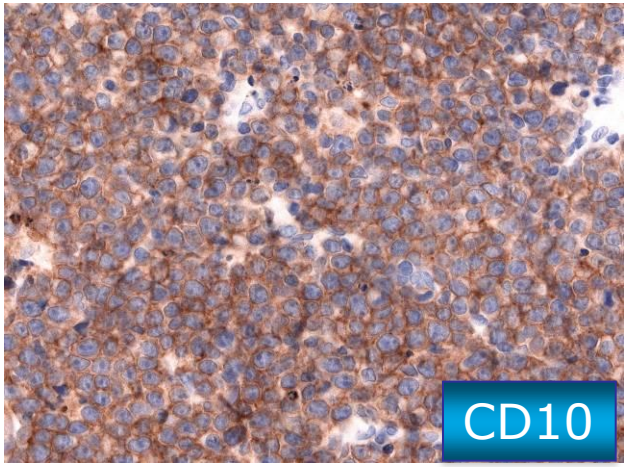
BCL2 expression matters

Johnson NA et al Blood 2009;114:2273-2279

When to test *MYC* in DLBCL?

- Selection of cases
- Clinical presentation
  - Extensive disease, CNS involvement, BM involvement and Leukemic presentation
- Morphology
  - All BCL-U morphology
  - All blastoid morphology
  - DLBCL of GCB type
    - Ki67 is not a good parameter
    - BCL2 > 50%
    - MYC > 40%
- FISH
  - Start with break-apart probes for *MYC* followed for *BCL2* and *BCL6*.





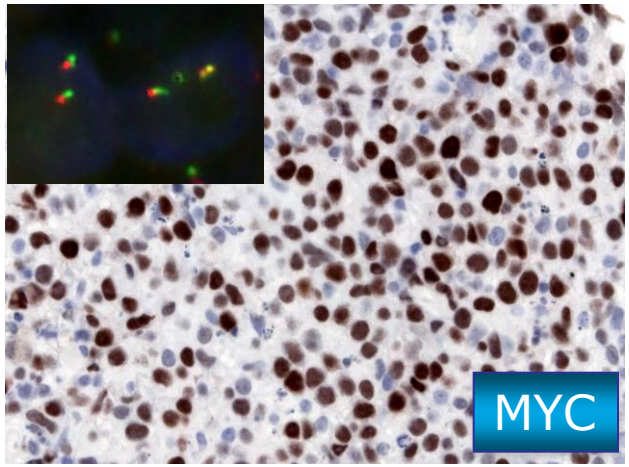
DLBCL with MYC expression without translocation

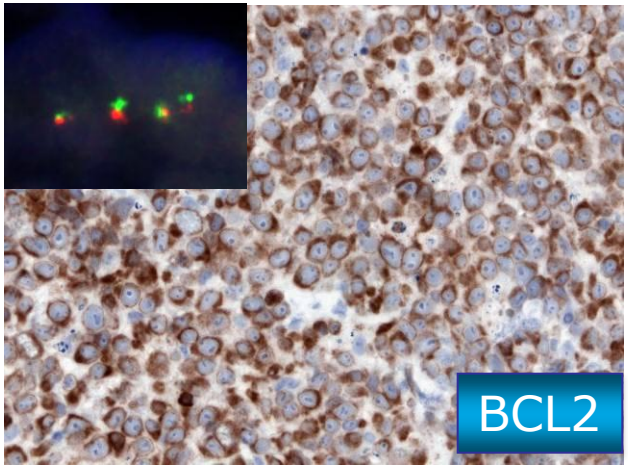
➢MYC protein expression is more frequent than genetic alteration in DLBCL

- *MYC* FISH rearranged in DLBCL 5-12%

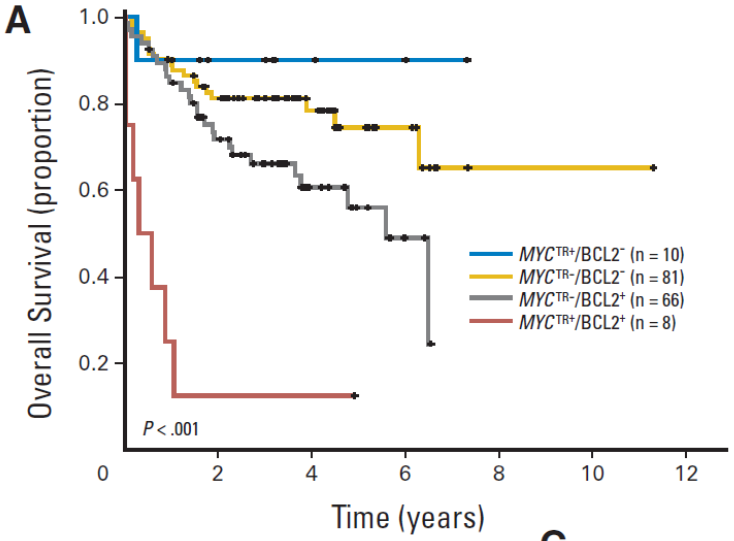
- MYC IHC+ 29-64%
- MYC+/BCL2+ IHC: 30%

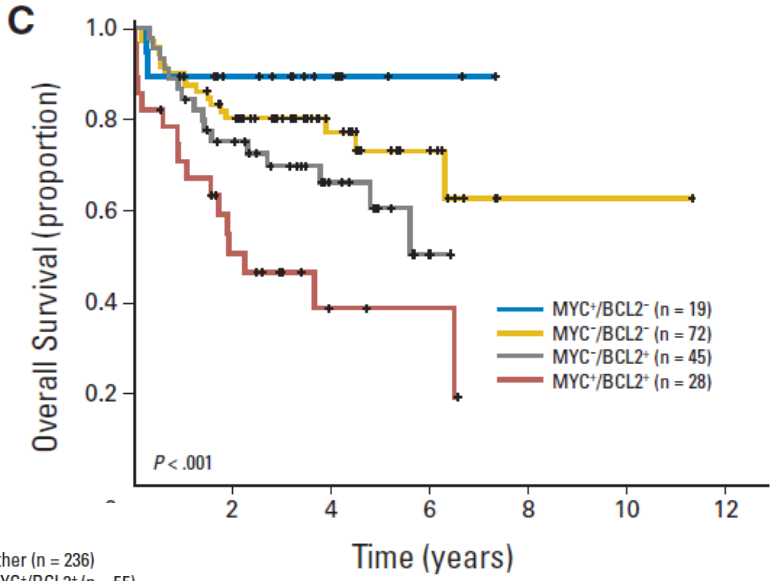
Author	Cases#	MYC/BCL2+
Johnson 2012	136	18%
Green 2012	185	29%
Horn 2013	141	28%
Hu 2013	466	34%
Valera 2013	120	27%



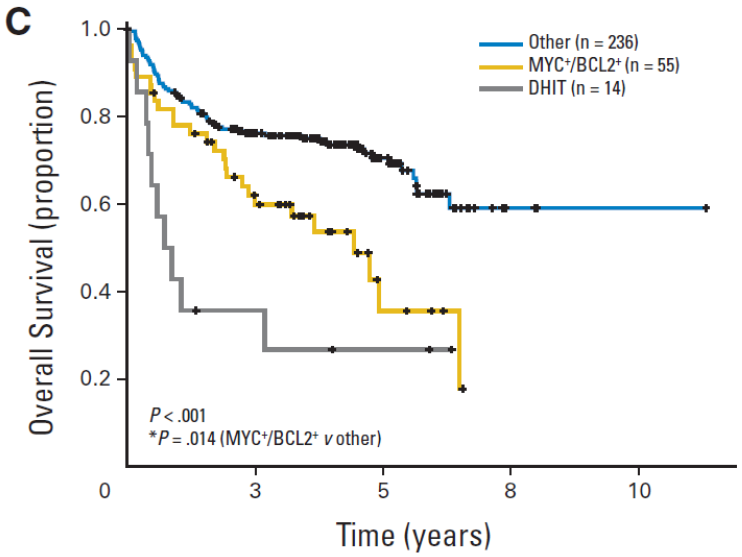


DLBCL with expression of MYC and BCL2





„Double expressor"



Johnson et al

VOLUME 30 · NUMBER 28 · OCTOBER 1 2012

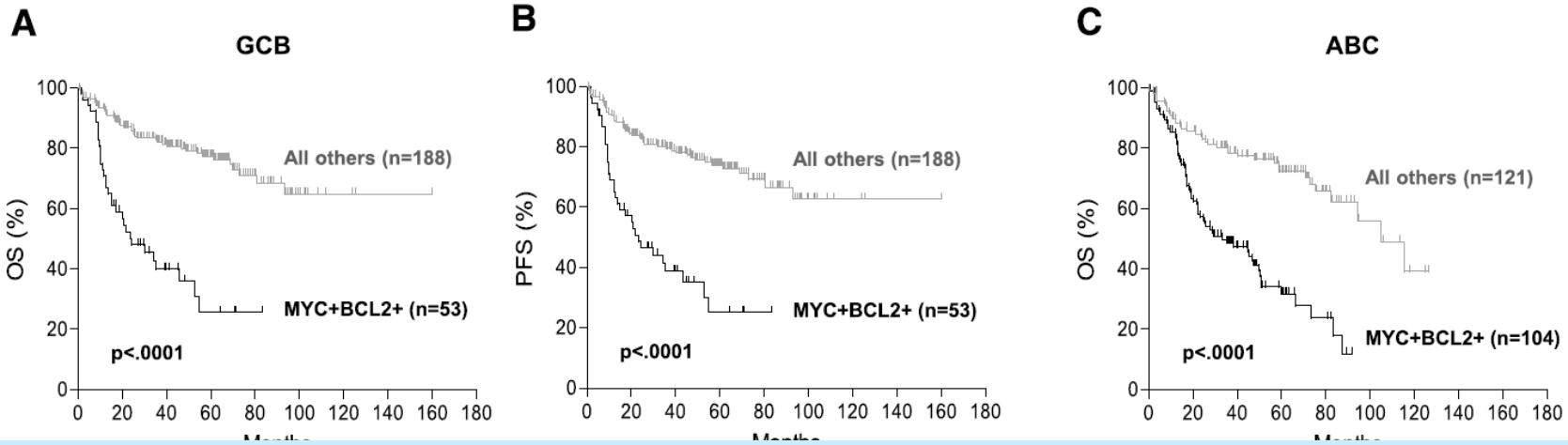
JOURNAL OF CLINICAL ONCOLOGY

Südwestdeutsches Tumorzentrum
Comprehensive Cancer Center Tübingen

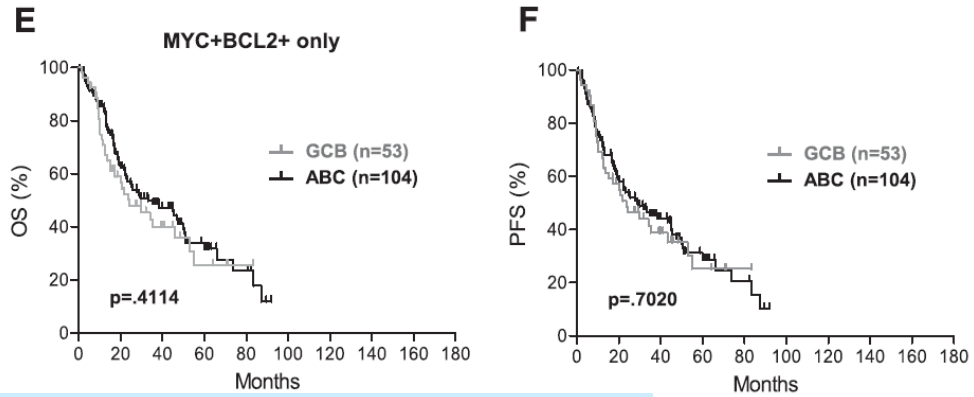
Eberhard-Karls-Universität UKT Universitätsklinikum Tübingen

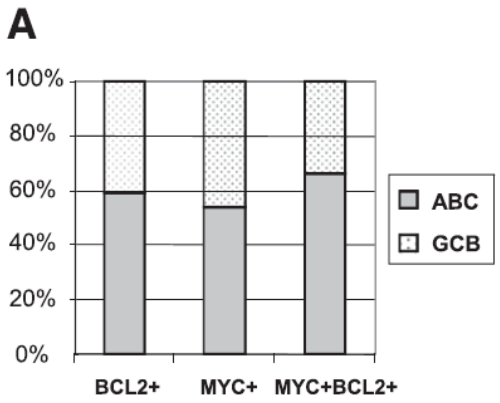
Eberhard Karls Universität Tübingen

ABC vs GCB "double expressors"



MYC/BCL2 coexpression, rather than cell-of-origin is a better predictor of prognosis in DLBCL





Double expression of BCL2/MYC is more frequently observed in non-GC phenotype (66% vs 39%)

BLOOD, 16 MAY 2013 • VOLUME 121, NUMBER 20

Diffuse Large B-cell lymphoma

- Changes in the 2016 revised WHO
  - Distinction of GCB vs ABC/non-GCB type required with use of immunohistochemical algorithm acceptable, may affect therapy
  - Coexpression of MYC and BCL2 considered new prognostic marker (double expressor lymphoma) (MYC>40% and BCL2>50%)
  - Mutational landscape better understood and might become part of the foundation for optimal patient care.

Prognostic factors
- Immunophenotypic: MYC/BCL2 IHC
- Genetic: *MYC, BCL2, BCL6 rearrangements*

"Grey zone lymphomas"

Diffuse large B-cell lymphoma (DLBCL), NOS	9680/3
T-cell/histiocyte rich large B-cell lymphoma	9688/3
Primary DLBCL of the CNS	9680/3
Primary cutaneous DLBCL, leg type	9680/3
EBV positive DLBCL of the elderly	9680/3
DLBCL associated with chronic inflammation	9680/3
Lymphomatoid granulomatosis	9766/1
Primary mediastinal (thymic) large B-cell lymphoma	9679/3
Intravascular large B-cell lymphoma	9712/3
ALK positive ~~DLBCL~~	9737/3
Plasmablastic lymphoma	9735/3
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease	9738/3
Primary effusion lymphoma	9678/3
Burkitt lymphoma	9687/3
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma	9680/3
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma	9596/3

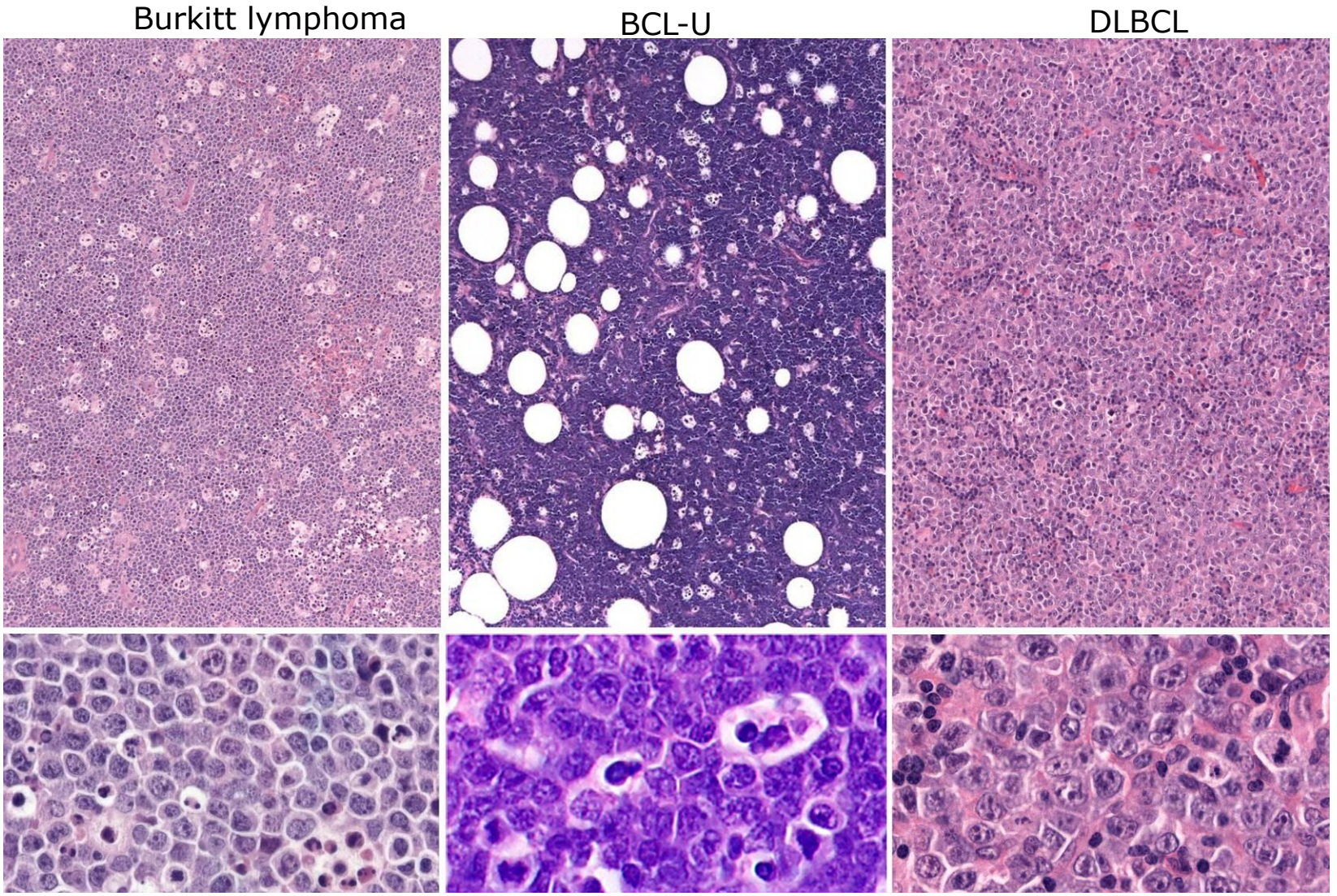
- Not an entitiy, but provisional groups that awaits further studies

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma	9680/3
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma	9596/3

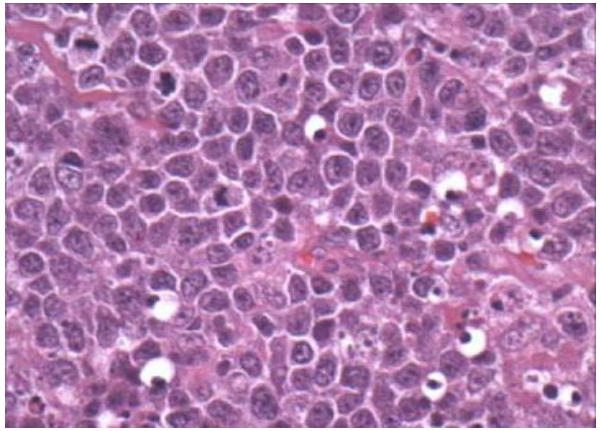
BCLU

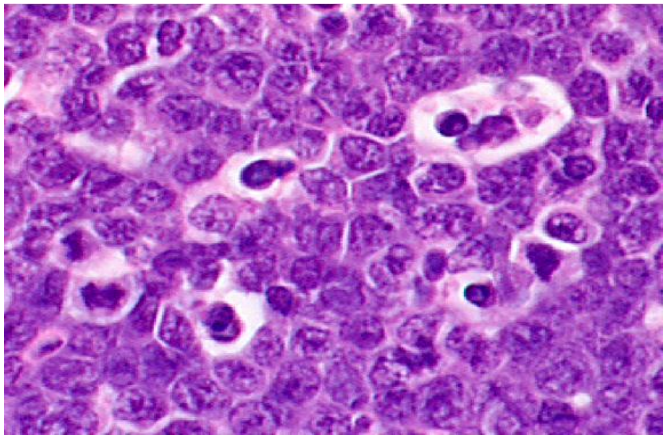
2008 WHO Classification

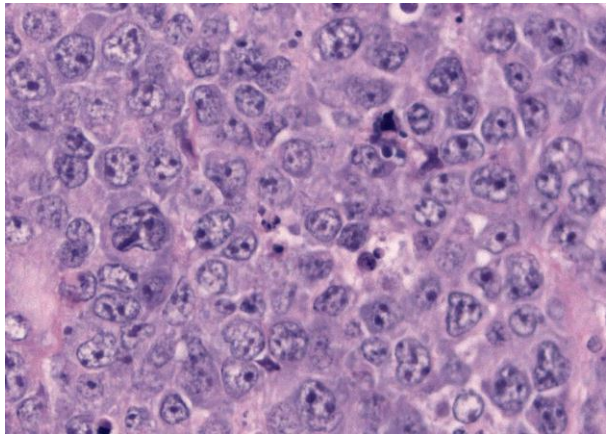
Differential diagnosis of DLBCL



Differential diagnosis of BL, B-unclassifiable, and DLBCL





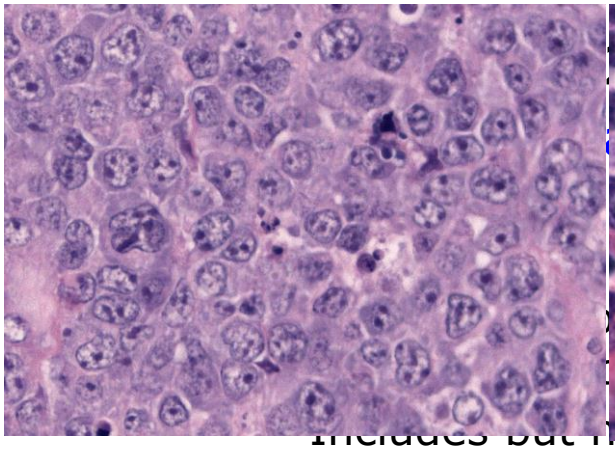
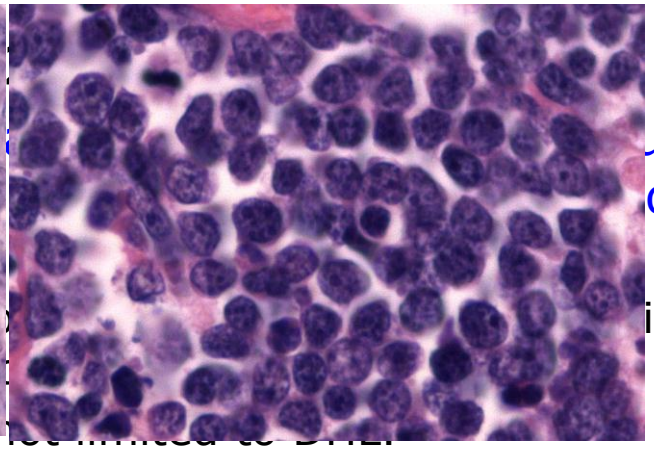
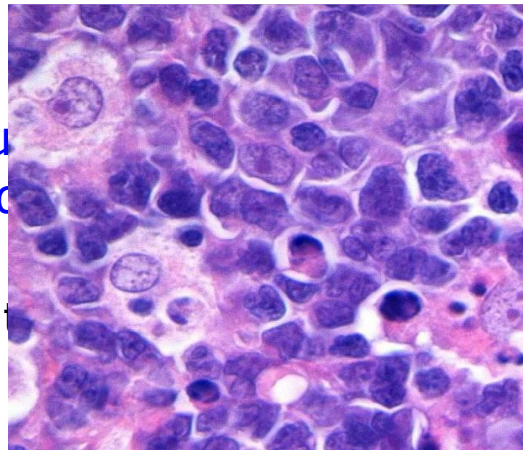


Burkitt
CD10+
BCL6+
BCL2-
MYC expression +
MIB1>98%
MYC-R simple
BCL2/BCL6-R –
ID3/TCF3 mutations +
EBV+/-

BCL-U category
CD10+
BCL6+/-
BCL2+
MYC expression +
MIB1<90%
MYC-R complex (54%)
BCL2/BCL6-R frequent
ID3 mutations rare
EBV-

GCB-DLBCL
CD10+
BCL6+
BCL2+/-
MYC expression +/-
MIB1 variable
MYC-R complex (5-15%)
BCL2/BCL6-R (2-6%)
ID3/TCF mutations -
EBV-

WHO 2016 Update
High grade B-cell lymphoma, NOS

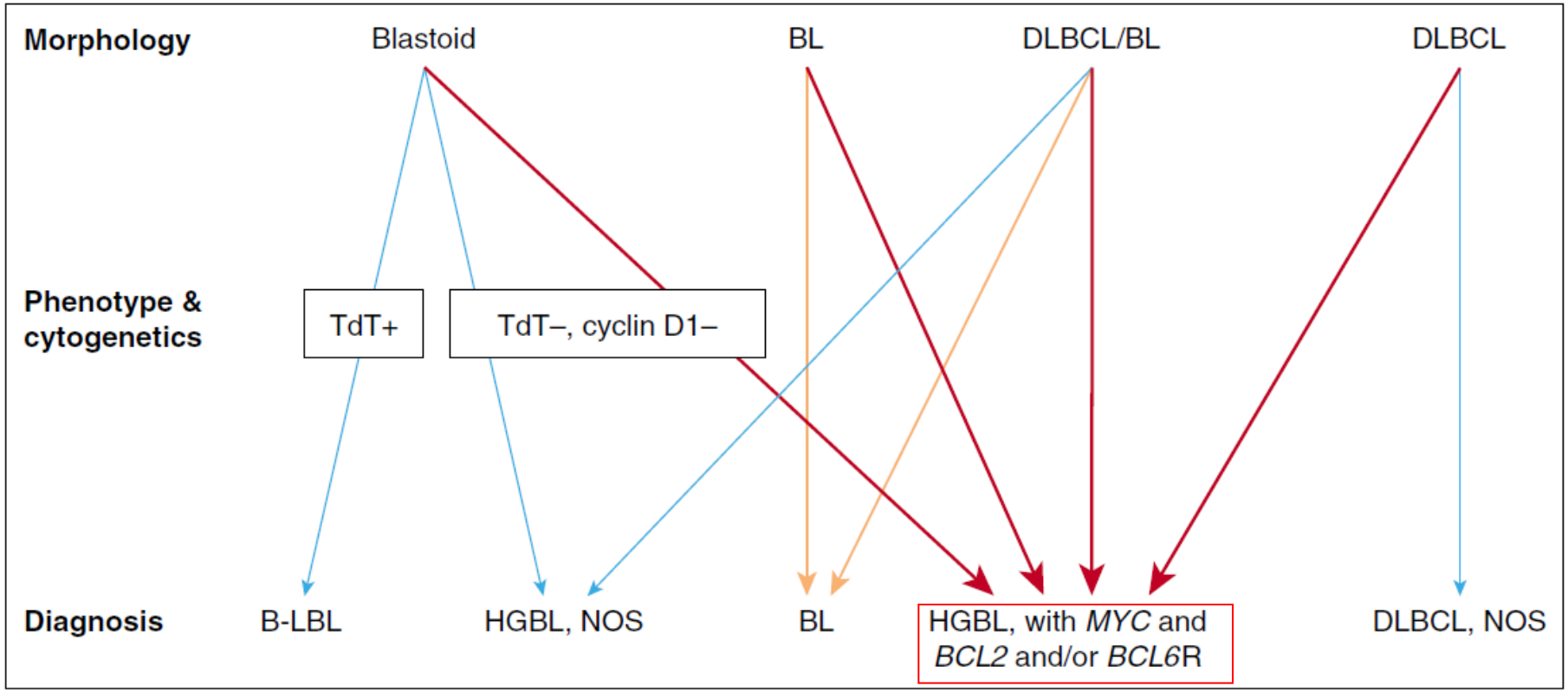
- WHO 4th edition update 2016:
  - High grade B-cell lymphomas
    - High-grade B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements (double or triple-hit)
      - Specifiy whether DLBCL, blastoid or BCLU morphology
      - Cases of FL or LBL with DH are not included!
    - High-grade B-cell lymphoma, NOS
      - Cases with BCLU or blastoid morphology or other high-grade features and no DH

Diagnostic approach to high grade B-cell lymphoma

HGBL with double or triple hit

BLOOD, 19 MAY 2016 • VOLUME 127, NUMBER 20

BCLu → HGBL, nos



The morphology appearance should be noted in a commnent

How I treat double-hit lymphoma

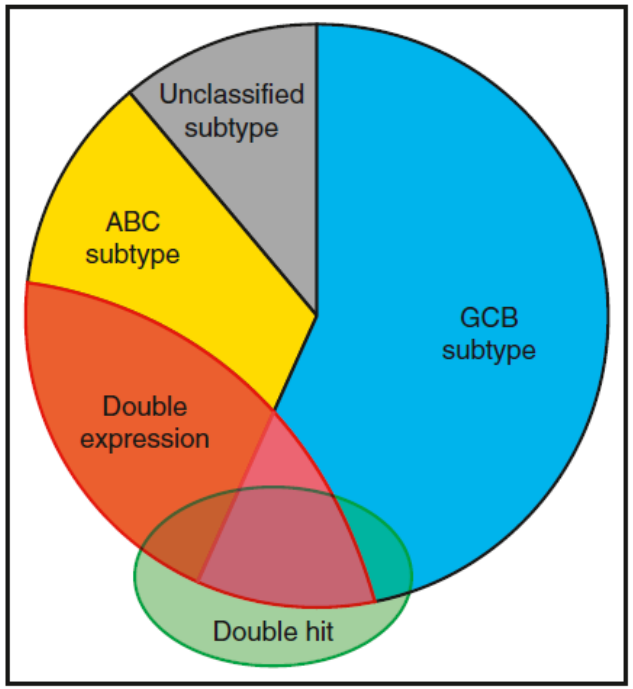
Jonathan W. Friedberg

James P. Wilmot Cancer Institute, University of Rochester, Rochester, NY

BLOOD, 3 AUGUST 2017 • VOLUME 130, NUMBER 5

Table 1. Terminology of myc-associated disease

Double-hit	High-grade lymphoma with rearrangements of *myc* and *bcl-2* or *myc* and *bcl-6*; must be diagnosed with FISH or more advanced genomic techniques.
Triple-hit	High-grade lymphoma with rearrangements of *myc* and *bcl-2* and *bcl-6*; must be diagnosed with FISH or more advanced genomic techniques.
Double-expressor	Protein expression of MYC and BCL-2 and/or BCL-6; measured by using an immunohistochemistry cutoff for the percentage of positive cells.

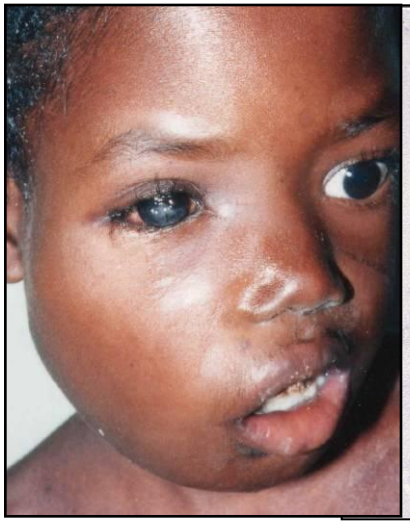


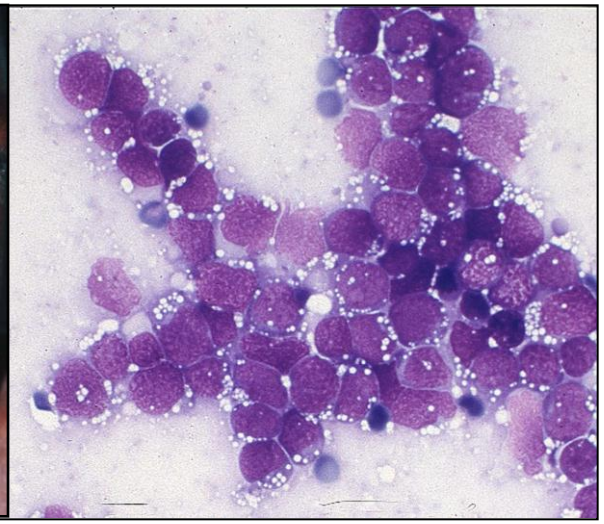
- One third of DLBCL are BCL2 and MYC+ by IHC (double expressors)
- Most of these cases are non-GCB DLBCL
- Double hit lymphomas are usually double expressors but not always
- Double hit lymphomas are GCB subtype (*BCL2/MYC*)

Burkitt lymphoma

Definition:

Highly aggressive lymphoma often presenting in extranodal sites, composed of monomorphic medium-sized B-cells with basopohilic cytoplasm and numerous mitotic figures.



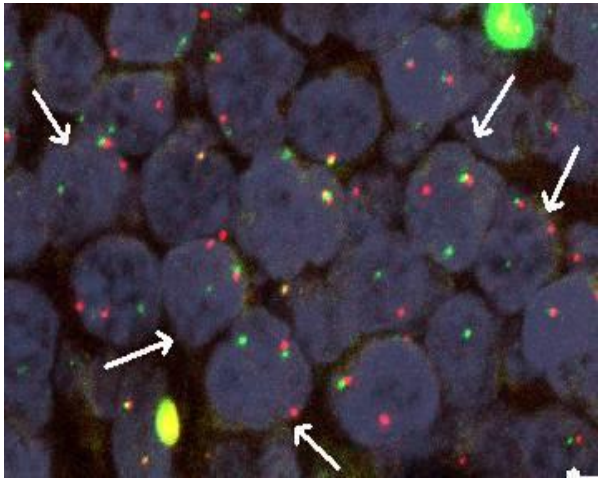


Epidemiology:

Endemic, sporadic and immunodeficiency associated

Genetics:

MYC translocation with simple karyotype, 100% EBV in endemic cases.



Break in c-myc locus

Dalla-Favera R, Science 1983;219:963

Classical Burkitt lymphoma without *MYC* alterations

- Approx 3-10% of all BLs including endemic and pediatric
- Considered cases that can be missed with FISH

  Cryptic insertions of *IG* into *MYC* locus

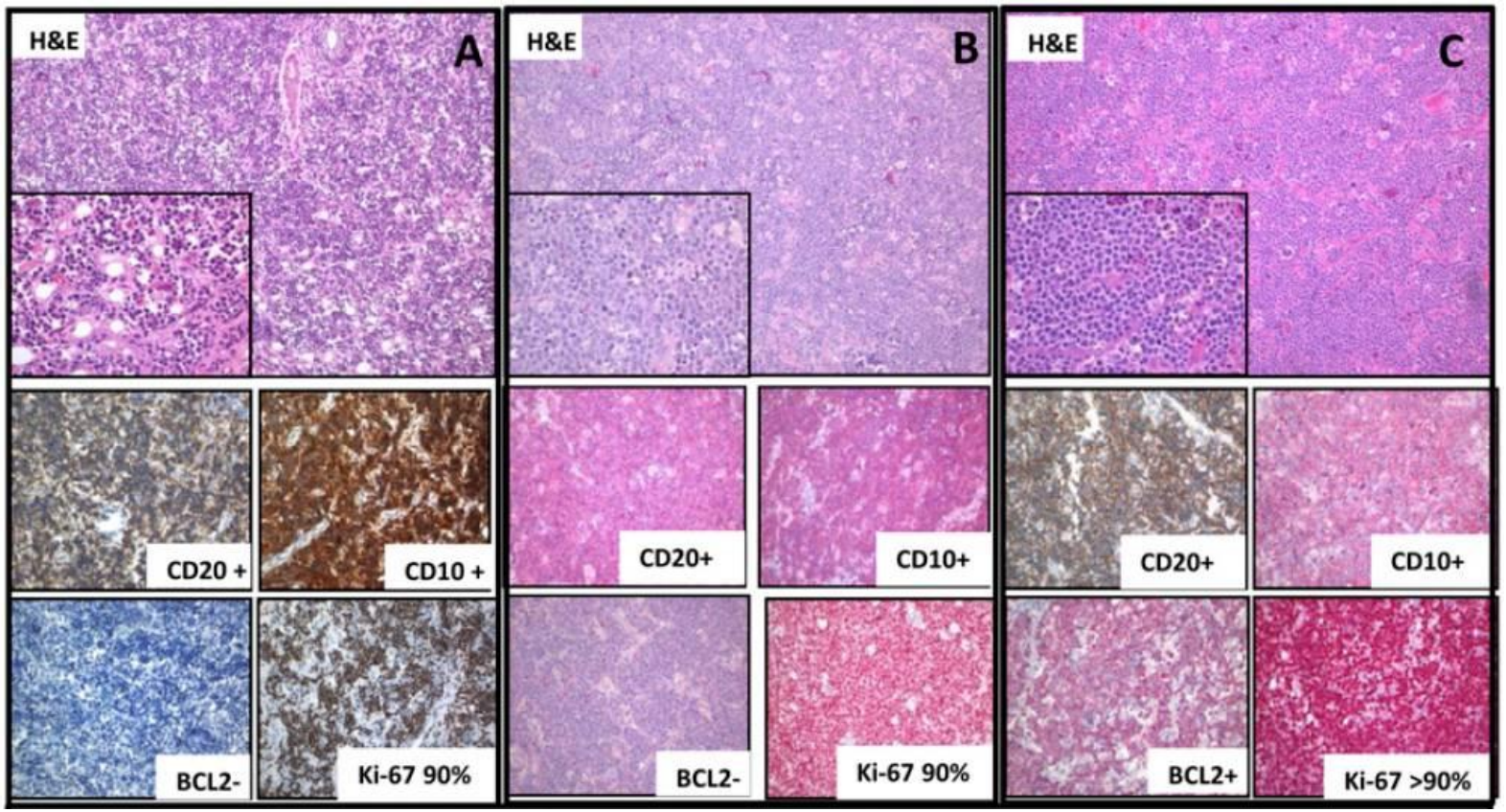
  distal 5´and 3´breaks
- Recurrent 11q alterations

  Candidate genes *FLI1*, *ETS1*

Aukema et al., Blood 2011,117:2319
Salaverria et al., JCO 2011
Salaverria et al, Blood 2014;123:1187

Burkitt -like lymphoma with 11q alterations

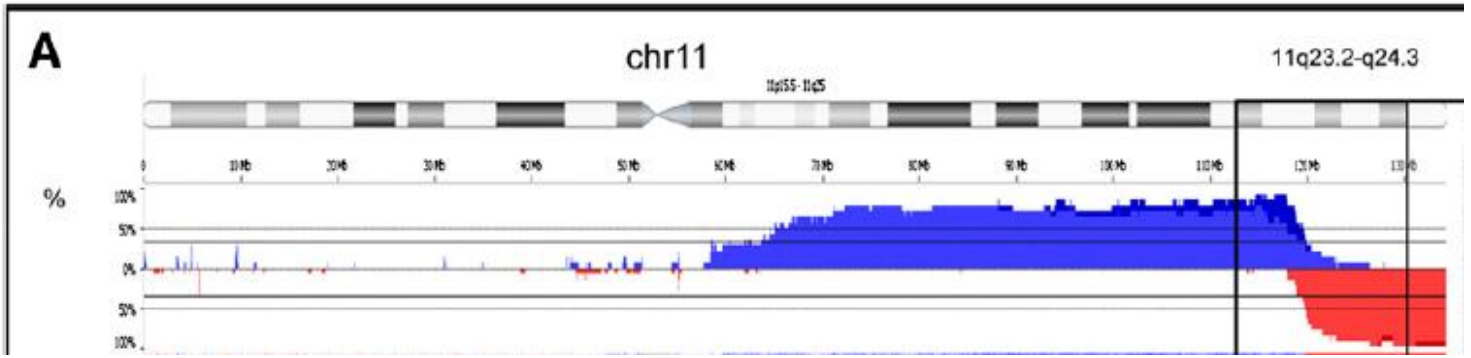
Some have Burkitt lymphoma morphology and phenotype



Salaverria et al, Blood 2014;123:1187

WHO 2016 update

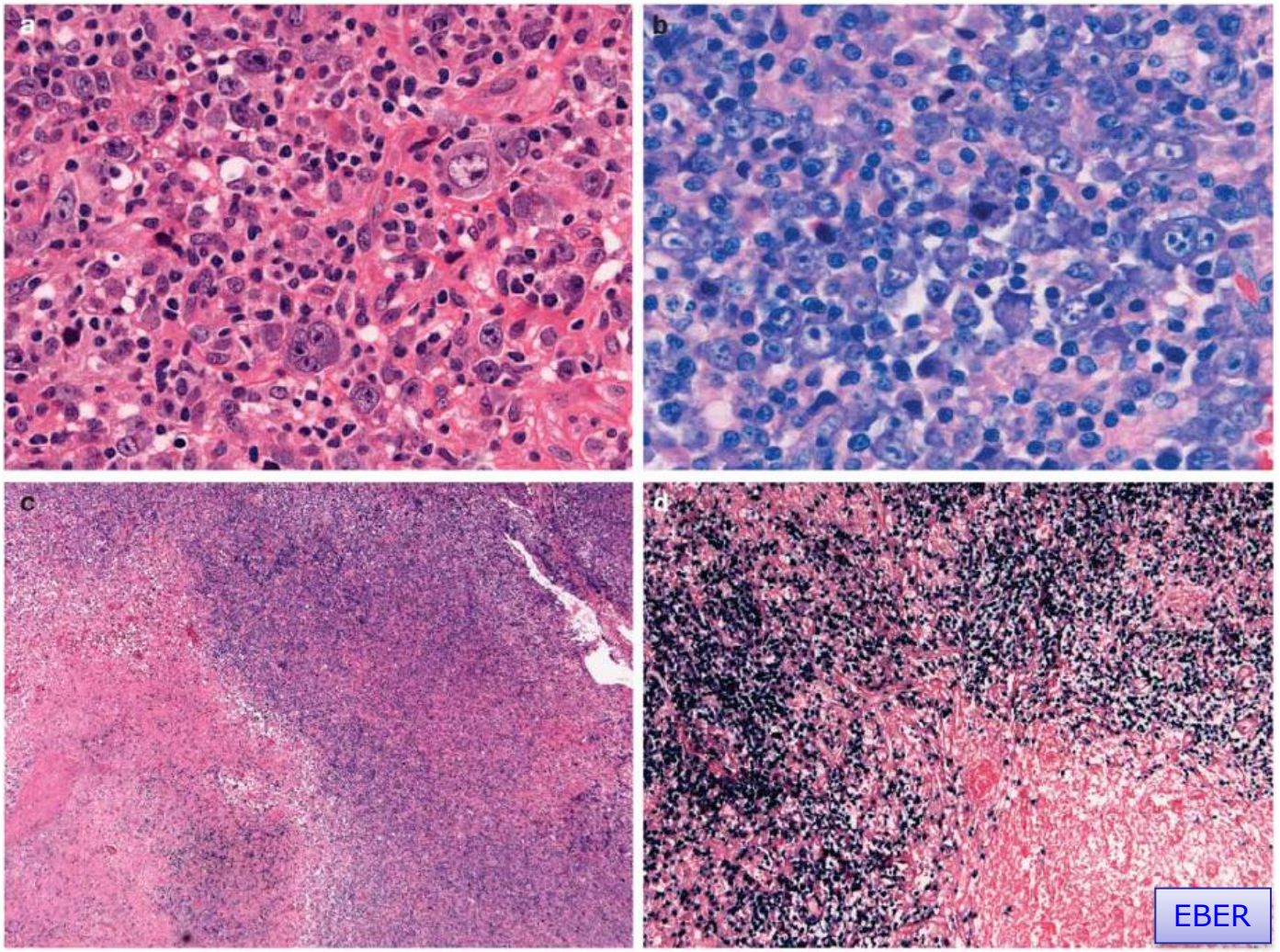
- New variant: *Burkitt-like lymphoma with 11q aberrations*



- Found in Burkitt lymphoma in the Post-transplant setting
- More complex karyotypes
- Nodal presentation
- <40 years at presentation
- *ETS1* or *FLI1* are involved

Ferreiro JF et al, Haematologica 2015;100:e275
Salaverria et al, Blood 2014; 123:1187
Pienkowska-Grela et al, Med Oncol 2011;28:1589

EBV+DLBCL , NOS



Hofscheier A et al, Mod Pathol 2011

EBV-positive large B-cell lymphomas in young patients: a nodal lymphoma with evidence for a tolerogenic immune environment

Alina Nicolae,[1] Stefania Pittaluga,[1] Shahed Abdullah,[1] Seth M. Steinberg,[2] Thu Anh Pham,[3] Theresa Davies-Hill,[1] Liqiang Xi,[3] Mark Raffeld,[3] and Elaine S. Jaffe[1]

Key Points

- EBV$^+$ LBCLs in young patients resemble those seen in the elderly, but usually have a good outcome.
- Tumor cells exhibit PD-L1 expression, with high indoleamine 2,3-dioxygenase–positive cell content, indications of a tolerogenic immune state.

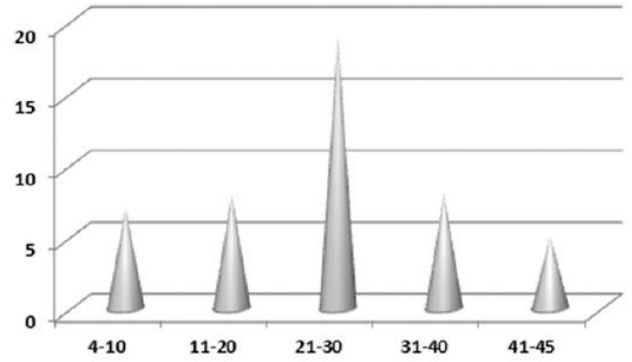
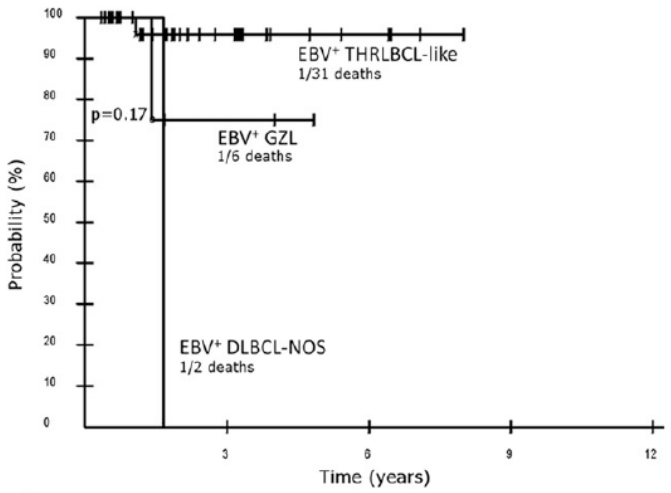
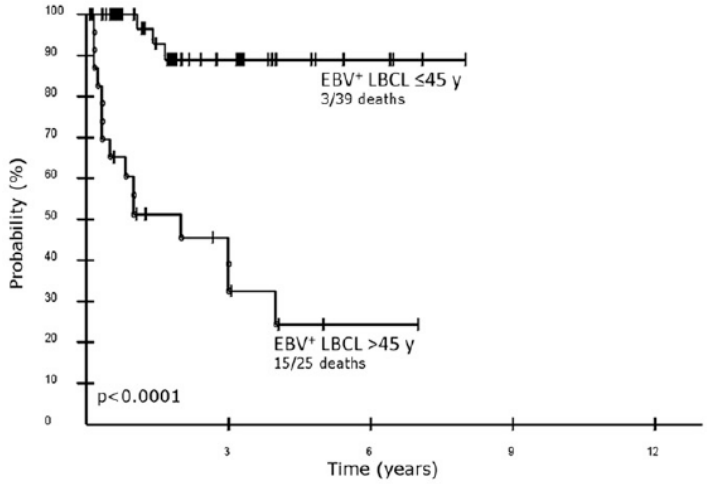


Figure 4. Distribution of EBV$^+$ LBCLs per age group. The age distribution followed a Gaussian curve, with a peak in the third decade of life (n = 19).





EBV Positive Mucocutaneous Ulcer—A Study of 26 Cases Associated With Various Sources of Immunosuppression

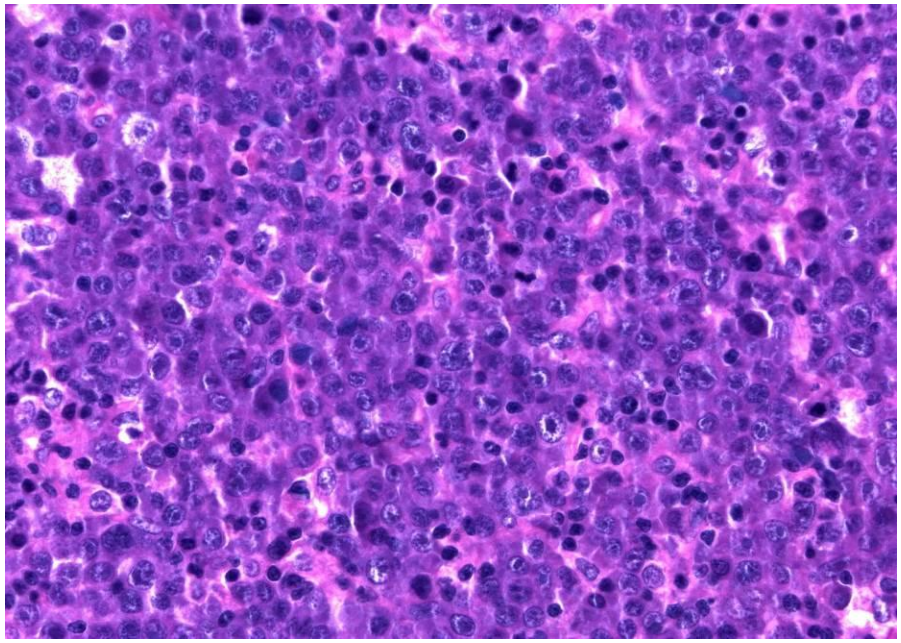
Stefan D. Dojcinov, MD, FRCPath, Girish Venkataraman, MD,† Mark Raffeld, MD,† Stefania Pittaluga, MD, PhD,† and Elaine S. Jaffe, MD†*

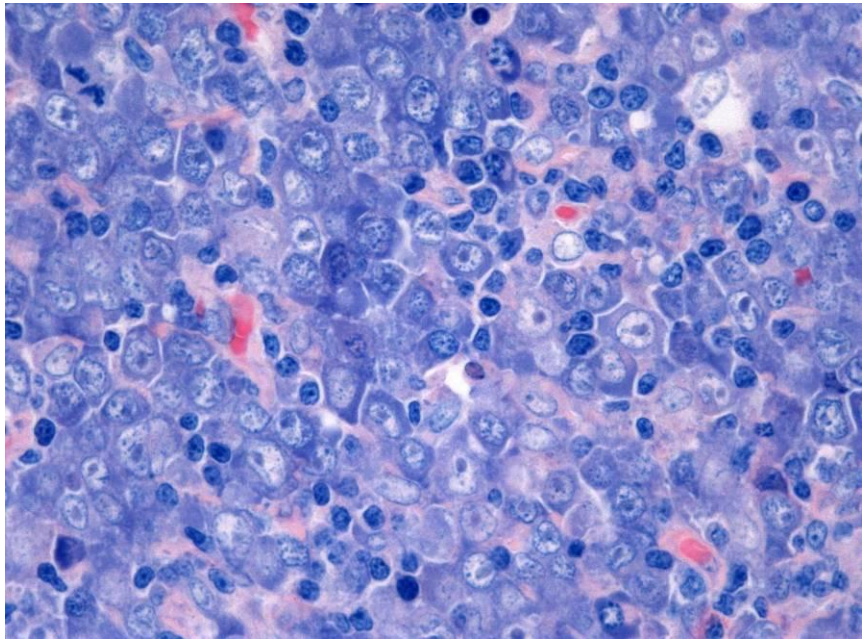
- EBV+ circumscribed ulcerative lesions
  - Immunosuppression associated
    - Azathioprine
    - Methotrexate
    - Cyclosporin-a
  - Age-related immunosenescence
- Clinical presentation: Oropharyngeal mucosa, skin or gastrointestinal tract
- Morphology: Resembles CHL
- Prognosis: excellent. Some cases regressed spontaneously

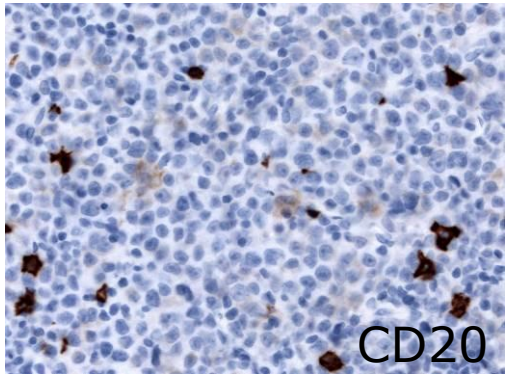
DLBCL with plasma cell phenotype

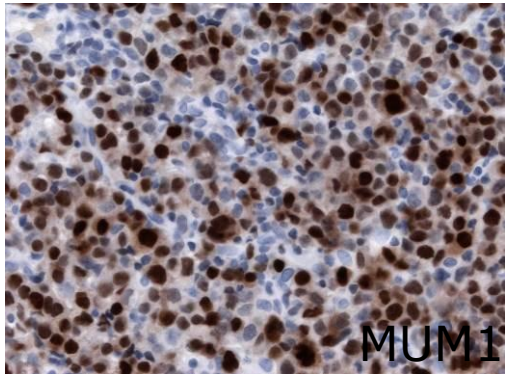
- ALK-positive DLBCL
  - Predominates in male, adults
  - The cells have plasmablastic morphology and phenotype
  - The t(2;17) involving *ALK* and clathrin
  - CD30 is negative
- Plasmablastic lymphoma (PBL)
  - Aggressive neoplasm
  - Immunodeficiency, HIV
  - EBV associated
  - *MYC* translocation
- Primary effusion lymphoma (PEL)
  - Young males with HIV infection
  - Human Herpes virus 8 (HHV-8) Kaposi sarcoma herpes virus
  - Usually co-infection with EBV

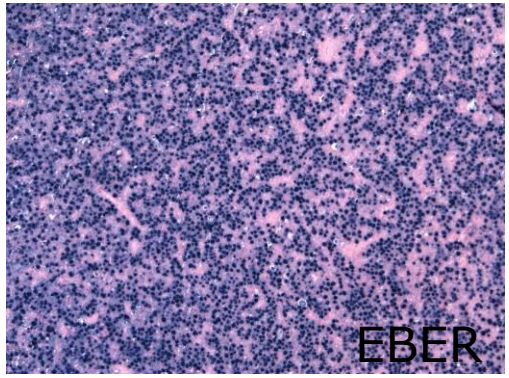
Plasmablastic lymphoma

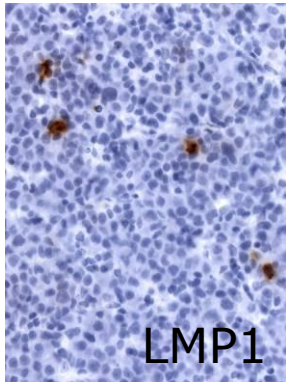












Primary effusion lymphoma

- Definition:

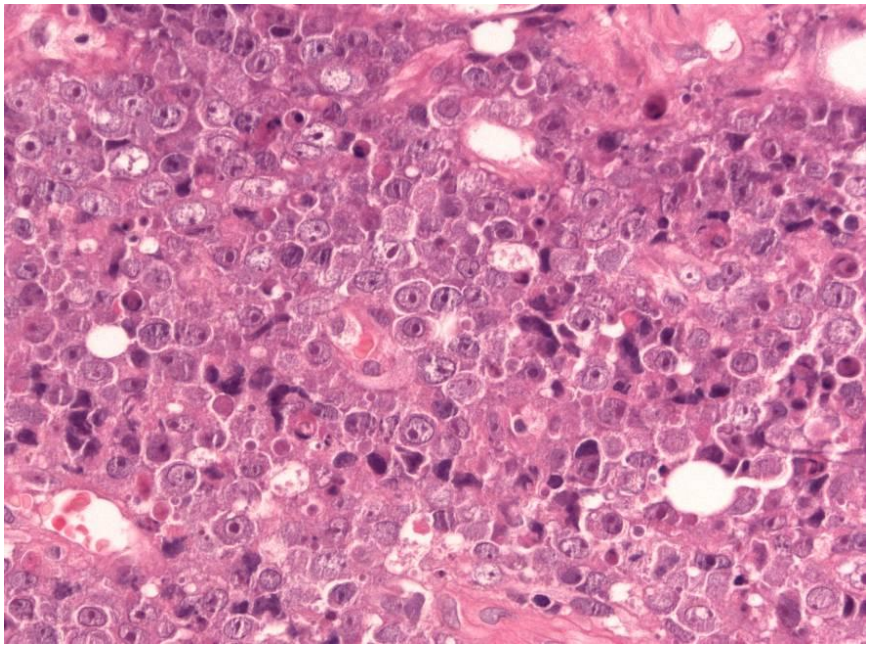
- PEL is a large cell lymphoma usually presenting as serous effusions without detectable mass.

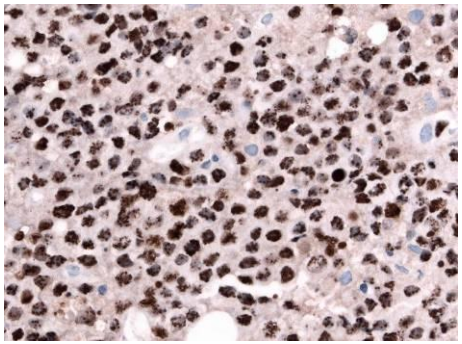
- It is universally associated with HHV8.

- It presents in immunodeficiency patients, mainly HIV+.

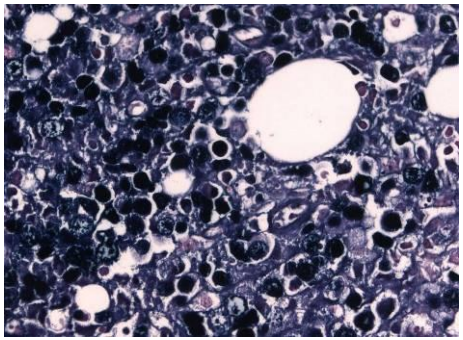
- Usually coinfected with EBV

WHO 2008, Said J and Cesarman E





HHV8 (LANA)



EBER

Conclusions: news in aggressive lymphomas

- WHO 4th Edition
- DLBCL
  - Subtypes required
    - T cell/histiocyte rich large B-cell lymphoma
    - Primary CNS DLBCL
    - Primary cutaneous DLBCL („Leg type“)
    - *EBV+DLBCL of the elderly*
- Burkitt lymphoma
- *BCLU*

- Update 2016
- DLBCL
  - Subtypes required
    - GCB vs ABC
    - T cell/histiocyte rich large B-cell lymphoma
    - Primary CNS DLBCL
    - Primary cutaneous DLBCL („leg type“)
    - EBV+ DLBCL, NOS
    - *EBV+ mucocutaneous ulcer**
- Burkitt lymphoma
- *Burkitt-like lymphoma with 11 q aberrations**
- High-grade B-cell lymphoma with DH/TH*
- High-grade lymphoma, nos*

Prognostic factors
- Immunophenotypic: MYC/BCL2 IHC
- Genetic: *MYC, BCL2, BCL6 rearrangements*

Conclusions

- The diagnosis of DLBCL needs to integrate, standard morphology, IHC, molecular techniques
  - Distincticton between GCB and ABC is required
    - RNA technology might have a role in the near future
    - IHC algorithms (Hans, Tally, etc)
  - IHC for MYC/BCL2 is required
  - FISH analysis if possible in all GCB-type, if not follow two-step approach

- Mutation analysis and targeted therapy will certainly influence the diagnosis and treatment of DLBCL in the near future
    - BTKi (Ibrutinib) or Bortezomib for relapsed ABC-DLBCL
    - EZH2i or BCL6i for relapsed GCB-DLBCL

➢ Stay tuned for **DLBCL with single *MYC*** rearrangements and

➢ **Other modulators of prognosis in** DH lymphomas and the **role of specific somatic mutations**