Lymphome B à grandes cellules: de la biologie à la clinique

SOCIETE ALGERIENNE
d'Hematologie et de Transfusion Sanguine

XIIIème congrès maghrébin d'Hématologie,
26-28 Mai 2016, Alger

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Lymphomes B à grandes cellules: vers une révolution…

La révolution de 1974: Les lymphomes sont des tumeurs des cell. Du système immunitaire

1990-…: L'ère du moléculaire…

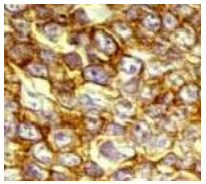
OMS 2016

Translocations

Epigenomics, miRNA

GEP
ABC / GCB..

NGS
mutations

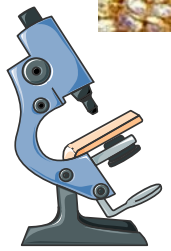


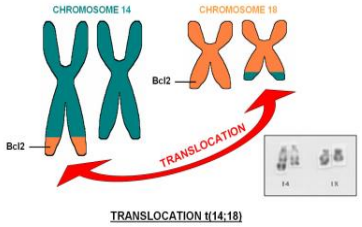
1985-1995

2000

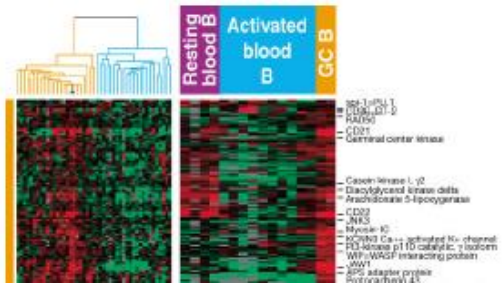
2009

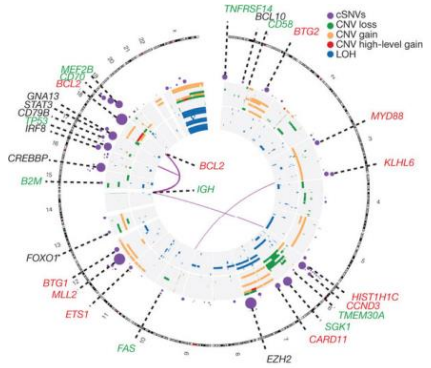
IgH- BCL2/BCL6/MYC



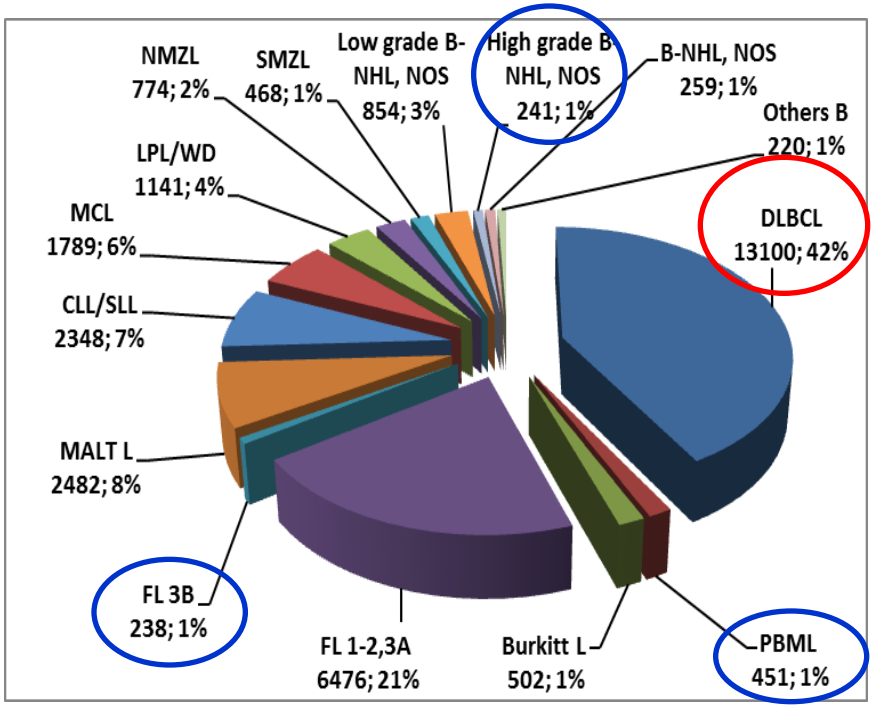


TRANSLOCATION t(14;18)





Lymphomes B à grandes cellules

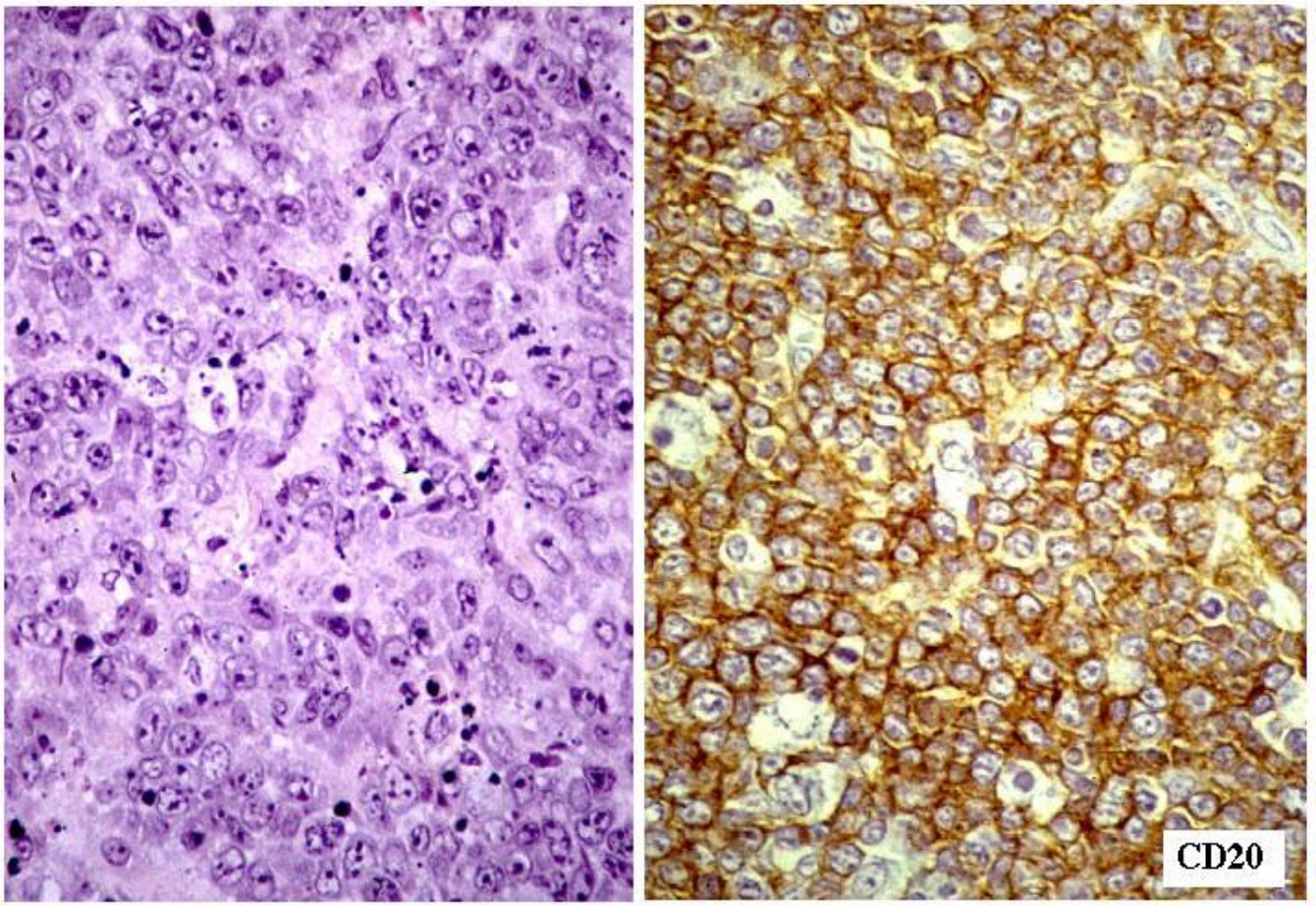


31343 lymphomes B

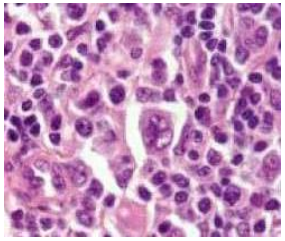
- 30-40 % des lymphomes de l'adulte
- 4 à 5000 nouveaux cas/an en France
- Grande hétérogénéité morphologique, clinique et biologique
- Evolution spontanée «agressive»

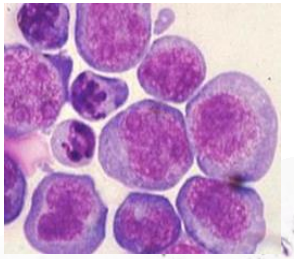
Source Lymphopath (2010-13)

Un diagnostic facile

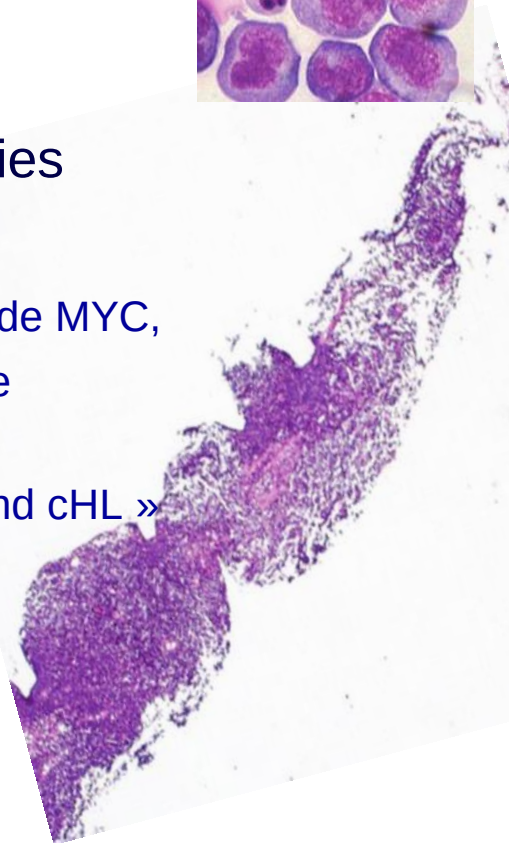


Avec cependant quelques pièges ….





- DLBCL ne ressemblant pas à des DLBCL (HTR-DLBCL)
- DLBCL n'exprimant pas CD20
  - Lymphome plasmoblastique,
  - Lymphomes des séreuses,…
- DLBCL ayant des aspects en commun avec d'autres lymphomes, le plus souvent reflet de processus ou voies oncogéniques partagés :
  - « High grade B-cell lymphomas » avec ou sans réarrangements de MYC, BCL2 et/ou BCL6 (Ex: « B-cell lymphoma with features intermediate between DLBCL and Burkitt (2008) »)
  - « B-cell lymphoma with features intermediate between DLBCL and cHL »
- **Prélèvement inadéquate (biopsies à l'aiguille!)**

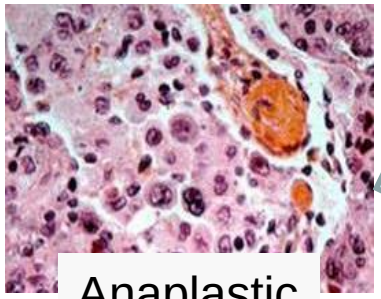


Update in the WHO lymphoma classification

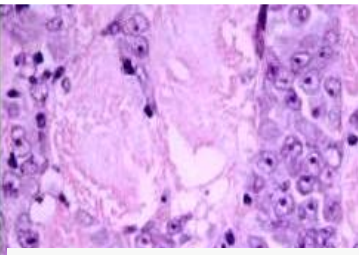
2014 WHO CAC Meeting, Chicago

Diffuse large B-cell lymphoma (DLBCL)

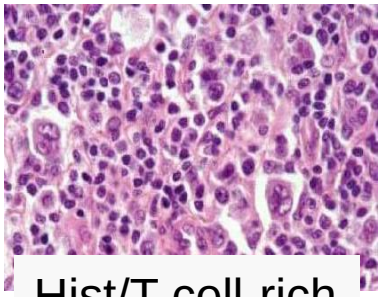
Centroblastic



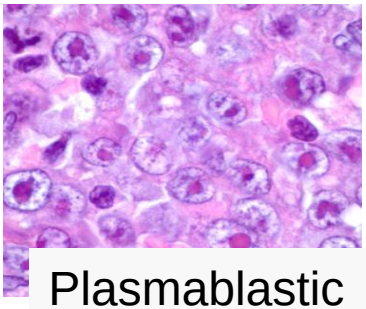
Anaplastic



Immunoblastic



Hist/T cell-rich



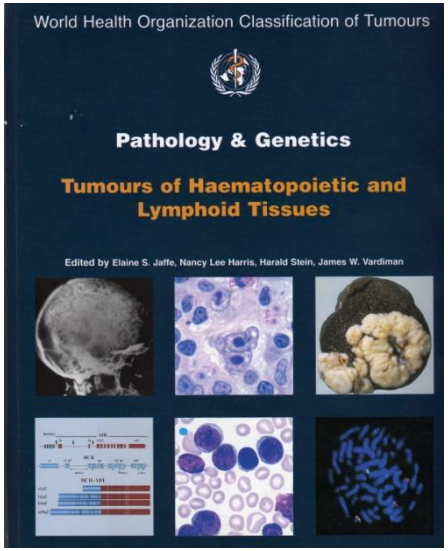
Plasmablastic

DLBCL

- Distinct entities
- Several molecular subtypes
- Prognostic /predictive biomarkers

An heterogeneous group of diseases

DLBCL WHO Classification (2008)



Diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS)

Germinal-centre B-cell-like (GCB)

Activated B-cell-like (ABC)

DLBCL subtypes

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

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

Epstein-Barr virus–positive DLBCL, NOS ~~of the elderly~~

EBV+ mucocutaneous ulcer

Primary mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

ALK-positive DLBCL

Plasmablastic lymphoma

Primary effusion lymphoma

HHV8-positive, DLBCL, NOS

B-cell lymphoma, with features intermediate between DLBCL and classical Hodgkin lymphoma

~~B-cell lymphoma, with features intermediate between DLBCL and Burkitt lymphoma~~

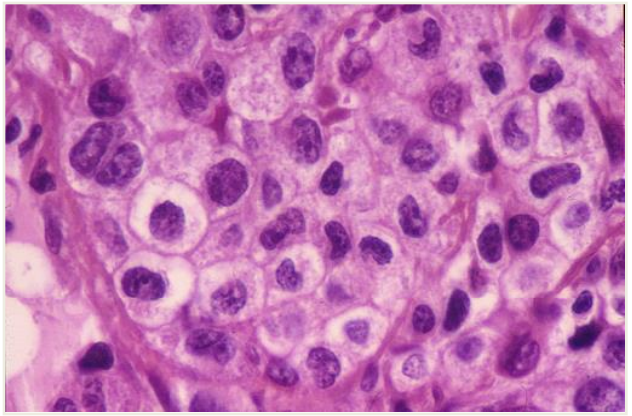
High grade B-cell lymphoma,

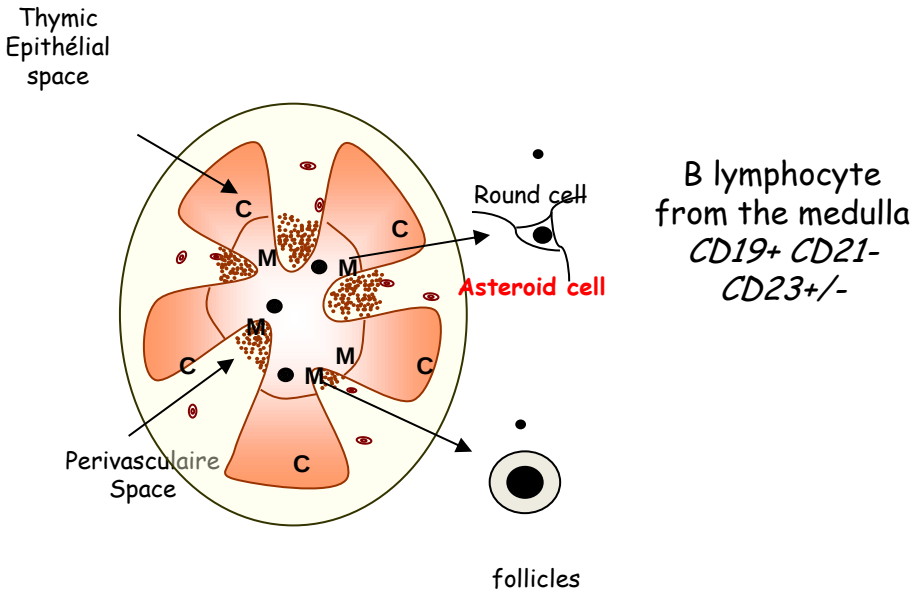
With MYC and BCL2 and/or BCL6 rearrangements

NOS

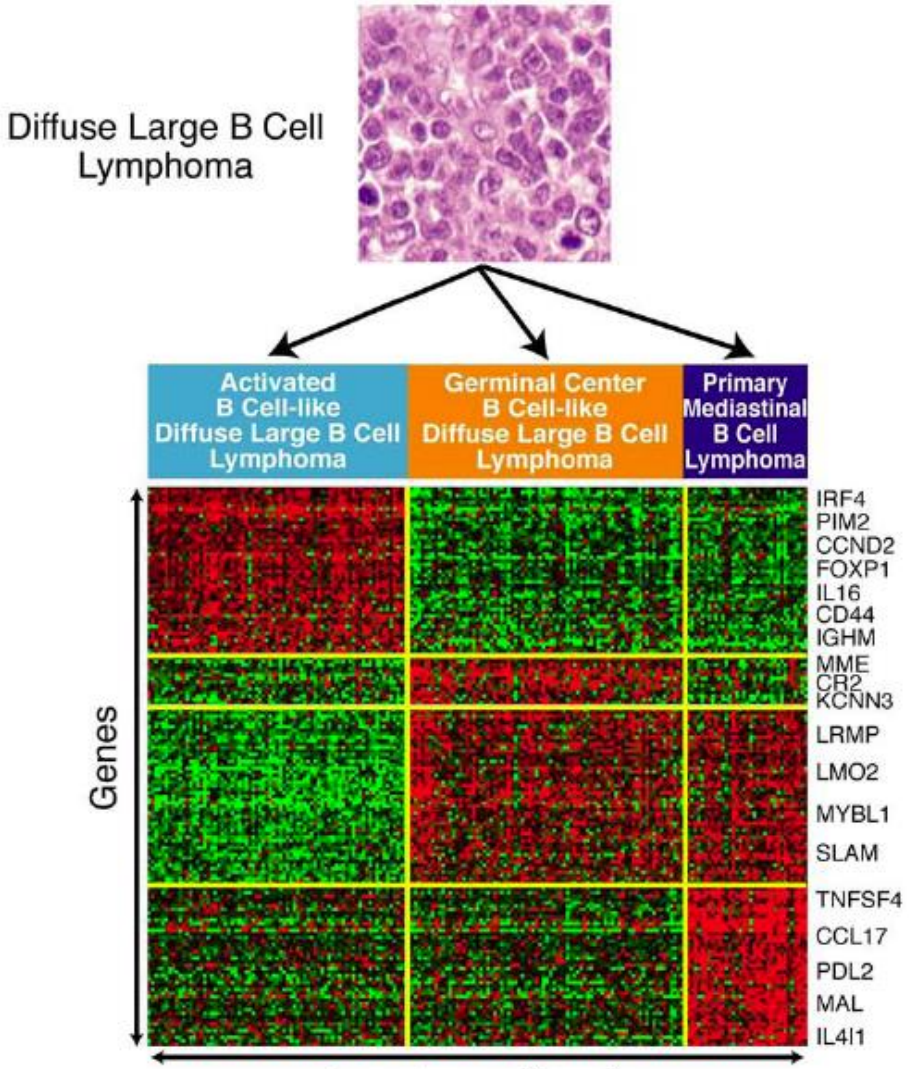
Lymphome B Primif du médiastin (thymique) (PMBL): une entité distincte

- Adultes jeunes (F>H), volumineuse masse mediastinale
- Grandes cell B (claires), fibrose
- **CD23+, CD30+, MAL+**, s/c Ig
- Origine : lymphocyte B thymique
- Signature moléculaire distincte (*MAL, IL4I1*)
- Gains **9p24** (*JAK2/PDL2/PDL1* locus), 2p,12q, réarrangements ***CIITA* (35%), mutations *PTPN1***
- Absence fréquente de MHC class I & II → « *immune privilege* »?



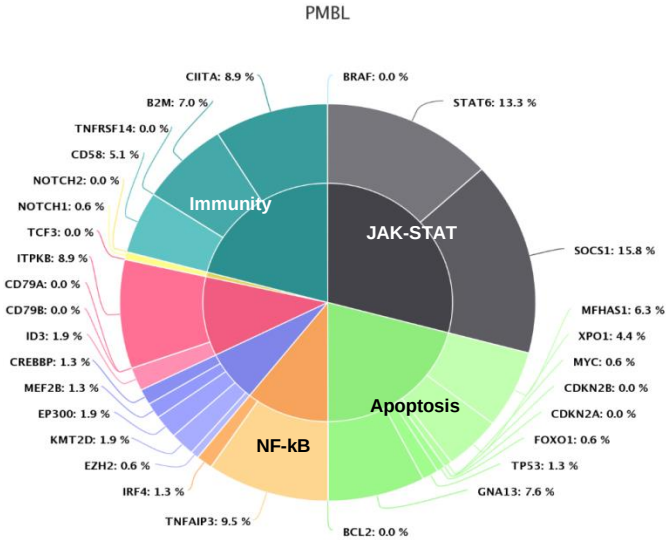


Lymphome B Primif du médiastin (thymique) (PMBL): une entité moléculaire distincte



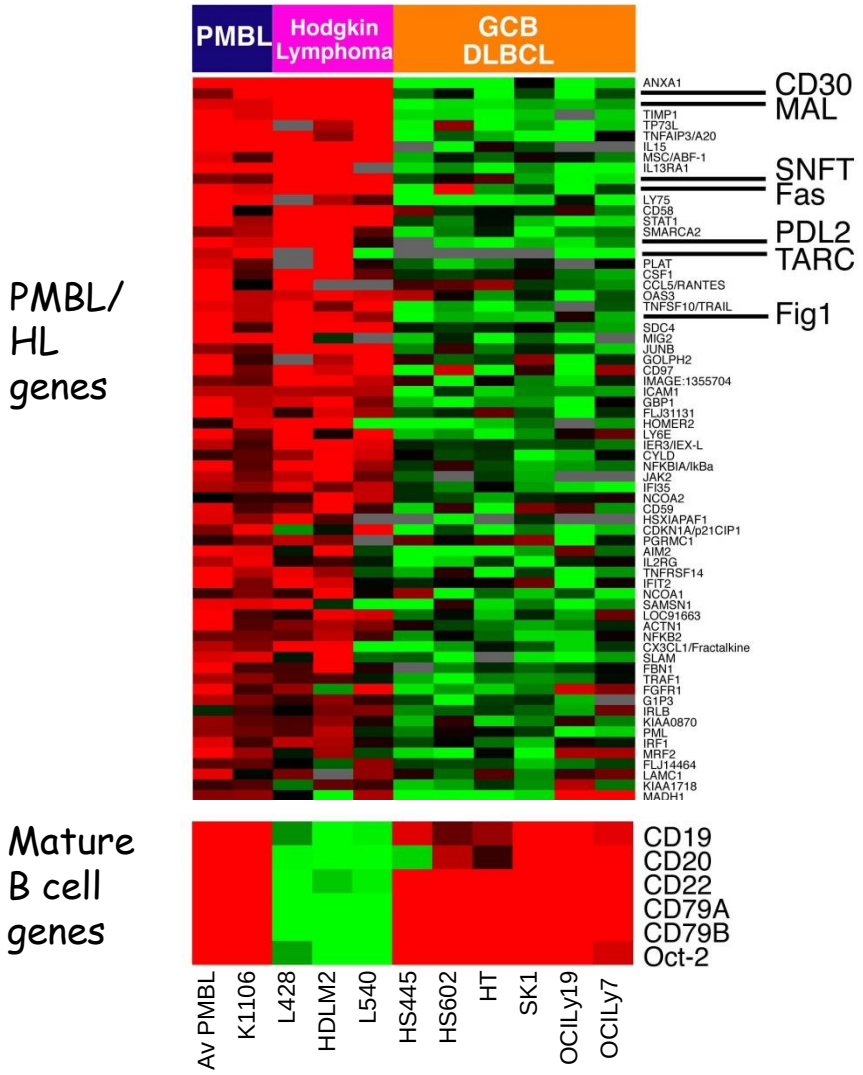
A Rosenwald et al. J Exp Med, 2003

- **Signature moléculaire distincte des DLBCL ABC ou GCB** (*MAL, CD23, IL4I1, JAK2,..*)
- **Activation constitutive des voies NF-kB et JAK-STAT (STAT6-P) activation**
  -Mutations/deletions of SOCS1
  (Melzner et al Blood, 2005; Weniger et al Oncogene 2005)
  - Mutations of STAT6
  (*Ritz , Guiter et al. Blood 2009)*
- **Paysage mutationnel distinct**

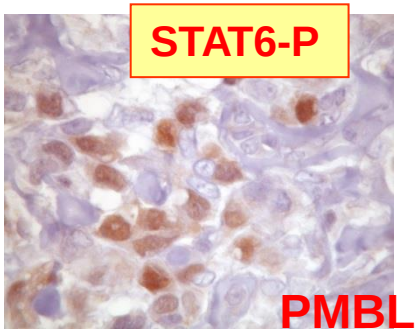


S Dubois, F Jardin. submitted

Extensive gene expression overlap between PMBL and cHL lines … but PMBL keep the expression of mature B-cell genes



Common pathogenic pathways in PMBL and in cHL :
NF-kB & STAT6 are activated in both lymphomas

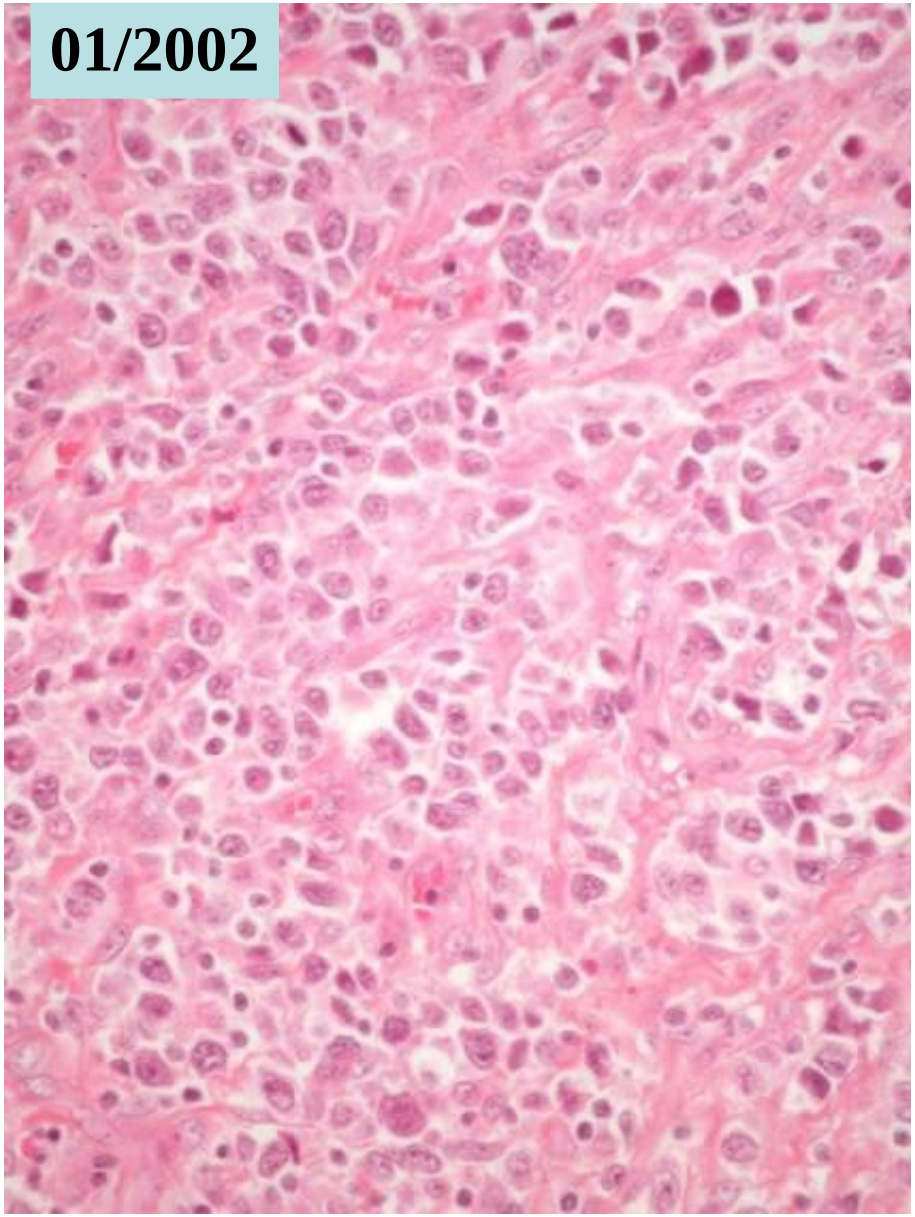


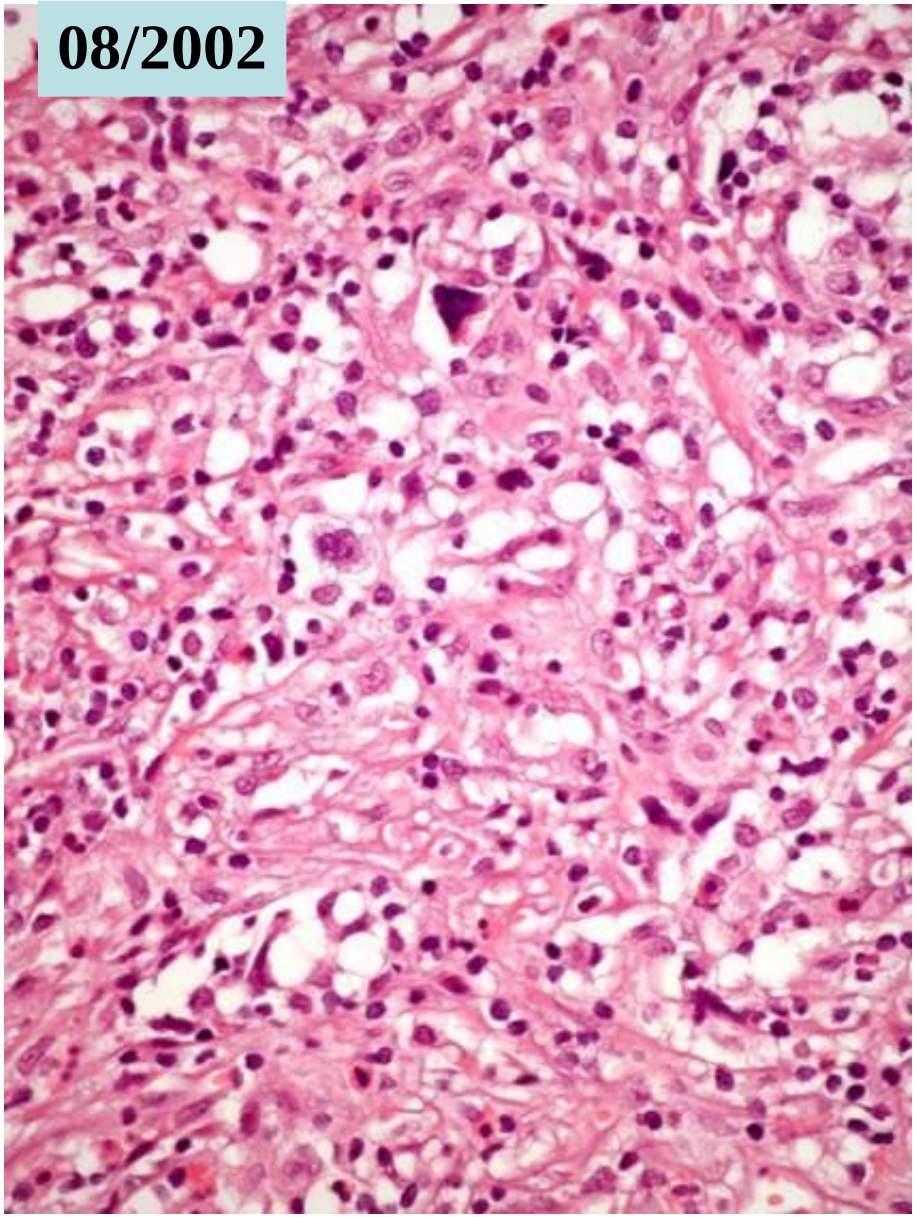
A Rosenwald et al. J Exp Med, 2003

Ritz, Guiter et al. Blood 2009

Guiter et al. Blood 2004

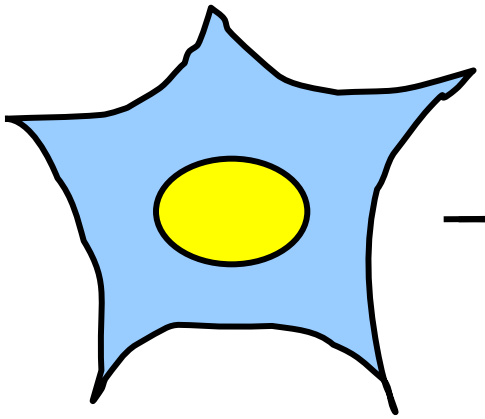
A 34 year-old woman, mediastinal mass





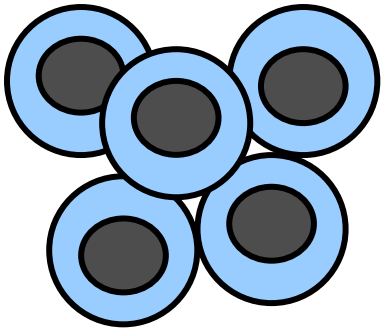
- « Mediastinal gray zone » lymphoma
- Mediastinal sequential cases
- Mediastinal composite cases

→ the missing link between NS-cHL and PMBL?



Thymic B cell

?



PMBL

B cell lymphoma with features intermediate between PMBL and cHL *(WHO 2008)*

cHL,ns

PMBL and cHL(NS) as related tumors

PMBL

Mediastinal presentation
Young patients (women)
Fibrosis
Response to Rxtherapy
Gains in chr 9p, 2p
Ig –
CD30+/- (w, het)
Activation of NF-kB pathway
STAT6 activation (mutations)
MAL +
Rearrangement CIITA (15%)
PDL1-PDL2 expression

Clear cells
No polymorphous infiltrate
CD20+, CD79a+
Oct-2+, BOB.1+, PU.1+
CD15-, EBV-

HL (NS)

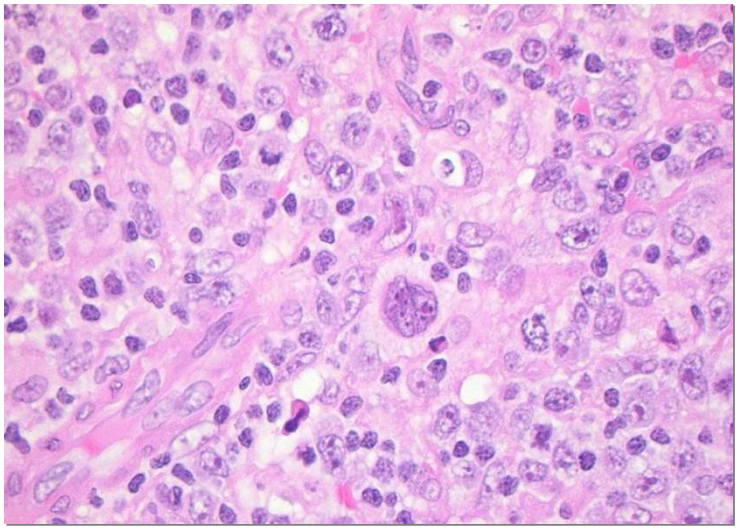
Mediastinal presentation
Young patients
Fibrosis
Response to Rxtherapy
Gains in chr 9p, 2p
Ig –
CD30+
Activation of NF-kB pathway
STAT6 activation (mutations?)
MAL (+/-)
Rearrangement CIITA (38%)
PDL1-PDL2 expression

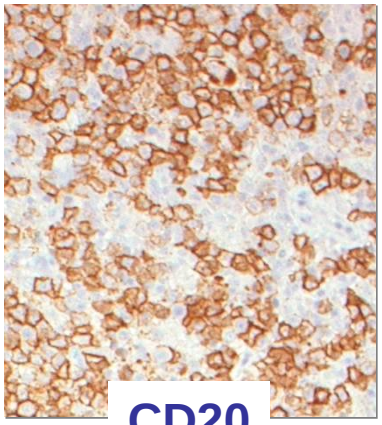
R-S cells
Polymorphous infiltrate
CD20-, CD79a-
Oct-2-, BOB.1-, PU.1-
CD15+, EBV +/-

Lymphome B à grandes cellules, NOS, EBV + (OMS 2016)

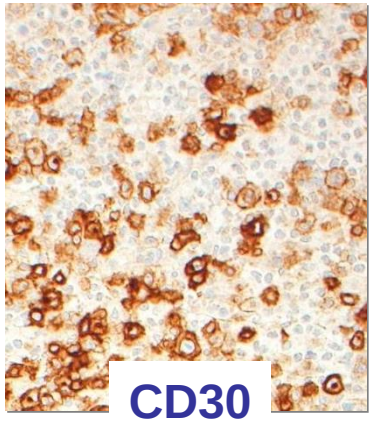
- **Prédilection sujet âgé (OMS 2008), mais possible à tt âge**
- à distinguer d'entités EBV+ spécifiques (type GL)
- Absence d'immunodépression connue
- Prolifération B clonale EBV+ > 50 ans, de novo
- aspect DLBCL , svt pléomorphe +/- cell. RS-like
- Souvent extra-ganglionnaire (70%)
- CD20+, CD30+, non GC/ABC
- EBV+: latence I ou II (LMP1+, EBNA2+)
- Pc initialement sombre (OS à 5 ans, 20%)

- !!! *Une entité particulière:* ***«Ulcère cutaneo-muqueux EBV+ »***



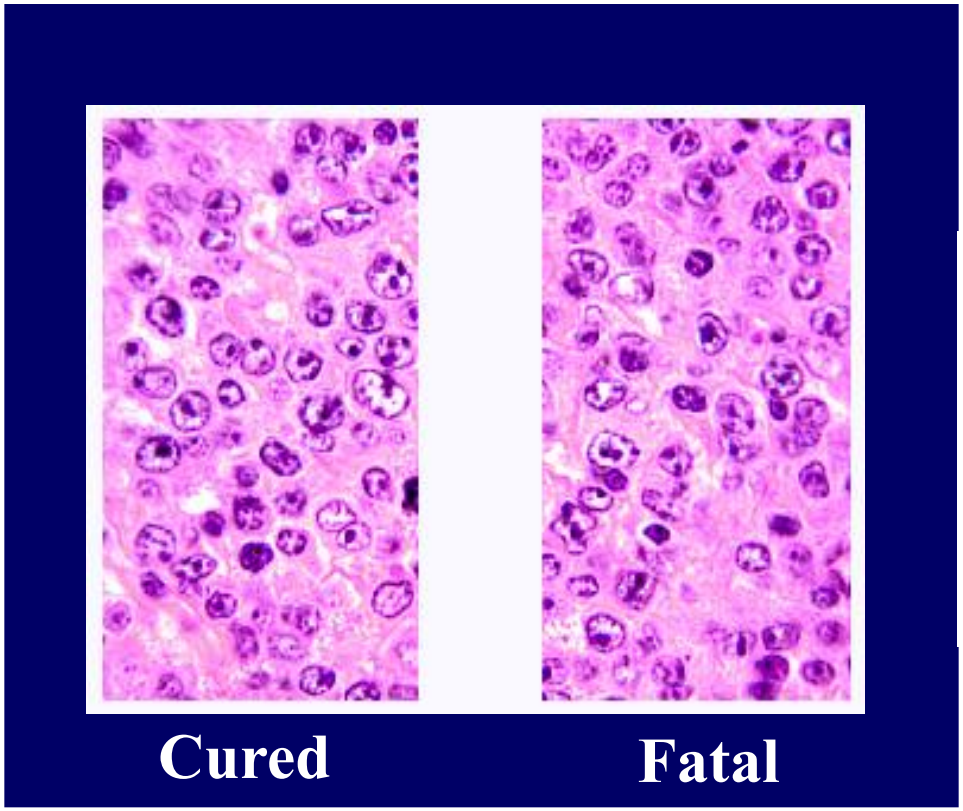


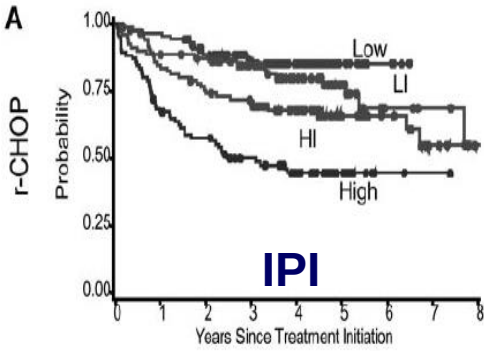
CD20



CD30

Besoin d'identifier les malades qui vont être réfractaires à R-chemo ou avoir une évolution fatale

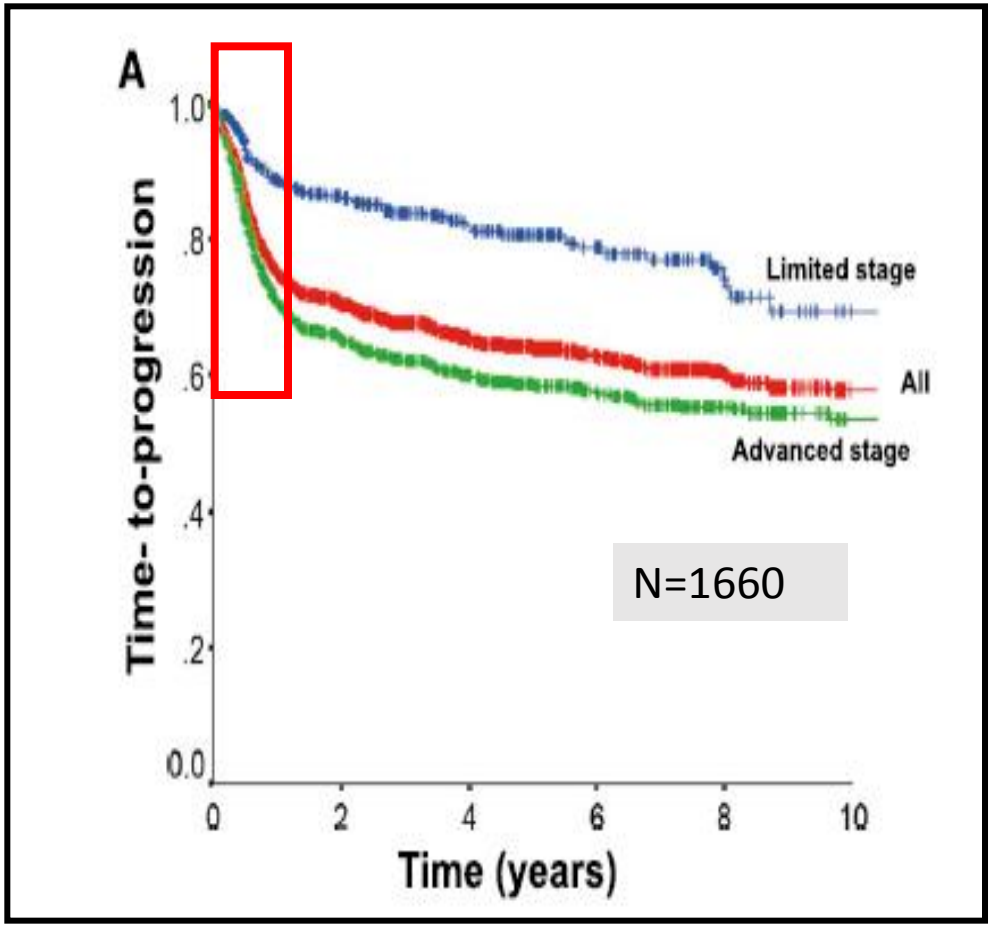


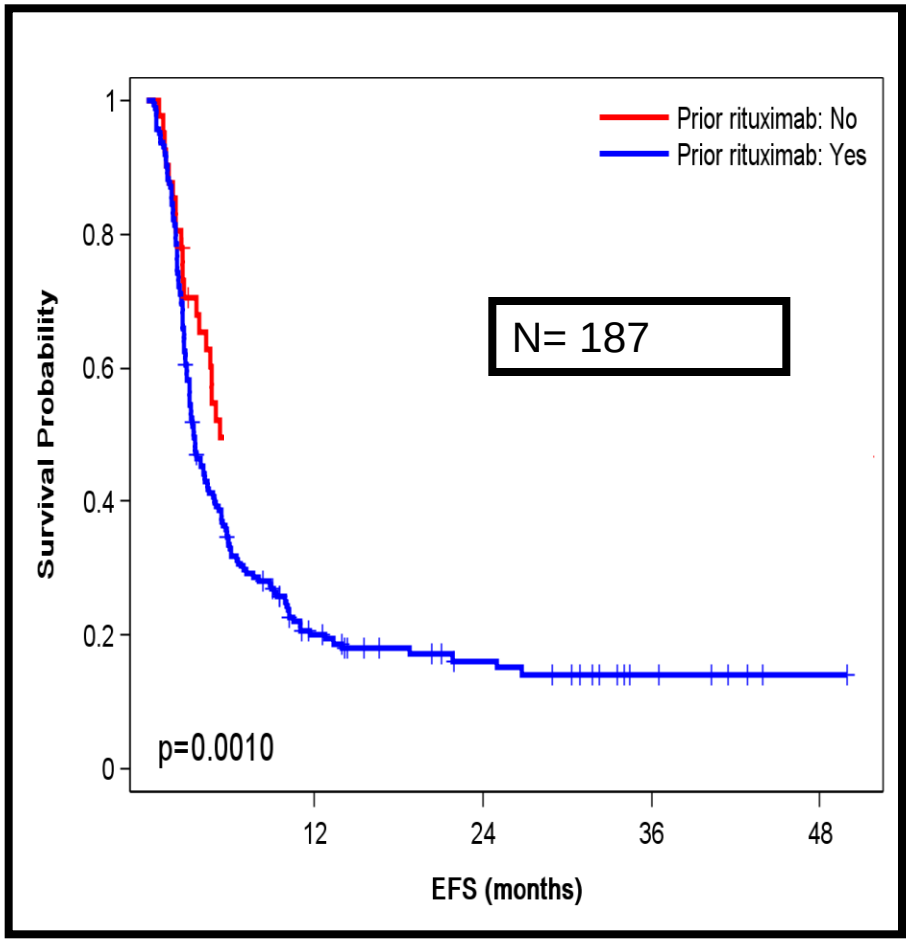


G Salles et al. Blood 2011

- Biomarqueurs pronostiques
- Biomarqueurs prédictifs de réponse au traitement

Même à l'ère du Rituximab, environ un tiers des DLBCL ne sont pas guéris…





AGGRESSIVE B-CELL LYMPHOMAS

Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity

Laurie H. Sehn and Randy D. Gascoyne

Blood 2015

Salvage Regimens With Autologous Transplantation for Relapsed Large B-Cell Lymphoma in the Rituximab Era

Christian Gisselbrecht, Bertram Glass, Nicolas Mounier, Devinder Singh Gill, David C. Linch, Marek Trneny, Andre Bosly, Nicolas Ketterer, Ofer Shpilberg, Hans Hagberg, David Ma, Josette Brière, Craig H. Moskowitz, and Norbert Schmitz

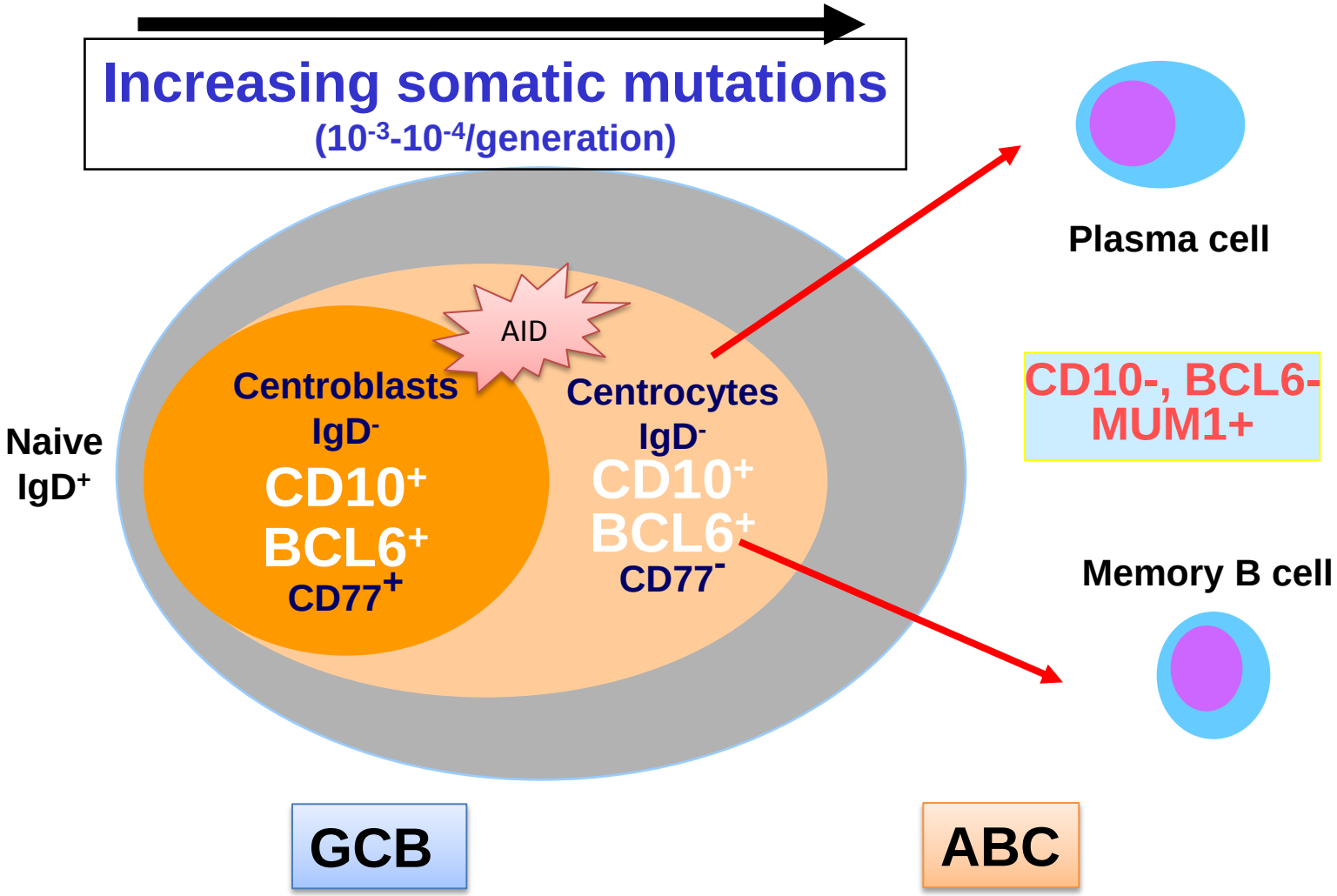
JCO 2010.

Many biological and pathological prognostic factors but only (very) few clinically relevant…!

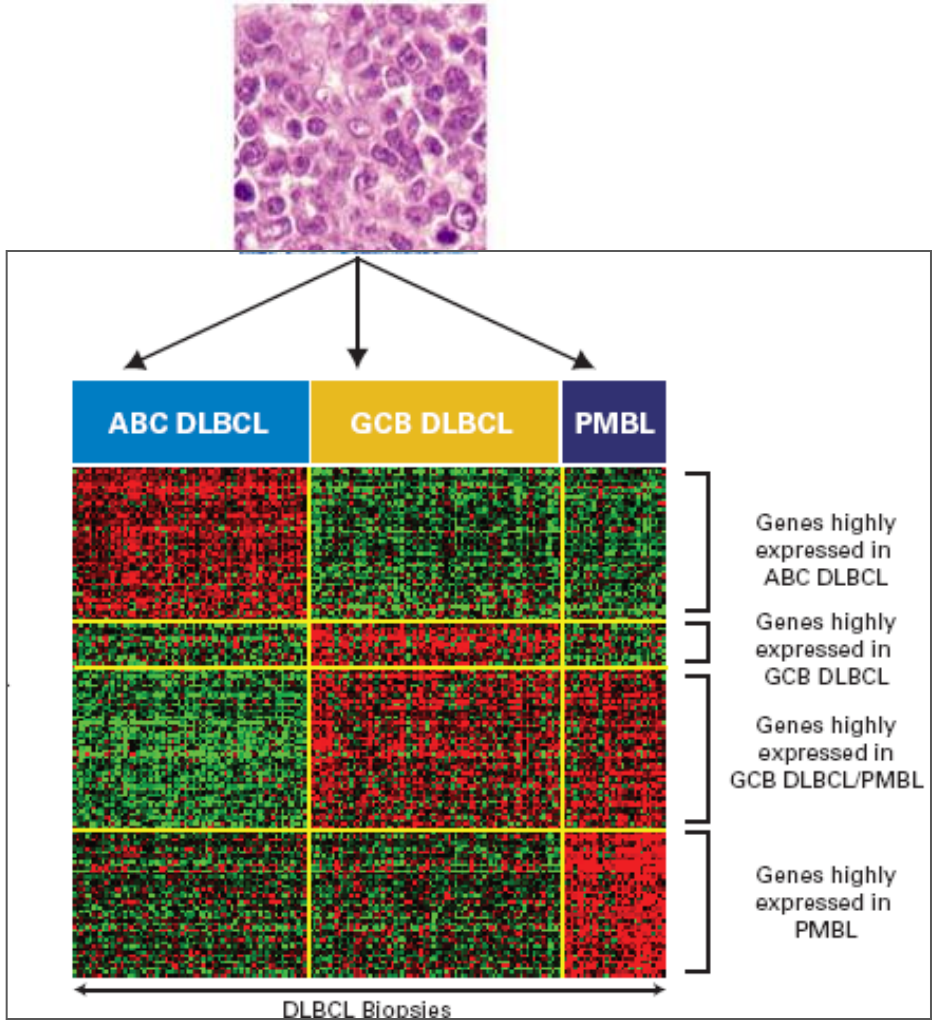
- Immunoblastic subtype
- CD5 expression
- CD30 expression
- BCL2 protein
- MYC protein
- **BCL2-MYC double expressor**
- *BCL6* translocation (~30%)
- *BCL2* translocation (~15-20%)
- ***MYC* translocation (6-9%)**
- **GCB/ABC phenotype (molecular)**
- GCB/non-GCB phenotype (immunohistochemistry)
- ……..

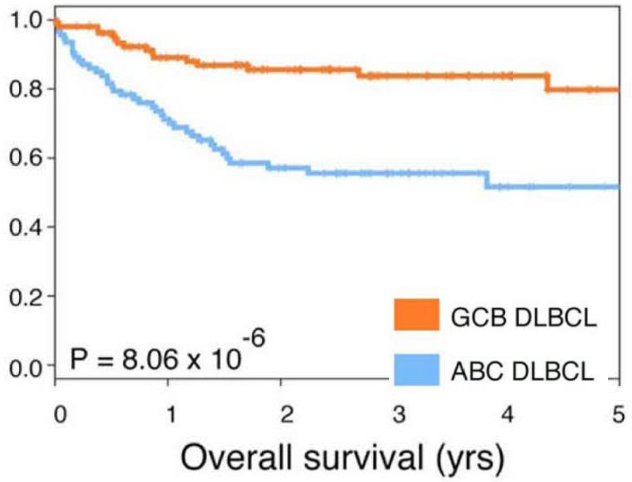
The ressemblance to normal cell stages is a major basis for classification of lymphomas

DLBCL are derived from cells that have migrated to or passed through germinal centers (GC)



DLBCL represents a heterogeneous disease with distinct molecular subtypes





- **Cell of origin**
- **Clinical relevance independently of the IPI**
- **Distinct genetic features & oncogenic pathways**

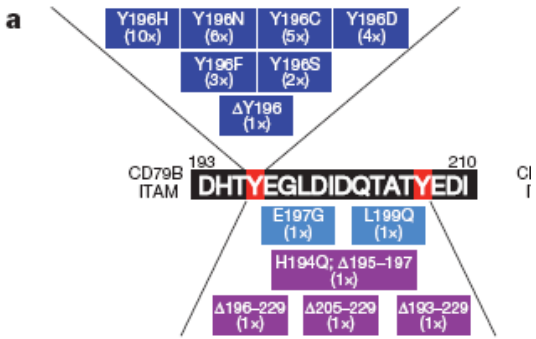
Rosenwald et al. NEJM 2002
Rosenwald et al. J Exp Med 2003
Lenz et al. 2008; H Nohai et al, J Clin Oncol 2011

The 3 DLBCL molecular subtypes show a distinctive pattern of driver mutations & oncogenic pathways

	Germinal center B cell-like (GCB DLBCL)	Activated B cell-like (ABC DLBCL)	Primary Mediastinal B Cell Lymphoma (PMBL)
Cell of Origin	Germinal center B cell	Post-Germinal Center B cell	Thymic B cell
Oncogenic Mechanisms	• PI3K activation • *BCL-2* translocation • *EZH2* mutations • Loss of PTEN • c-rel amplification **"Tonic BCR"**	•NF-kB activation •CRD11 mutations •MYD88 mutations •CD79B mutations •A20 deletions **"Chronic active BCR"**	•Constitutive activation •of STAT6 & NF-kB •CIITA translocation •9p24 amplification •REL amplification
Clinical Outcome	Favorable ~80% 3-yr survival	Poor 45% 5-yr survival	Favorable ~95% 5-yr survival
Potential targets	BCL6 EZH2 PI3K/AKT	**BCR** IRAK4 JAK-STAT	JAK-STAT PD1

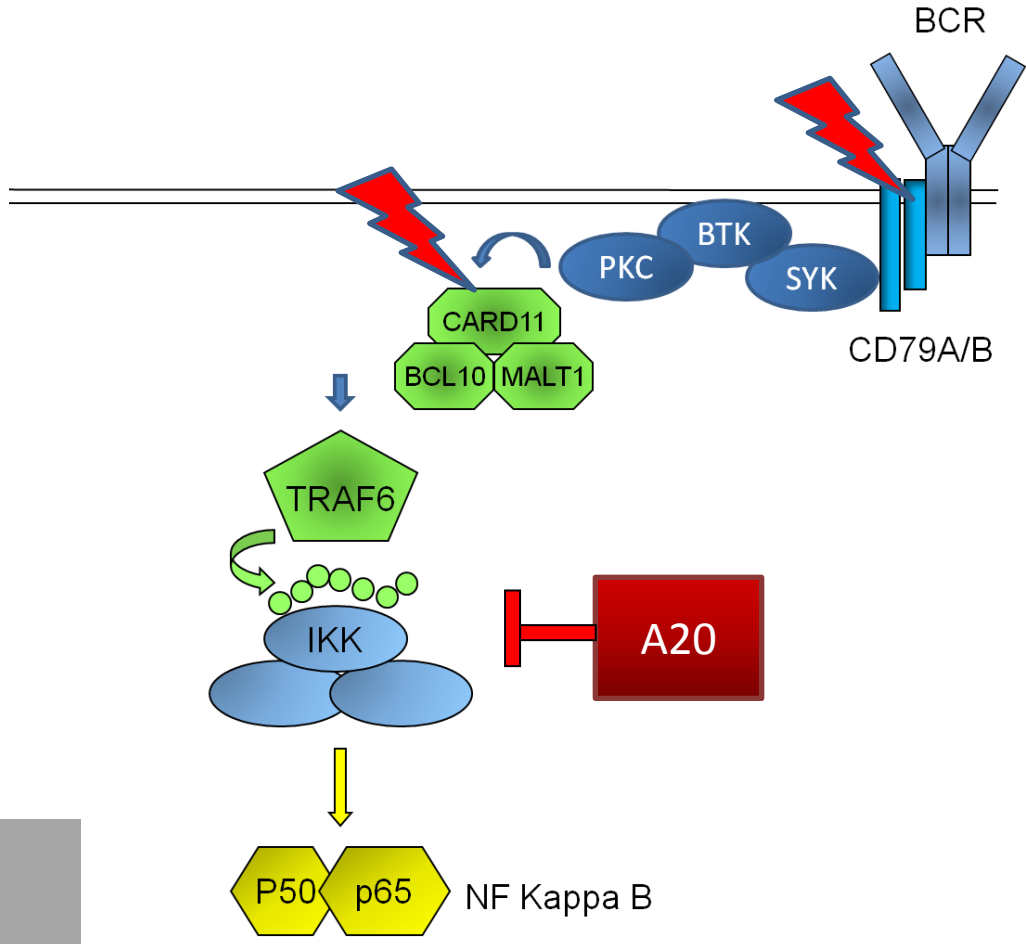
« ABC » DLBCL display a chronic active B-cell receptor signalling with recurrent mutations in genes involved in the BCR signaling pathway

20% mutations *CD79B*
Davis et al, Nature 2010



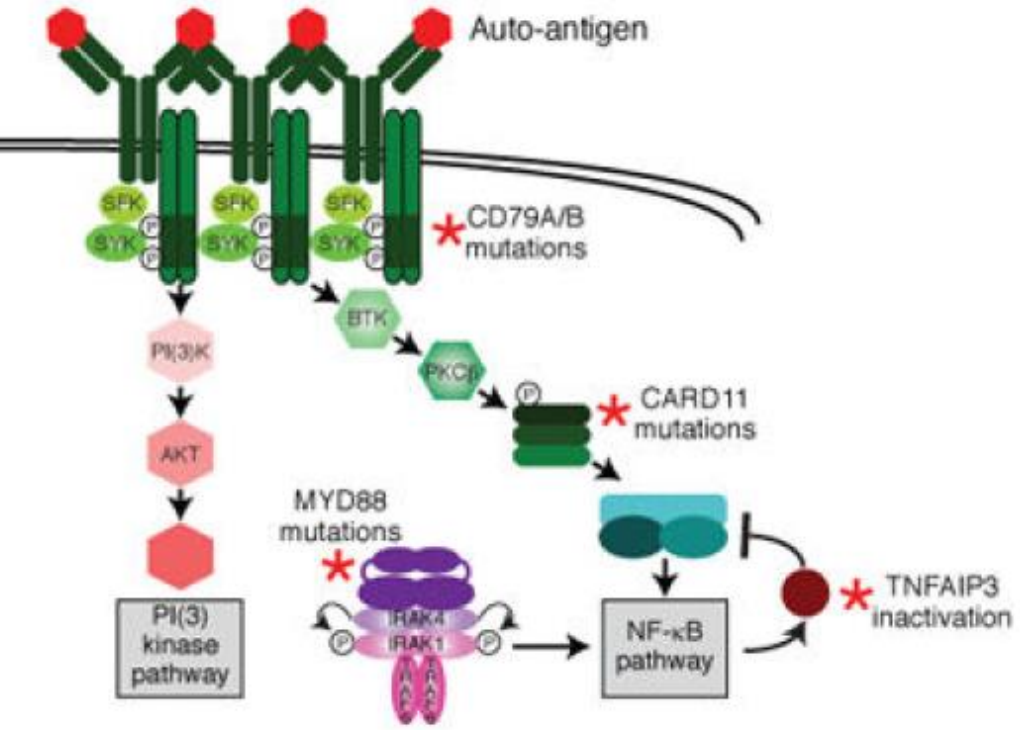
10% mutations *CARD11* (CARMA1)
Lenz et al, Science 2008

30% mutations/deletions *TNFAIP3* (A20)
Compagno et al, Nature 2009

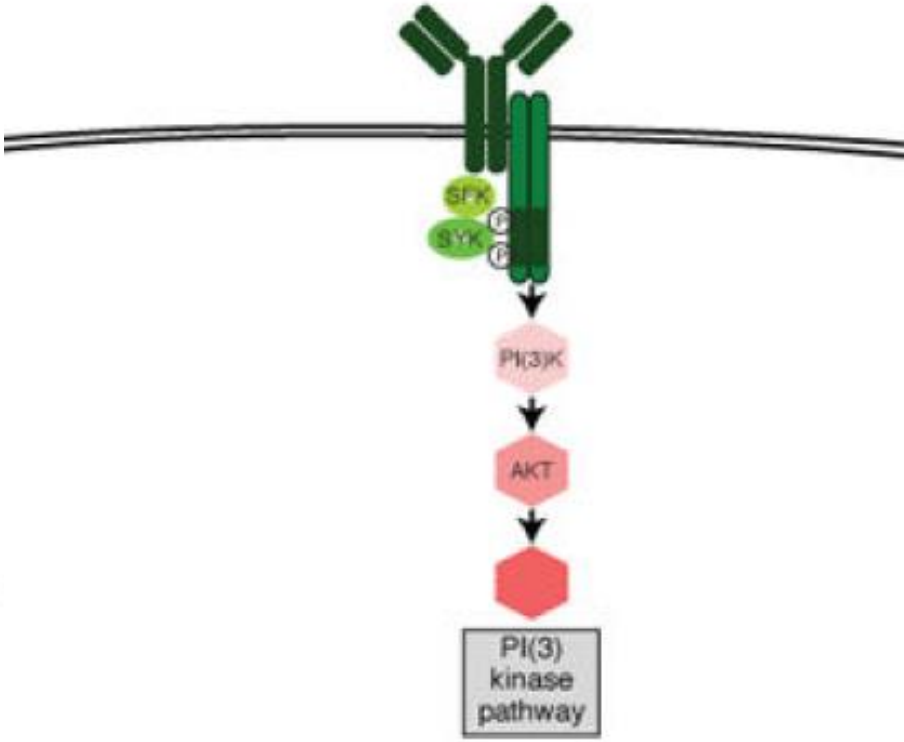


More than 50% ABC DLBCL carry mutations in positive or negative regulators of NFkB (*Compagno et al. Nature 2009*)

Chronic active BCR
ABC DLBCL



Tonic BCR
GCB DLBCL



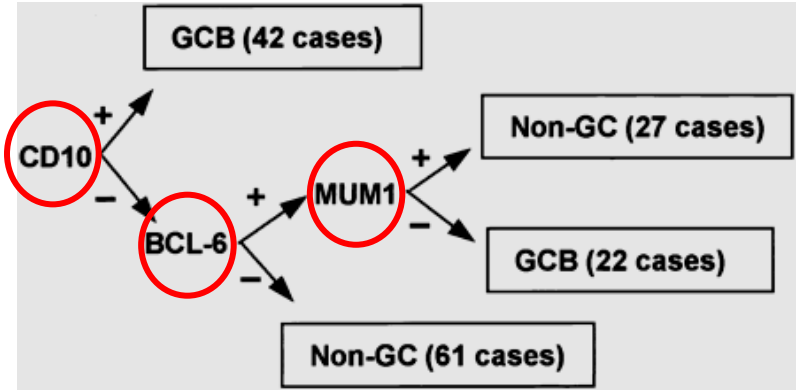
Adapted from Young et al. Sem Hematol 2015

Determination of the cell of origin of DLBCL is highly relevant in clinical practice

- Cell of origin (GCB / non GCB) represents a separation of 2 different disease entities,
- Cell of origin (GCB / non GCB) can predict outcome of DLBCL (R-CHOP) patients
- **Most likely, this parameter will be included in DLBCL future trials**
- **The updated 2016 WHO classification will require the identification of these 2 subtypes**

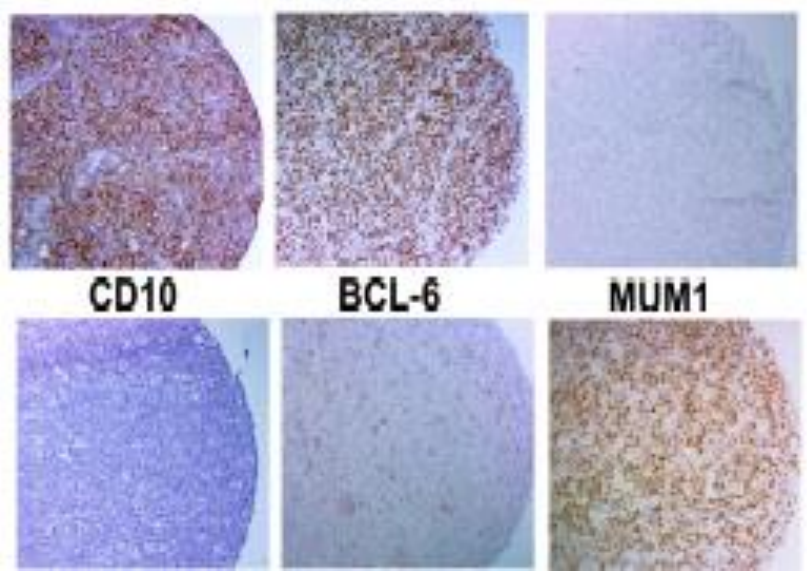
→**Which robust technique in daily practice (FFPE) ?**
→Which optimal biomarker(s), which algorithms?

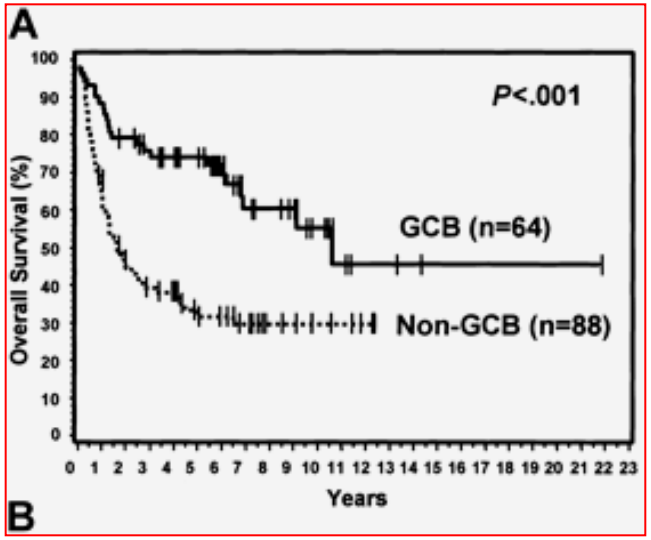
An example: can immunostochemistry be a surrogate for the GC/ABC classification ?



GC

Non-GC

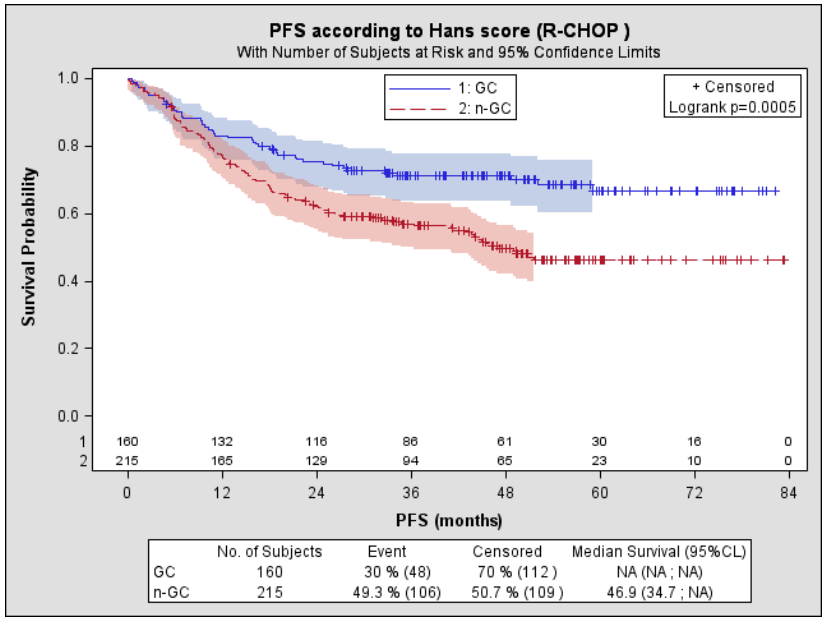


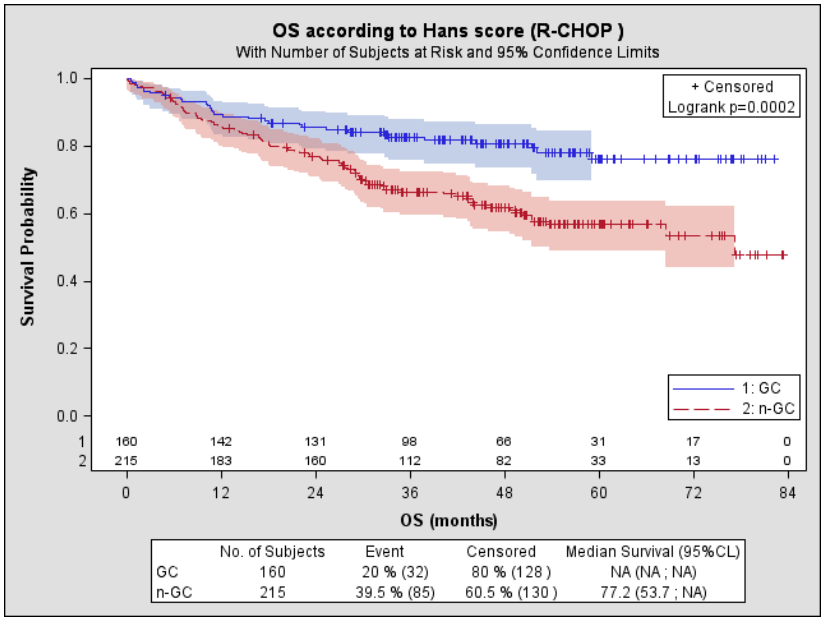


C Hans et al, Blood 2004, 103:275

Hans algorythm predicts survival in phase III clinical trials for R-CHOP treated DLBCL patients

- ***375 de novo*** previously untreated DLBCL patients
- enrolled into the LNH03-2B & LNH03-6B trials
- **R-CHOP**
- TMAs IHC + Affy U132Plus 2.0





- **Hans algorithm correlates with GCB/ABC profile by GEP in 84%**
- **Hans algorythm and BCL2 expression predicts outcome, but not MYC nor MYC-BCL2 double expression**

TJ Molina et al. unpublished

Improved GCB/ABC classifiers…

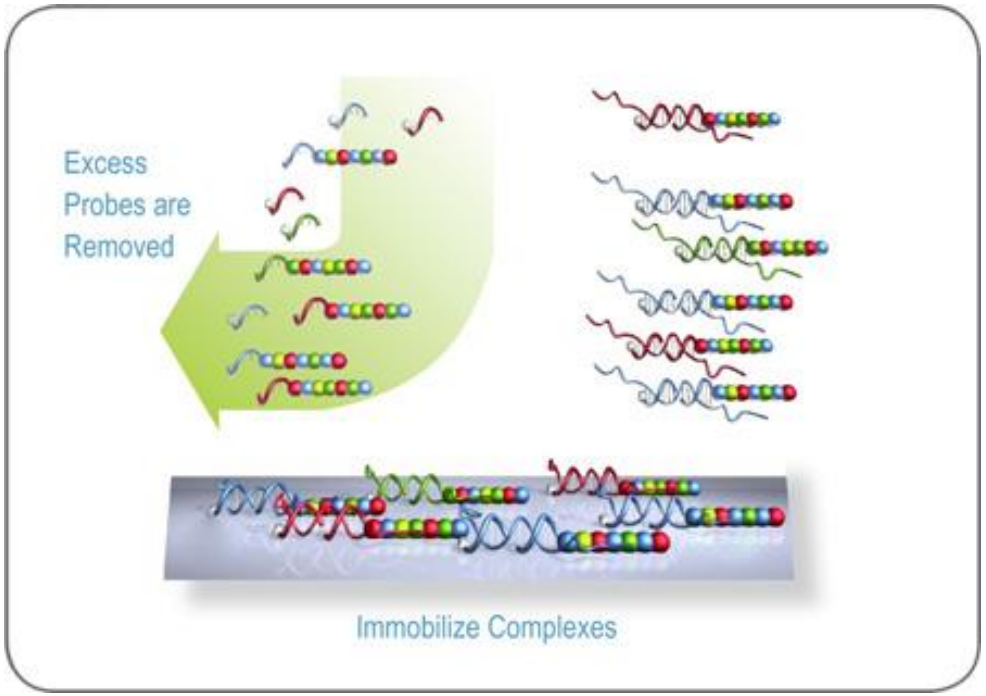
- **Immunohistochemistry**
  - *"Hans" algorithm, Hans et al, Blood 2004*
  - *"Choi" algorithm, Choi et al, Clin Can Res 2009*
  - *"Tally" algorithm, Meyer et al, JCO 2011*
  - *LM02, Natkunam et al, JCO 2008*

- **RT-PCR**
  - *Hsi et al, Blood abstract 2013 (frozen, PrimeraDx)*

- **Gene Expression Profiling**
  - *Lenz et al, NEJM 2008, (frozen tissue, Affy)*
  - *Gascoyne et al, JMD 2010 (FFPE, Affy)*
  - *Rimsza et al, CCR 2010 (FFPE, ArrayPlate-HTG)*
  - *Gutierrez-Garcia et al. Blood 2011 (FFPE, Affy)*
  - ***D Scott et al. Blood 2014 (nCounter system)***
  - ***Mareschal S et al. J Mol Diag 2015 (RT-MLPA assay)***

Improved GCB/ABC classifiers : Nanostring nCounter system

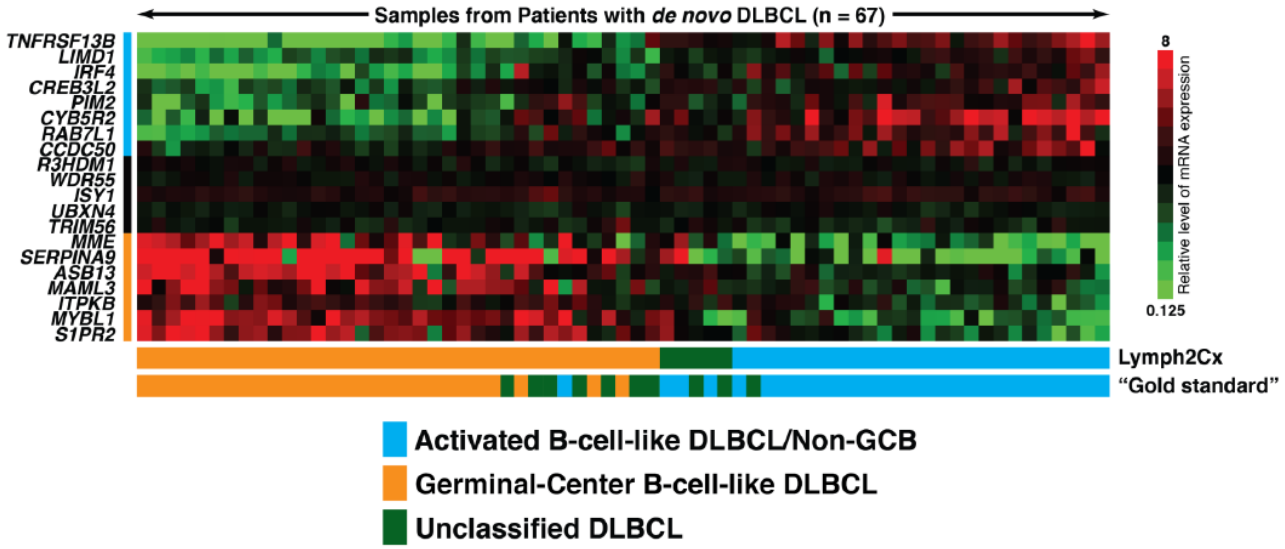
- ✓ Capture and reporter probes with fluorescent “bar codes”
- ✓ Solution phase hybridization
- ✓ Immobilization
- ✓ Digital scanning

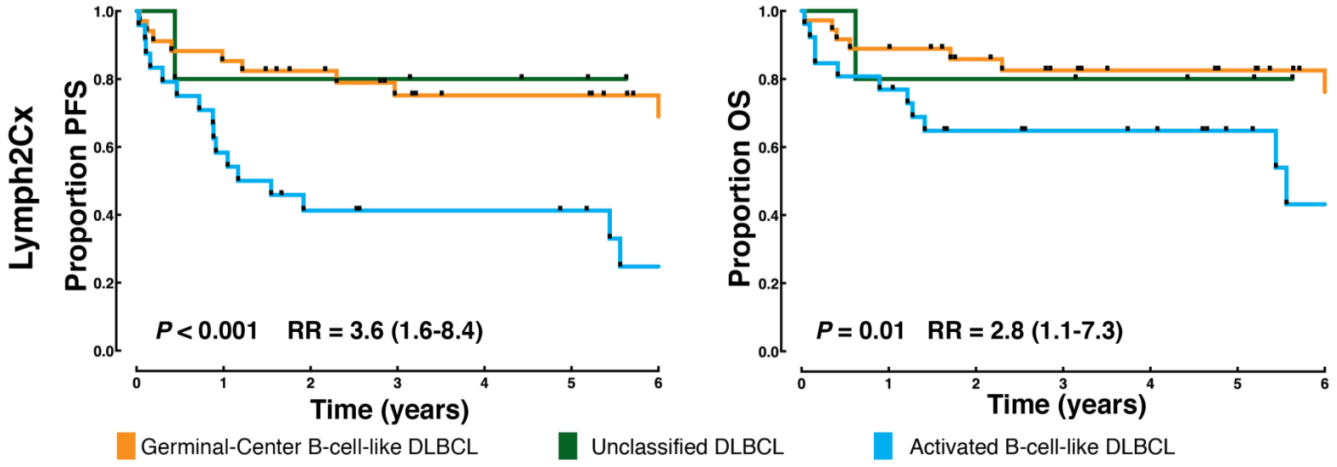


- Multiplex assay for up to 800 targets
- Applicable to FFPET
- No amplification or enzymes
- 36 hour turn-around-time
- Manufactured under GMP/ISO 13485
- PAM50-based Progsigna Breast Cancer Assay

Courtesy: Rimza L

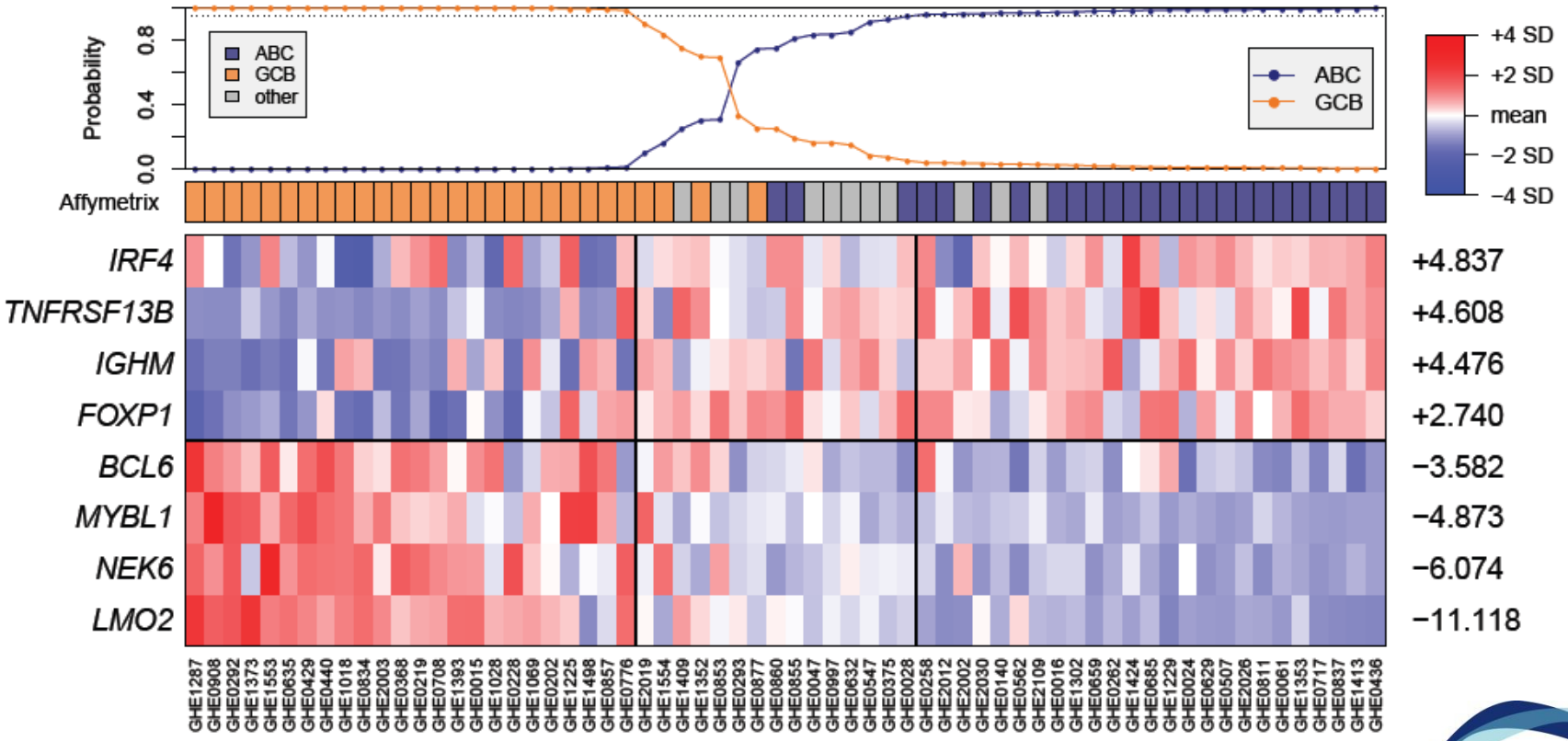
The Lymph2Cx Assay compared to Affymetrix





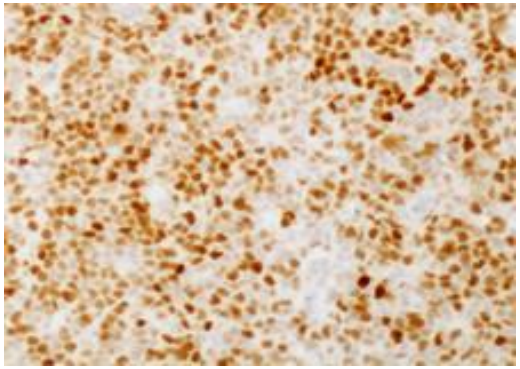
D. Scott et al, Blood 2014

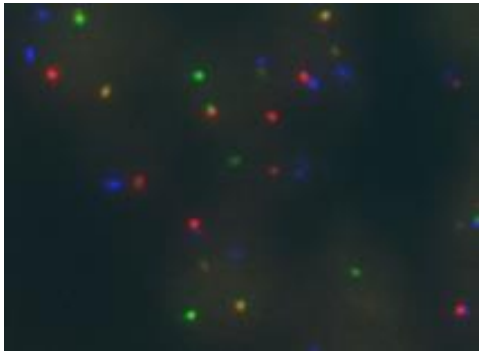
LYSA experience: RT-MLPA assay



Mareschal S et al. J Mol Diag 2015.

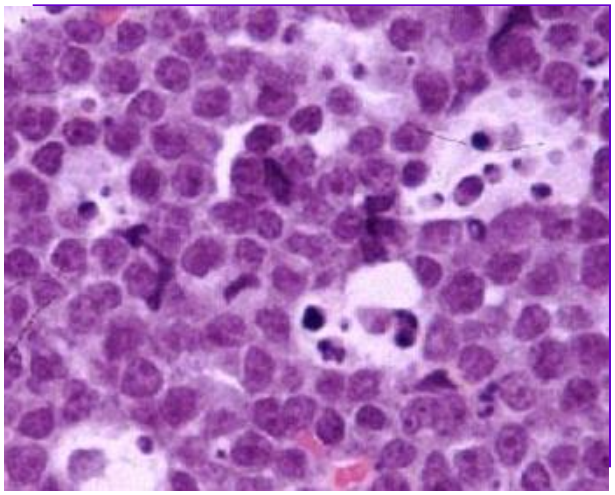
MYC or *MYC* ?



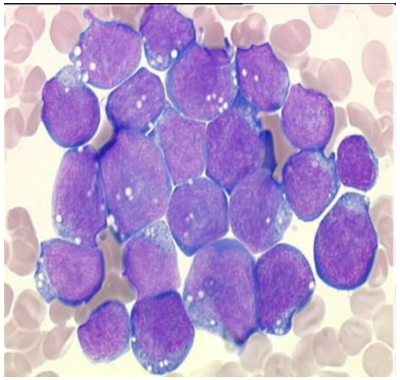


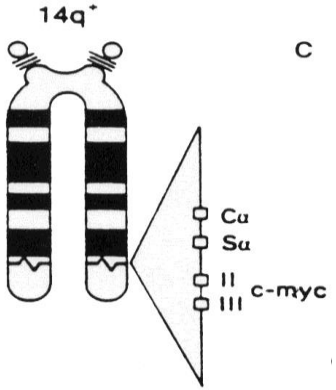
In adult DLBCL, *MYC* Break more frequent in non Burkitt lymphoma
MYC (protein) expression does not correlate with *MYC* break

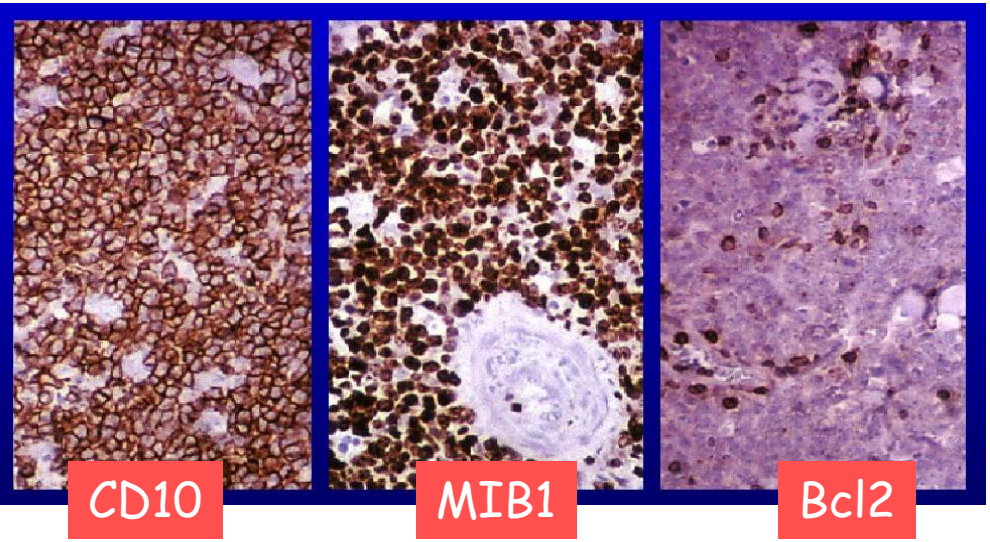
Burkitt lymphoma

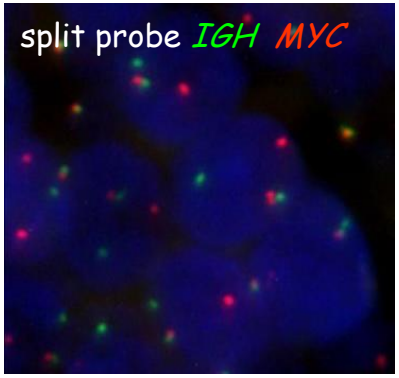


t(8;14)(q24;q32)(*MYC-IGH*)
variants t(2;8) ou t(8;22)
- **the genetic hallmark of BL**

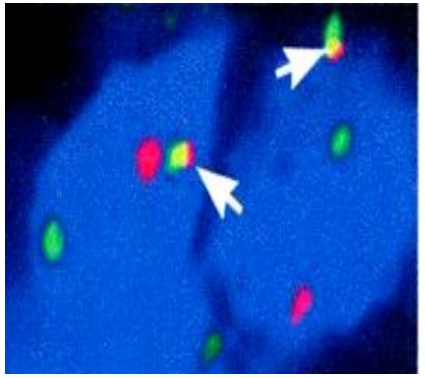








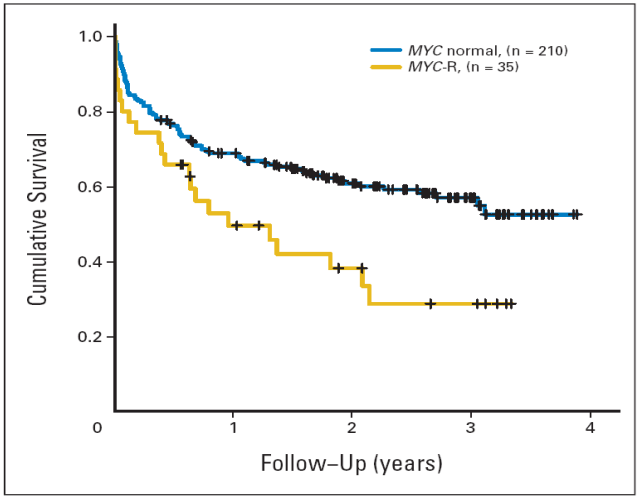
fusion probe *IGH* *MYC*



MYC in DLBCL

MYC break (FISH)

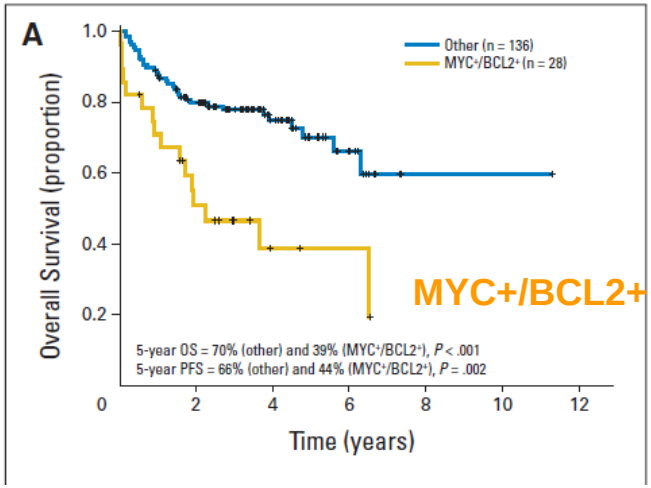
- 8-10% of *de novo* DLBC
- include « single hits » & « double hits » (the later being regarded as very aggressive diseases)
- inferior survival in most studies higher risk of CNS relapse



Barrans et al, J Clin Oncol 2010

MYC protein (IHC)

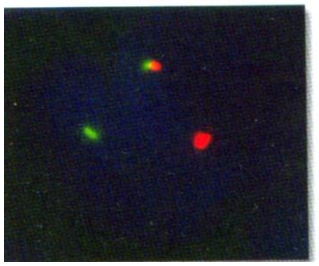
- 15-30% (pending the 70%-40% threshold)
- Variable impact on survival
- **MYC-BCL2 double expressor phenotype may associate with a poor outcome**



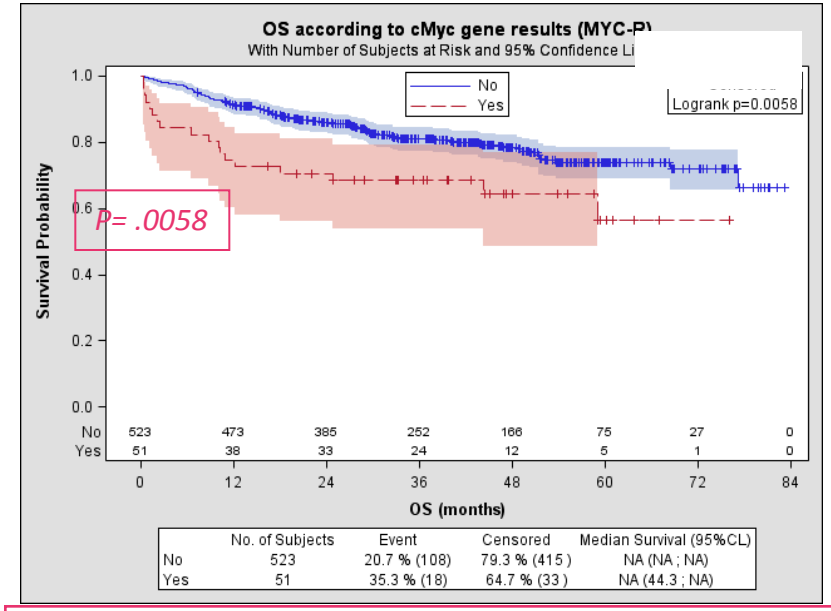
Johnson NA et al. J Clin Oncol 2012

MYC break associates with poor outcome

- ***574 de novo*** previously untreated DLBCL patients
- enrolled into the LNH01-5B & LNH03-B trials
- **R-chemotherapy**



Parameter	N	%
Chromosomal break points		
BCL2/18q21	122 /574	21,3
BCL6/3q27	157/573	27,4
MYC/8q24	51/574	8,9
***MYC-R* subtypes**		
MYC-SH	19/51	37,3
MYC-DH	32/51	62,7
MYC-BCL2	19	
MYC-BCL6	7	
MYC-BCL2-BCL6	6	
MYC partner gene (°)		
MYC-R		
IG	24/50	48
non-IG	26/50	52



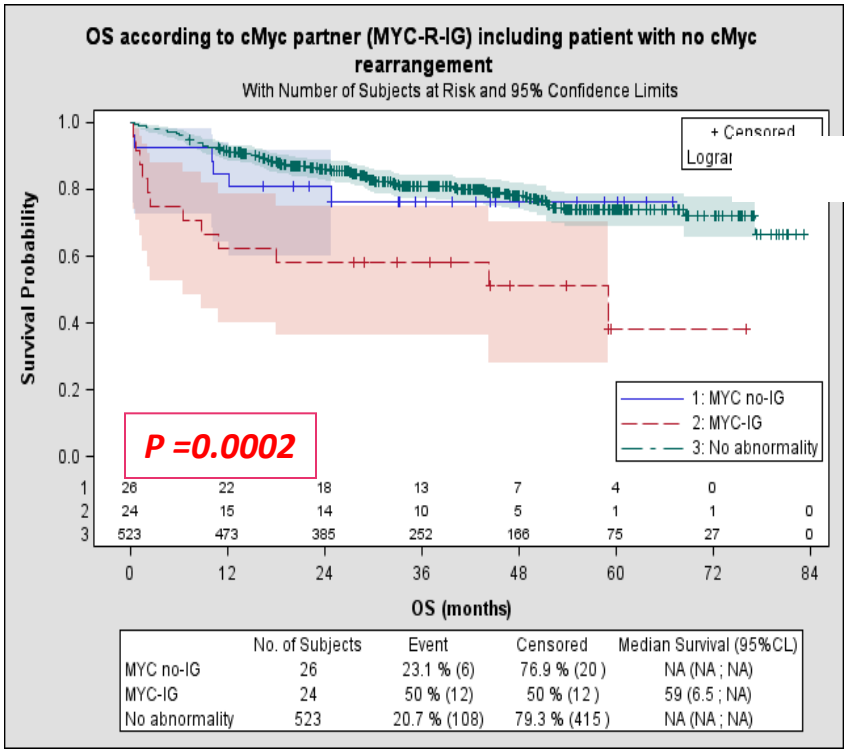
Significant impact of *MYC-R* on OS (*P= .0058*)

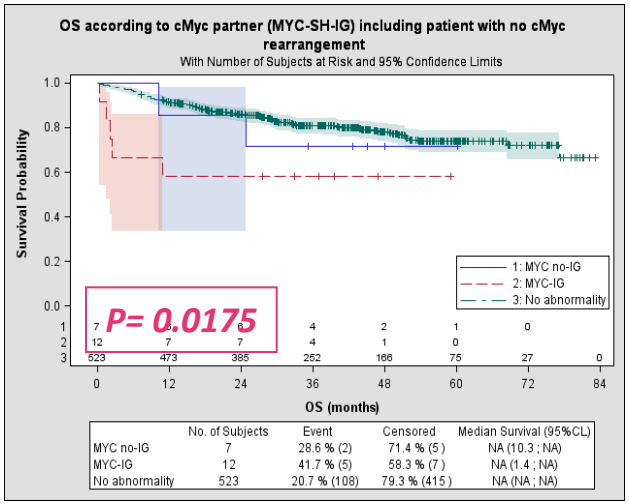
MYC-SH= MYC single hit
MYC-DH= MYC double hit

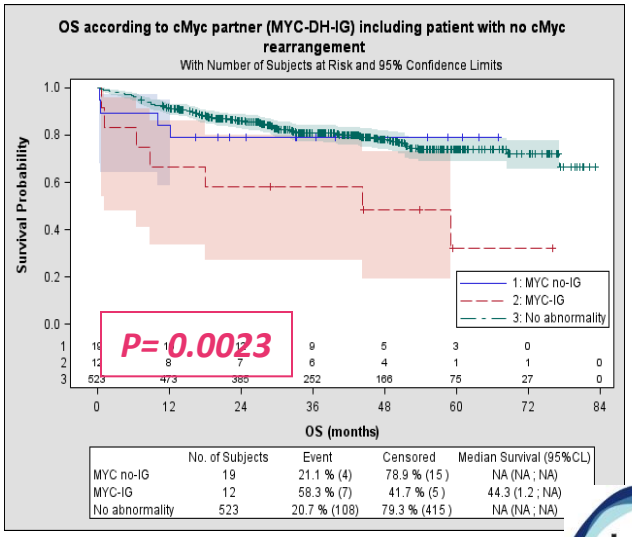
(°) in one case, the MYC partner could not be determined

C Copie-Bergman et al. Blood 2015

MYC-IG patients, but not *MYC-non-IG* DLBCL had a shorter survival as compared to *MYC*-negative DLBCL







- **This adverse prognostic effect of *MYC-IG* was maintained in *MYC-SH* and *MYC-DH* DLBCL**
- **Was observed in the GC subgroup, not in the non GC subgroup**
- **Overall, *MYC-IG* patients show similar clinical features as compared to *MYC-non-IG* DLBCL (except for age and n° of extranodal sites)**

C Copie-Bergman et alBlood 2015

MYC or *MYC* ?

• In ***de novo* DLBCL patients** treated with R-chemotherapy, converging data to support the adverse prognostic impact of *MYC-R* in DLBCL :
- associated with clinical parameters of aggressiveness;
- both in so-called *SH* and *DH* subgroups,
- in the GC DLBCL
- **likely to be strongly related to the *MYC-IG* partner gene**

- WHO 2016:
  - search for *MYC* translocation by FISH in DLBCL recommanded, at least in the GCB subgroup with further investigation of the partner gene in *MYC-R* DLBCL
  - investigate MYC-BCL2 expression by IHC

What has become the « BCL-U » category ?

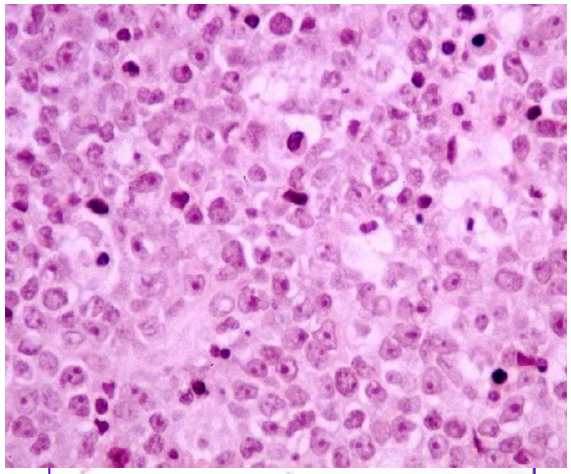
High grade B-cell lymphoma « double hit » With *MYC* and *BCL2* (or *BCL6*) R

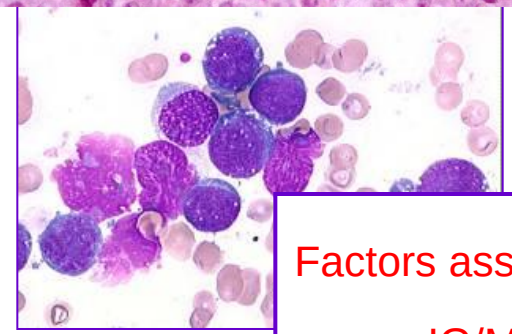
All « double-hit » LBCL (FL or LBS excluded) Morphology to be noted in a comment

High grade B-cell lymphoma NOS

Cases with blastoid appearance
Cases with equivocal morphology

High grade B-cell lymphoma with MYC and BCL2 R « double hit »





- ~4% of NHLs (54/1260)

- extranodal disease, BM , PBL, CNS involvement

- Sometimes preexisting FL

- variable morphology: intermediate, DLBCL, blastoid
  high proliferation index >80%
  germinal center phenotype (CD10+)

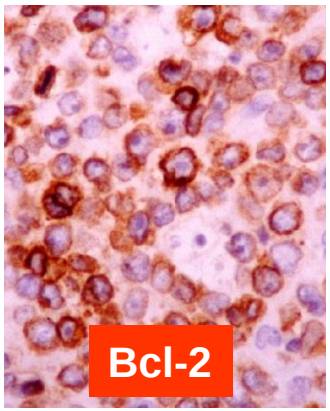
;22), t(2;8)
1p36,

rdel et al)

- Resistance to conventional therapies including intensified therapy

Factors associated with a more favorable outcome

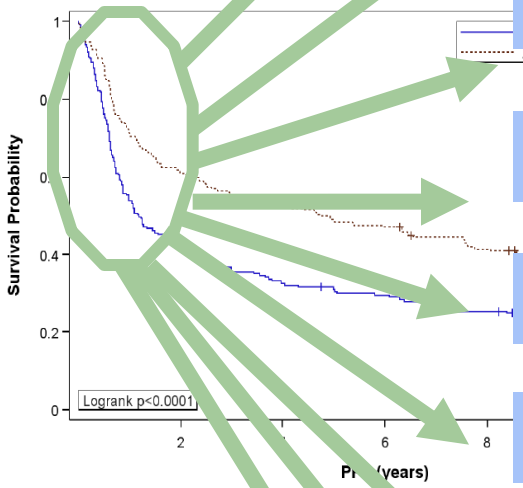
- o non-IG/MYC translocation partner
- o absent BCL2 protein expression
- o treatment with rituximab-based chemotherapy



Kanungo et al, Mod Pathol 2006, Le Gouill et al, Haematologica 2007, Johnson NA, Blood 2009, Snurdel M, Am J Surg Pathol 2010

Future directions in DLBCL: toward a personalized medecine…?

DLBCL: Moving forward CHOP: ongoing trials

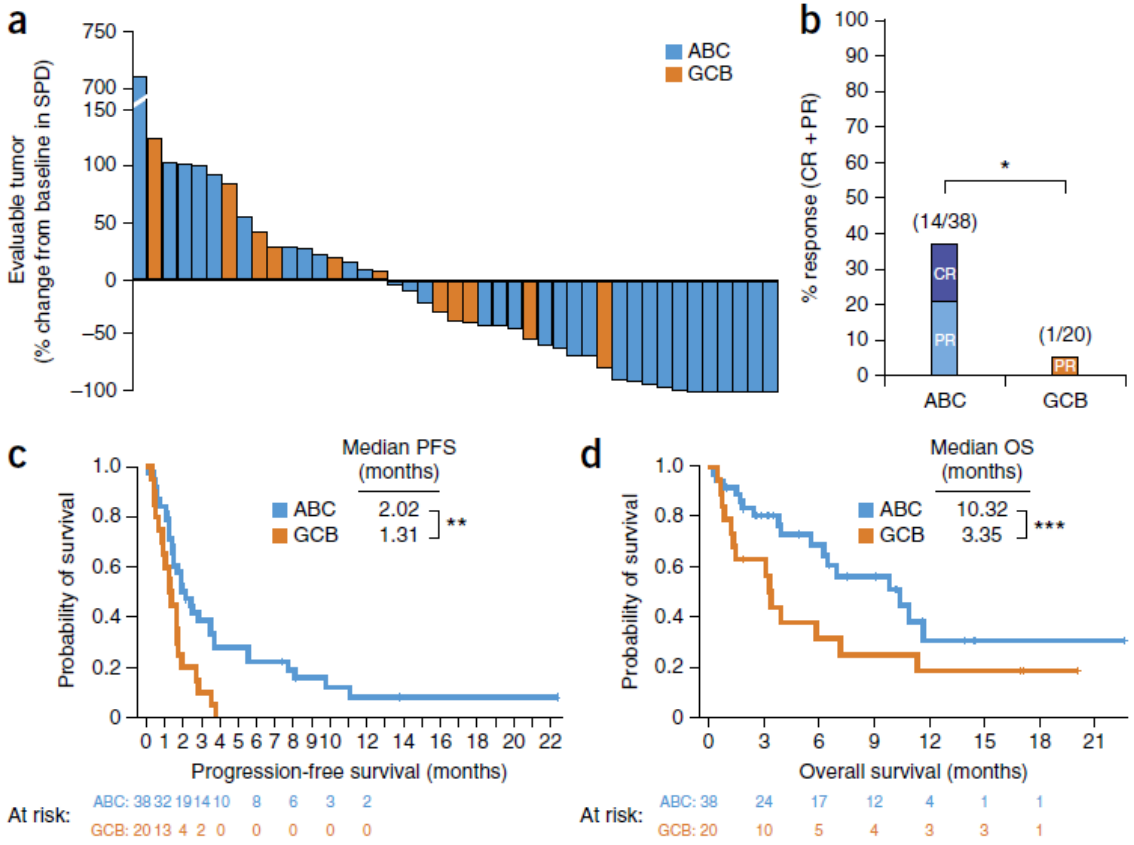


Lenalidomide	R2-CHOP / R-CHOP-> R/R2 Confirmatory studies ongoing
BTKi / ibrutinib	>> Activity in non-GC Ph II ORR 100% / CR 91% / Ph III ongoing
Bortezomib	>> Activity in non-GC Rand studies ongoing R-CHOP-BTZ in ABC
BCL2 / ABT-199	Ongoing Ph II G-CHOP/R-CHOP + ABT-199
PKCβ / enzastaurin	PRELUDE trial – no difference EFS, DFS, OS
mTOR	PILLAR2 trial rand maint Everolimus in CR post R-CHOP, ongoing
PI3K	Idelalisib, IPI-145, Copanlisib
Epigenetics	Azacytidine + R-CHOP
EZH2	GCB…Non-GCB

COO is a major determinant of treatment response both in first line and in relapsed/refractory DLBCL

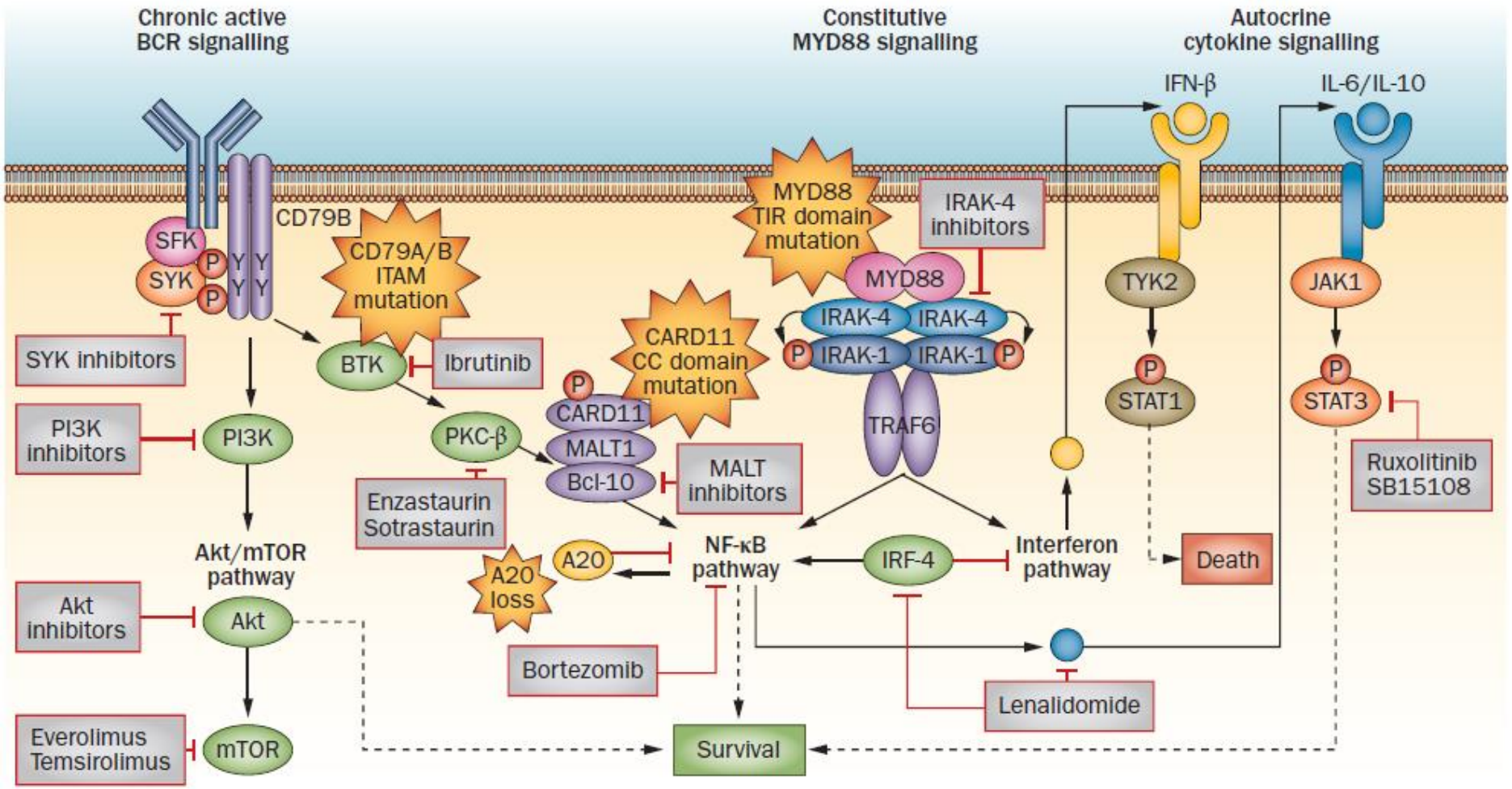
ABC DLBCL has a ~40% cure rate with currently available therapies

Response to Ibrutinib therapy in relapsed/refractory DLBCL patients



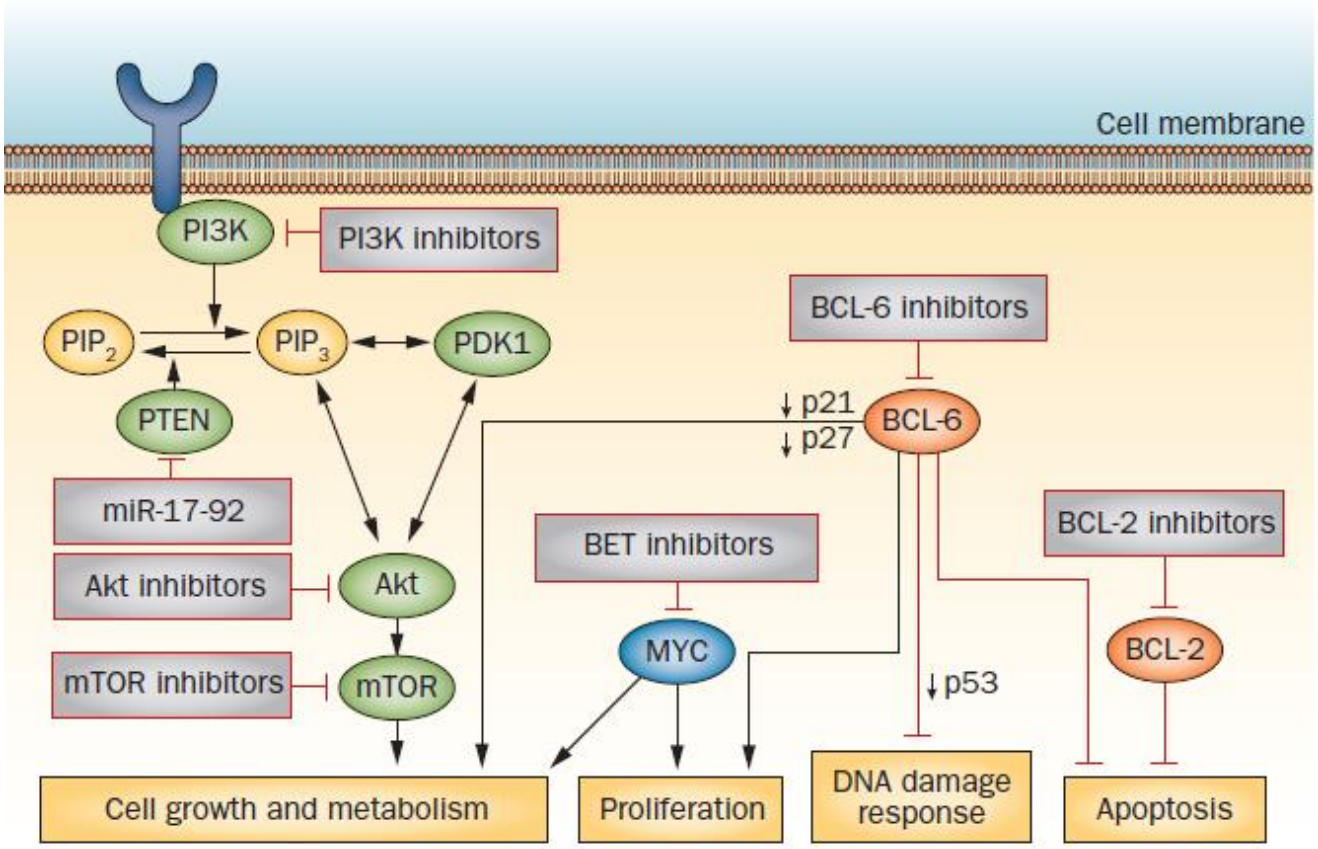
W Wilson et al. Nature Med 2015

The key signaling pathways in ABC DLBCL with targeted novel agents



Roschewski et al. Nat Rev Clin Oncol 2014

The key signaling pathways in GCB DLBCL with targeted novel agents



Roschewski et al. Nat Rev Clin Oncol 2014

Recurrent somatic mutations in genes with roles in histone modification (methylation *MML2,* ***EZH2***; acetylation ***MEF2B****, CREBBP, EP300*,…)

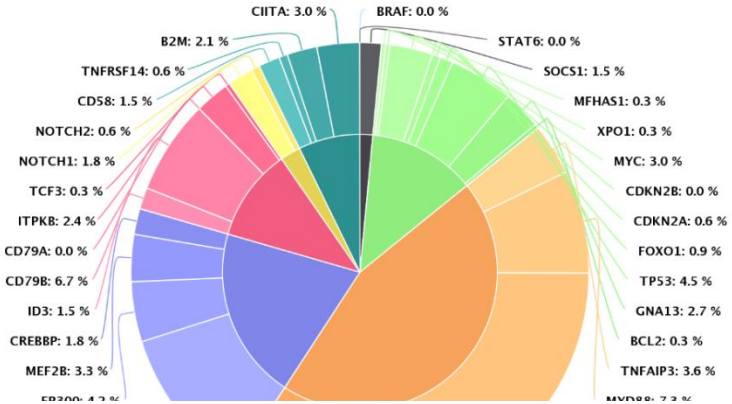
Morin et al. Nature Genetics 2010; Pasqualucci et al. Nature 2011; Morin et al. Nature 2011

DLBCL: Present & Future

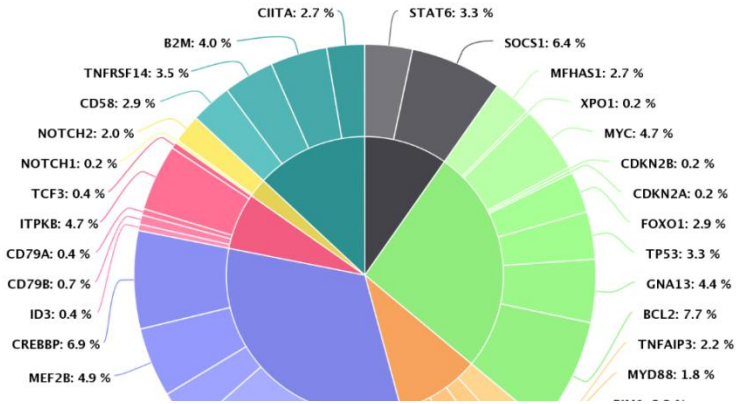
- GCB and ABC are DLBCL different diseases with important clinical impact →Determination of the COO is a requirement in the 2016 updated WHO classification
- BCL2 expression, MYC R (with IG partner), BCL2-MYC double expressor likely are prognostic factors ; controversies whether they should be investigated in every DLBCL patient?
- Other predictive biomarkers are emerging which could drive specific therapies (IHC, NGS,…)

From exome sequencing to targeted Next Generation Sequencing (NGS) in clinical practice

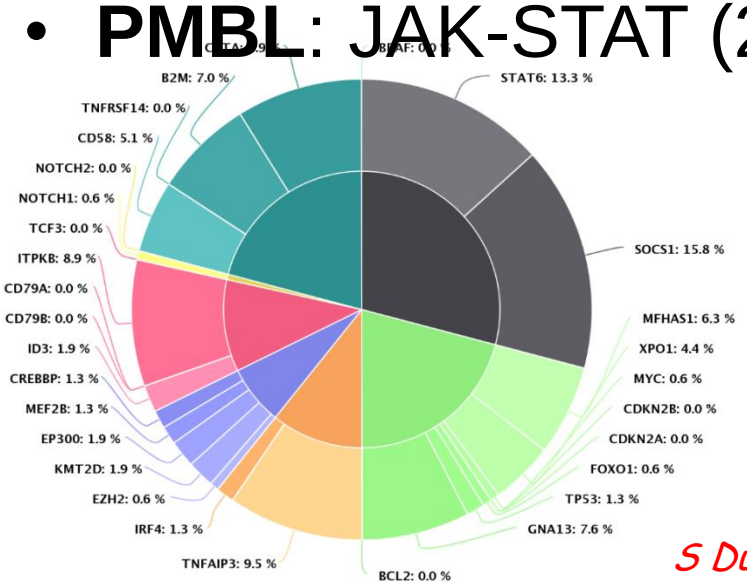
ABC

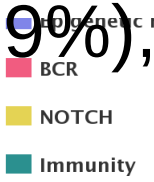


GCB



- **ABC**: NFkB (45%), Epigenetics (20%)
- **GCB**: Epigenetics (32%), Apoptosis (26%)
- **PMBL**: JAK-STAT (29%), Immunity (21%)

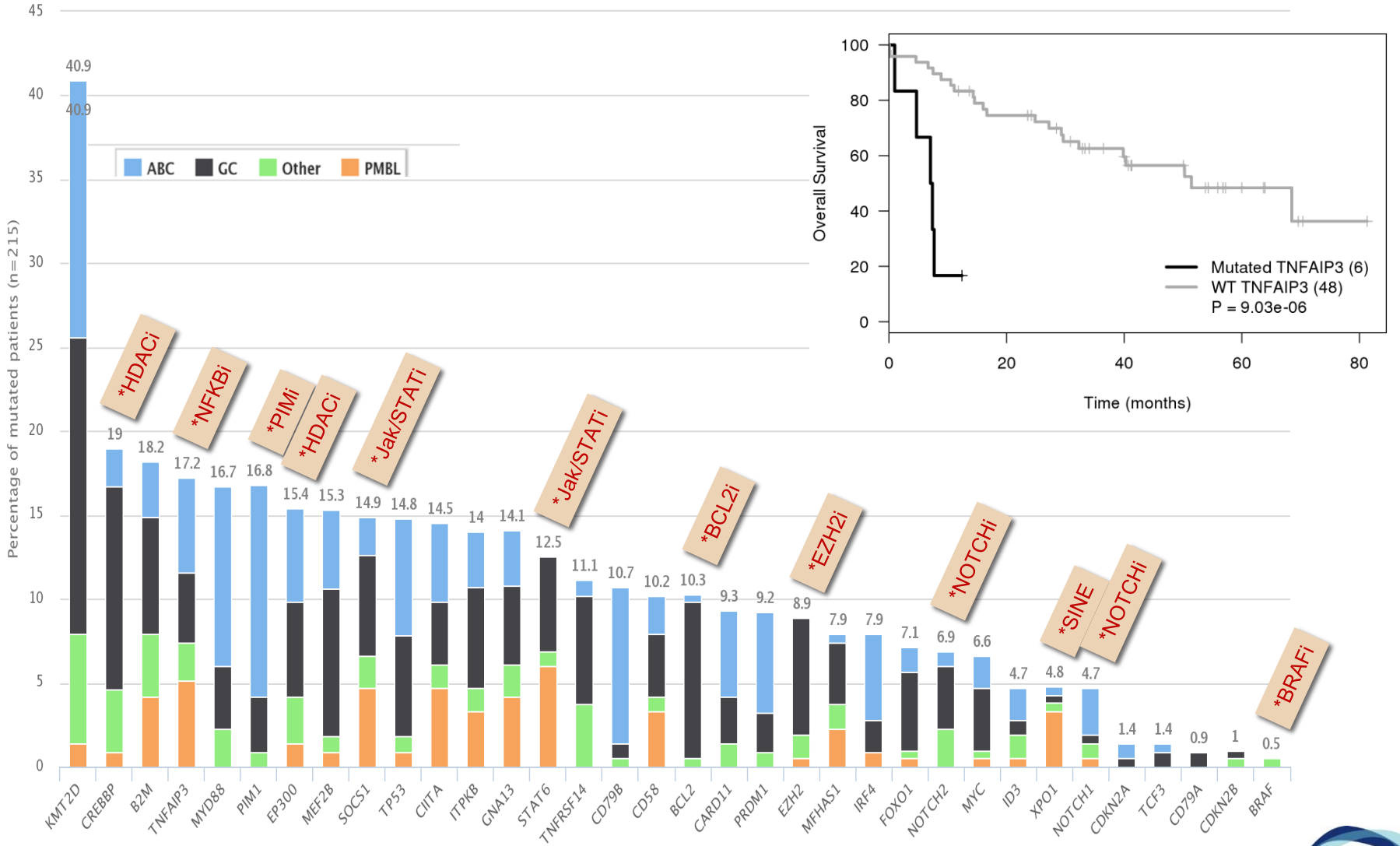




- 216 DLBCL pts
- clinical trials
- R-chemo
- FFPE
- 34 genes (relevant, targetable,…)

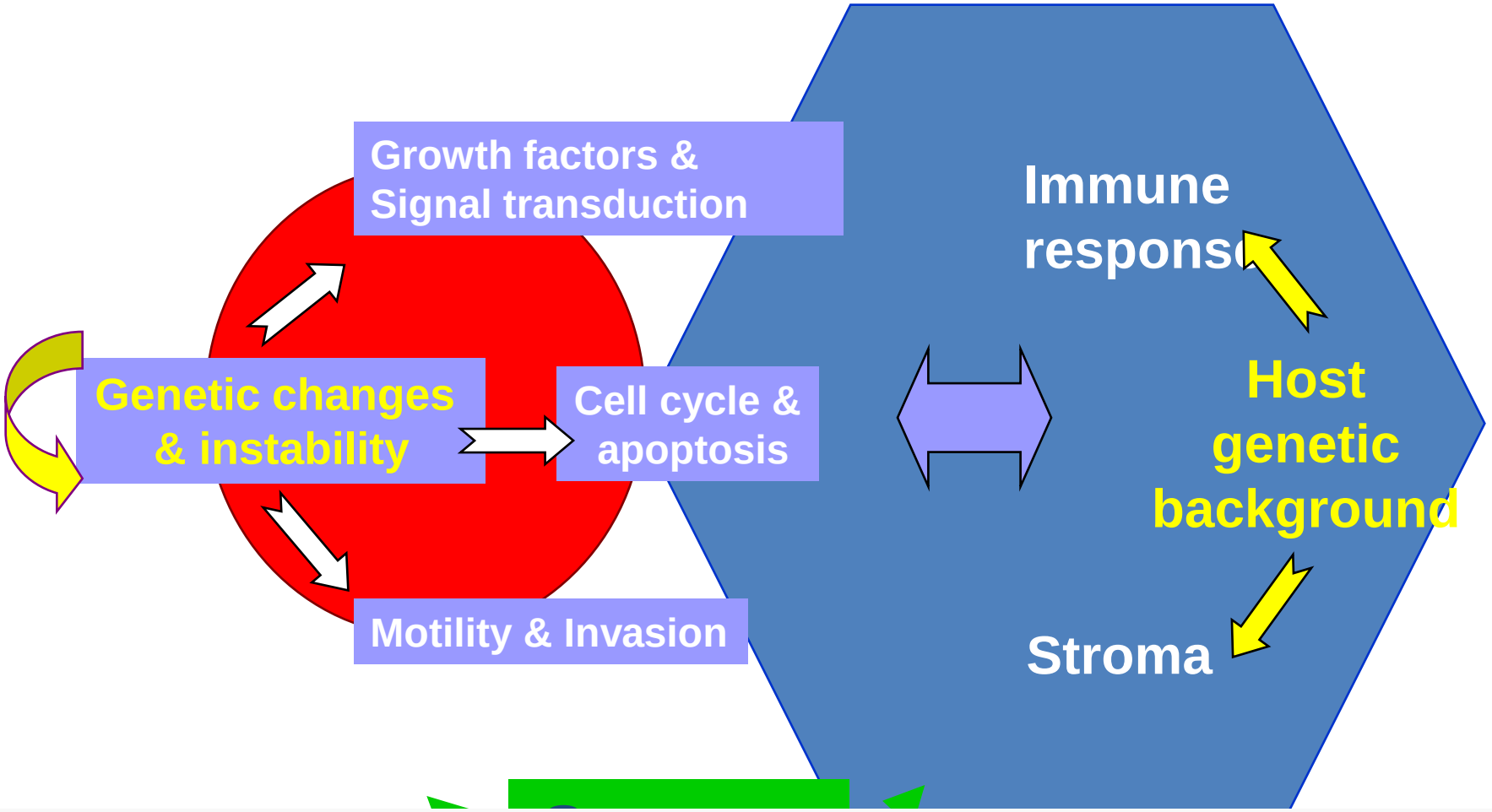
S Dubois, F Jardin. Clin Cancer Res 2015

From exome sequencing to targeted Next Generation Sequencing (NGS) in clinical practice

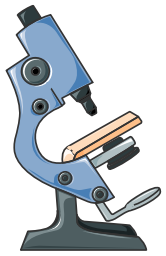


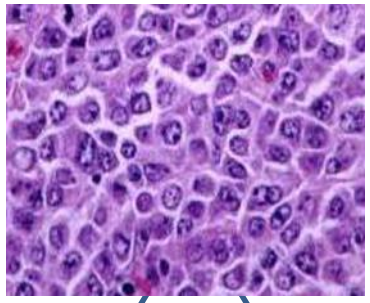
S Dubois, F Jardin. Clin Cancer Res 2015

Microenvironement & host response



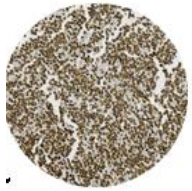
Lymph node signature predicts outcome in R-CHOP DLBCL pts *(Lenz et al. NEJM, 2008)*
Serum PDL1 level with prognostic impact *(Fest et al. Leukemia 2014)*
Immune checkpoint inhinitors in PMBL and DLBCL, NOS?

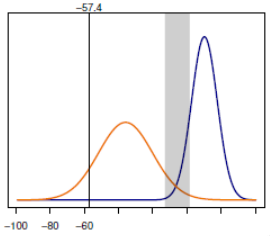




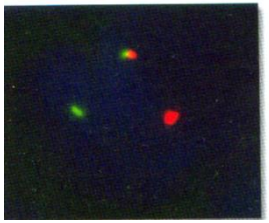
DLBCL (CD20)
DLBCL subtypes
EBV
HHV8
PMBL…

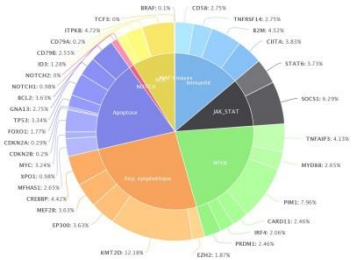
COO
DLBCL molecular subtypes
GCB/ABC/PMBL





DLBCL genetic/Pc subtypes
FISH: MYC +/- BCL2/BCL6
IHC: MYC/BCL2 protein





DLBCL Predictive Frs
COO
CD30?
« NGS gene panel »
EZH2
CD79B
MYD88, ..

- 5-15 tissue sections FFPE
- 20-200 ng DNA FFPE
- Limited bioinformatics
- In pts at diagnosis and at relapse (clonal heterogenity)

DLBCL: present & future

1. Diagnosis usually easy; do not miss cases that are CD20-negative or that do not ressemble DLBCL
2. ~30-35% of the patients not cured by R-chemo (>50% of ABC DLBCL)
3. **WHO 2016**: DLBCL GC and ABC are different diseases; determination of GCB/ABC subtypes to be done → which technique [Molecular techniques (Nanostring, RT-MLPA) more robust than IHC]
4. Prognostic biomarkers : c-MYC-Ig (FISH), DHL, BCL2-MYC double expressor?
5. The BCL-U category has eveloved to « High grade B-cell lymphomas with or without MYC and BCL2 and/or BCL6 rearrangements
6. **Toward targeted therapies** (refractory patients> de novo), several compounds within pipelines -→ molecular characterization to be expected (at diagnosis or at relapse?)

Any way for the pathologist ….?

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