

# Médecine génomique de précision en onco- hématologie : Lymphomes B diffus à grandes cellules

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*Unité d'innovation en génétique et épigénétique oncologique*

*INSERM 1231 - UBFC*

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DES d'Hématologie

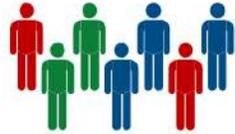
22 Novembre 2019

Paris



# Médecine de précision

**Médecine actuelle :**  
Approche « *one-size fits all* »



Traitement identique

**Médecine du futur :**  
Traitement personnalisé



Caractérisation moléculaire  
des maladies



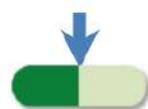
Répondant au  
médicament A



Traitement A



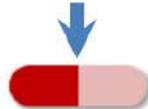
Répondant au  
médicament B



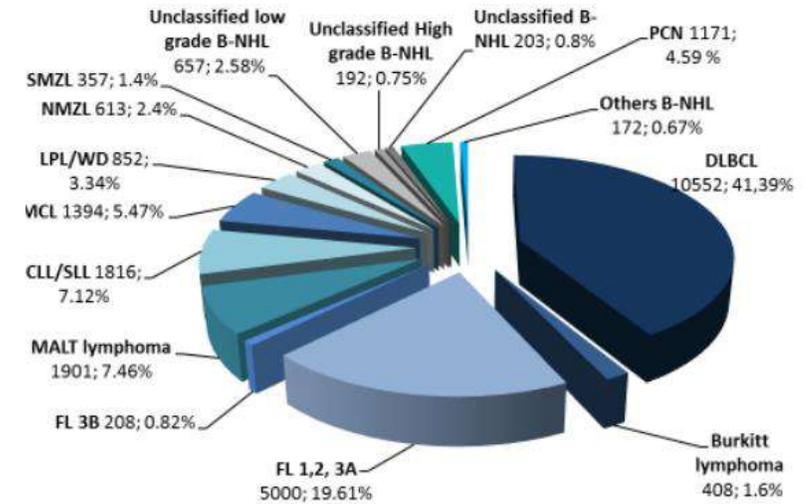
Traitement B



Répondant au  
médicament C



Traitement C



# Lymphomes agressifs à grandes cellules B

**WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues**

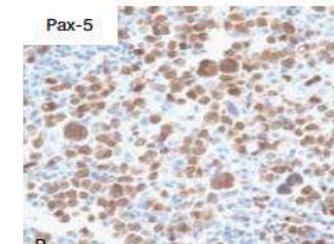
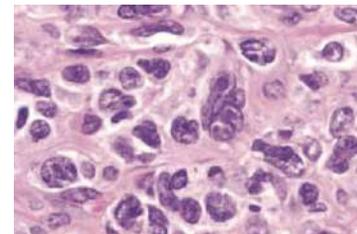
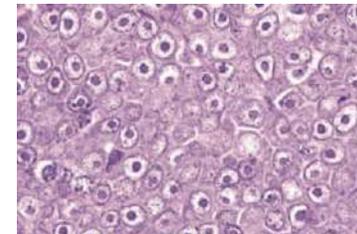
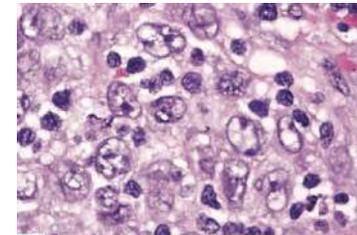
Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri, Harald Stein, Jurgen Thiele, Daniel A. Arber, Robert P. Hasserjian, Michelle M. Le Beau, Attilio Orazi, Reiner Siebert

The grid contains the following images:

- Top row: Gross pathology specimen, PET scan showing a hypermetabolic lesion, and a gross pathology specimen.
- Middle row: Three histological sections showing different lymphoma morphologies.
- Bottom row: Cytogenetic analysis showing chromosome 11 and 13, a flow cytometry plot, and a fluorescence in situ hybridization (FISH) image.

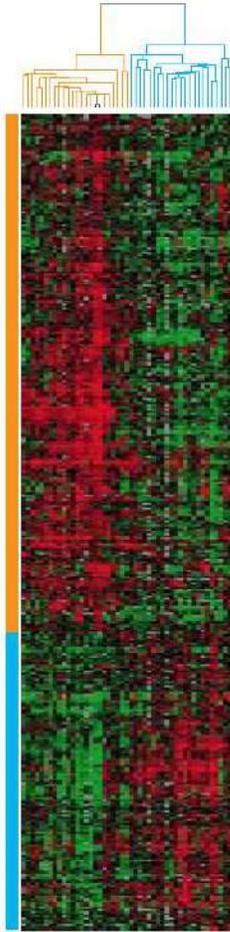
WHO

Diffuse large B-cell lymphoma (DLBCL), NOS	9680/3
Germinal centre B-cell subtype	9680/3
Activated B-cell subtype	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, NOS	9680/3
EBV-positive mucocutaneous ulcer	9680/1*
DLBCL associated with chronic inflammation	9680/3
Fibrin-associated diffuse large B-cell lymphoma	
Lymphomatoid granulomatosis, grade 1,2	9766/1
Lymphomatoid granulomatosis, grade 3	9766/3*
Primary mediastinal (thymic) large B-cell lymphoma	9679/3
Intravascular large B-cell lymphoma	9712/3
ALK-positive large B-cell lymphoma	9737/3
Plasmablastic lymphoma	9735/3
Primary effusion lymphoma	9678/3
Multicentric Castlemann disease	
HHV8-positive DLBCL, NOS	9738/3
HHV8-positive germinotropic lymphoproliferative disorder	9738/1*
Burkitt lymphoma	9687/3
Burkitt-like lymphoma with 11q aberration	9687/3*
High-grade B-cell lymphoma	
High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements	9680/3
High-grade B-cell lymphoma, NOS	9680/3
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma	9596/3



## Phénotype GCB/ABC : rappel historique (2)

(LymphoCHip)



GCB

~ 375 gènes

Phénotype « *Germinal center B cell like* » (GCB)  
Gènes exprimés lors de la différenciation dans le CG:  
BCL6, GCET2, SERPINA9, CD10...

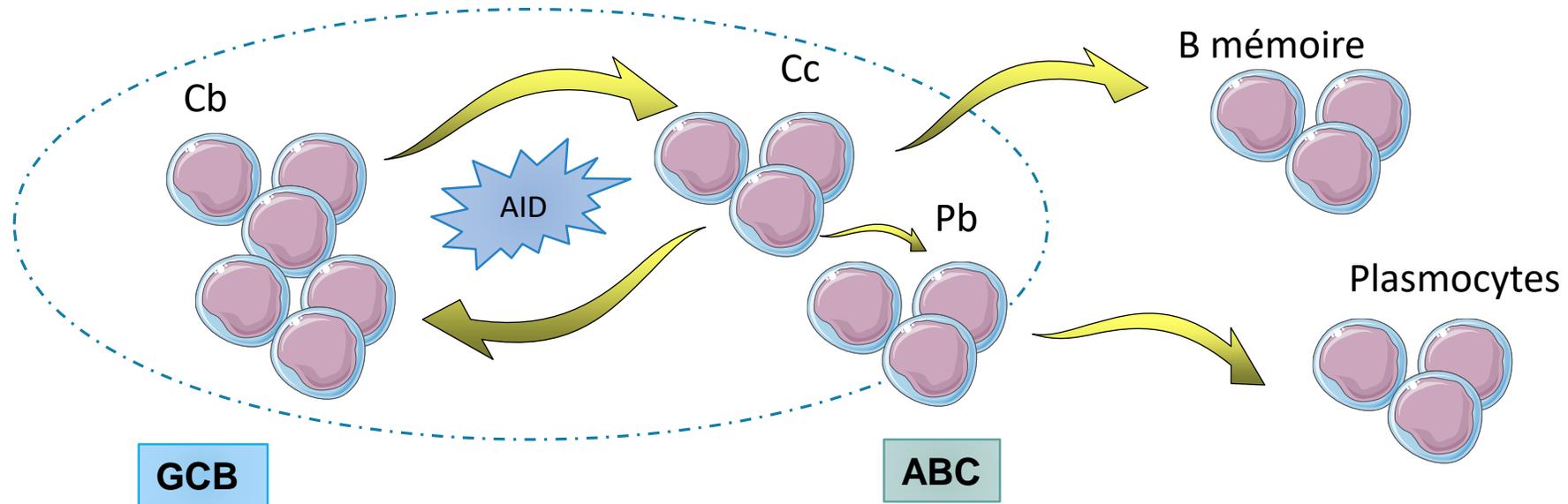
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Phénotype « *Activated B cell like* » (ABC)  
Gènes exprimés lors de l'activation des lymphocytes B in vitro:  
MUM1/IRF4, BCL2...

ABC



# Phénotype GCB/ABC : pertinence physiopathologique (1)



t(14;18) +/- MYC

Del 1p, 10q, 13q  
Gain 2p, 12q, 13q

t(3;14) (switch)

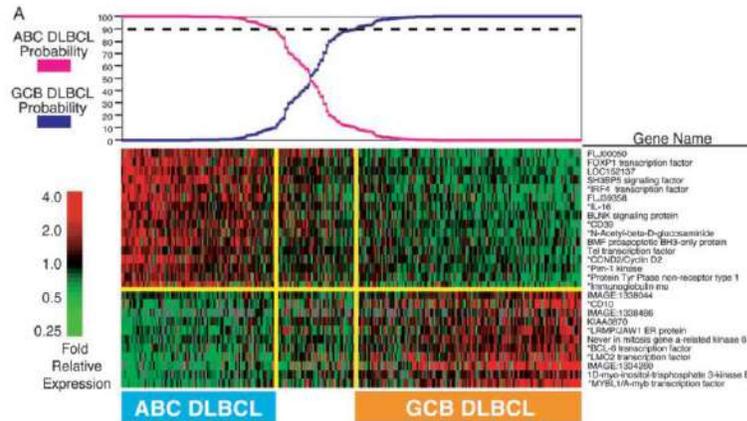
Del 6q, 9p21  
Trisomie 3, Gain 3p, 3q, 18q21, 19q



# Classification 'cellule d'origine' : intérêt diagnostique et clinique

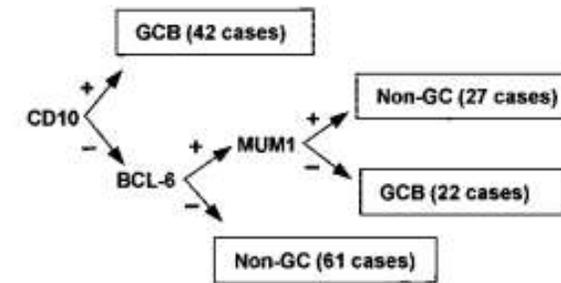
Gene expression-based (RNA)

IHC COO ('simple' test for routine diagnostics)

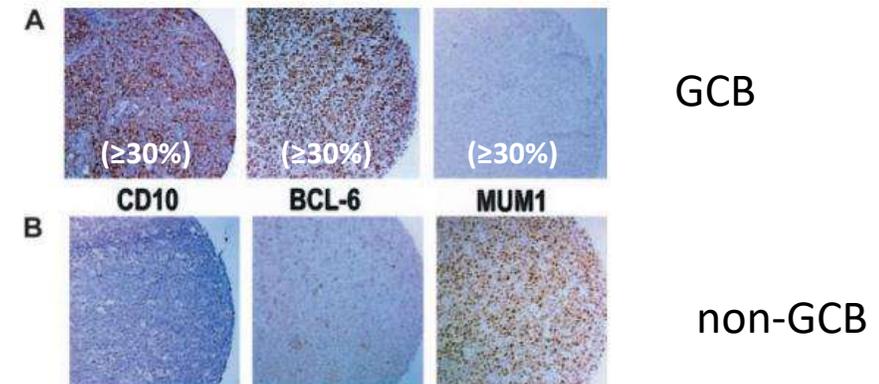


**Fig. 4.** Differences in survival between DLBCL subgroups. The Kaplan-Meier plots display the survival of patients in the GCB and ABC DLBCL subgroups defined by using gene expression data from Lymphochip (2) (Left) or Affymetrix (3) (Right) microarrays. A log-rank test was used to calculate the P values.

Wright G, PNAS 2003



**Figure 1.** Decision tree for immunoperoxidase TMA classification of DLBCL.

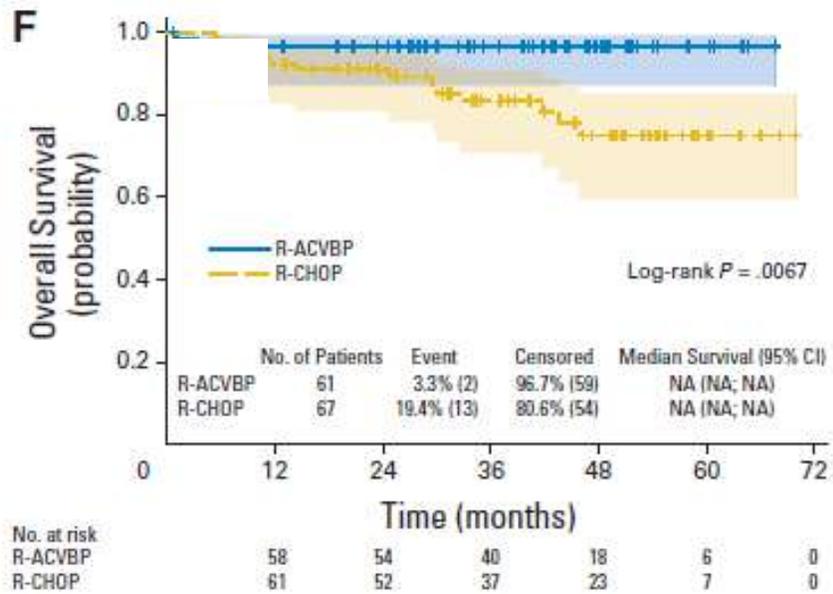


**Figure 2.** Results of immunoperoxidase staining. (A) Immunoperoxidase stains of a GCB case that is positive for CD10 and bcl-6 but negative for MUM1. (B) Immunoperoxidase stains of a non-GCB case that is negative for CD10 but shows rare bcl-6<sup>+</sup> cells and is positive for MUM1. Original magnification, × 100.

Hans C et al, Blood 2004

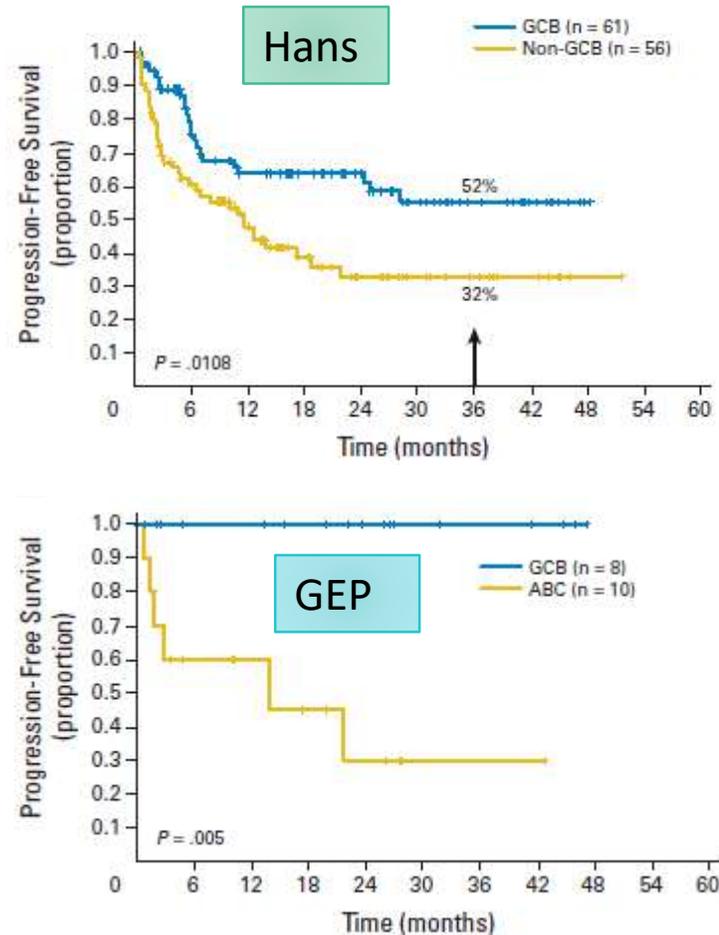
DLCBL 45 ans, IPIaa = 1  
 Hans : CD10 -, BCL6 +, IRF4 - = GCB

Hans (non-GC tumours according to treatment arm)



R-ACVBP ou R-CHOP ?

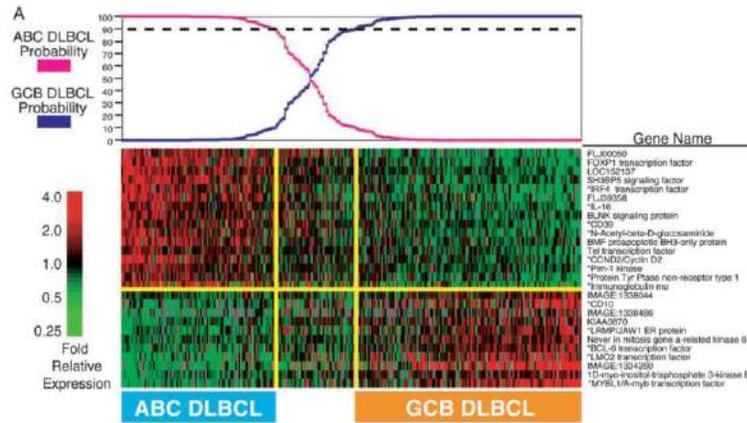
R-DHAP ou R-ICE ?



# Classification 'cellule d'origine' : intérêt diagnostique et clinique

Gene expression-based (RNA)

RT-MLPA COO ('simple test for routine diagnostics')



5 GCB Genes :

*NEK6*  
*LMO2*  
*BCL6*  
*MYBL1*  
*TNFRSF9*

5 ABC Genes:

*IRF4*  
*IGHM*  
*FOXP1*  
*TACI*  
*CCND2*

Double Hit :

*MYC*  
*BCL2*

Controls :

*CCND1*  
*CD20*  
*(MS4A1)*

21 Marqueurs

6 Marqueurs GCB  
*NEK6*  
*LMO2*  
*BCL6*  
*ITPKB* ←  
*MAML3*  
*MYBL1*  
*MME (CD10)* ←

5/6 Marqueurs ABC  
*IRF4*  
*IGHM*  
*FOXP1*  
*TNFRSF13B (TACI)*  
*LMD1* ←  
*MYD88L265P* ←

3 Marqueurs PMBL  
*CD30* ←  
*CD23* ←  
*MAL* ←

Double Hit  
*MYC*  
*BCL2*  
*CEREBLON (CRBN)*  
*EBER (EBV)*  
*CD5*  
*TNFRSF9*  
*CCND1*  
*CCND2*  
*MS4A1 (CD20)*

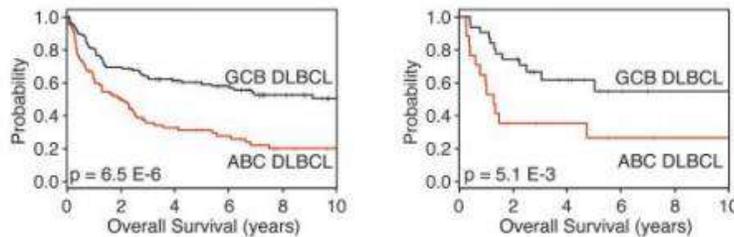
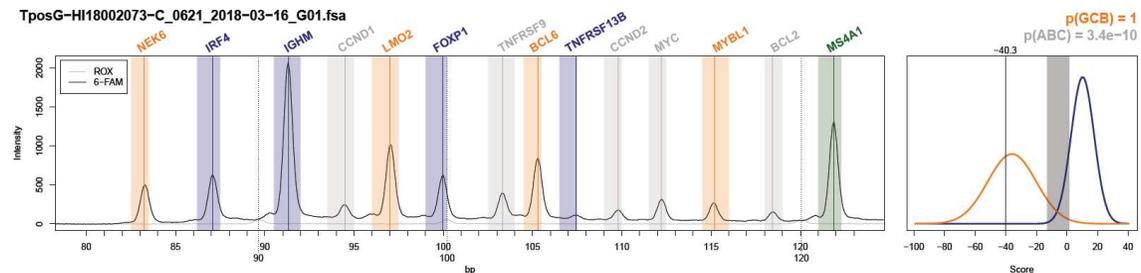
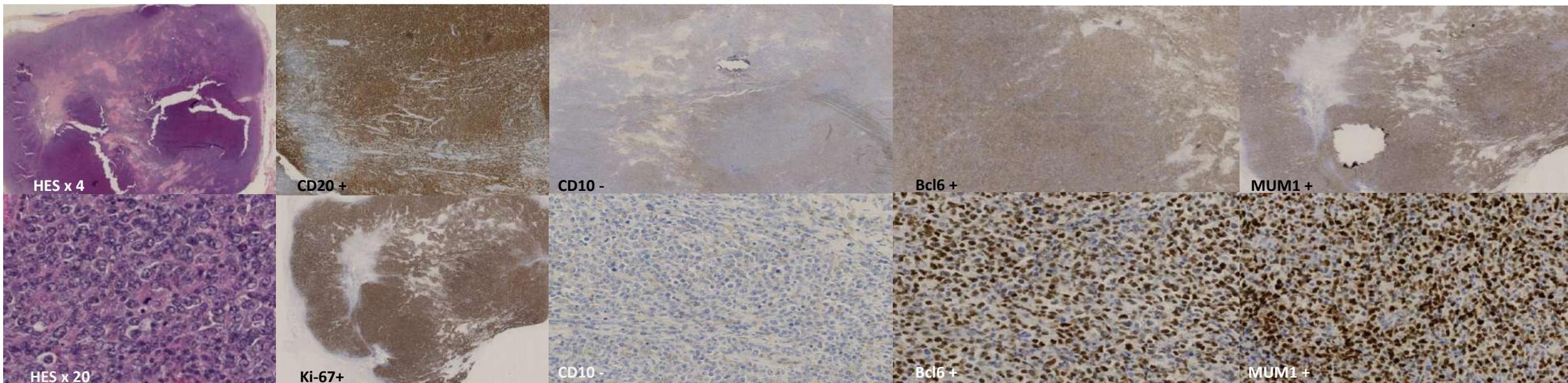
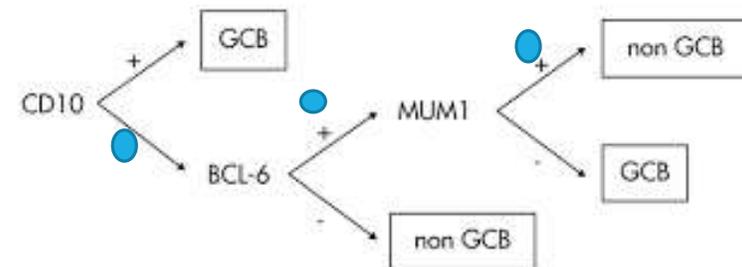


Fig. 4. Differences in survival between DLBCL subgroups. The Kaplan-Meier plots display the survival of patients in the GCB and ABC DLBCL subgroups defined by using gene expression data from Lymphochip (2) (Left) or Affymetrix (3) (Right) microarrays. A log-rank test was used to calculate the P values.





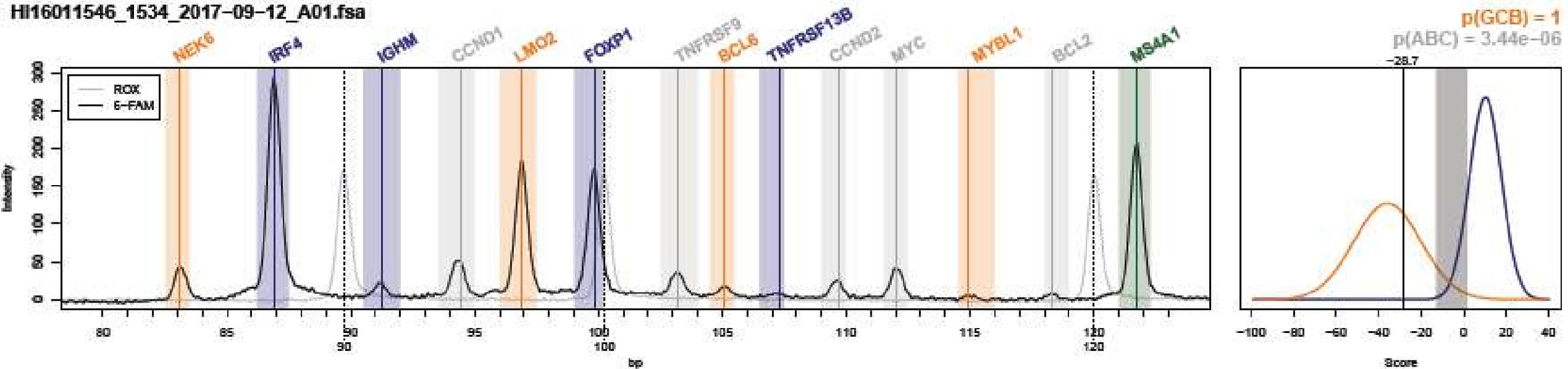
# HI16011546 - Hans vs RT-MLPA



**non GC**

# HI16011546

HI16011546\_1534\_2017-09-12\_A01.fsa

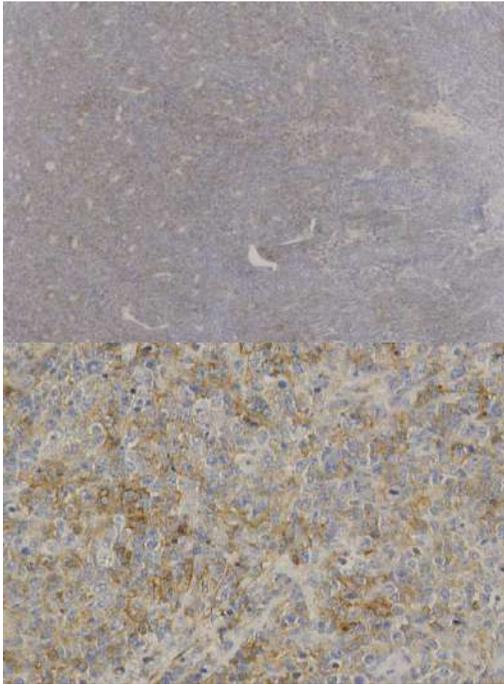


**GCB**

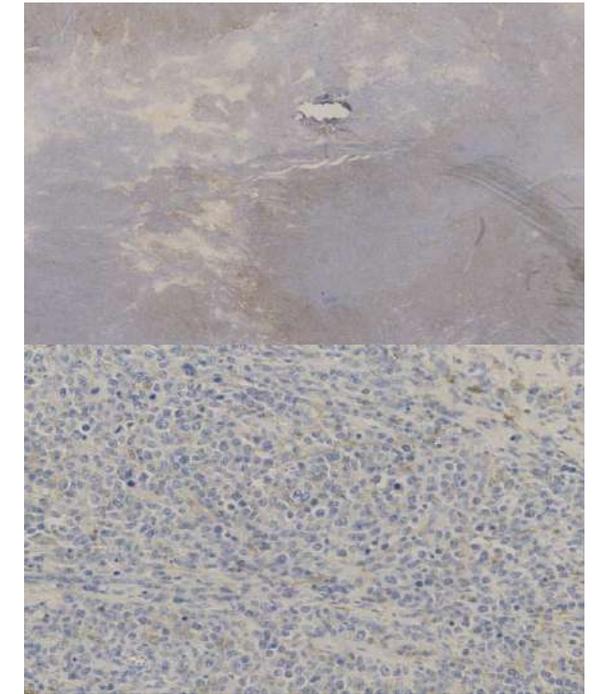
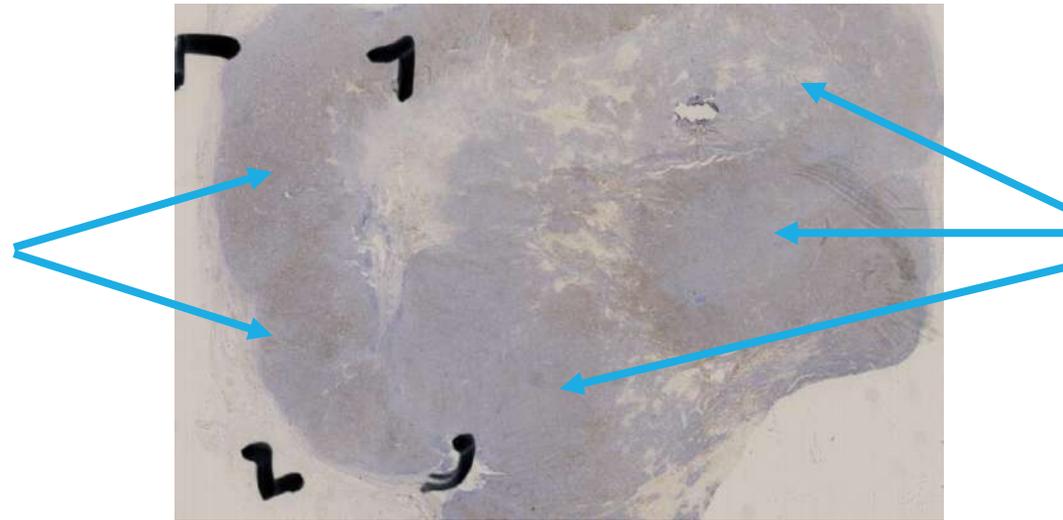


# Non GC (Hans) vs GCB (RT-MLPA) ?

zone macro disséquée

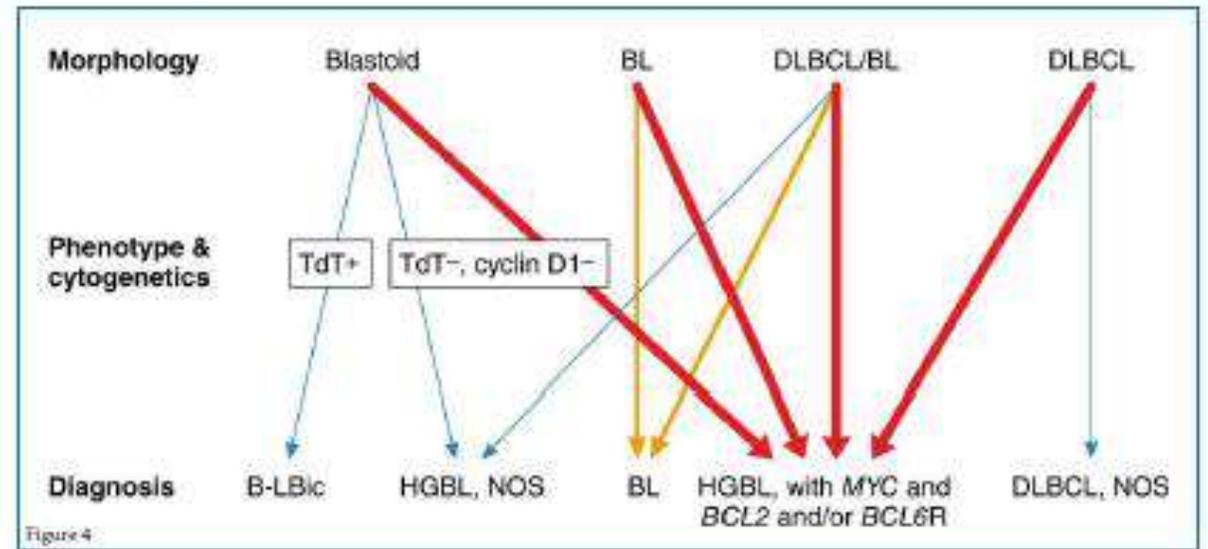
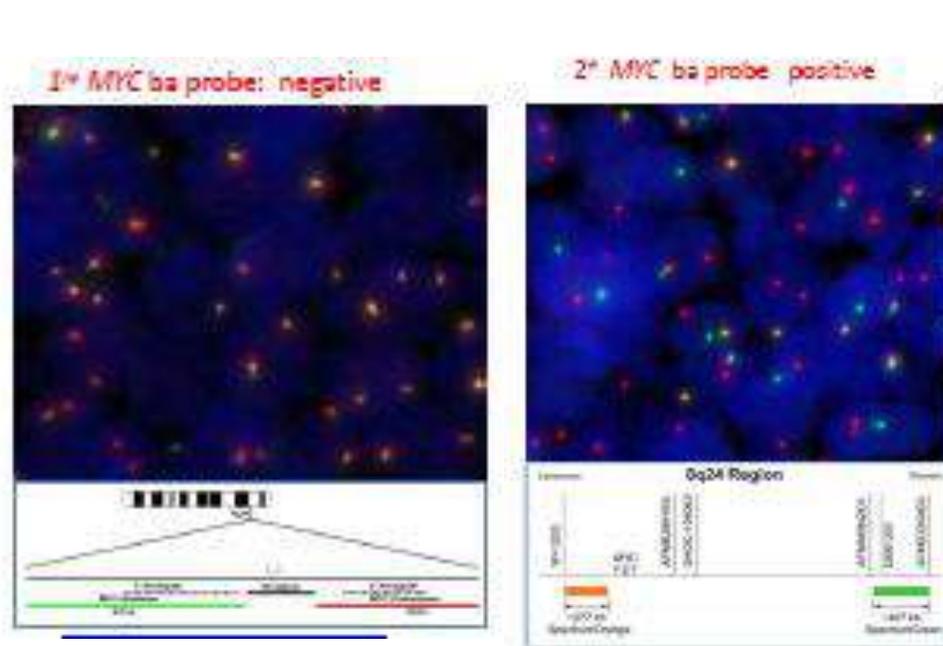


CD10 +

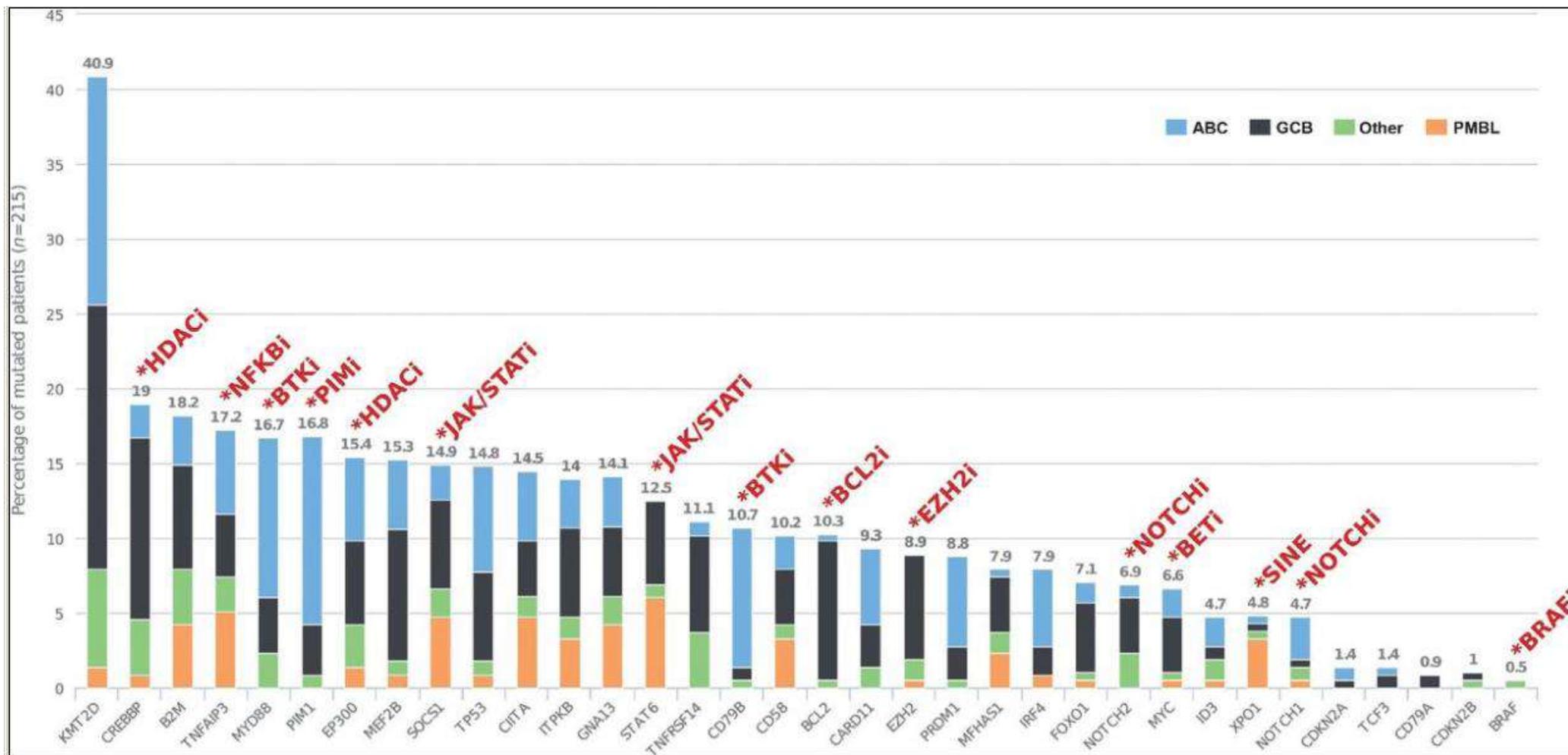


CD10 -

# FISH (*BCL2*, *BCL6* et *MYC*) : intérêt diagnostique



# Séquençage exome/« whole genome » des DLBCL : intérêt diagnostique et théranostique



Dubois S, Clin Cancer Res 2016



UBFC





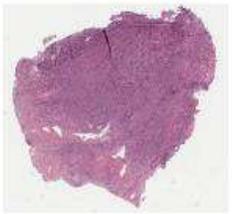
# Bilan

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RT3 (phase 1) : 85% cas complet < 38j

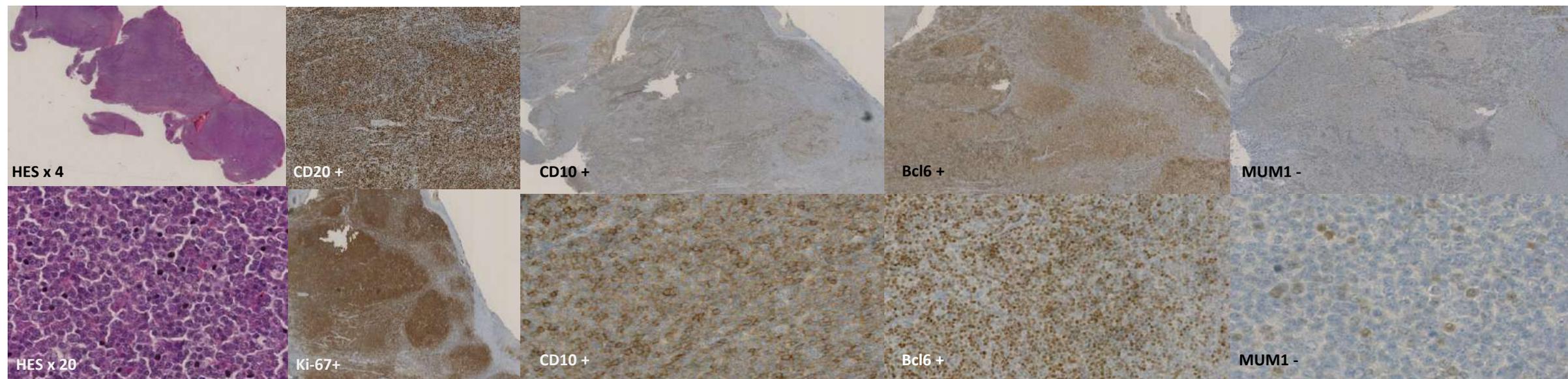
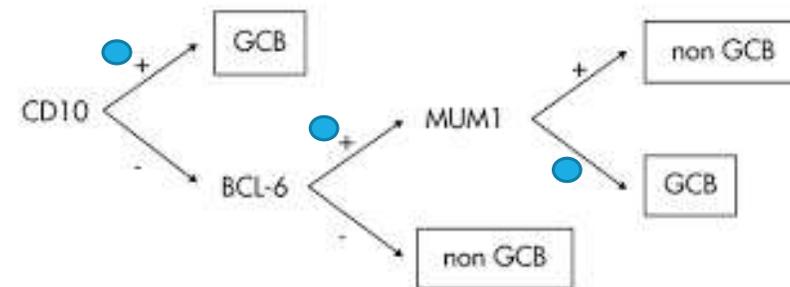
CHU Dijon (2018)

- 43 cas CHU / 7 cas hors CHU (Lymphopath)
- 43 biopsies / 7 exérèses chirurgicales (14%)
- 24 ganglions / cavum (48%), 6 cerveaux, 6 organes, 5 os / pbo, 4 médiastin / thymus, 4 sous cutanés, 1 palais
- 44 DLBCL, 5 PMBL, 1 B riche en T
- 10 cas (20%) bloc épuisé (pas de FISH et RT-MLPA)
- 15 cas (30%) FISH et/ou RT-MLPA non contributive





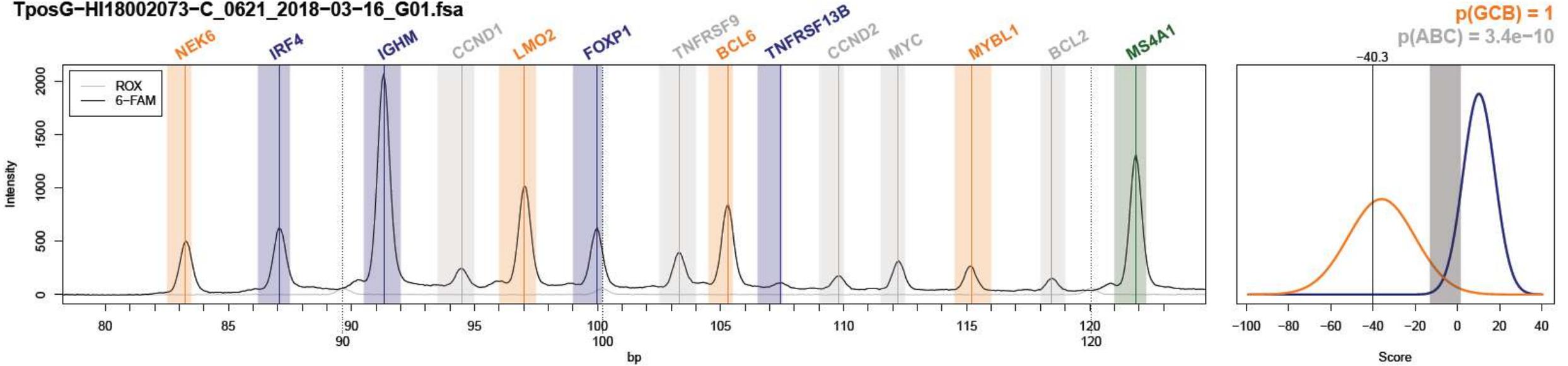
HI14003608 – cas n°1



**Sous type GC**

# HI14003608 – cas n°1 COO RT MLPA

TposG-HI18002073-C\_0621\_2018-03-16\_G01.fsa



Sous type GC



# Lymphopanel : NGS 'capture' / séquençage illumina

## Cas 1 (HI140003608) (sous-type GCB)

The screenshot displays the Lymphopanel NGS analysis interface. The top navigation bar includes 'PROJECT Interpretation 1', 'SAMPLE #200092822 PM14001622 < 1/16 >', 'RUN 03/08/2018 NextSeq\_Lymphopanel\_Run1', and 'PM14001622 S1 --'. The user 'Caroline Chapusot' is logged in. The interface shows tabs for 'Overview', 'OncoPortal', and 'Variants'. A 'Low Coverage' warning is present. The 'Variants' tab is active, showing a variant list sorted by VF%. The variant list includes two entries:

P	...	P...	★	!	T...	Gene	Coding consequence	c.DNA	Protein	VF%	Depth	altNum	ref	alt	Chr...	Position in Exon	ClinVar ...	SIFT	POLYPHEN2	GERP	dbSNP	ExAC
B						PAX5	missense	c.4G>C	p.Asp2His	61.4	10018	6035	C	G	9	-4		0.999	0.455	6.08		
B						CIITA	missense	c.2342_2345de...	p.Ser781_Val782delins...	47.4	23699	11450	CGGT	TGGC	16	-316						

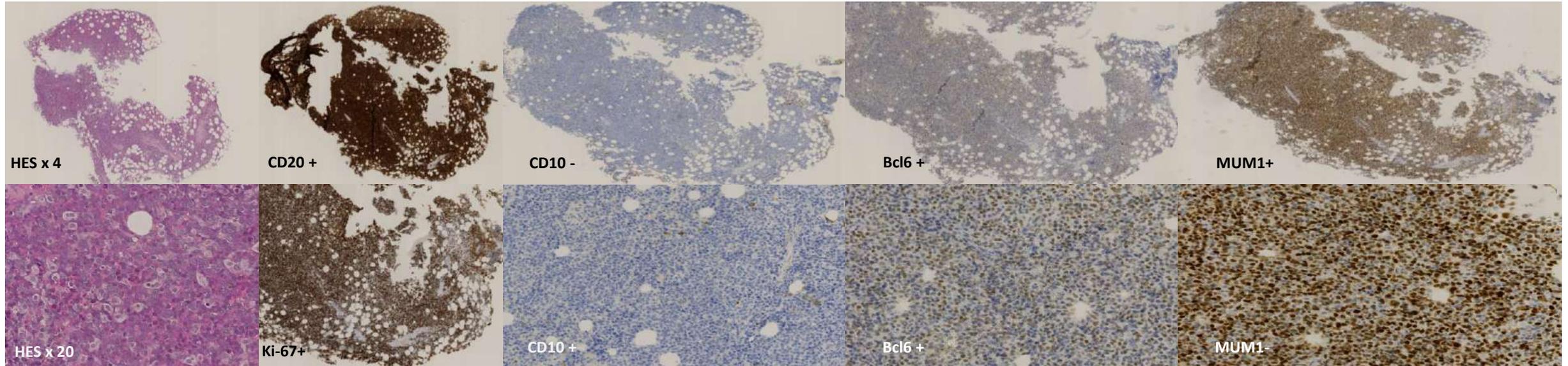
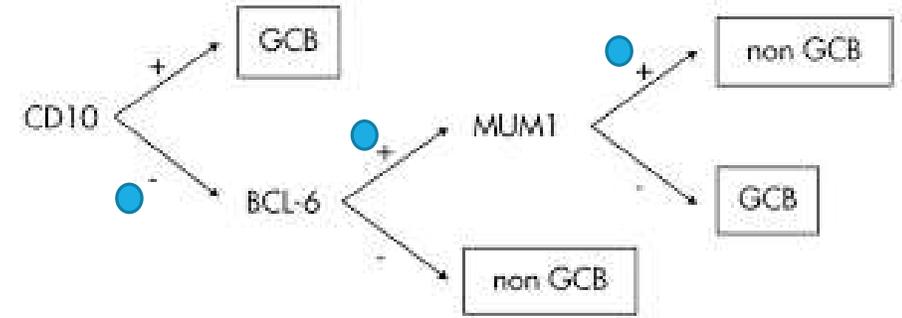
- Absence de mutations spécifiques de sous-type DLBCL-ABC / GCB
- Présence de mutations *CIITA* (GCB ou non-GC) et *PAX5* (inhabituel dans les DLBCL)

**CIITA** encodes MHC class II transcriptional transactivator  
**PAX5** = transcription factor.





HI16000264 – cas n°2

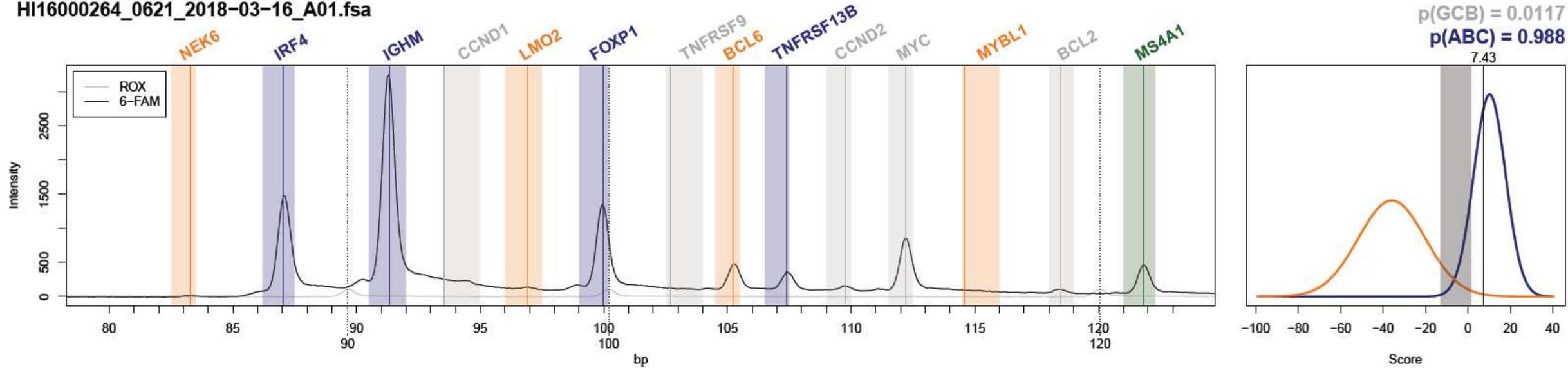


**Sous type NGC**



# HI16000264 – cas n°2 COO RT MLPA

HI16000264\_0621\_2018-03-16\_A01.fsa



Sous type ABC



# Lymphopanel : NGS 'capture' / séquençage illumina

## Cas 2 (HI160000264) non-GC

SNVs/INDELs																	Interpretation Scope					
																	LYMP_DIJ_v1					
Variant List - sorted by: VF%																	save column preferences					
P...	P...	★	⚠	T...	Gene	Coding consequence	c.DNA	Protein	VF%	Depth	alt/lum	ref	alt	Chr...	Position in E...	ClinVar ...	SIFT	POLYPHEN2	GERP	dbSNP	ExAC	
A					PRDM1	frameshift	c.474_486delGATGCGCTATGTG	p.Trp158*fs*1	80.0	2001	1334	CTGGA...	C	6	63							
B					PRDM1	missense	c.83G>A	p.Gly28Glu	78.5	2414	1896	G	A	6	41		0.905	0.0	-0.875			
C					PIM1	synonymous	c.969G>A	p.= (p.Leu323Leu)	78.0	2089	1630	G	A	6	89							
A		★			CD79A	splice_acceptor_cd...	c.568-2_610del	p.?	70.0	6184	3121	ATCCC...	A	19	0							
B					PLCG2	missense	c.1916A>G	p.Asn639Ser	49.7	4885	2428	A	G	16	-19		0.903	0.017	2.69	rs377753289	1.0E-4	

*PRDM1 p.Trp158\*fs ; p.Gly28Glu (argument en faveur d'un DLBCL non-GC)*

*PIM1 (synonymous) – non rapporté.*

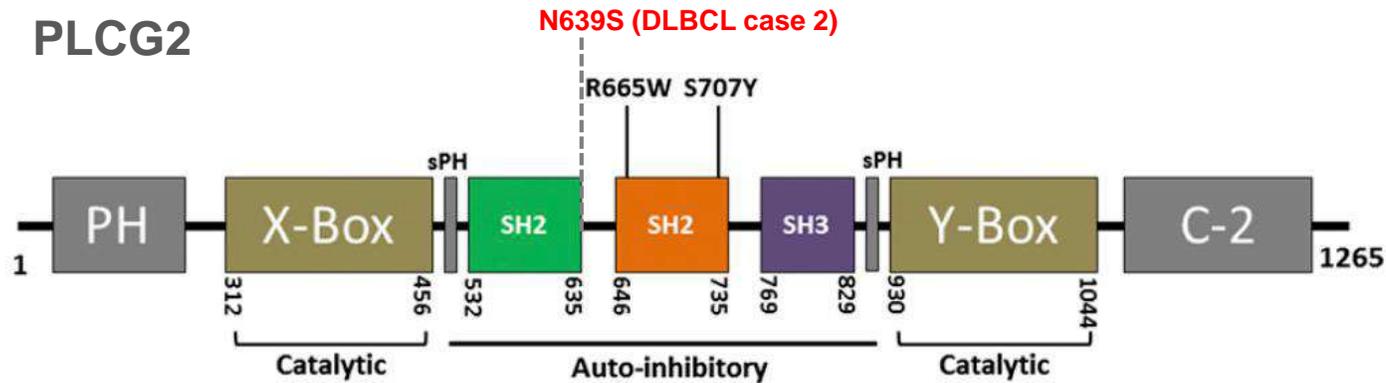
*CD79A (splice acceptor site) - GC*

*PLCG2 p.Asn639Ser – variant constitutionnel rare ou événement somatique ?*



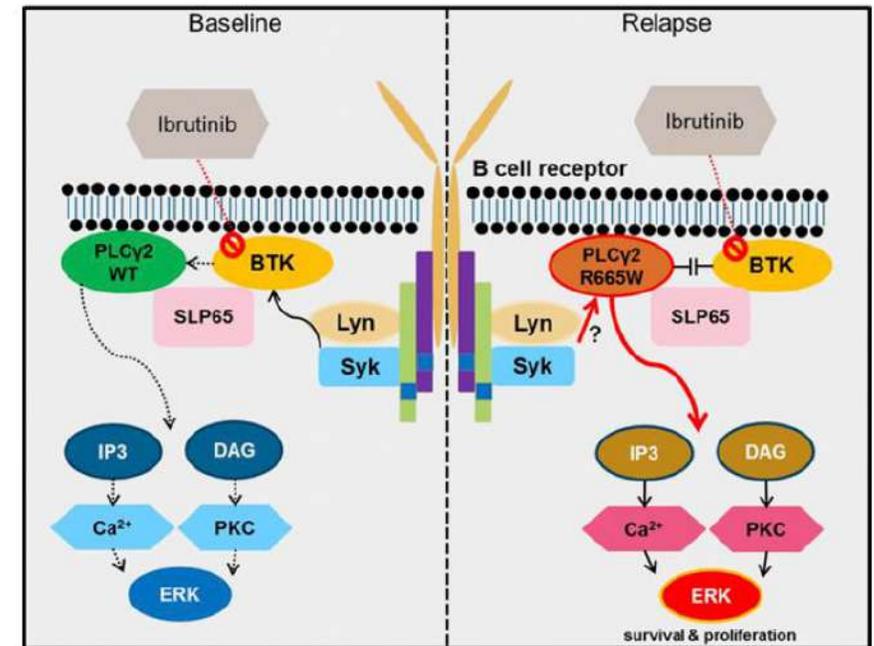
# Cas 2 (HI160000264) non-GC

## Signification fonctionnelle du variant PLCG2 p.Asn639Ser ?



Quelle recommandation au RCP moléculaire ?  
 Explorer la nature somatique versus constitutionnelle ?

*iBTK resistance in CLL : p.Arg665Trp / R665W*



**Figure 6.** The diagram illustrates PLCG2<sup>R665W</sup>-mediated ibrutinib resistance. In treatment naïve CLL, proximal BCR signaling triggers downstream BTK activation. PLCγ2 is consequently activated in a BTK-dependent manner. Targeting BTK by ibrutinib can abrogate PLCγ2-initiated downstream survival signal (left panel); in contrast, mutant PLCγ2 (R665W) can be activated via SYK or LYN, bypassing BTK dependency in the resistant CLL, thereby propagating downstream survival signals despite ibrutinib treatment (right panel). PKC, protein kinase C.

Liu et al, Blood 2015.



ORIGINAL ARTICLE

## Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma

R. Schmitz, G.W. Wright, D.W. Huang, C.A. Johnson, J.D. Phelan, J.Q. Wang, S. Roulland, M. Kasbekar, R.M. Young, A.L. Shaffer, D.J. Hodson, W. Xiao, X. Yu, Y. Yang, H. Zhao, W. Xu, X. Liu, B. Zhou, W. Du, W.C. Chan, E.S. Jaffe, R.D. Gascoyne, J.M. Connors, E. Campo, A. Lopez-Guillermo, A. Rosenwald, G. Ott, J. Delabie, L.M. Rimsza, K. Tay Kuang Wei, A.D. Zelenetz, J.P. Leonard, N.L. Bartlett, B. Tran, J. Shetty, Y. Zhao, D.R. Soppet, S. Pittaluga, W.H. Wilson, and L.M. Staudt

- Génomique des DLBCL en évolution constante. Mutations, transcriptome, épigénétique...avec impact clinique.

- Défi pour le déploiement dans les soins  
Coûts ?

RCP génomique ?

Recommandations en temps réels ...

## Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes

Bjoern Chapuy<sup>1,2,18</sup>, Chip Stewart<sup>3,18</sup>, Andrew J. Dunford<sup>3,18</sup>, Jaegil Kim<sup>3</sup>, Atanas Kamburov<sup>3</sup>, Robert A. Redd<sup>4</sup>, Mike S. Lawrence<sup>2,3,5</sup>, Margaretha G. M. Roemer<sup>1</sup>, Amy J. Li<sup>6</sup>, Marita Ziepert<sup>7</sup>, Annette M. Staiger<sup>8,9</sup>, Jeremiah A. Wala<sup>3</sup>, Matthew D. Ducar<sup>10</sup>, Ignaty Leshchiner<sup>3</sup>, Ester Rheinbay<sup>3</sup>, Amaro Taylor-Weiner<sup>3</sup>, Caroline A. Coughlin<sup>1</sup>, Julian M. Hess<sup>3</sup>, Chandra S. Pedamallu<sup>3</sup>, Dimitri Livitz<sup>3</sup>, Daniel Rosebrock<sup>3</sup>, Mara Rosenberg<sup>3</sup>, Adam A. Tracy<sup>3</sup>, Heike Horn<sup>8</sup>, Paul van Hummelen<sup>10</sup>, Andrew L. Feldman<sup>11</sup>, Brian K. Link<sup>12</sup>, Anne J. Novak<sup>11</sup>, James R. Cerhan<sup>11</sup>, Thomas M. Habermann<sup>11</sup>, Reiner Siebert<sup>13</sup>, Andreas Rosenwald<sup>14</sup>, Aaron R. Thorner<sup>10</sup>, Matthew L. Meyerson<sup>2,3</sup>, Todd R. Golub<sup>2,3</sup>, Rameen Beroukhi<sup>2,3</sup>, Gerald G. Wulf<sup>15</sup>, German Ott<sup>9</sup>, Scott J. Rodig<sup>2,16</sup>, Stefano Monti<sup>4</sup>, Donna S. Neuberg<sup>2,4</sup>, Markus Loeffler<sup>7</sup>, Michael Pfreundschuh<sup>17</sup>, Lorenz Trümper<sup>15</sup>, Gad Getz<sup>2,3,5,19\*</sup> and Margaret A. Shipp<sup>1,2,19\*</sup>



# Recommandations Françaises sur les panel de gènes NGS des hémopathies lymphoïdes matures



## Définition d'un panel minimal de gènes pour la prise en charge des hémopathies lymphoïdes matures

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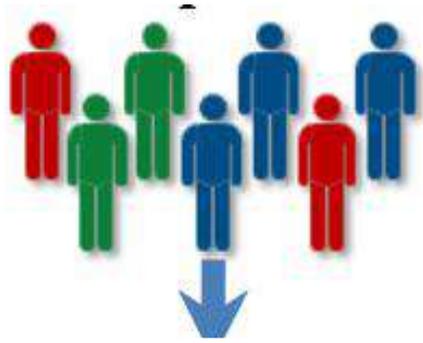
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Etudes médico-économiques

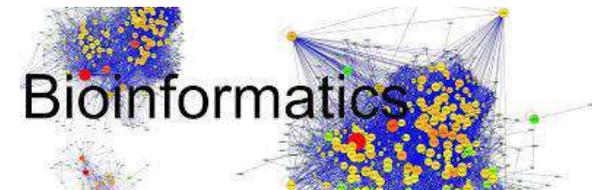
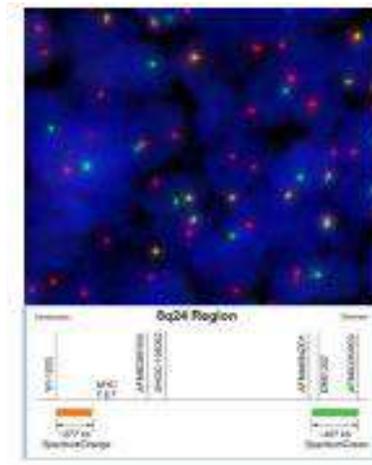
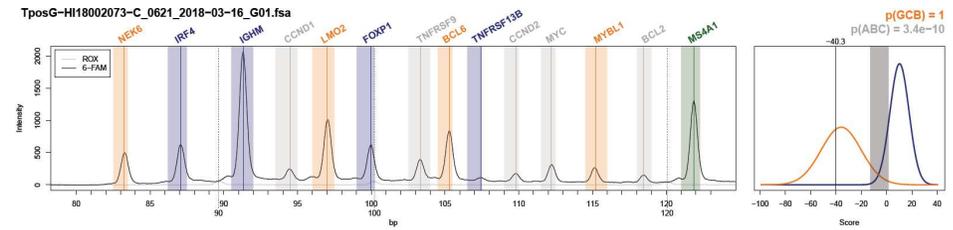
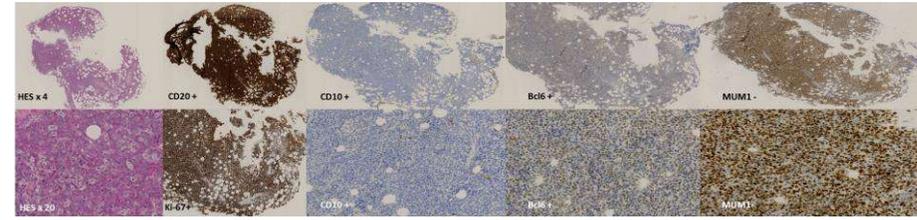
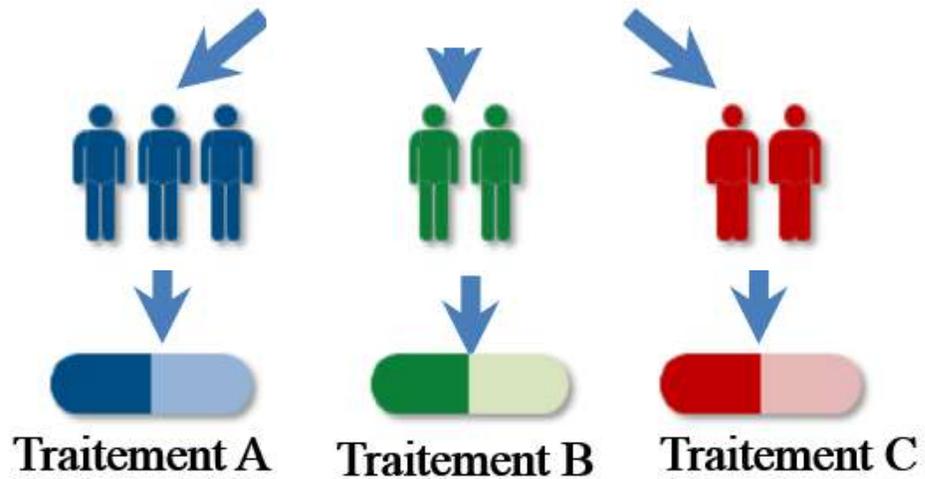
Programme PRME NGS en onco-hématologie  
E Macintyre / C Preudhomme





# Caractérisation histo-moléculaire

## Compte rendu intégré



# Conclusions / Perspectives

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- Diagnostic histo-moléculaire intégré
- Identification de cibles moléculaires « actionnables »
- Mise en place d'une RCP moléculaire
- Extension à d'autres types de lymphome (FL, MCL, MZL,..)
- Place de l'exome / WGS ?
- Génomique intégré ? (MRD, épigénétique,...)
- Importance critique de la bioinformatique oncologique



# Remerciements

## Service de pathologie

Laurent Martin  
Selim Ramla  
Benjamin Tournier  
Caroline Chapusot

## Unité d'innovation en génétique et épigénétique oncologique

Mary Callanan  
Caroline Chapusot  
Sylviane Ragot  
Cyril Fournier Bioinformaticien

## Service d'hématologie

### Biologie

Marc Maynadié  
Julien Guy  
Francois Bailly

### Clinique

Olivier Casasnovas  
Cédric Rossi  
Marie Lorrain Chrétien  
Jean Noel Bastie  
Denis Caillot



## INSERM 1231 Lipides, Nutrition, Cancer (Carmen GARRIDO, DRE)

Labex Lipstic

Hématopoïèse normal et leucémique

Laurent Delva DR

Romain Aucagne Post-doc

Benedicte Burlet M2R

Jean Noel Bastie

Mary Callanan

