

Workshop Alger, mai 2007

Cytométrie et Hémopathies malignes

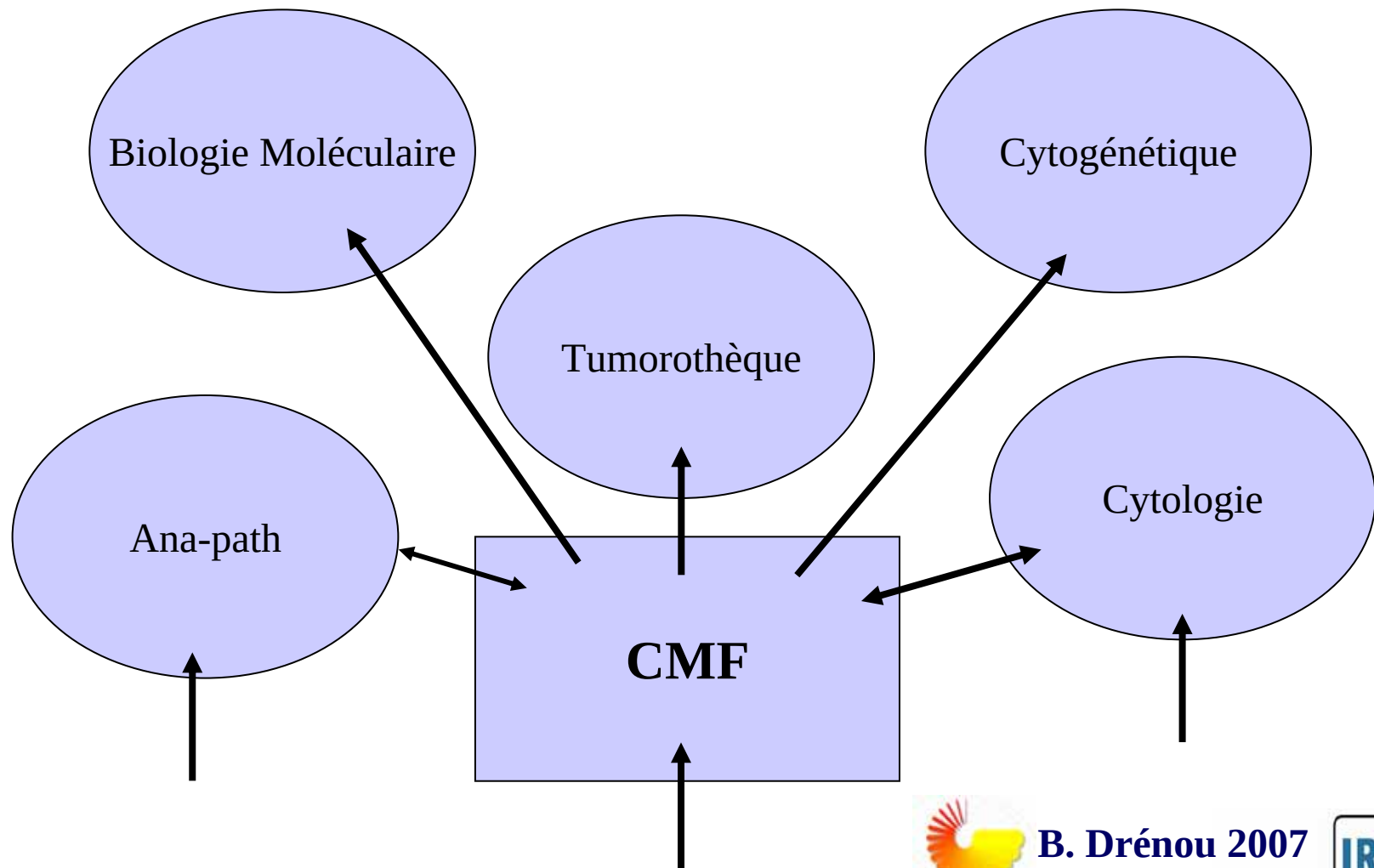


Bernard Drénou
Hématologie Mulhouse



Institut de Recherche en Hématologie et Transplantation
Mulhouse - France

Position de la CMF



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Méthodologies

- **Ficoll « rapide »** (avec « filtre»)
- **Lavage / marquage**
- **Panel orientation : 2 tubes 6 couleurs**
- **Second panel : diagnostique**
- **Cytomètre**



Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Stratégies variables selon appareillages
 - Lignée
 - Différenciation dans la lignée
 - Clonalité
 - Tumorigénicité (trou phéno. Marqueur aberrant)
 - Pronostic
 - Thérapeutique

Suivi

- Stratégies multi-couleurs



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Intérêt de la CMF dans les Hémopathies malignes

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- Lignée
- Différenciation dans la lignée
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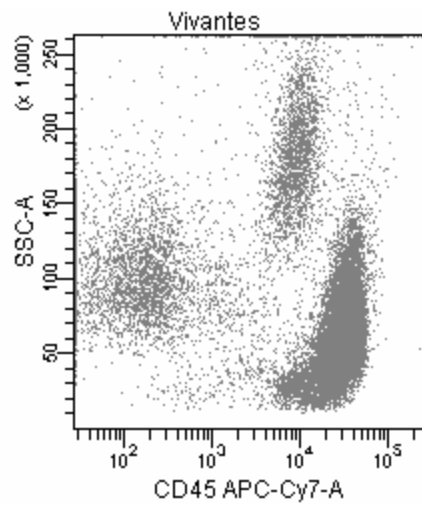
Suivi

– Stratégies multi-couleurs

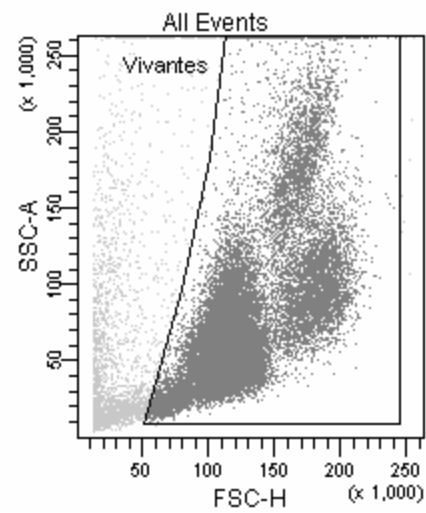


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CD45



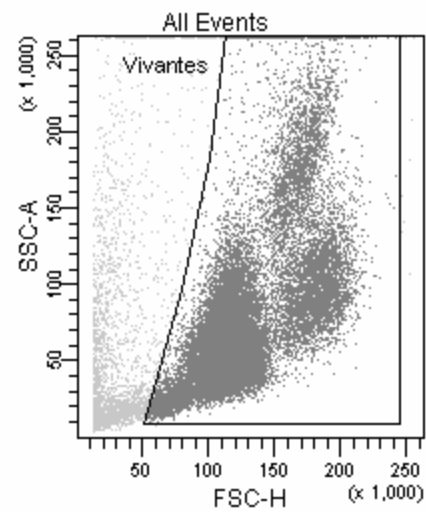
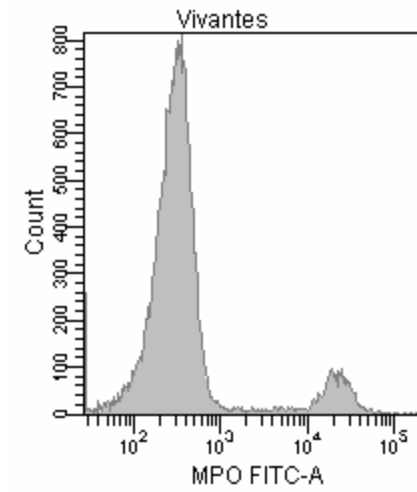
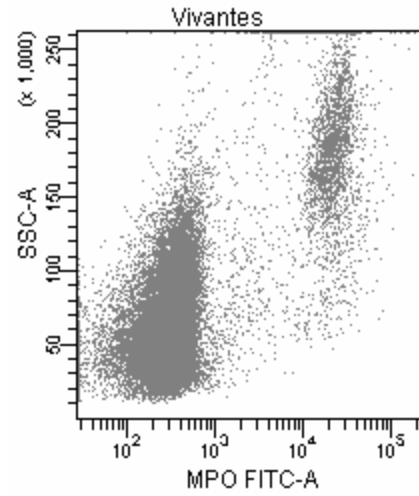
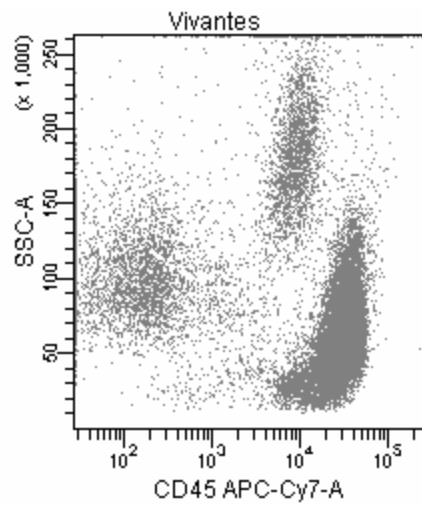
SSC

FSC



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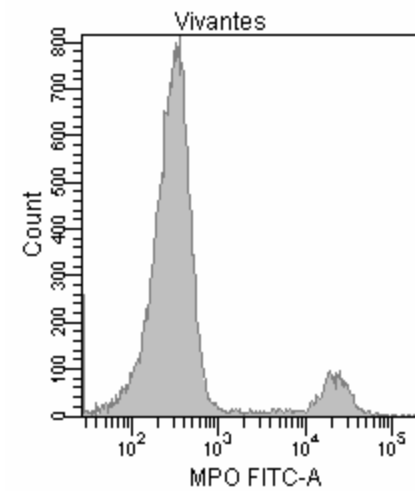
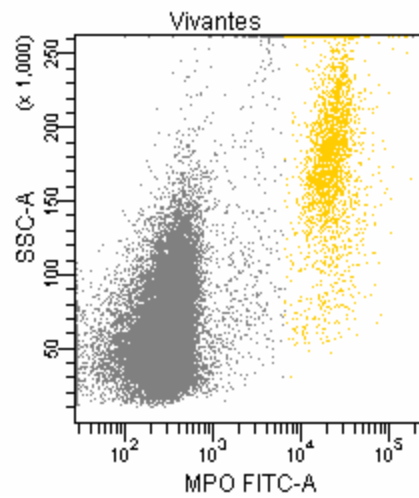
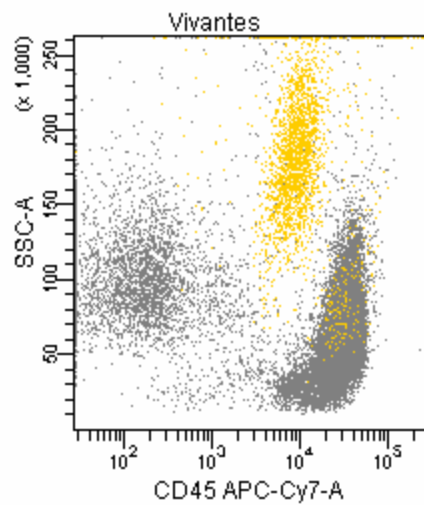


MPO

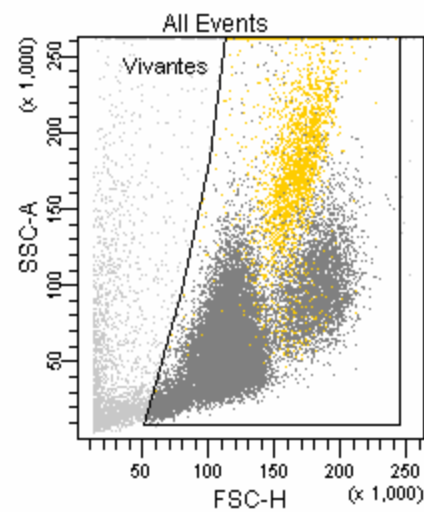


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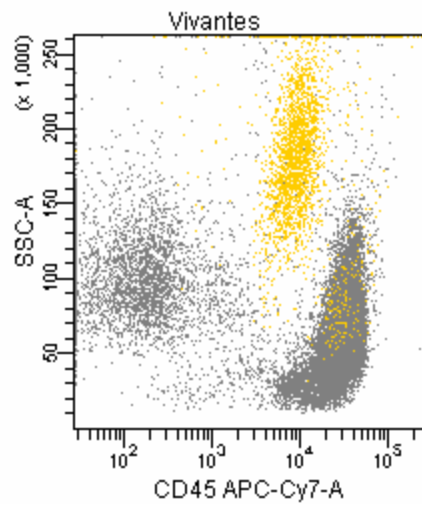


MPO

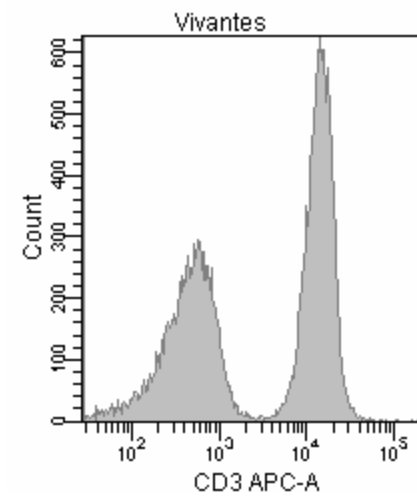
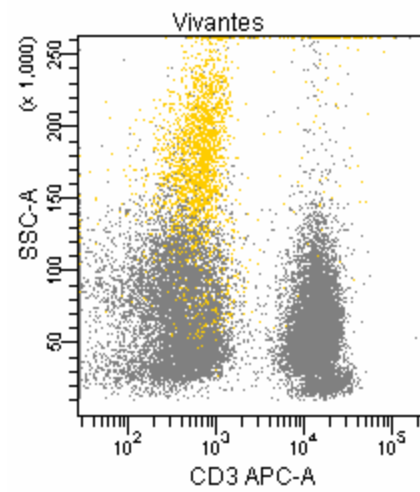
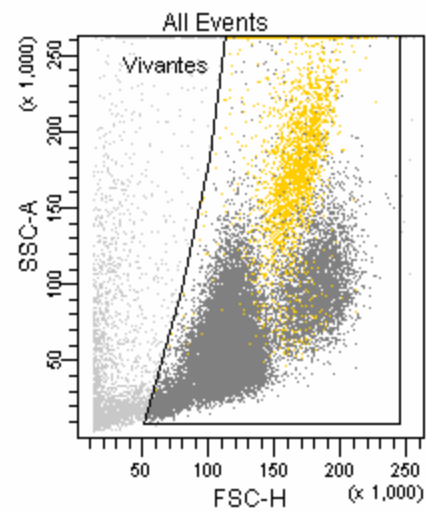


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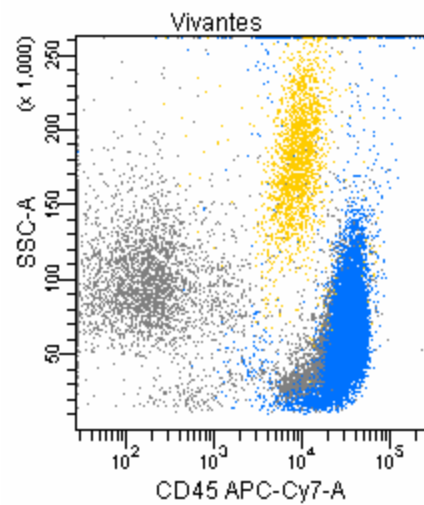


CD3

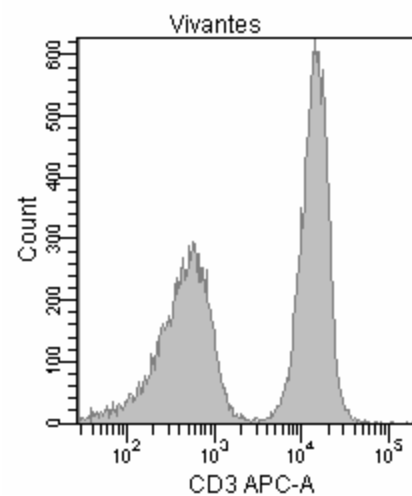
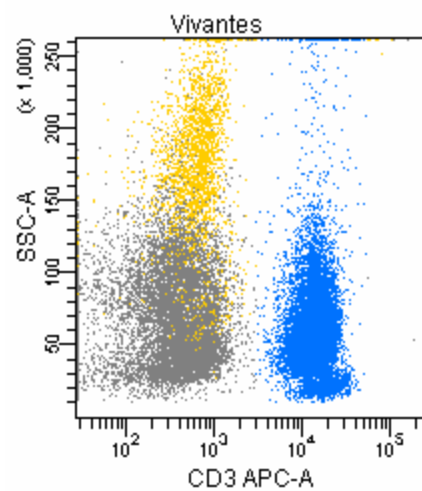
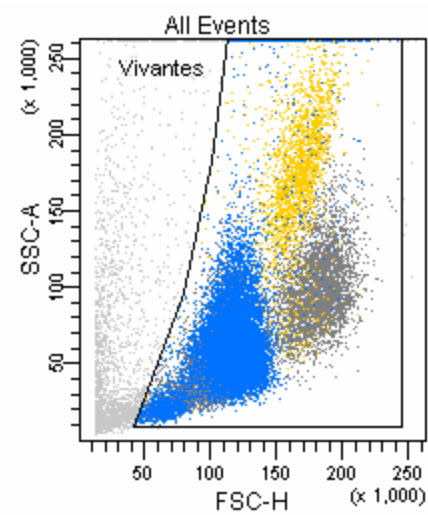


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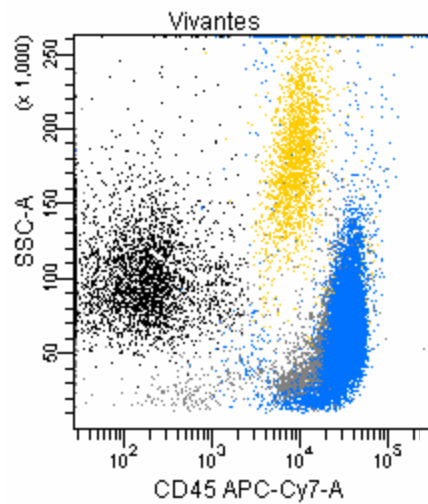


CD3

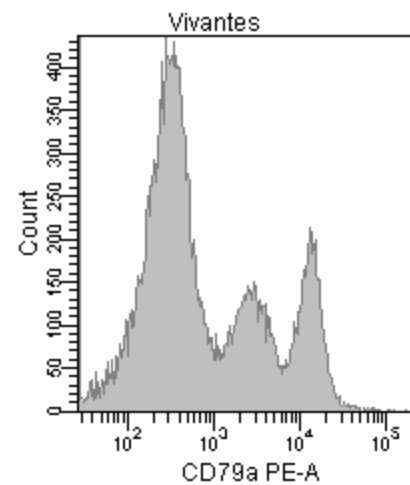
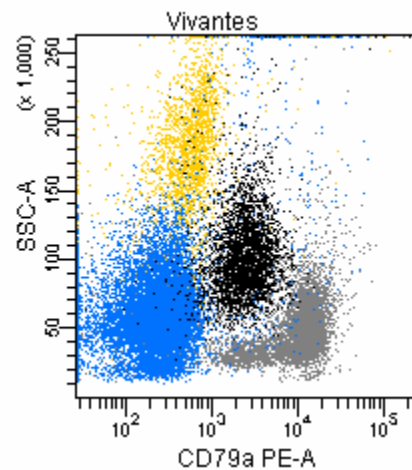
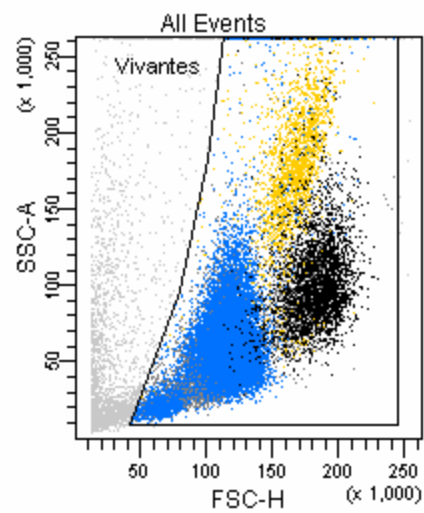


B. Drénou 2007



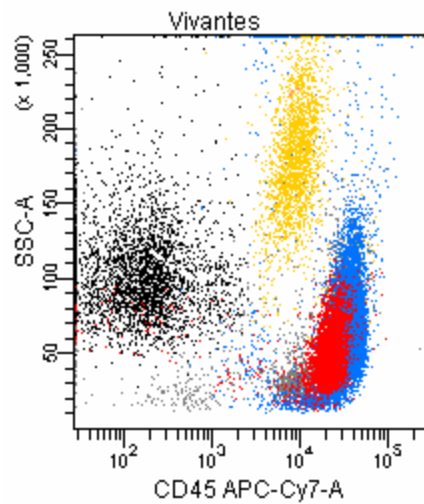


CD79a

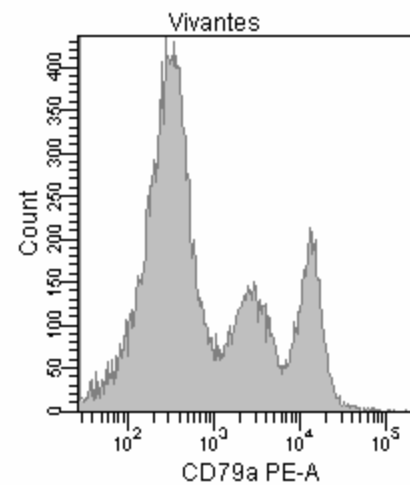
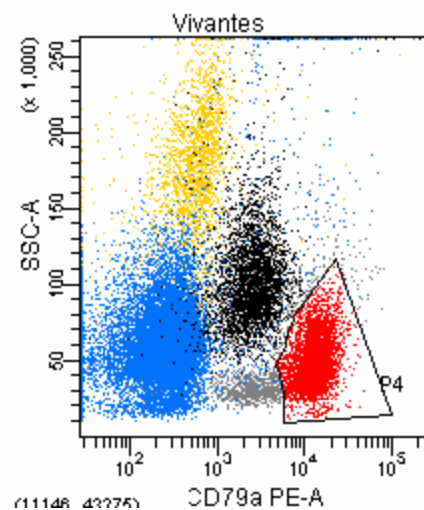
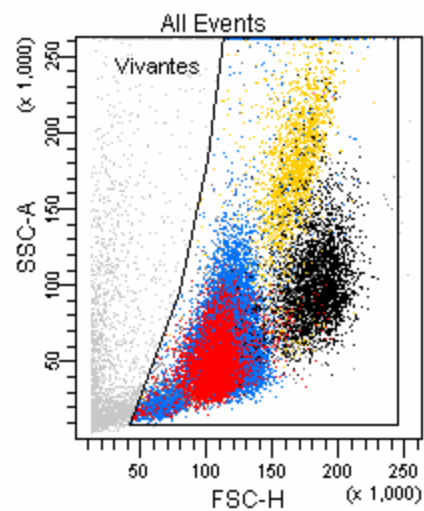


B. Drénou 2007



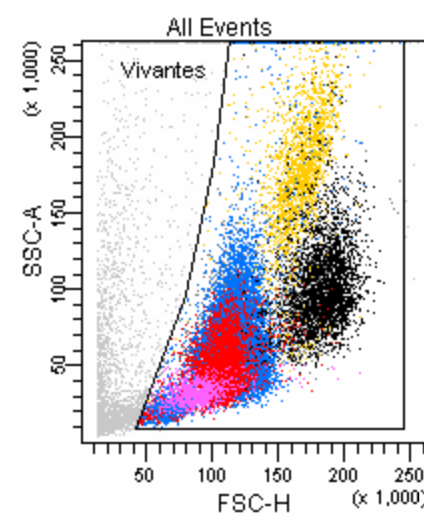
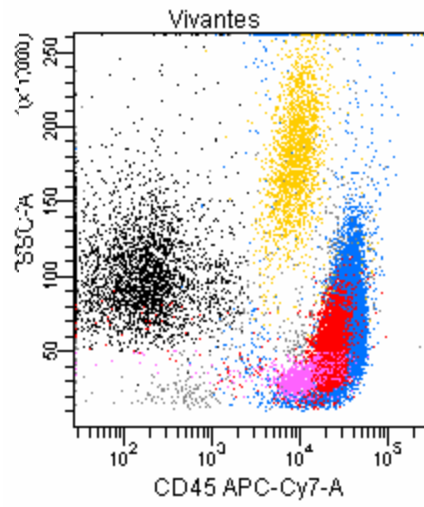


CD79a



B. Drénou 2007



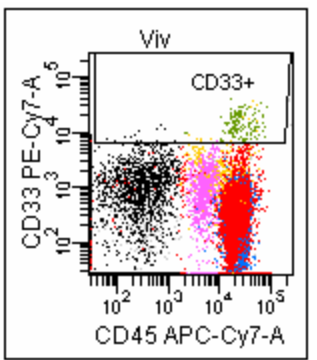
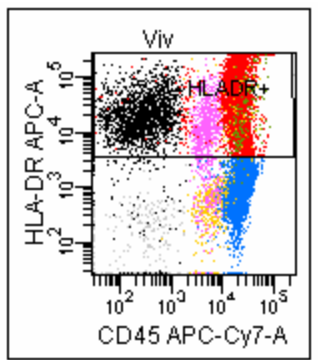
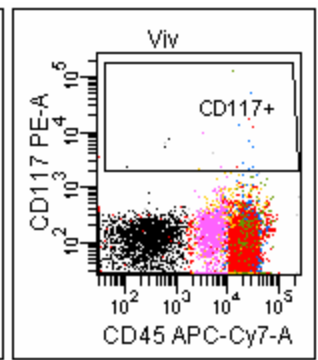
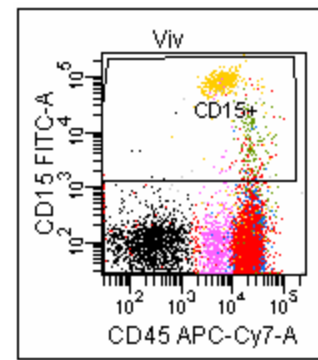


CD15

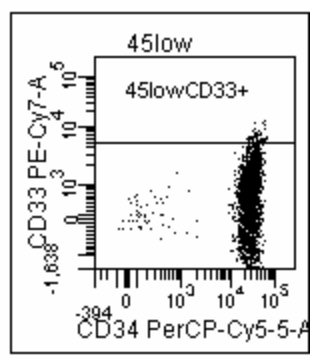
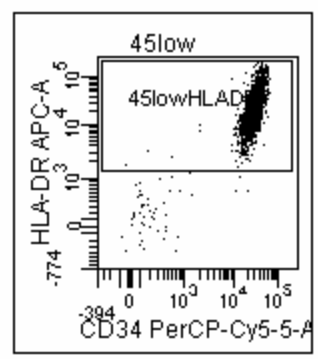
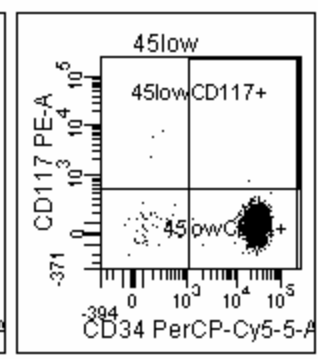
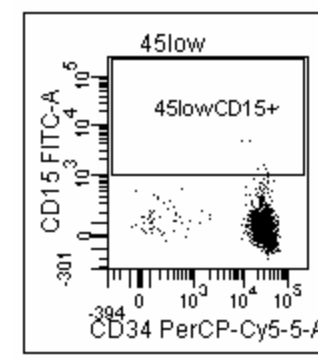
CD117

HLA-DR

CD33

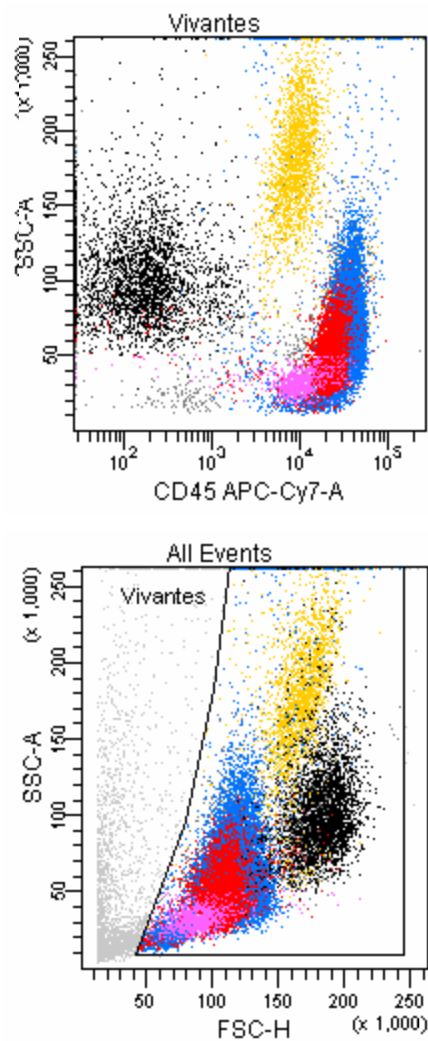


CD45 Low



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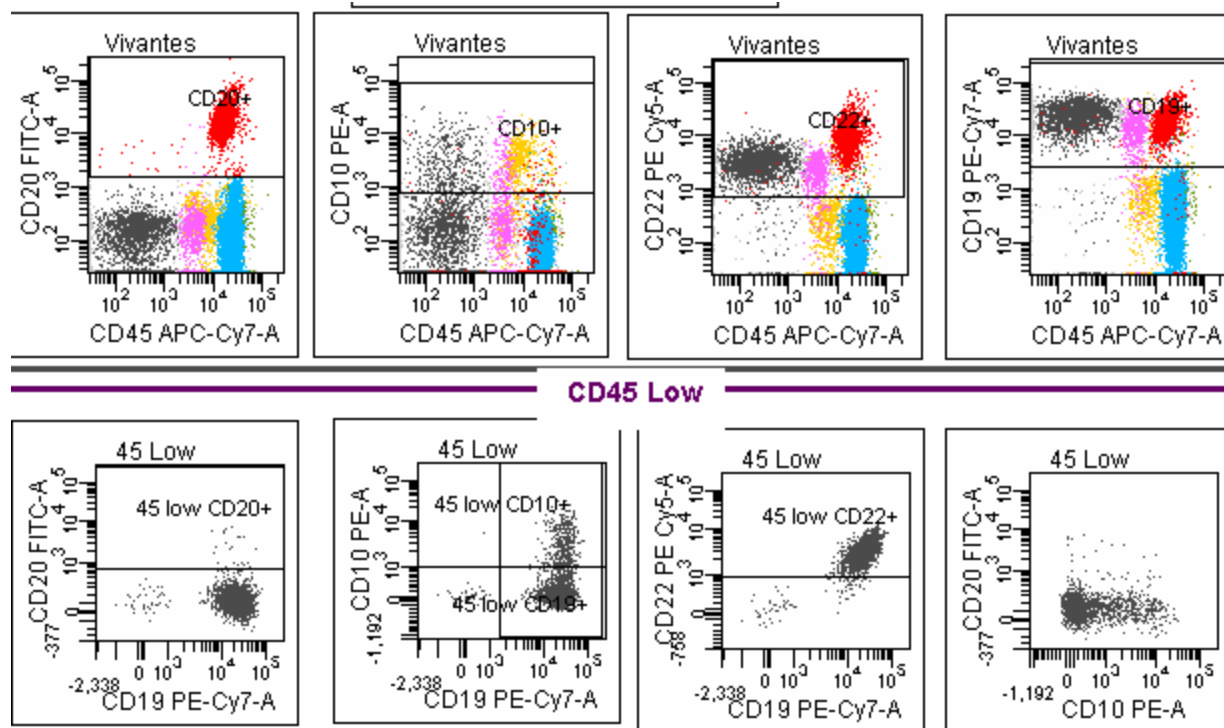


CD20

CD10

CD22

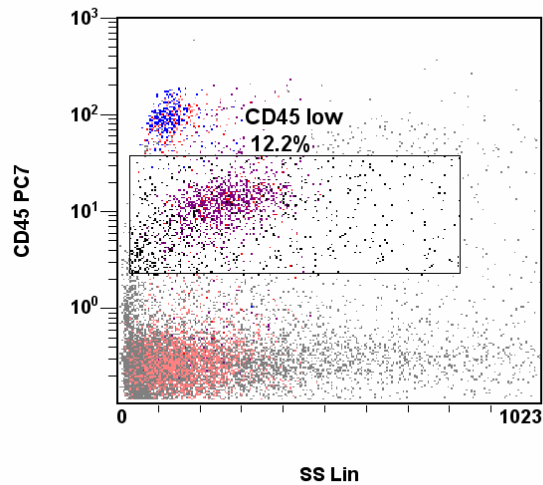
CD19



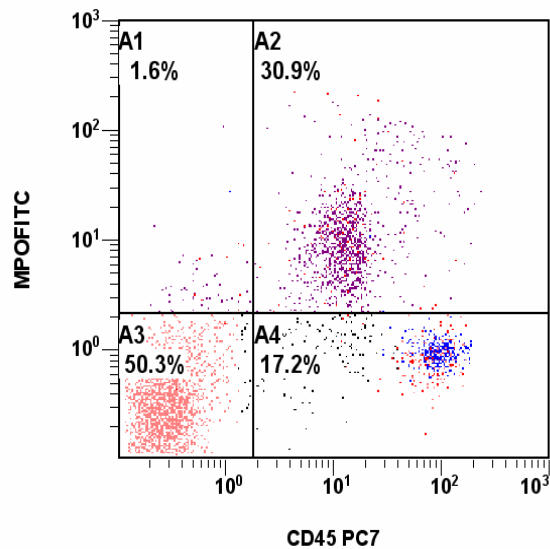
B. Drénou 2007



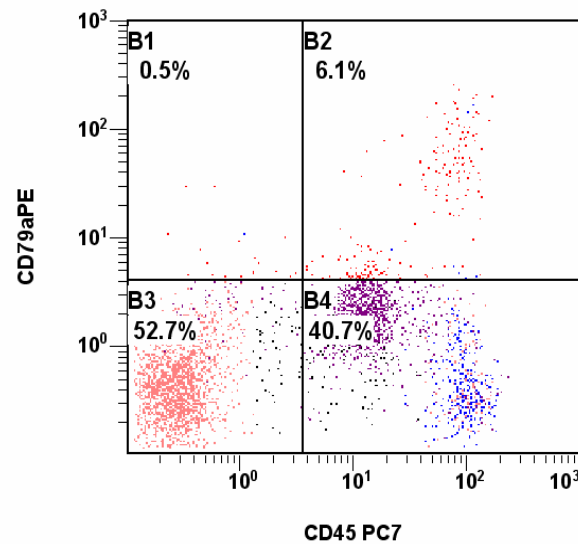
Exemple d'une LAM



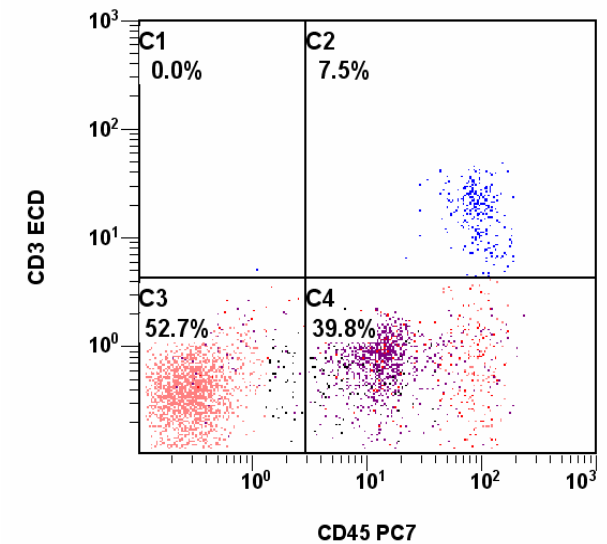
MPO



CD79a



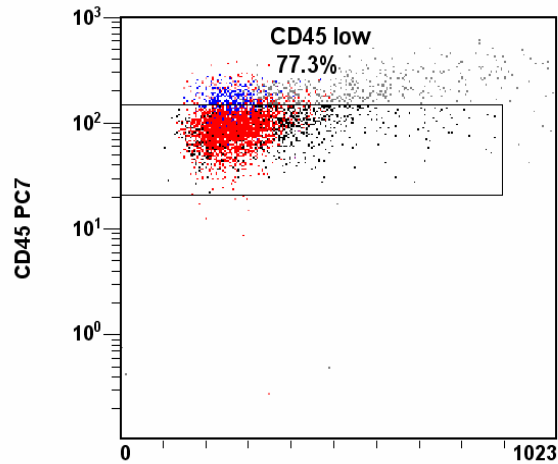
CD3



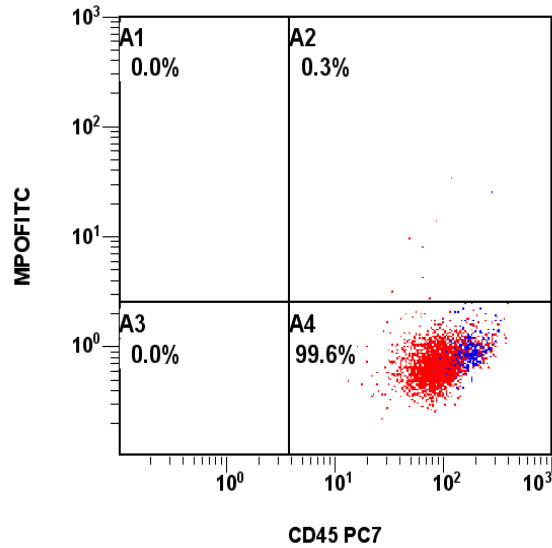
B. Drénou 2007



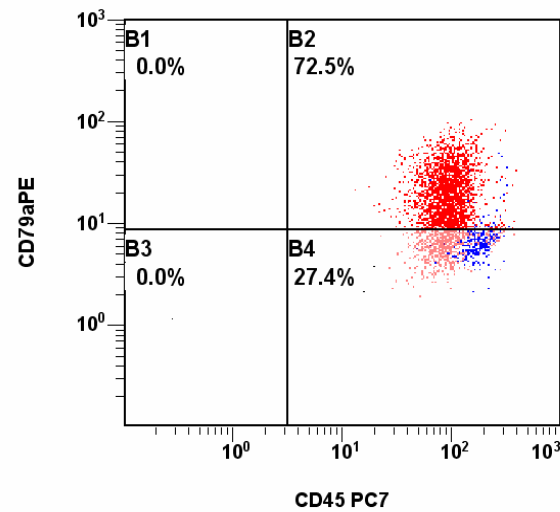
Exemple d'une LAL B



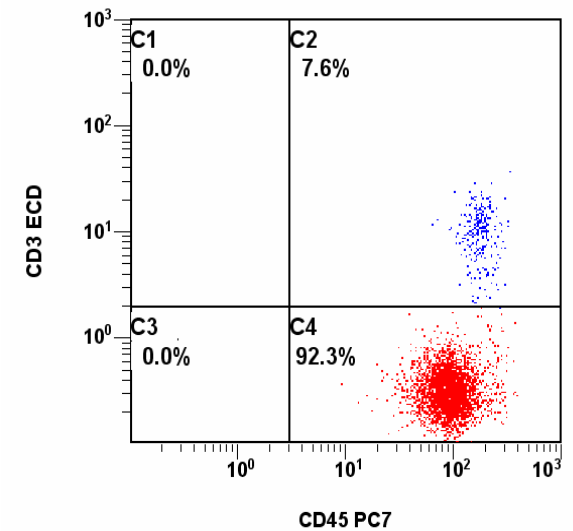
SS Lin
MPO



CD79a



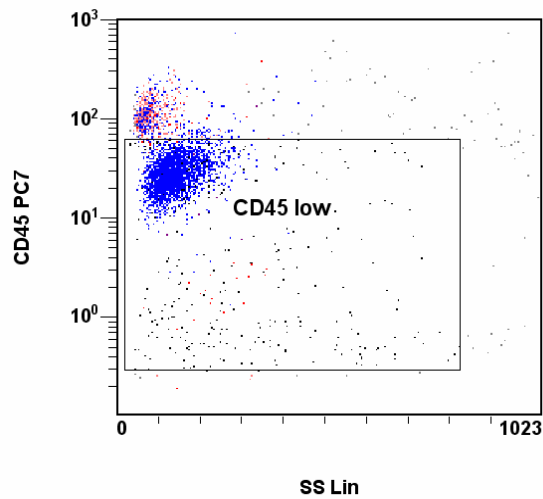
CD3



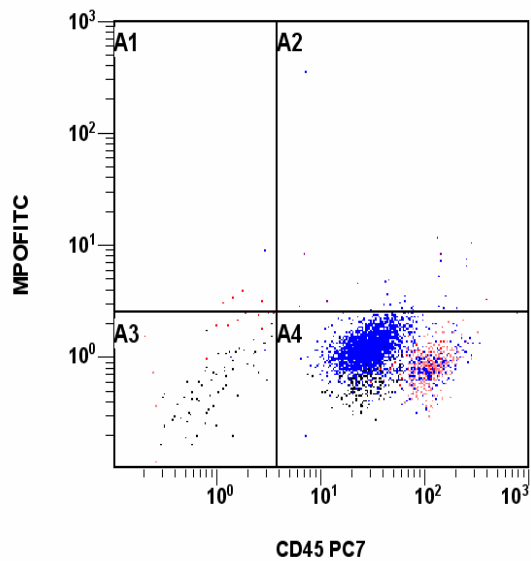
B. Drénou 2007



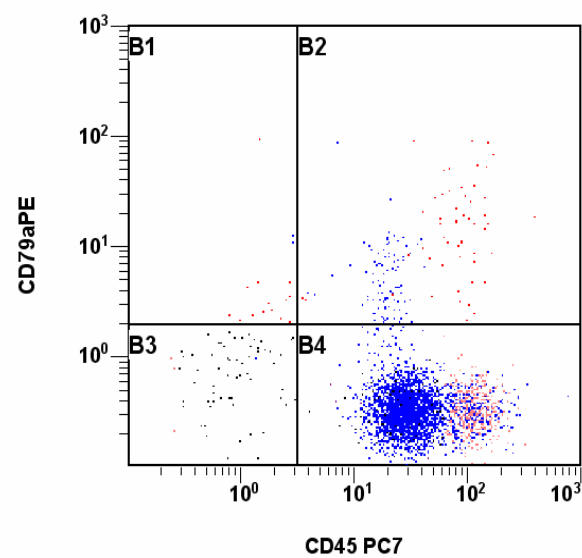
Exemple d'une LAL T



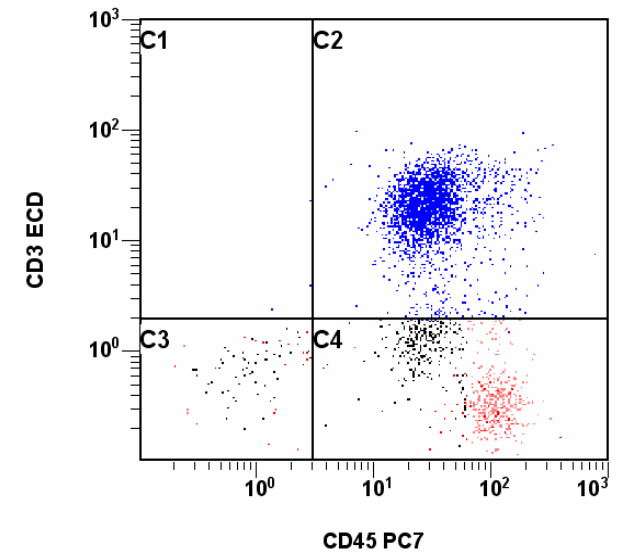
MPO



CD79a



CD3



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Autres marqueurs spécifiques de lignée:

HLA classe II (DR), CD34	Cellules souches
cytCD79a , cytCD22, CD19, CD10, sCD22, CD20, cytIgM (μ), slg	Lignée lymphocytaire B
cytCD3 , CD2, CD5, CD7, CD1a, sCD3, CD4, CD8, TCR	Lignée lymphocytaire T
Myéloperoxydase , cytCD13, CD13, CD33, CD117, CD14, CD15, CD65, CD11b, CD64	Lignée myéloïde
CD41, CD42, CD61	Lignée plaquettaire
Glycophorine A, CD36	Lignée érythroïde

Recommandation de l'EGIL et du GEIL:

présence d'au moins deux marqueurs spécifiques d'une lignée
absence d'au moins deux marqueurs des autres lignées



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leucémie biphénotypique

La **BAL** (bi-phenotypic acute leukemia)
est définie quand le score est **supérieur à 2** dans deux lignées

	Lignée B	Lignée T	Lignée myéloïde
2 points	cCD79a, cCD22, cμ	CD3, TCR	MPO, Lysozyme
1 point	CD19, CD10, CD20	CD2, CD5, CD8, CD10	CD13, CD33, CD65, CD117
0.5 point	TdT, CD24	TdT, CD7, CD1a	CD14, CD15, CD64



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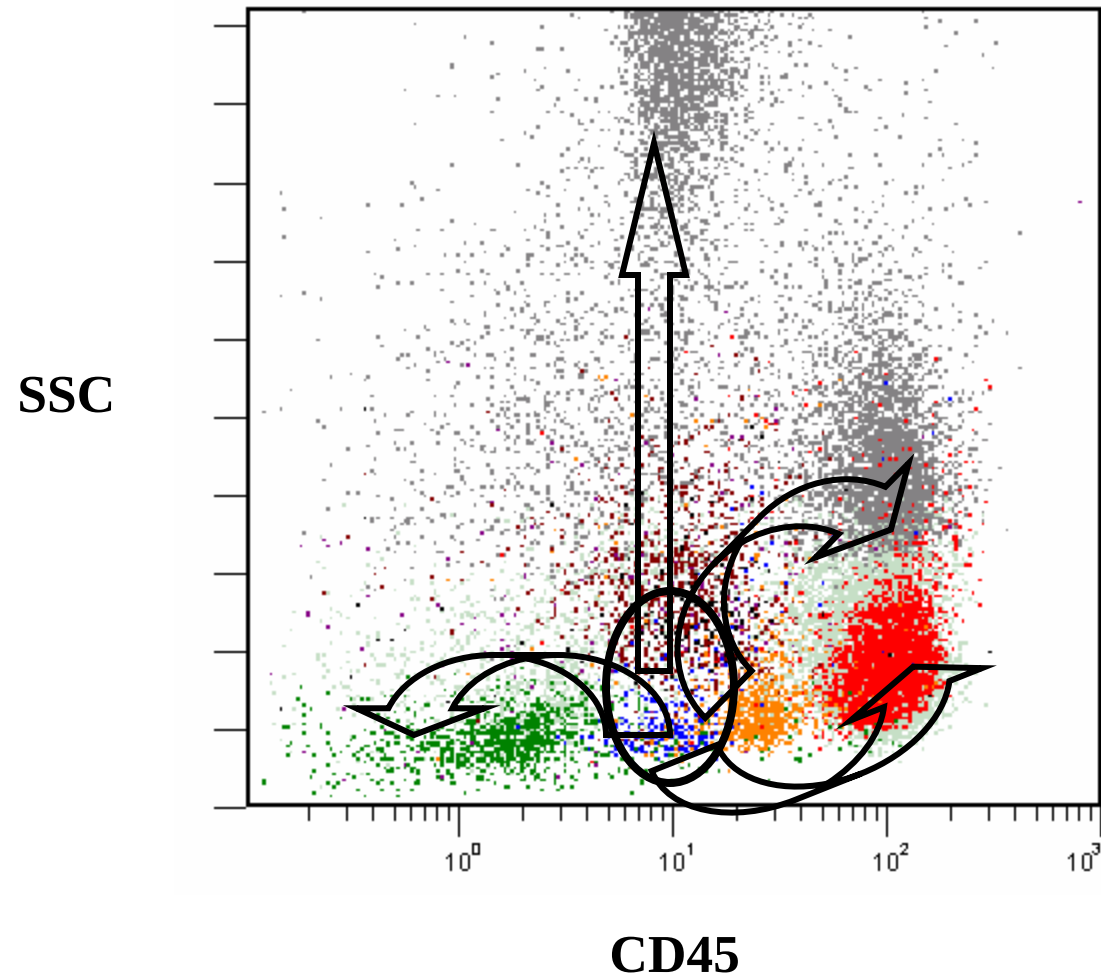
Suivi

– Stratégies multi-couleurs



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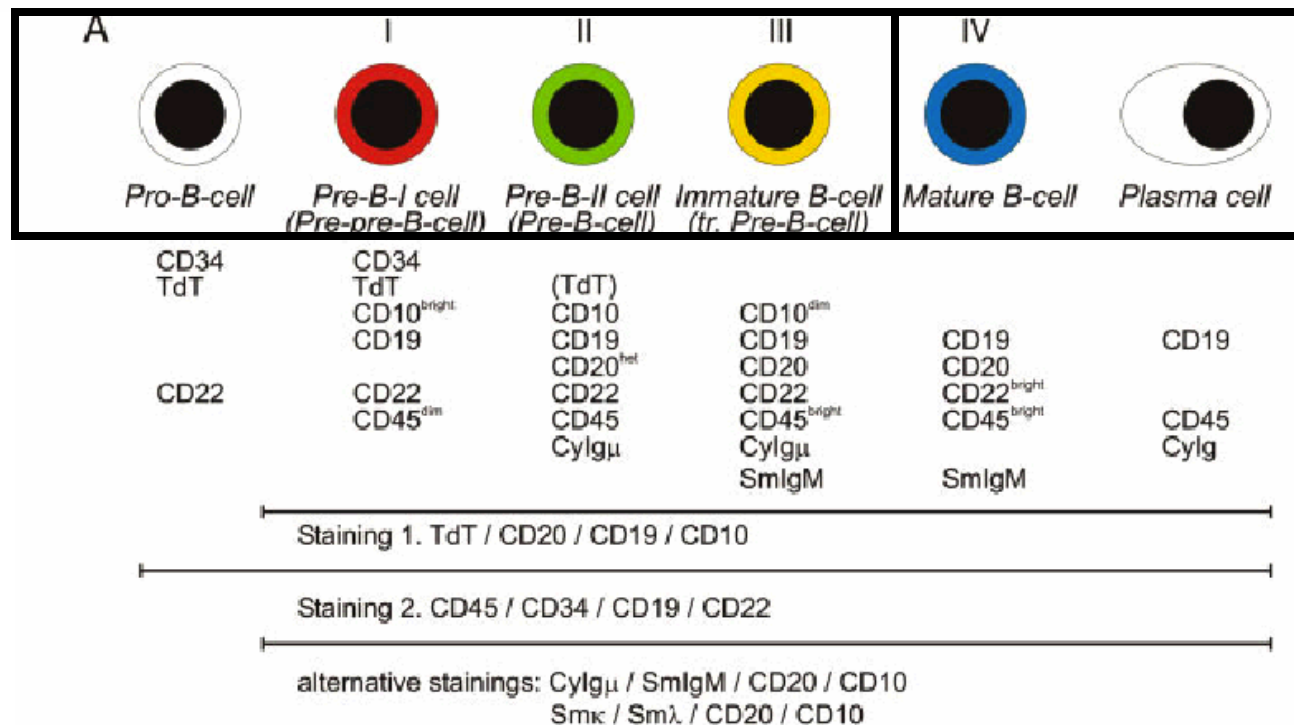


Improving Lab Practice

Immunophenotypic Differentiation Patterns of Normal Hematopoiesis in Human Bone Marrow: Reference Patterns for Age-Related Changes and Disease-Induced Shifts

E.G. van Lochem,* V.H.J. van der Velden, H.K. Wind, J.G. te Marvelde,
N.A.C. Westerdaal, and J.J.M. van Dongen

Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

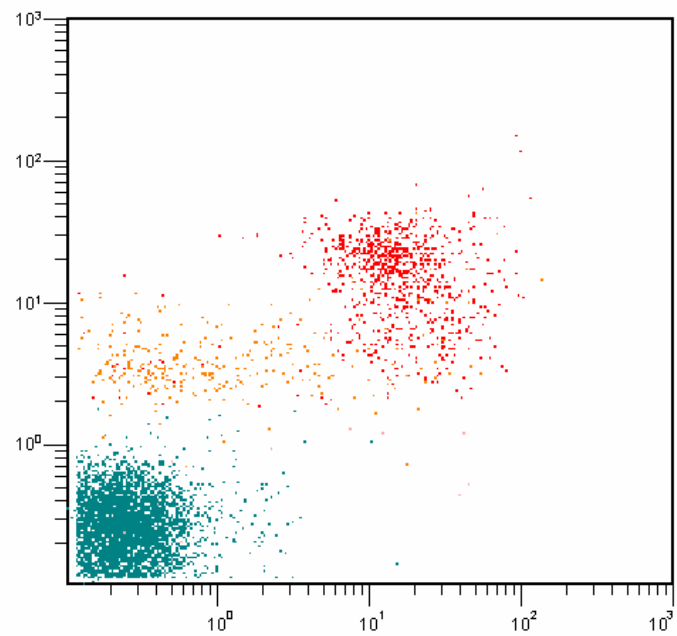


B. Drénou 2007



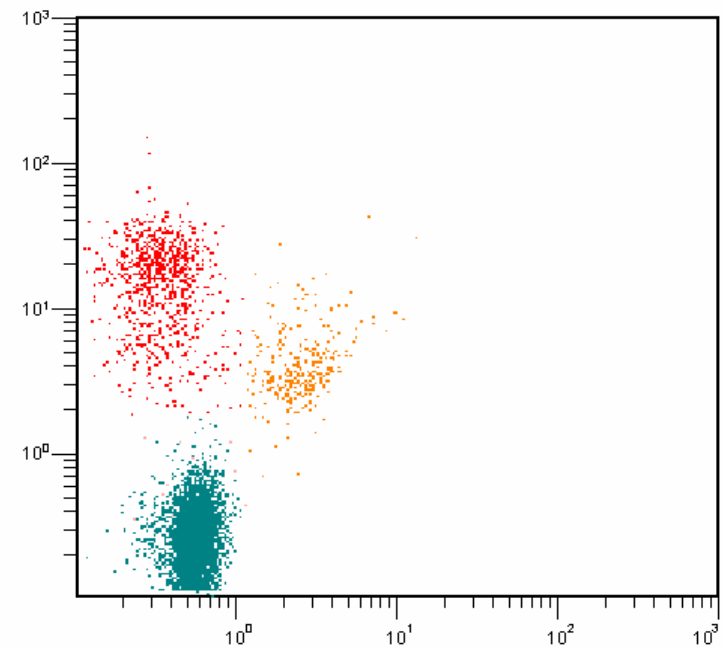
CD20 / CD22 / CD10

CD22



CD20

CD22



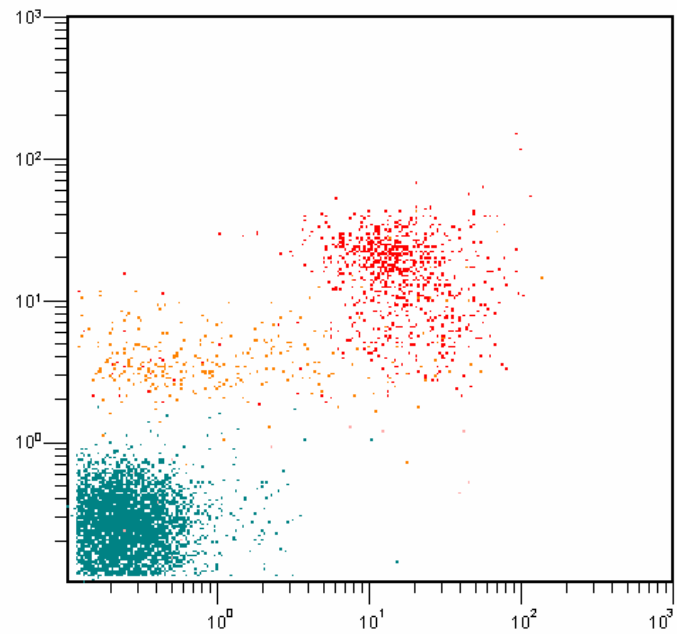
CD10



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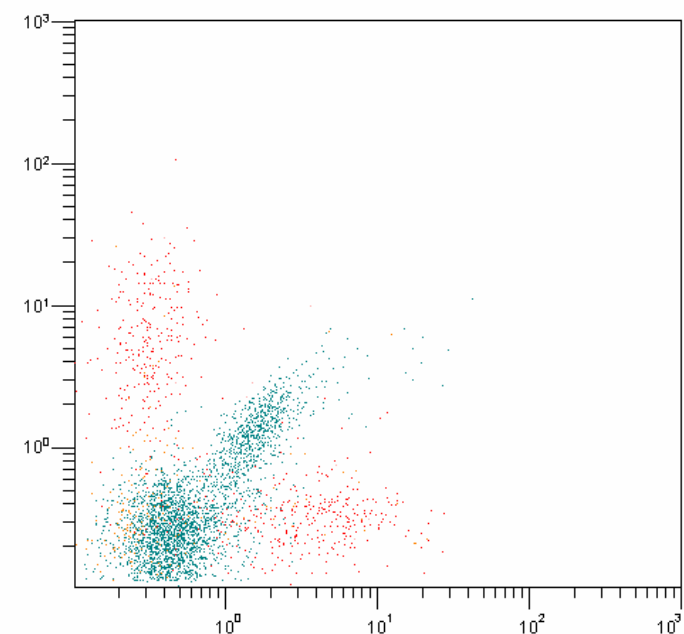


CD22



CD20

Lambda



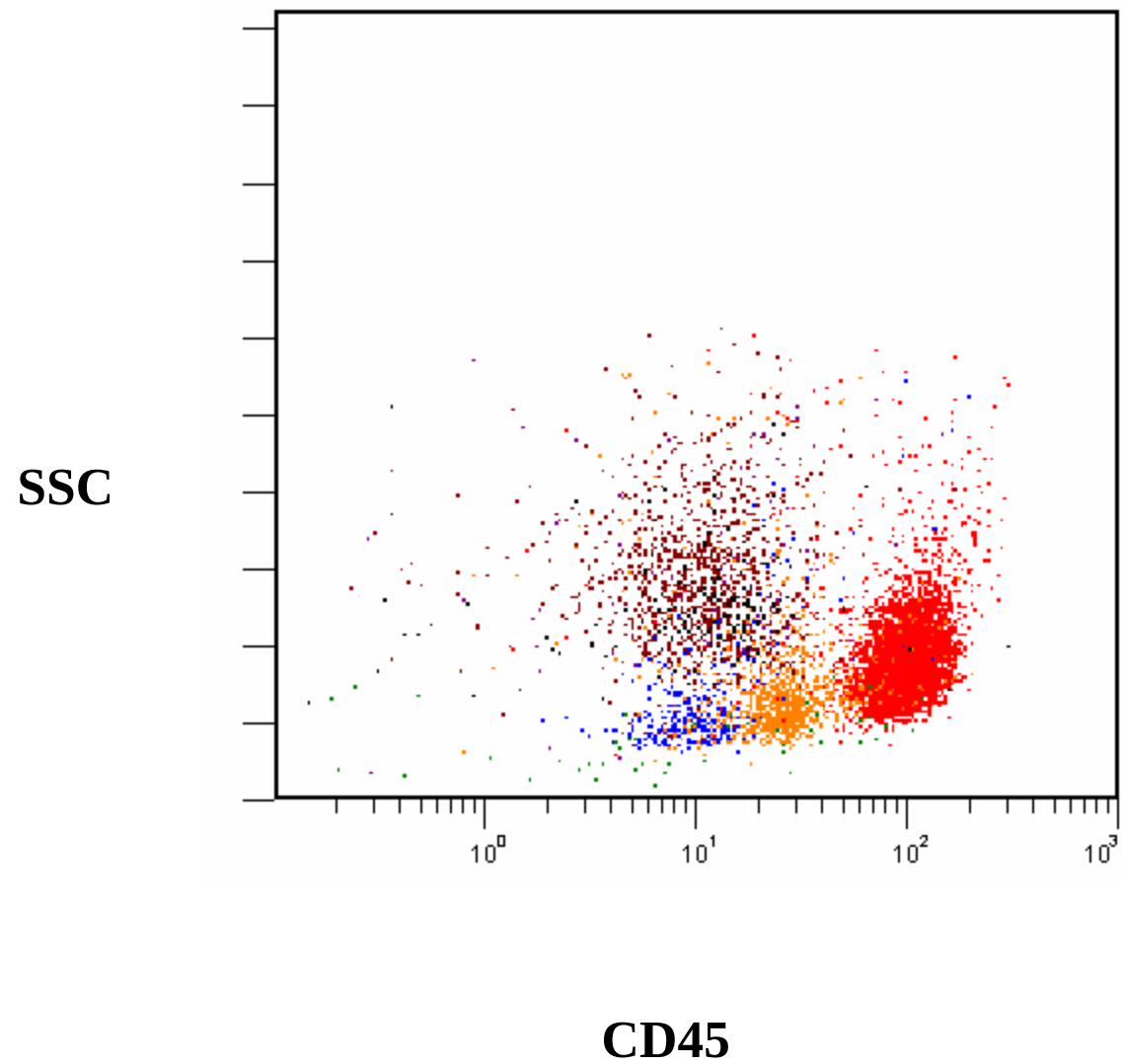
Kappa



B. Drénou 2007



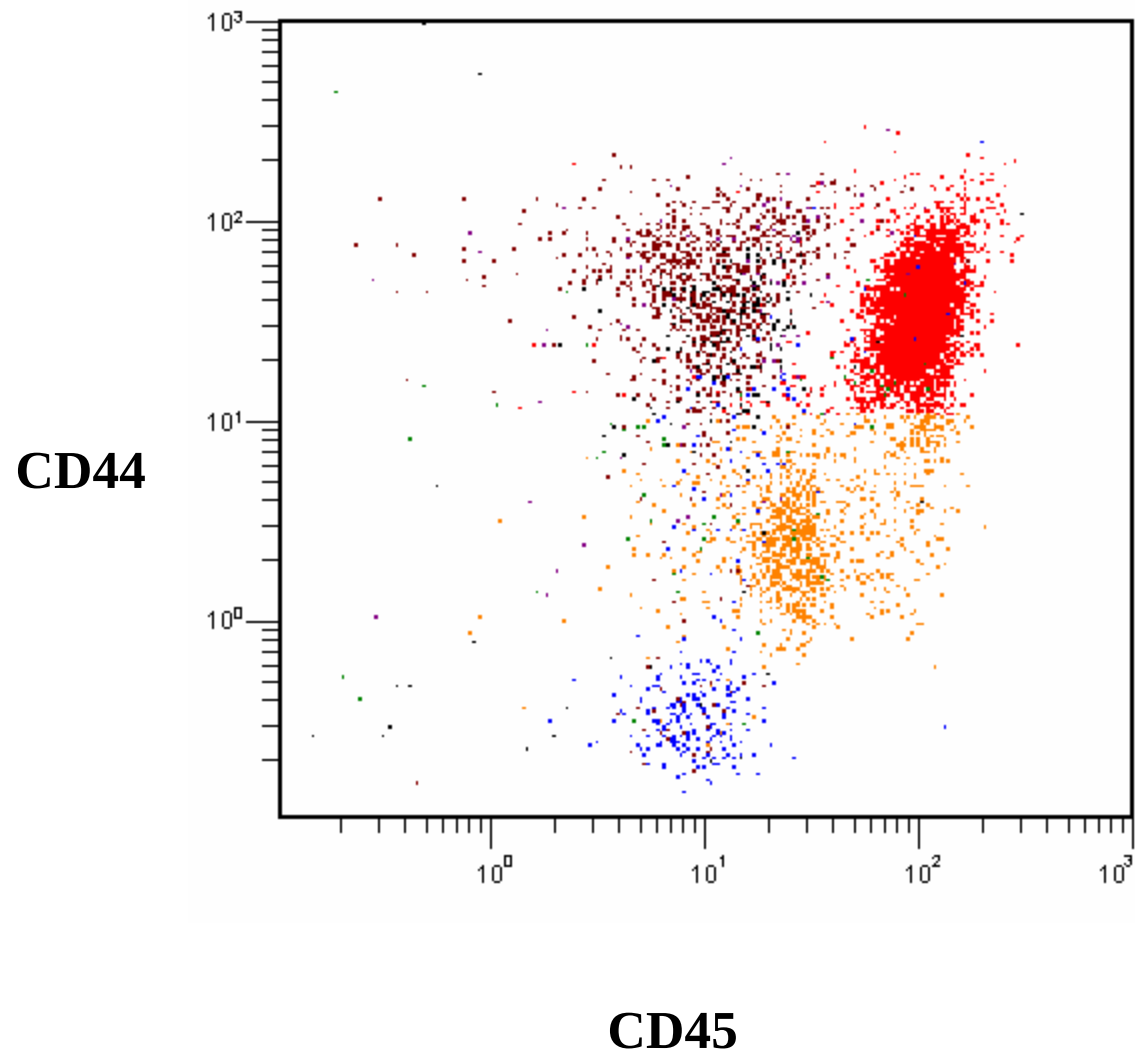
Moelle osseuse normale



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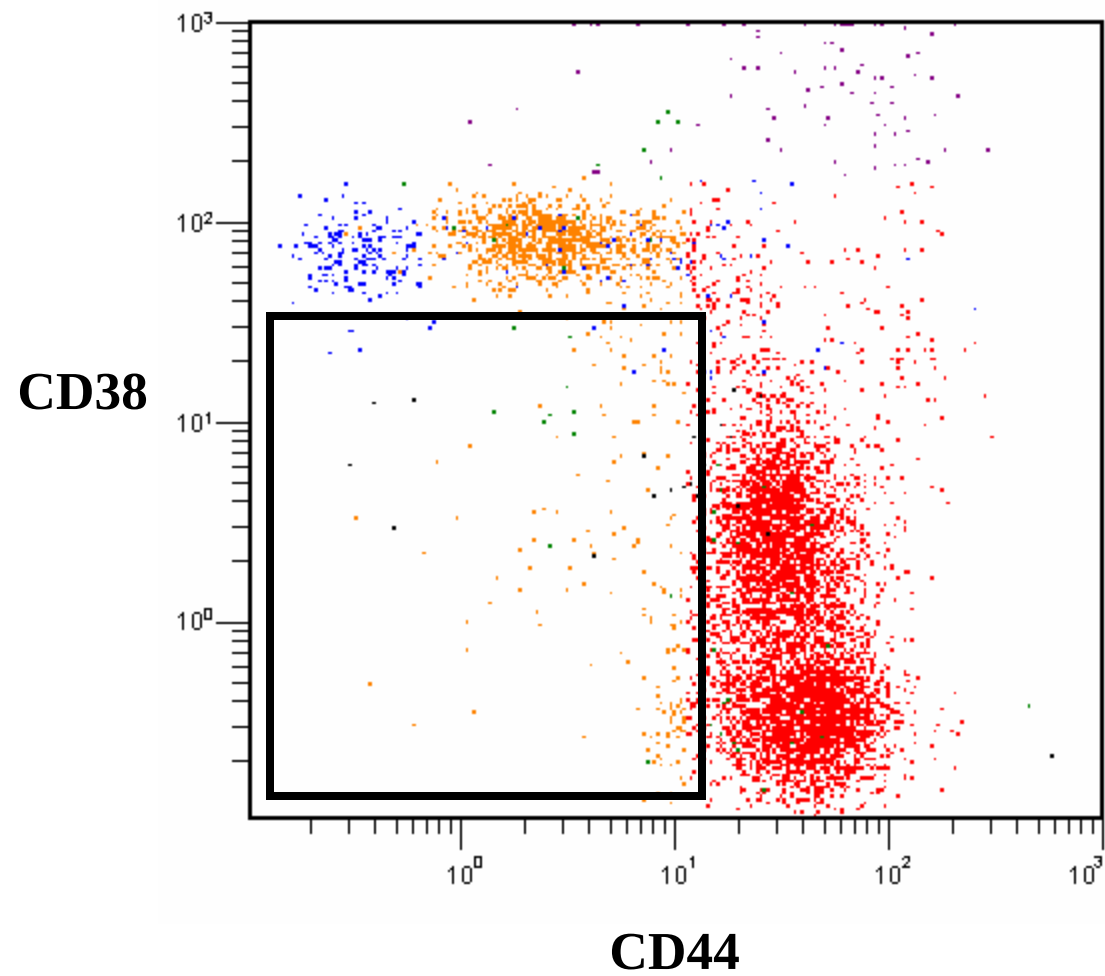
Moelle osseuse normale



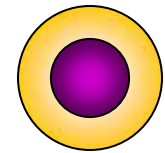
B. Drénou 2007



Moelle osseuse
Fenêtrage CD19

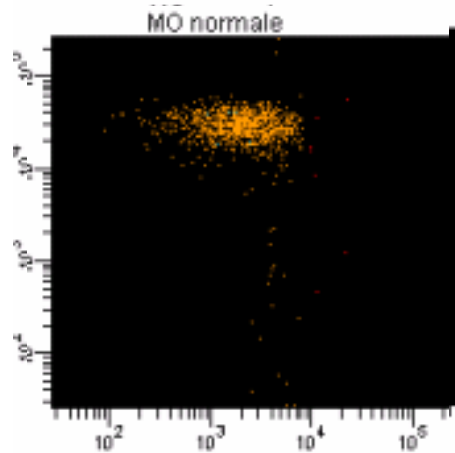


Moelle Osseuse

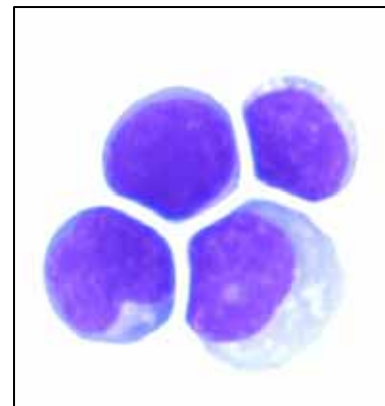
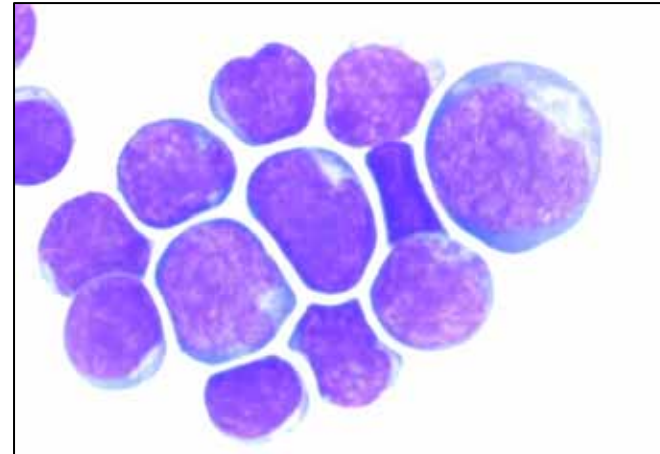


Fenetrage CD19

CD38



CD44



Tri sur Facsaria



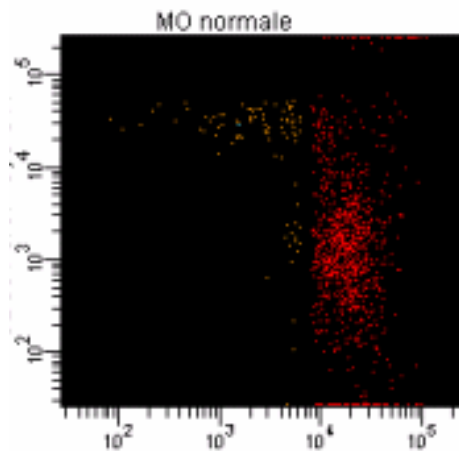
B. Drénou 2007



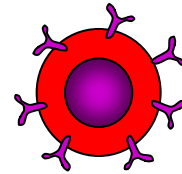
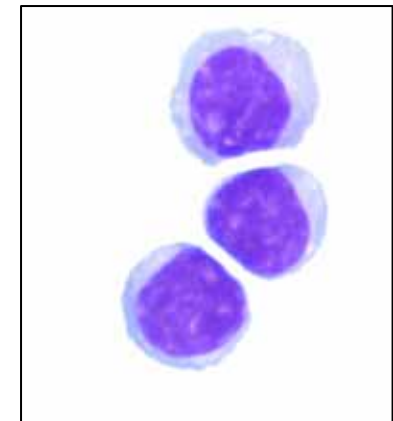
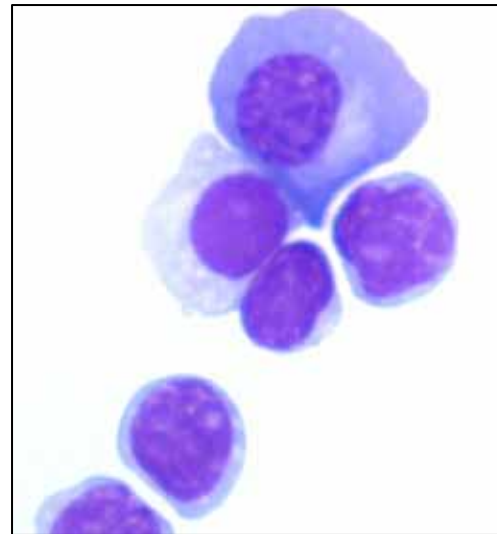
Moelle Osseuse

Fenetrage CD19

CD38



CD44



Tri sur Facsaria

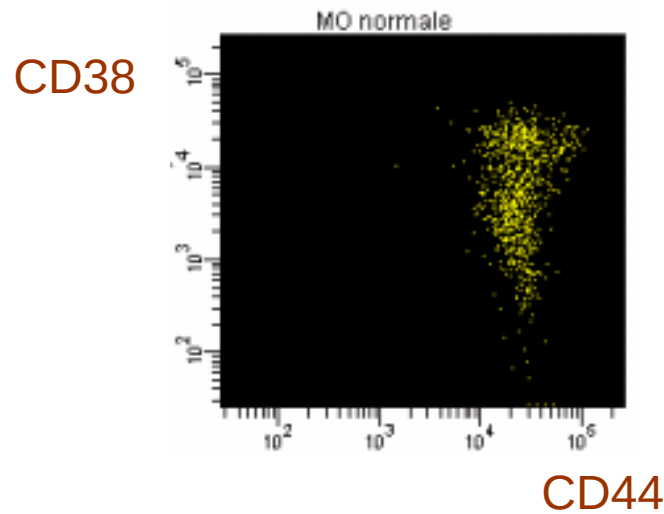
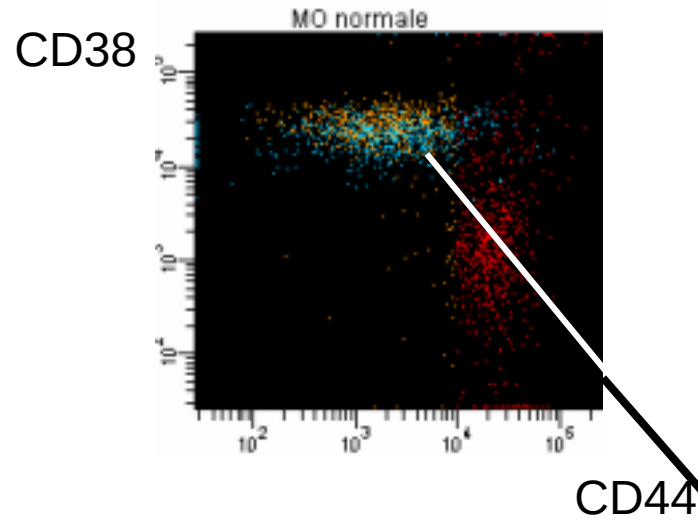


B. Drénou 2007

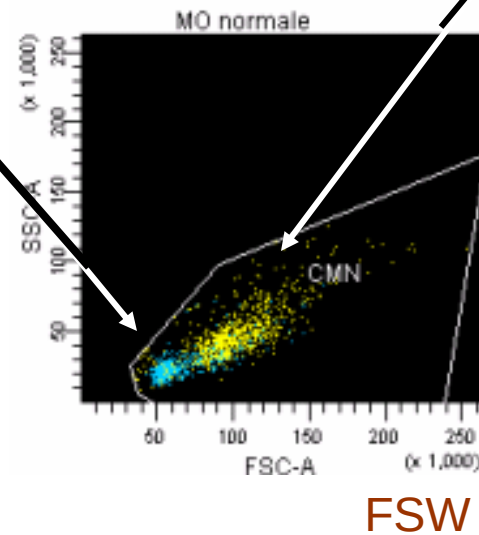


Moelle Osseuse

Fenetrage CD19



SCC



Fenetrage CD34

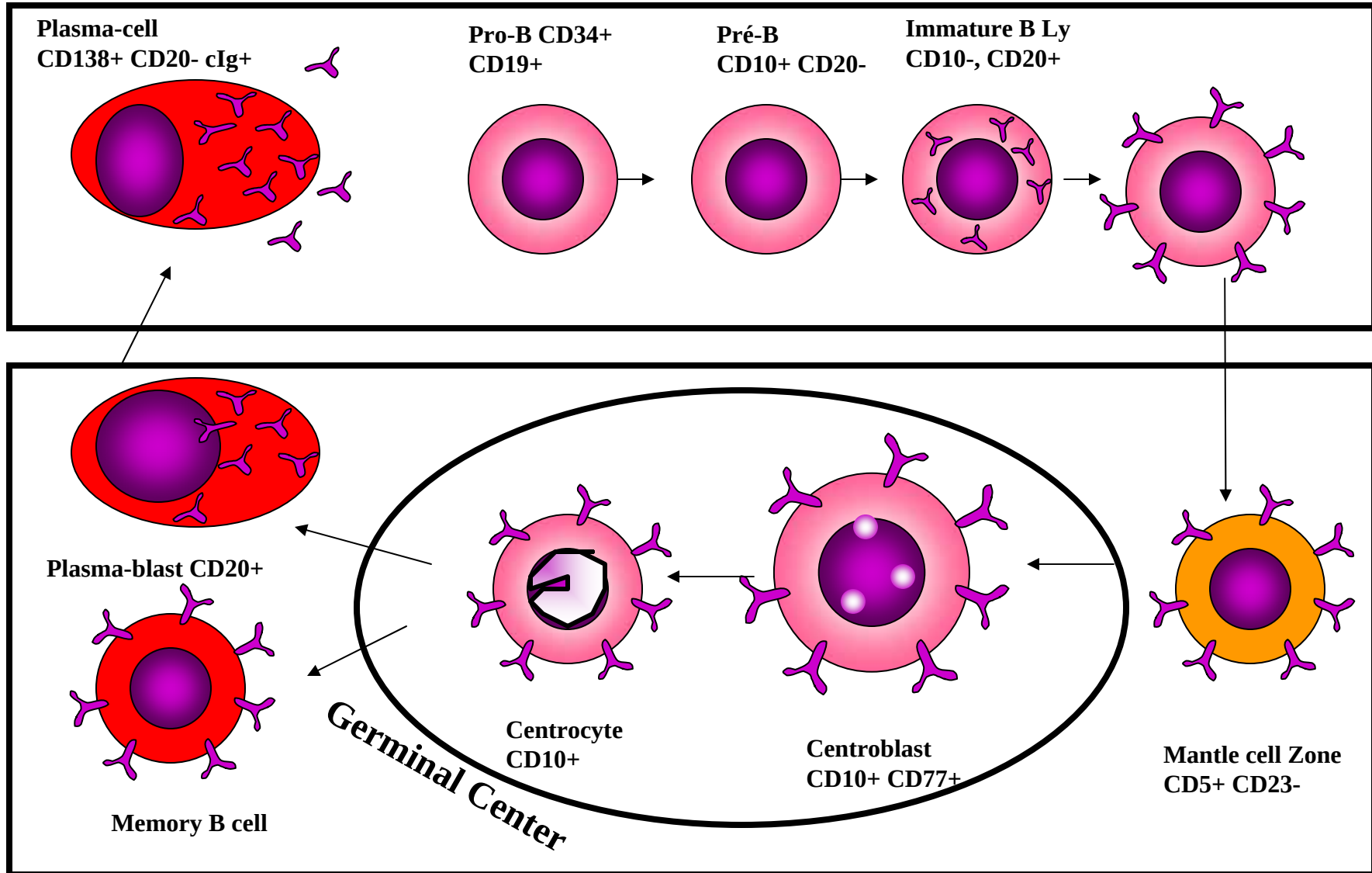
Tri sur Facsaria



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Bone-marrow



Peripheral lymphoid organs



B. Drénou 2007



LAL B

	CD19 + / CD22 + (s ou c) / cCD79 + (au moins deux)		
	CD10	cμ	slg κ ou λ
B-I (Pro-B)	-	-	-
B-II (Common ALL)	+	-	-
B-III (Pre-B)	±	+	-
B-IV (Mature B)	±	±	+



LAL T

	cCD3 +			
	CD7	CD2 et/ou CD5 et/ou CD8	CD1a	sCD3
T-I (Pro-T ou early T)	+	-	-	-
T-II (Pre-T)	+	+ (au moins un)	-	-
T-III (Cortical T ou intermediate T)	+/-	+ (au moins un)	+	+/-
T-IV (Mature T) Groupe a : TCR α/β + Groupe b : TCR γ/δ +	+/-	+ (au moins un)	-	+



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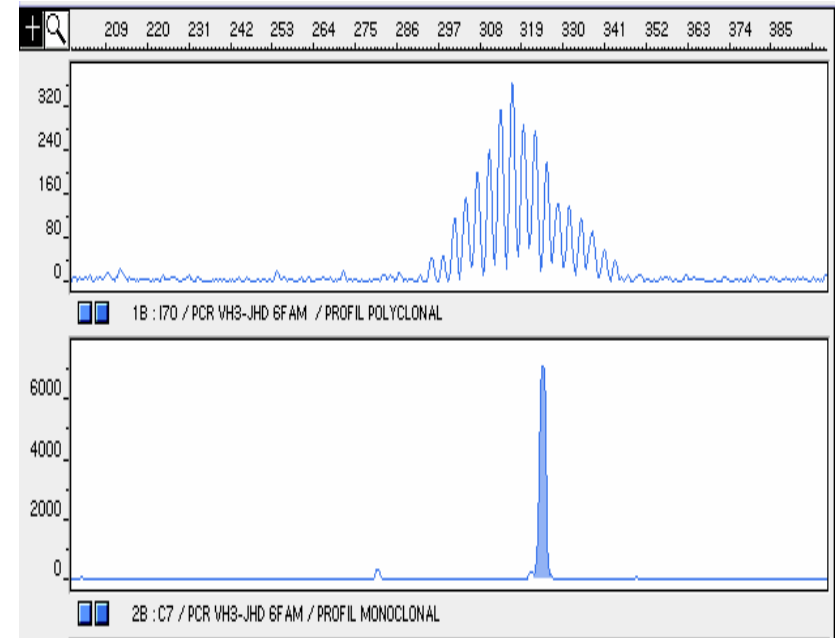
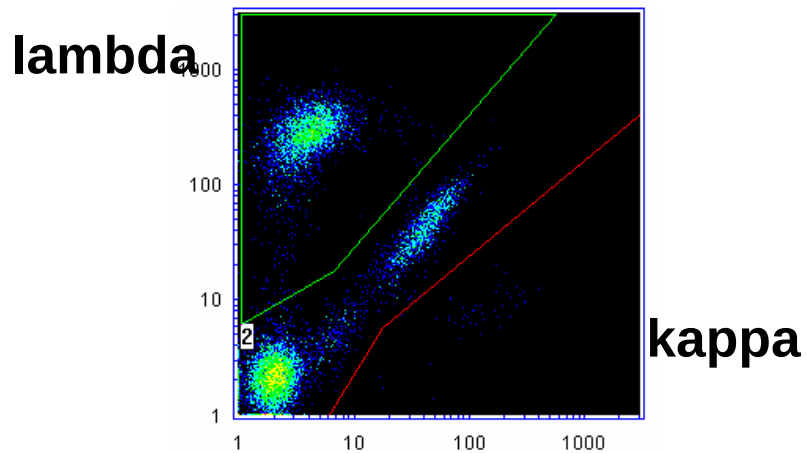
– Stratégies multi-couleurs



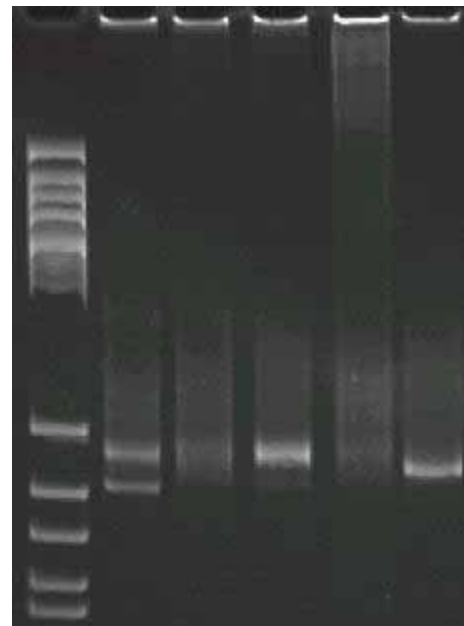
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- Clonalité B



- Clonalité T



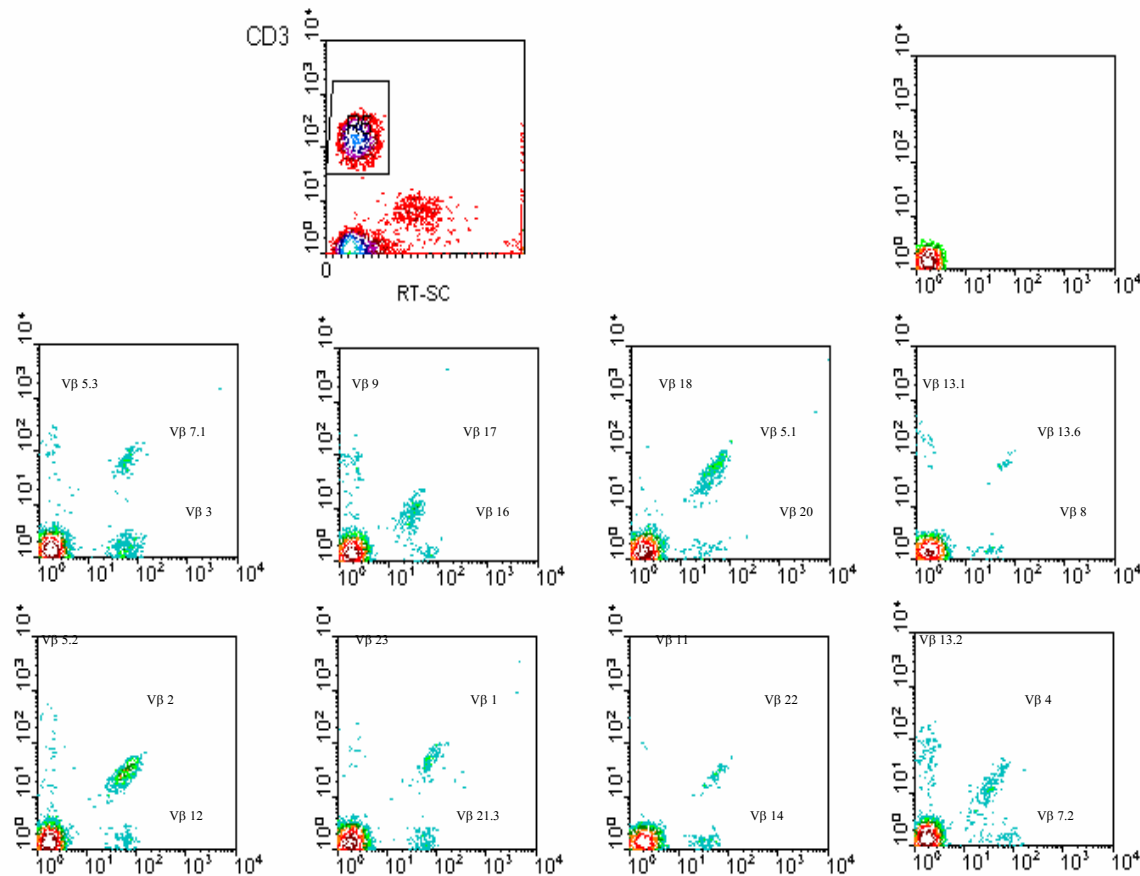
TCR γ



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Répertoire normal



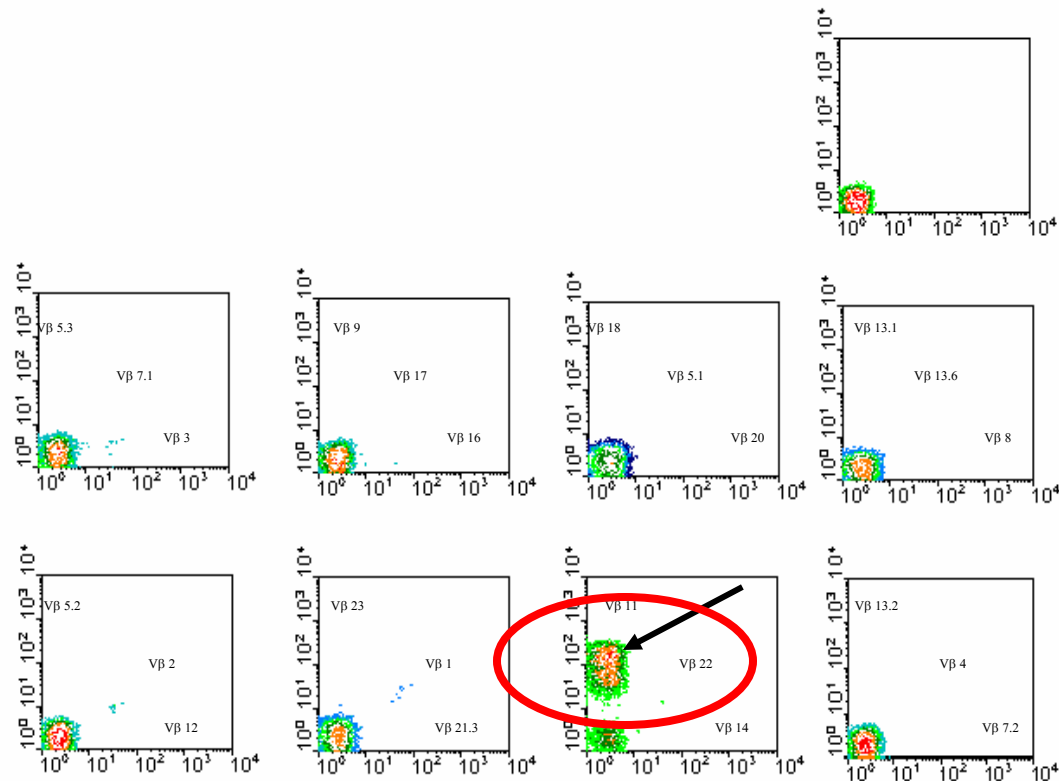
Pas d'expansion Vβ
 $\Sigma V\beta / \Sigma TCR\alpha\beta = 70 \pm 30\%$



B. Drénou 2007



Répertoire de type « Monoclonal »



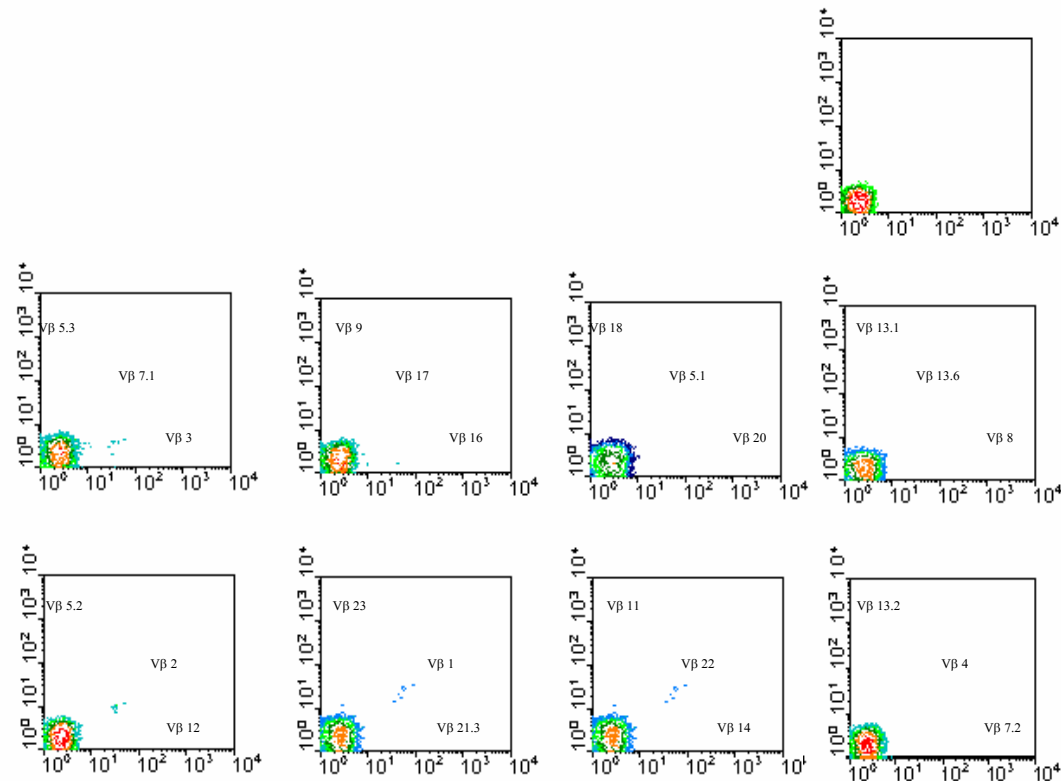
Vβ d'hyperexpression (Vβ11 = 80%)



B. Drénou 2007



Répertoire défectif



Pas d' expansion Vβ
 $\Sigma V\beta / \Sigma TCR\alpha\beta < 40\%$

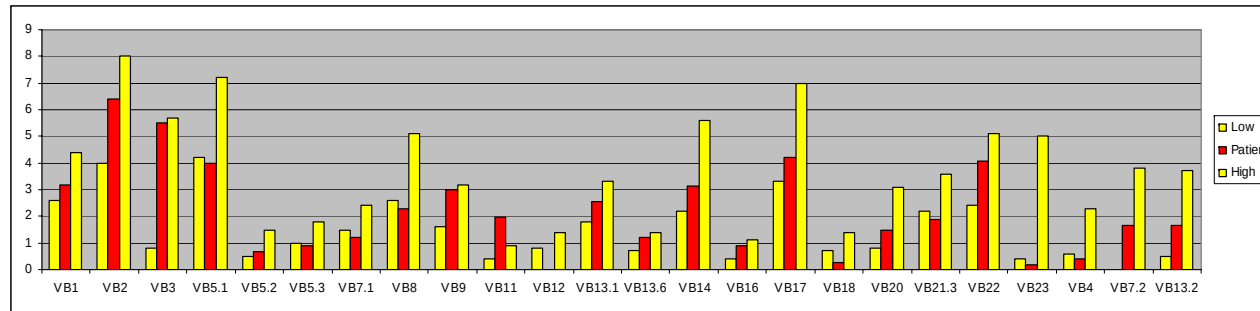
AFC 2002 / SFH 2003



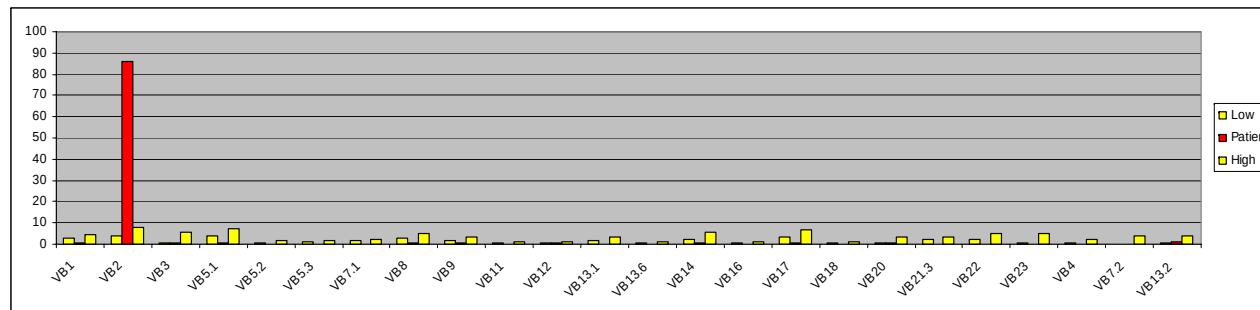
B. Drénou 2007



Repertoire V β Polyclonal



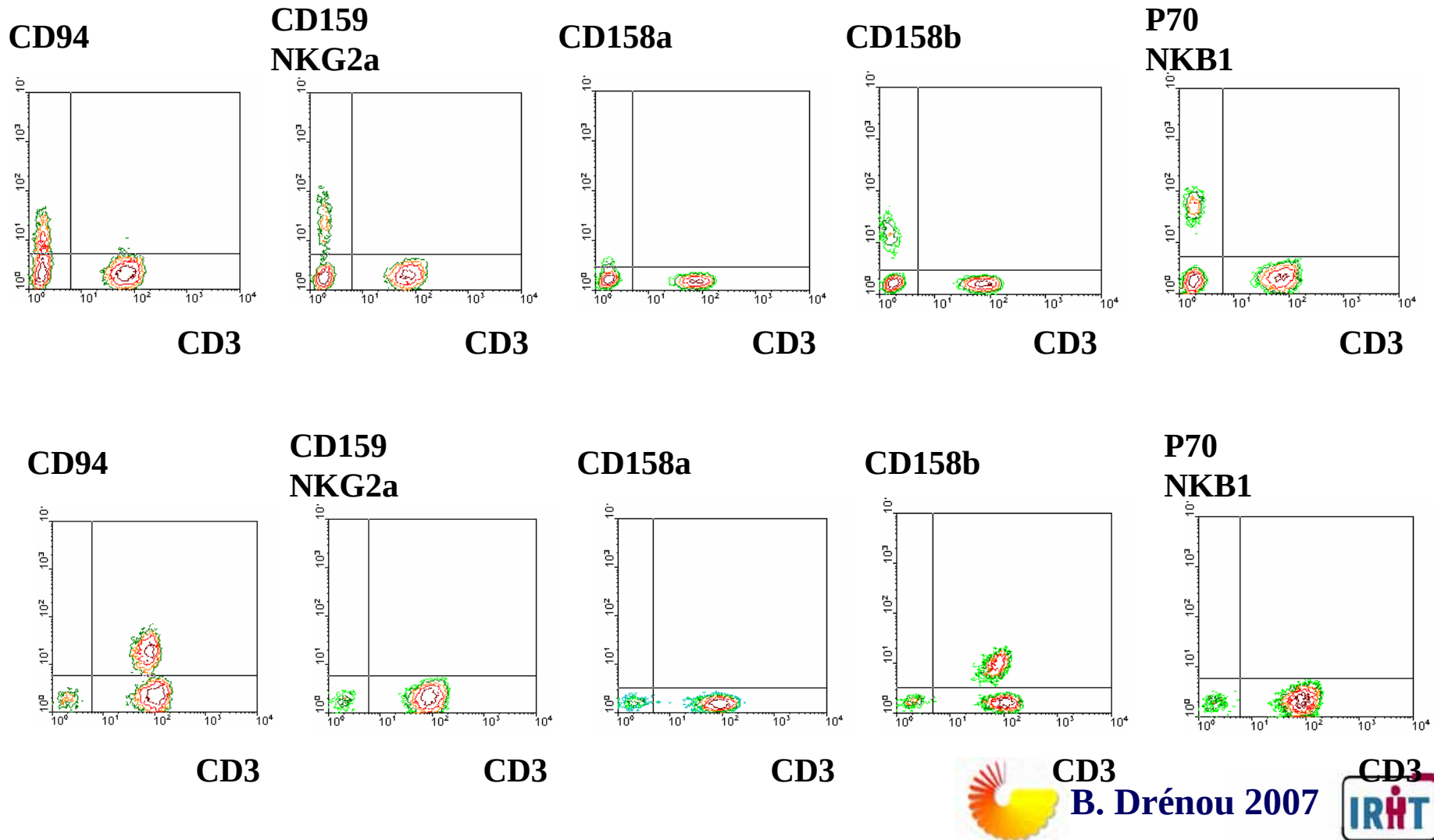
Repertoire V β Monoclonal



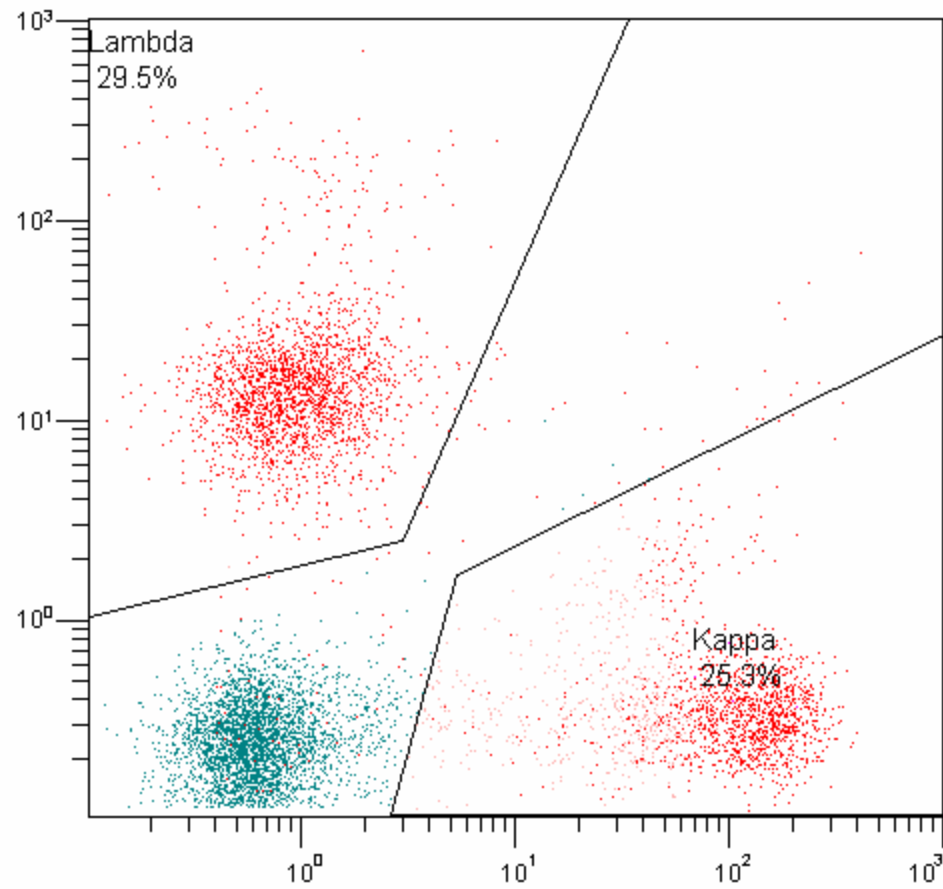
B. Drénou 2007



Use of KIR for T-NK clonality



Lambda



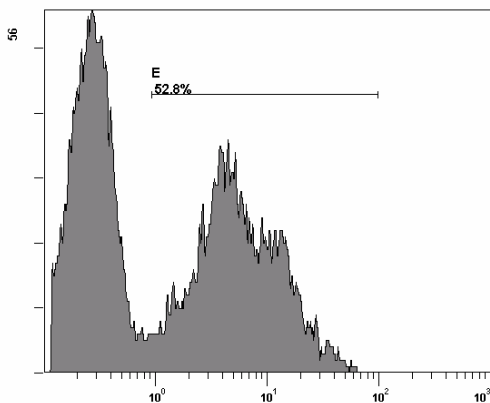
Kappa



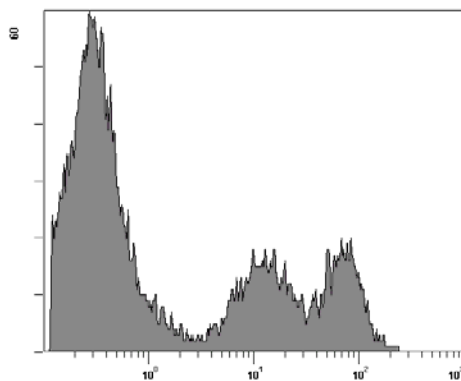
B. Drénou 2007



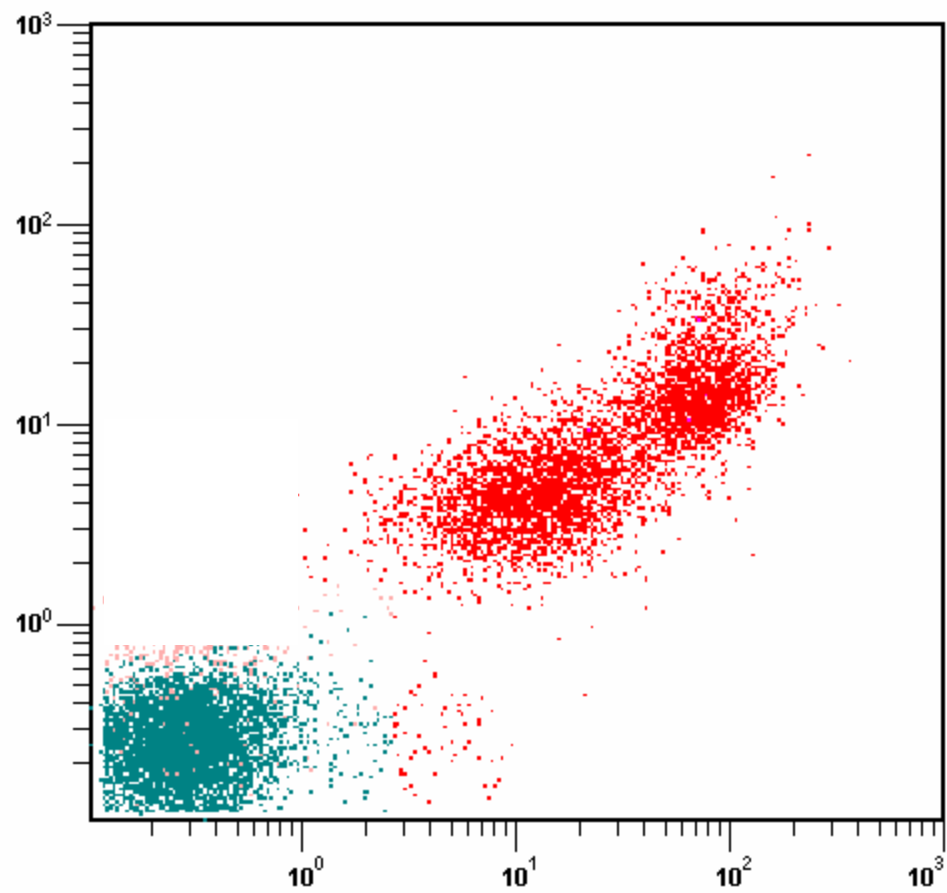
CD22 (PCy5)



CD22



CD20



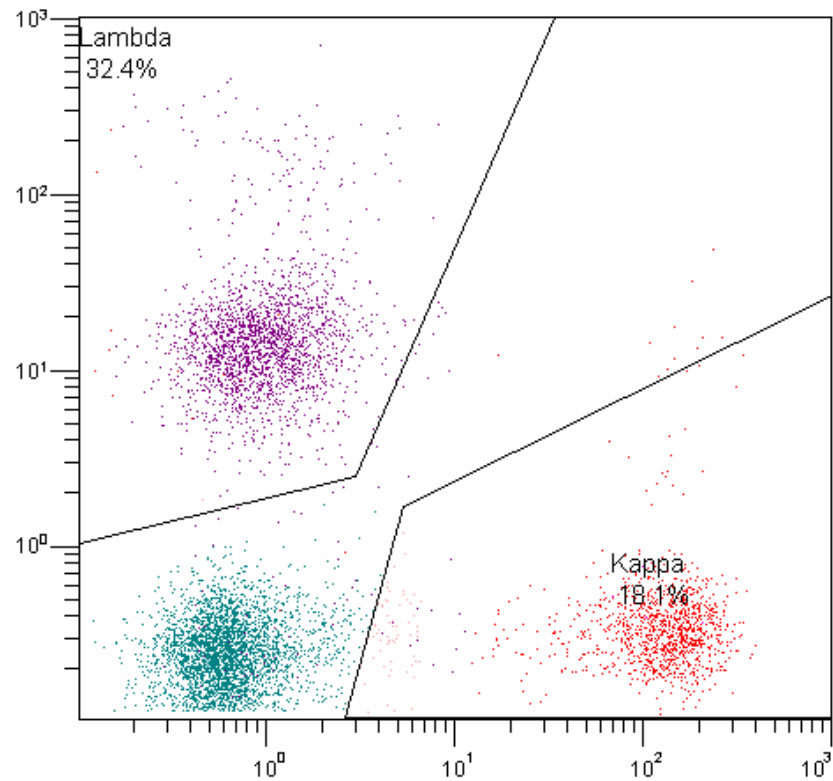
CD20 (PCy7)



B. Drénou 2007

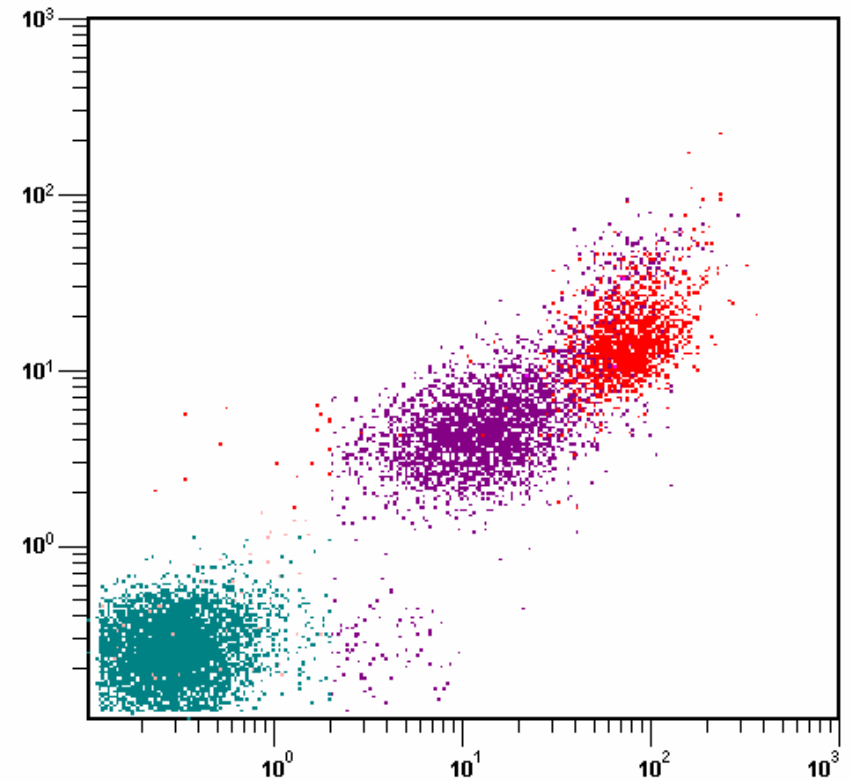


Lambda



Kappa

CD22 (PCy5)



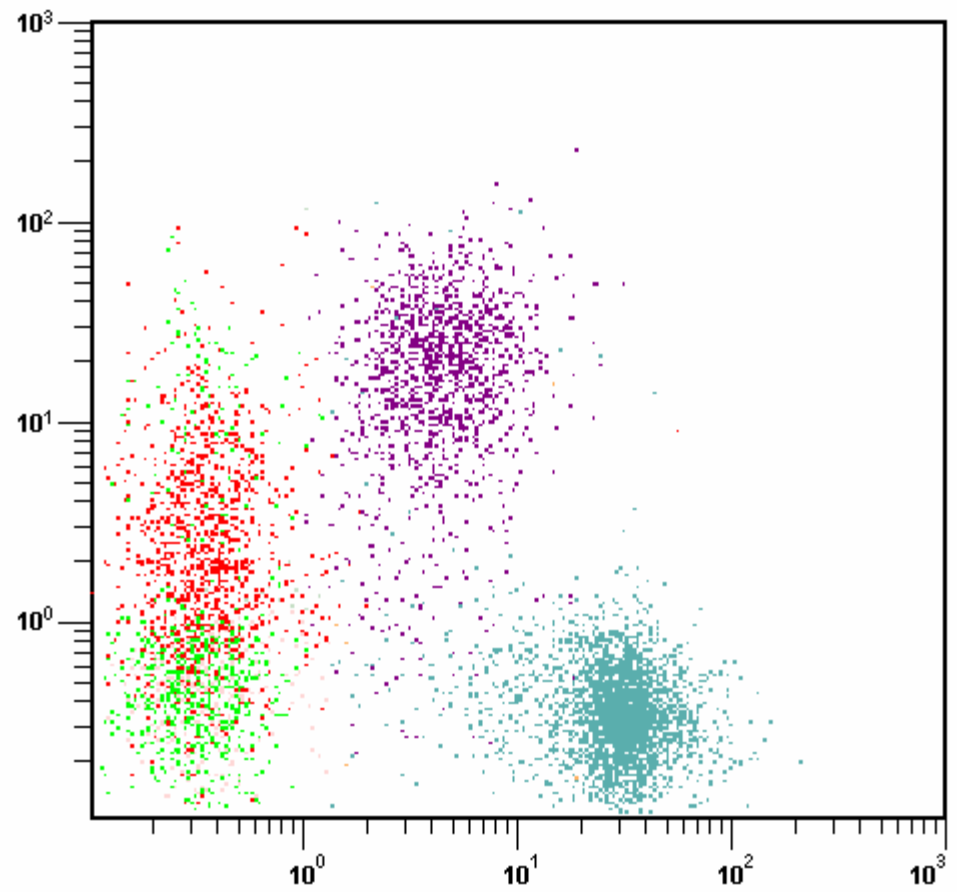
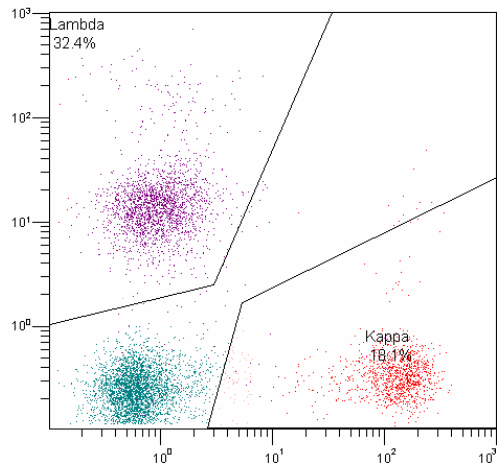
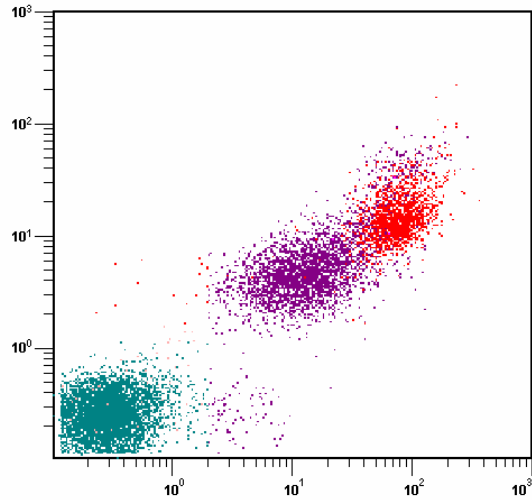
CD20 (PCy7)



B. Drénou 2007



CD19



CD5



B. Drénou 2007



Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
- Pronostic
- Thérapeutique

Suivi

– Stratégies multi-couleurs



B. Drénou 2007



Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
 - Myélome
 - Lymphopathie T
- Pronostic
- Thérapeutique

Suivi

- Stratégies multi-couleurs

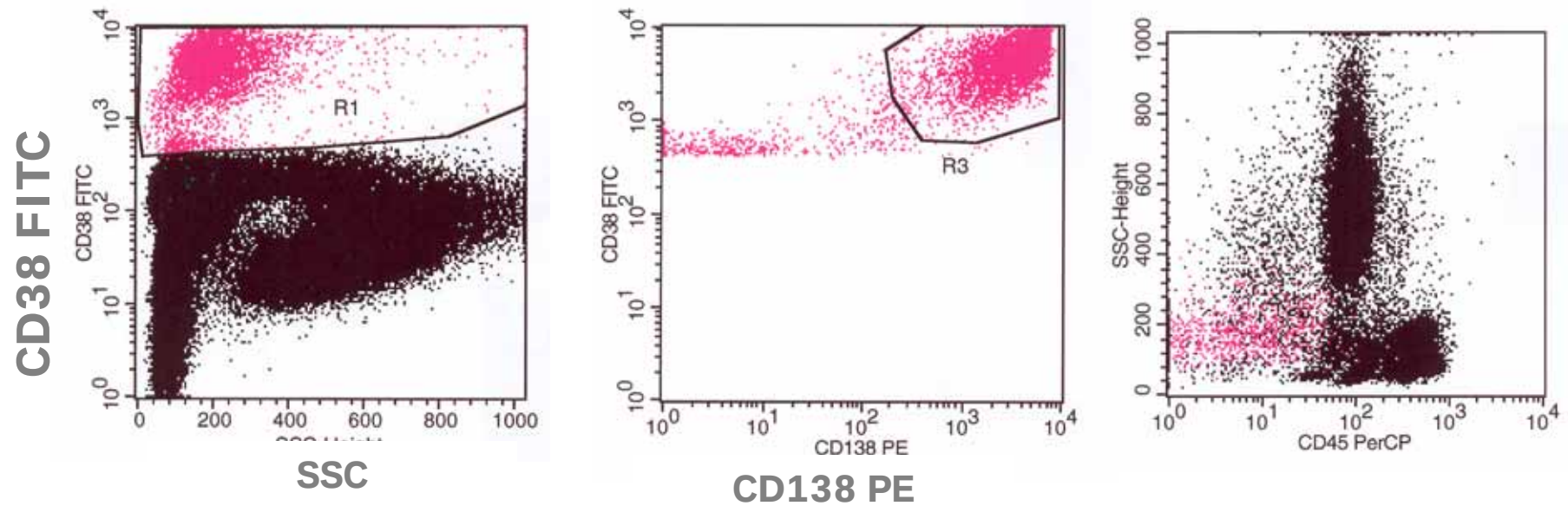


B. Drénou 2007



Exemple des plasmocytes

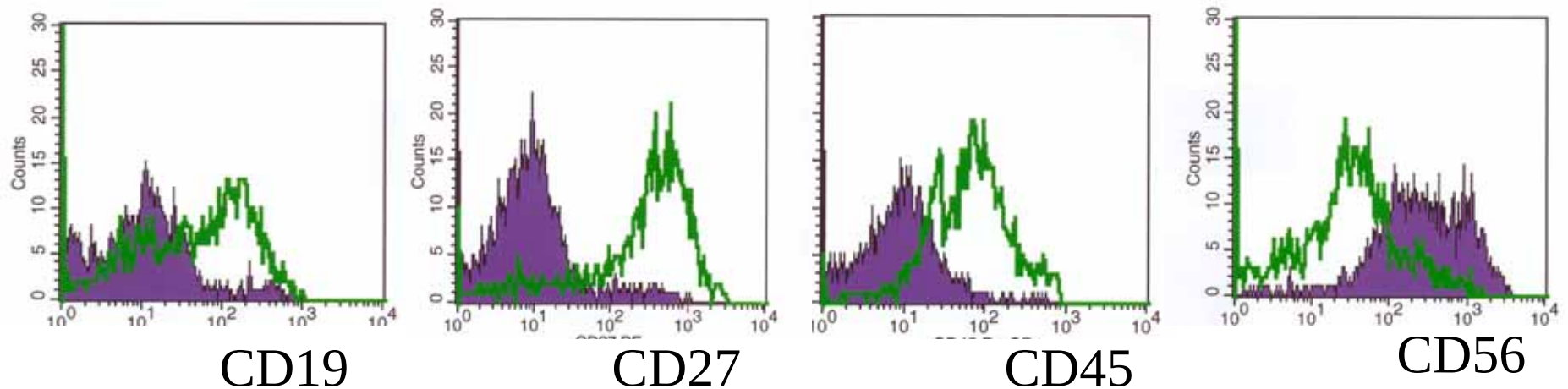
Méthodes



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Phénotype des plasmocytes: myélome vs témoin



Profil phénotypique des plasmocytes tumoraux (retrouvé
dans 54% des MM)

⇒ **CD19-CD27- CD45-CD56+**



B. Drénou 2007



Etablissement d'un score simple de phénotypage pour les gammopathies monoclonales

Score en fonction du pourcentage de plasmocytes exprimant le CD19, CD27 et CD45

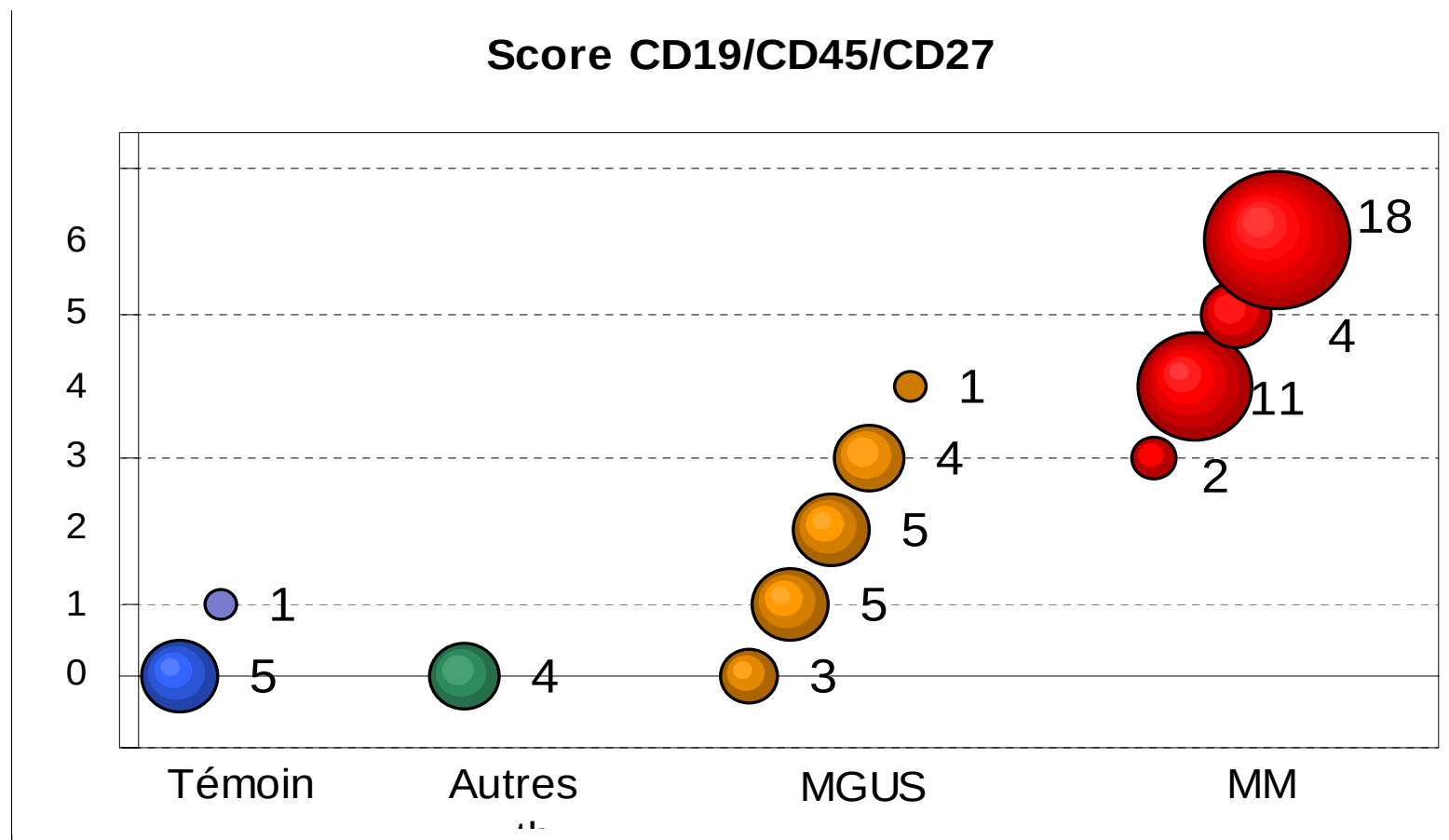
% de plasmocytes exprimant CD19,27,45	Score
< à 30%	2 points
30%-60%	1 point
> à 60%	0 point



B. Drénou 2007



Score pour les gammopathies monoclonales

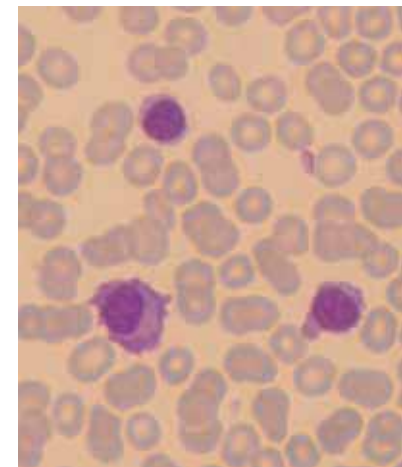
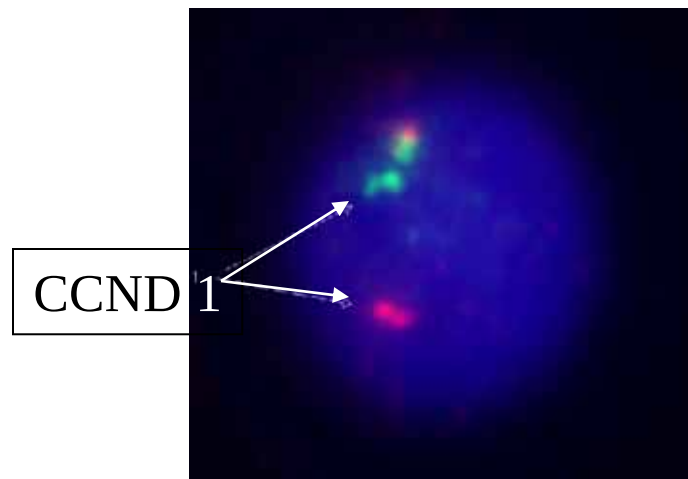


Cas des Myélomes CD20+

(4/35)

Cytologie : petit plasmocytes matures

FISH : t(11,14)



X 400



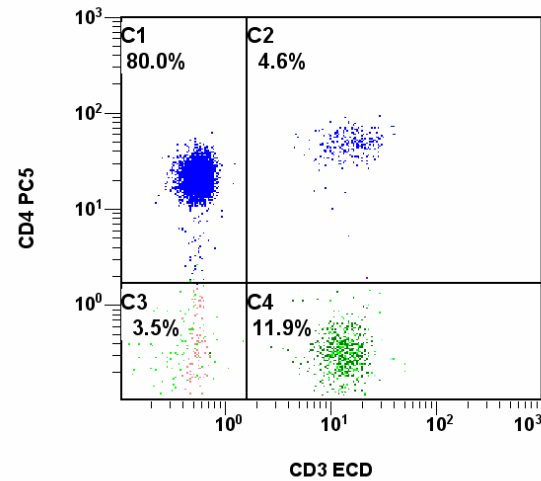
B. Drénou 2007



Les Lymphomes T:

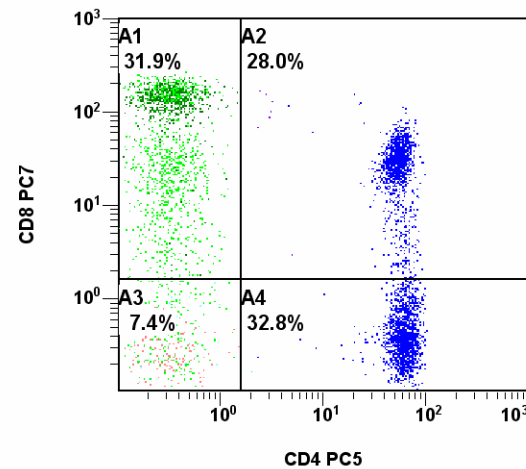
- Ly CD3- mais CD4+

CD4



CD3

CD8



CD4

- Coexpression CD4/CD8

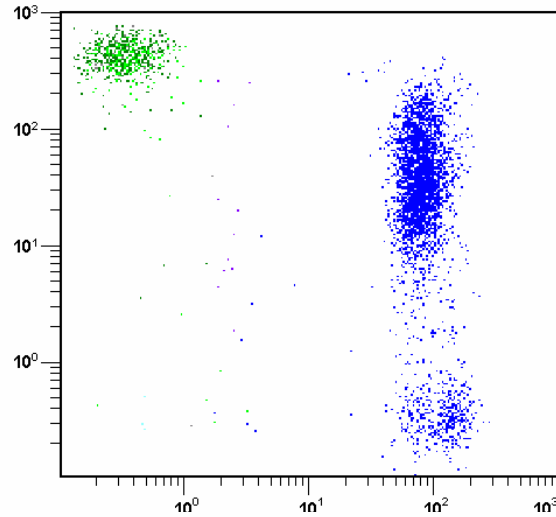


B. Drénou 2007



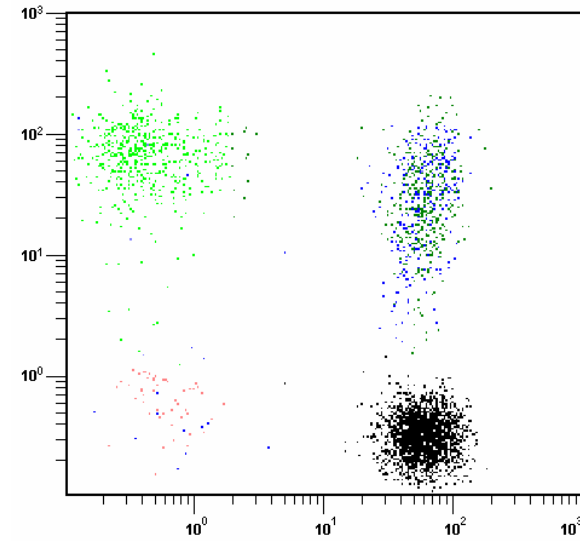
Sezary

CD8



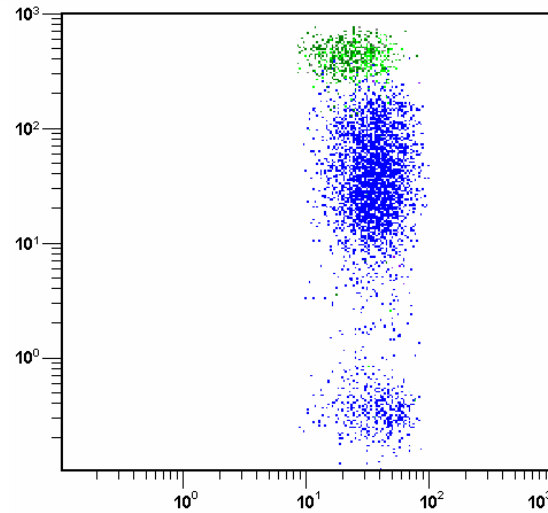
CD4

CD7

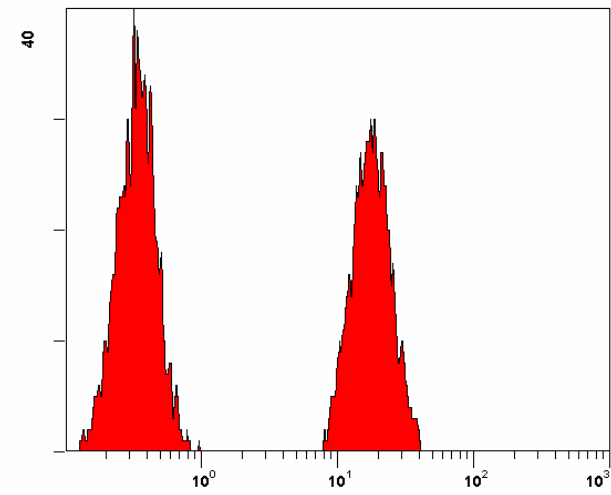


CD3

CD8



CD3



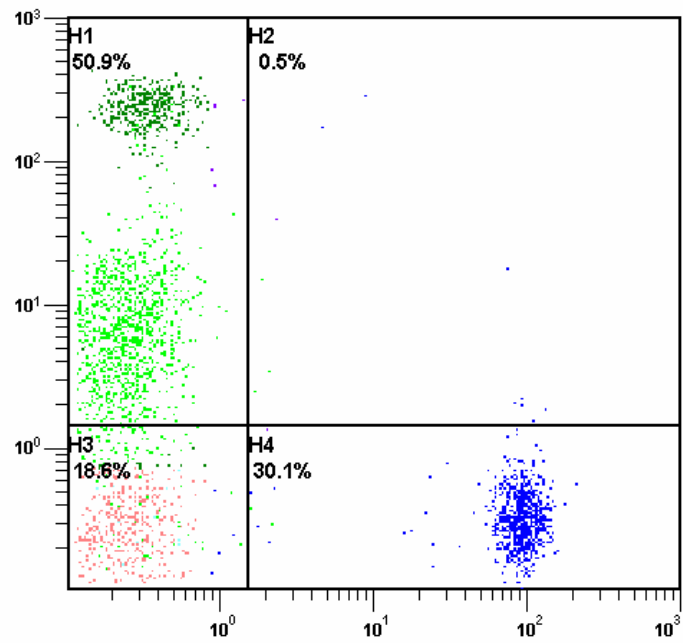
V β 2



B. Drénou 2007

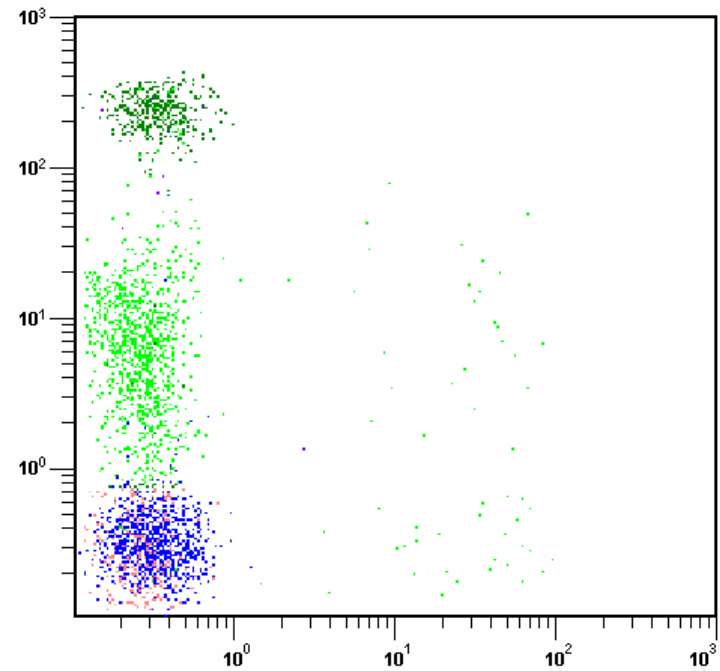


CD8



CD4

CD8



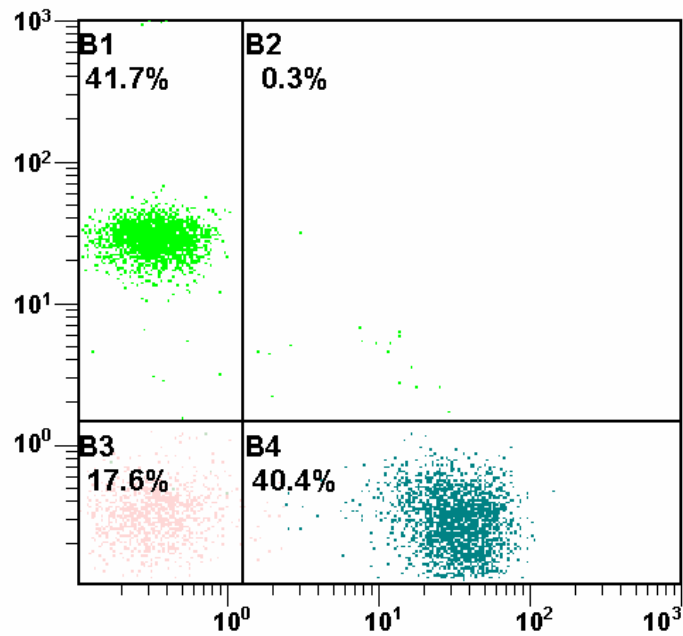
CD16



B. Drénou 2007

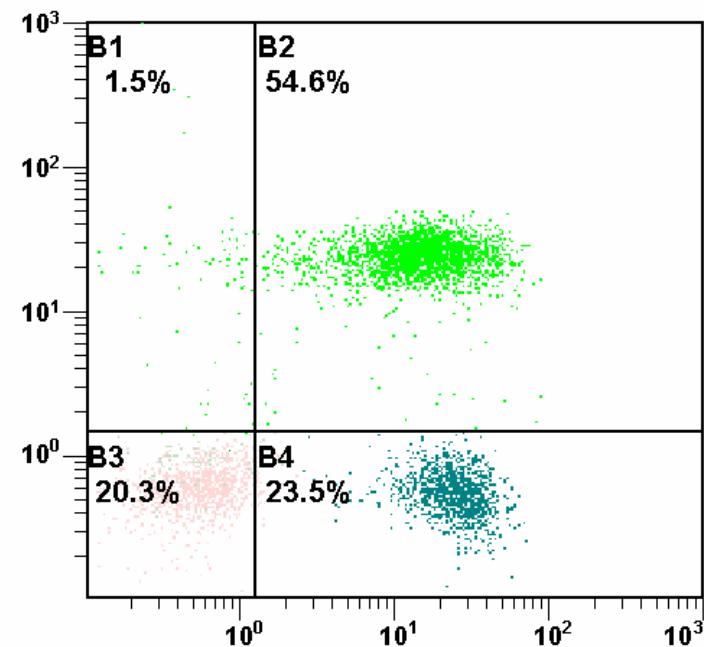


CD103



sCD3

CD103



cCD3

LNH du grêle dans le cadre d'une maladie Coeliaque



B. Drénou 2007



Intérêt de la CMF dans les Hémopathies malignes

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- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
- Pronostic
- Thérapeutique

Suivi

– Stratégies multi-couleurs



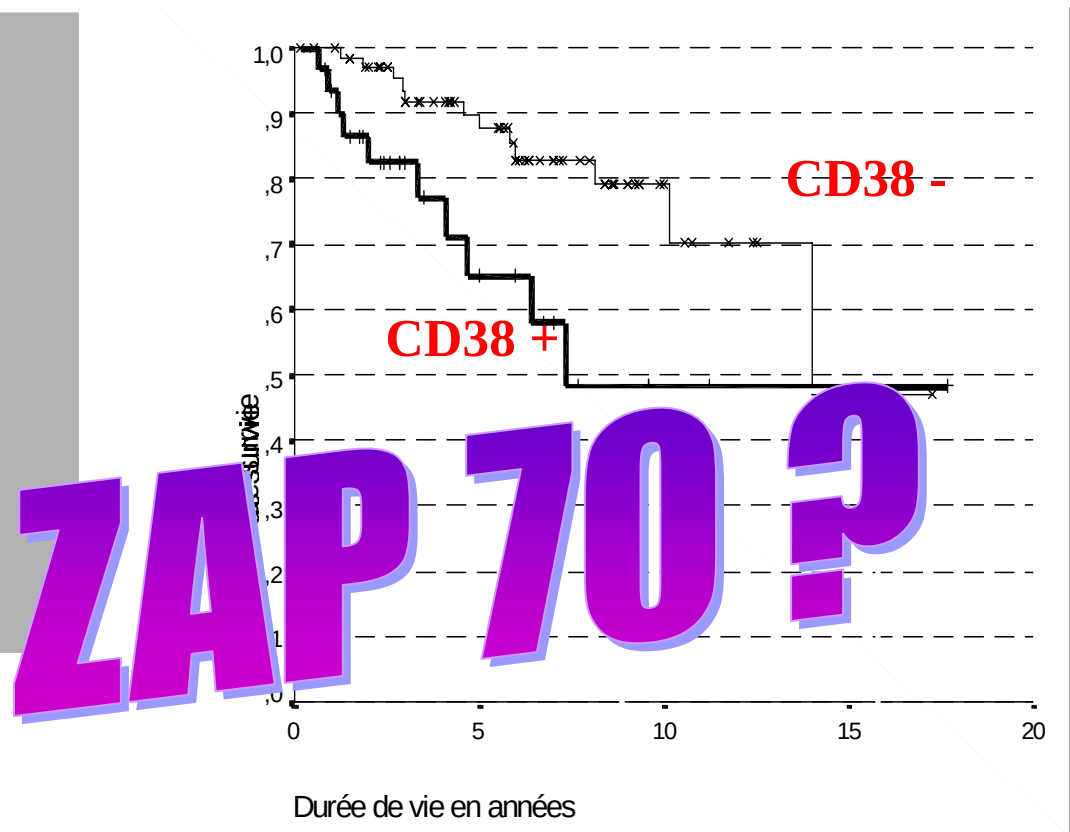
B. Drénou 2007



CD38: valeur dans la LLC

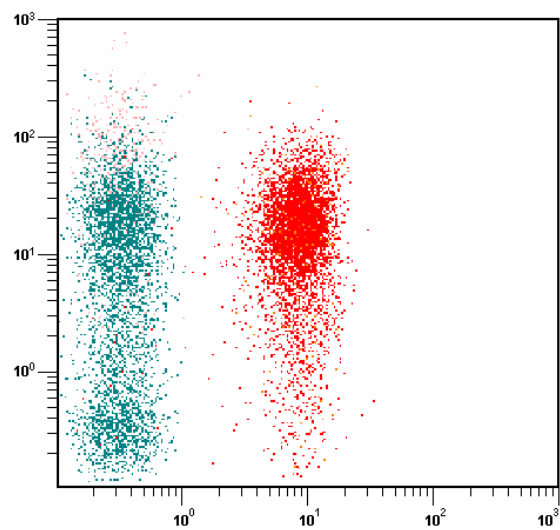
	n	(%)	Survie	P
CD38 (+)	31	(30,7 %)	7,3 ans	0,018
CD38 (-)	70	(69,3%)	14 ans	

	CD38 (+)	CD38 (-)	p
Age :	58	61	0,3
Sexe (H) :	68%	54%	0,2
Binet A :	16(52%)	57(81%)	0,008
B+C :	15(48%)	13 (19%)	
Cyto AT:	16(52%)	17(24%)	0,007
Tdd<12 :	25(80%)	26(38%)	<10 ⁻⁴



LLC

CD38



CD19



B. Drénou 2007



Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
- Pronostic
- Thérapeutique

Suivi

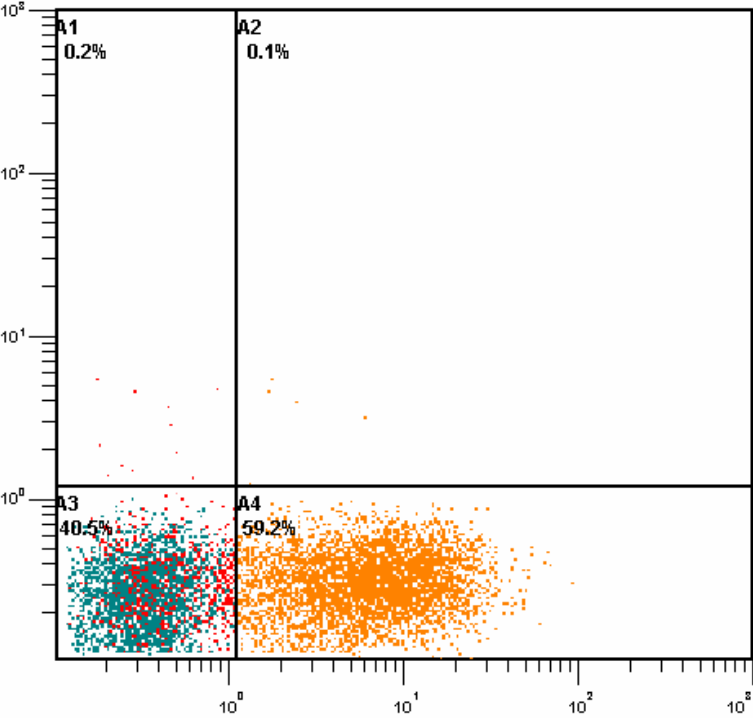
– Stratégies multi-couleurs



B. Drénou 2007

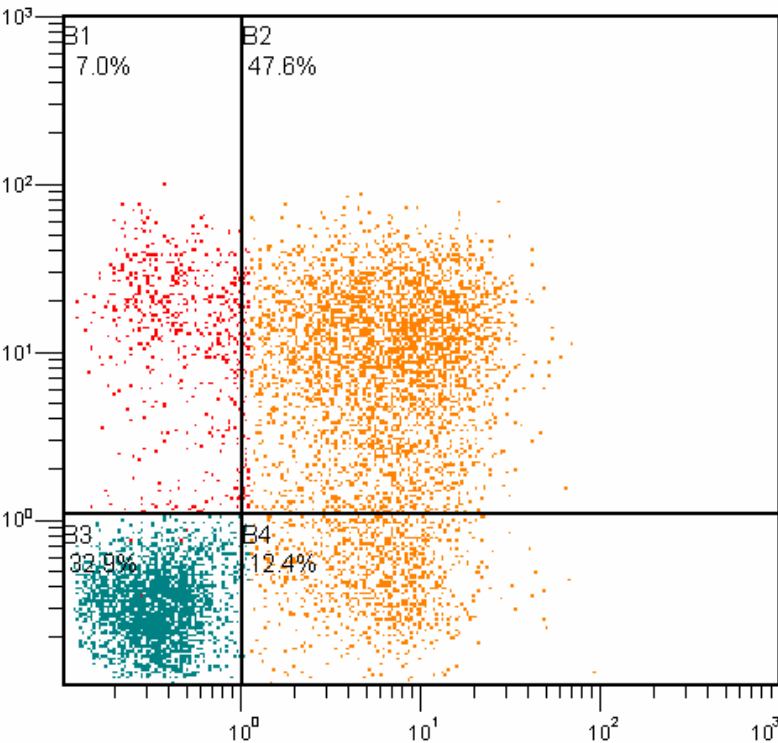


CD20



CD10

CD22



CD10

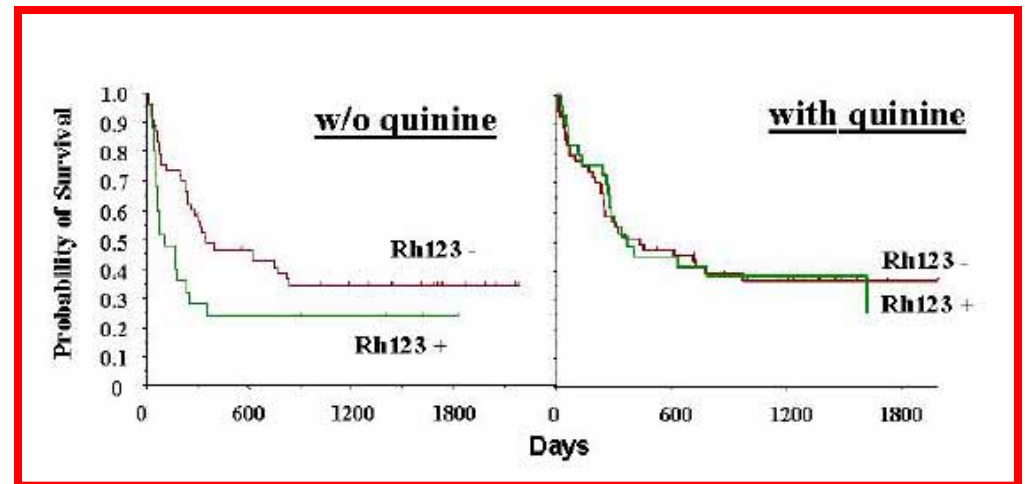
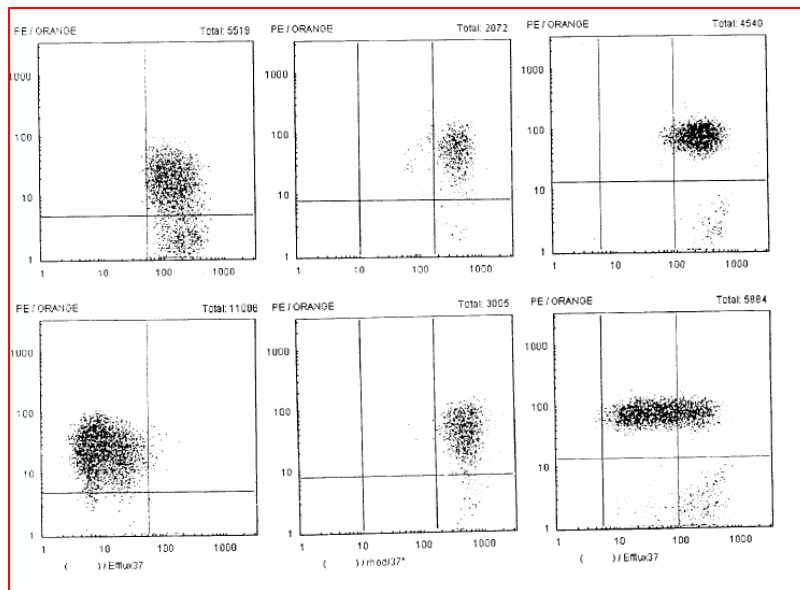


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MDR: efflux de rhodamine 123

PgP



LAM Blood 2003

LNH NK Blood 1997

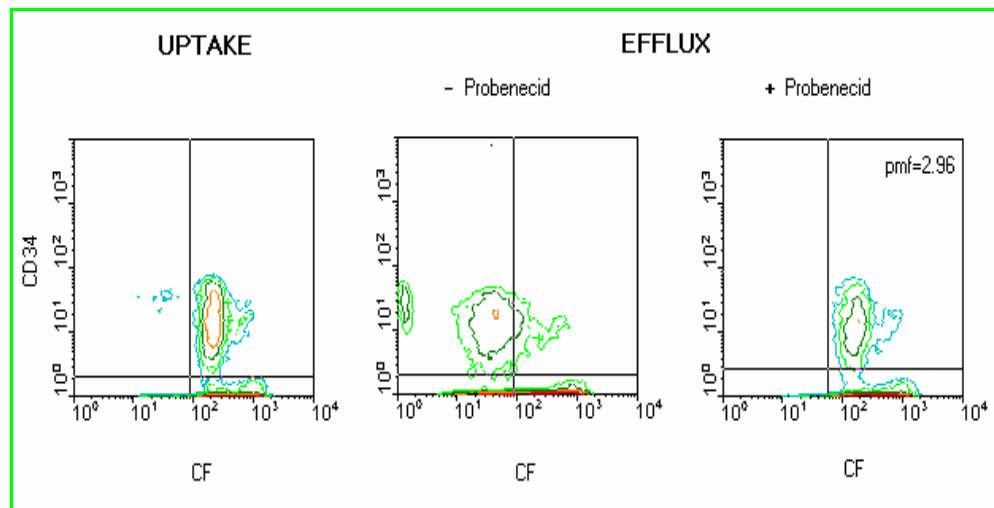


B. Drénou 2007

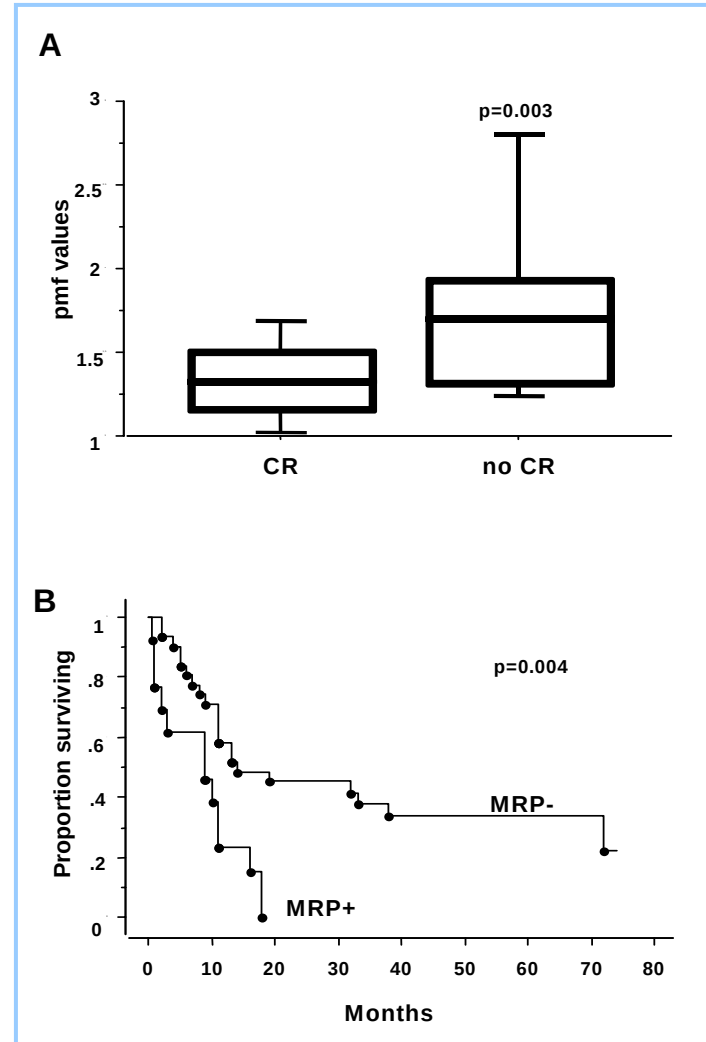


MDR

MRP



Br. J Haematol 2001



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Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
- Pronostic
- Thérapeutique

EN PRATIQUE

Suivi

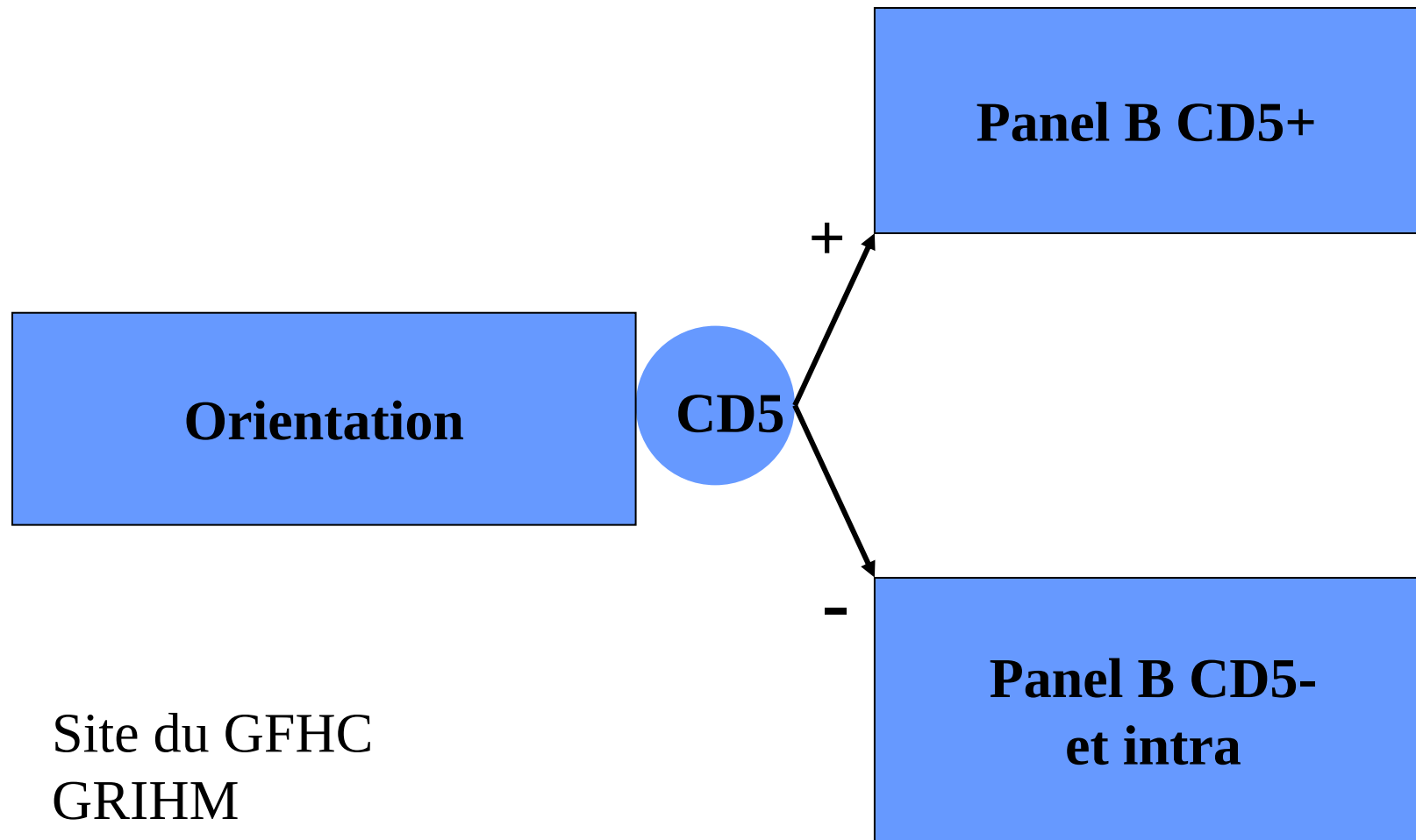
– Stratégies multi-couleurs



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Stratégie pour les lymphopathies



Site du GFHC
GRIHM
Sponsor: BD



B. Drénou 2007



Panels 4C (GRIHM)

PANEL ORIENTATION HYPERLYMPHOCYTOSE				
N°				
1	3	16+56	19	45
2	Kappa	Lambda	19	5
3	3	7	8	4

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	FMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG

PANEL COMPLEMENT LYMPHOS B CD5-				
Tube n°				
1	20	22 *	19	23
2	44	10	19	45
3 intra contrôles	Crtf c	Crtf c	19	44
4 intra	Ki67 c	Bcl2 c	19	44

Les * correspondent aux fluorochromes « forts »



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Panel Orientation 4C

PANEL ORIENTATION HYPERLYMPHOCYTOSE				
N°				
1	3	16+56	19	45
2	Kappa	Lambda	19	5
3	3	7	8	4

CD45

- Vérification de la Lyse
- Rendu possible sur 100% des lymphocytes
- En cas de MO repérage des hématogones (comarquage avec CD19)
- Diagnostic d'immaturité devant une blastose difficile à caractériser



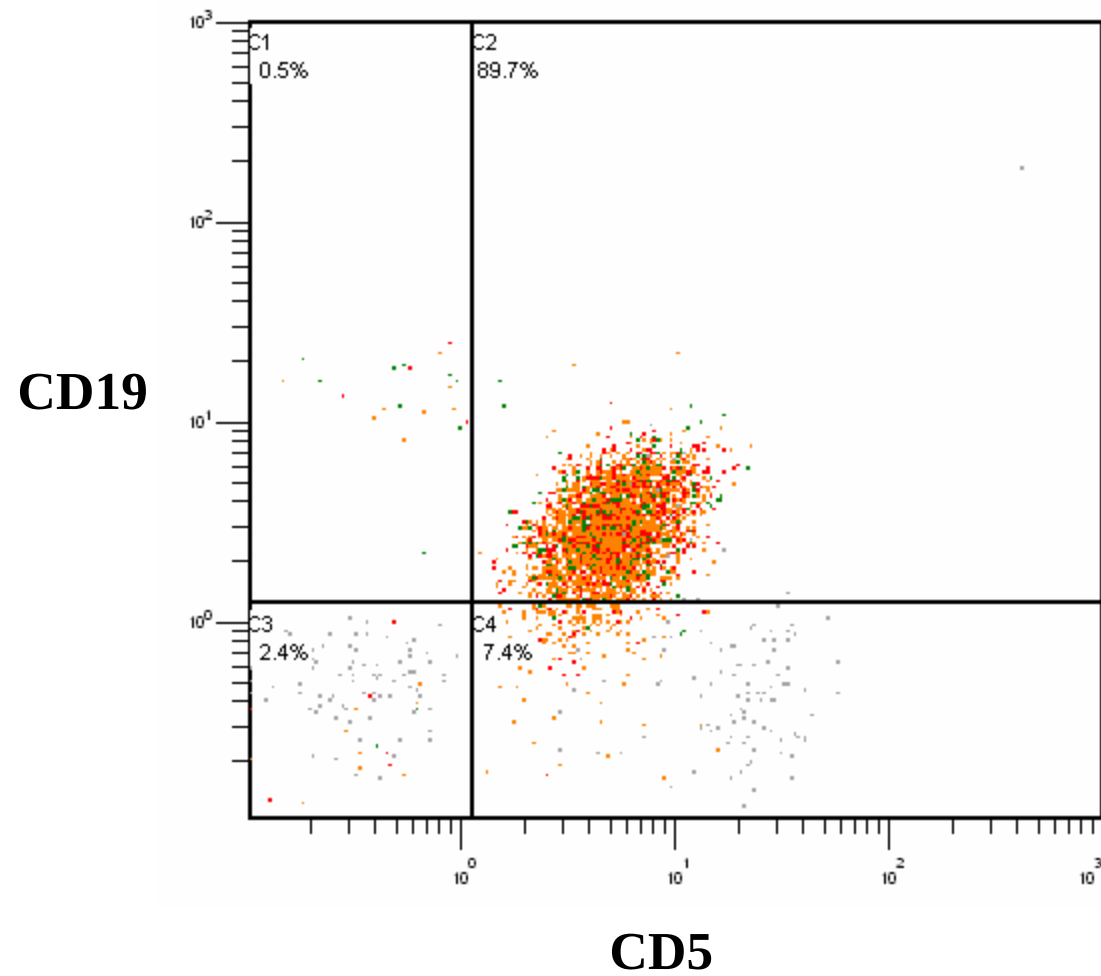
Panel Orientation : tube B

PANEL ORIENTATION HYPERLYMPHOCYTOSE				
N°				
1	3	16+56	19	45
2	Kappa	Lambda	19	5
3	3	7	8	4

- Mise en évidence de population B CD5+
- Etude de la clonalité de cette population



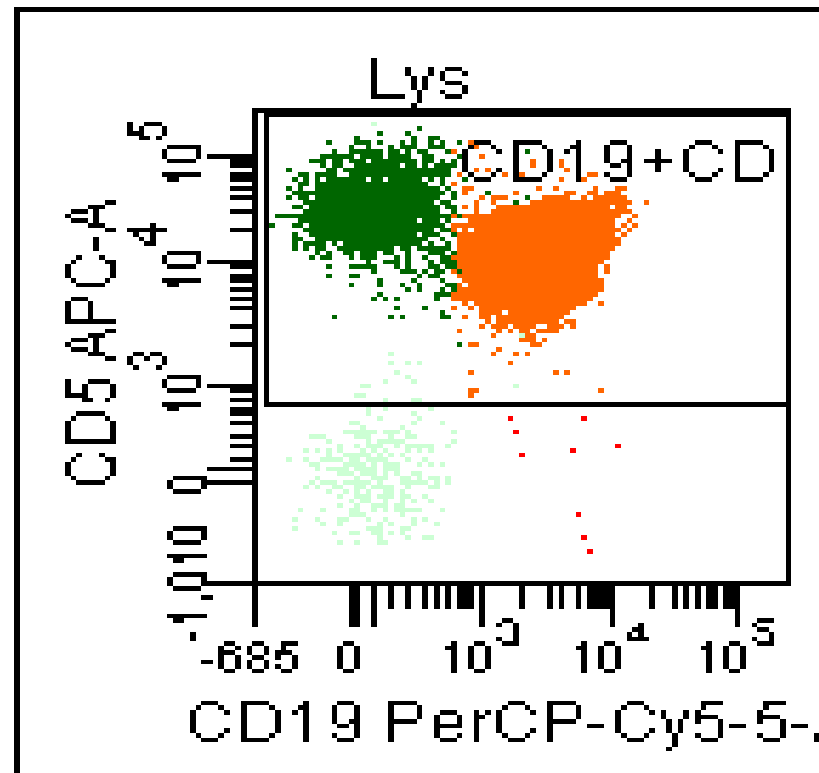
MCL



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LLC



Panel Orientation : tube B

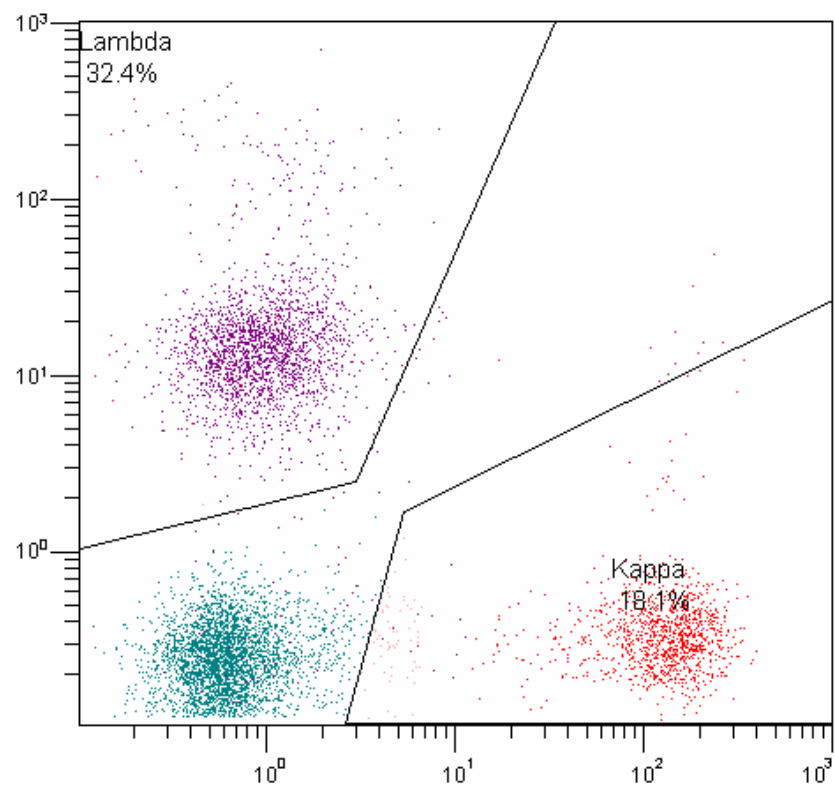
PANEL ORIENTATION HYPERLYMPHOCYTOSE					
N°					
1	3	16+56	19		45
2	Kappa	Lambda	19		5
3	3	7	8		4

- Kappa / Lambda avec CD19 (intensité comparative entre les différents B)
- Clonalité sans fixation non spécifique (NK)
- Même tube que CD5 d'où clonalité sur sous population B CD5+



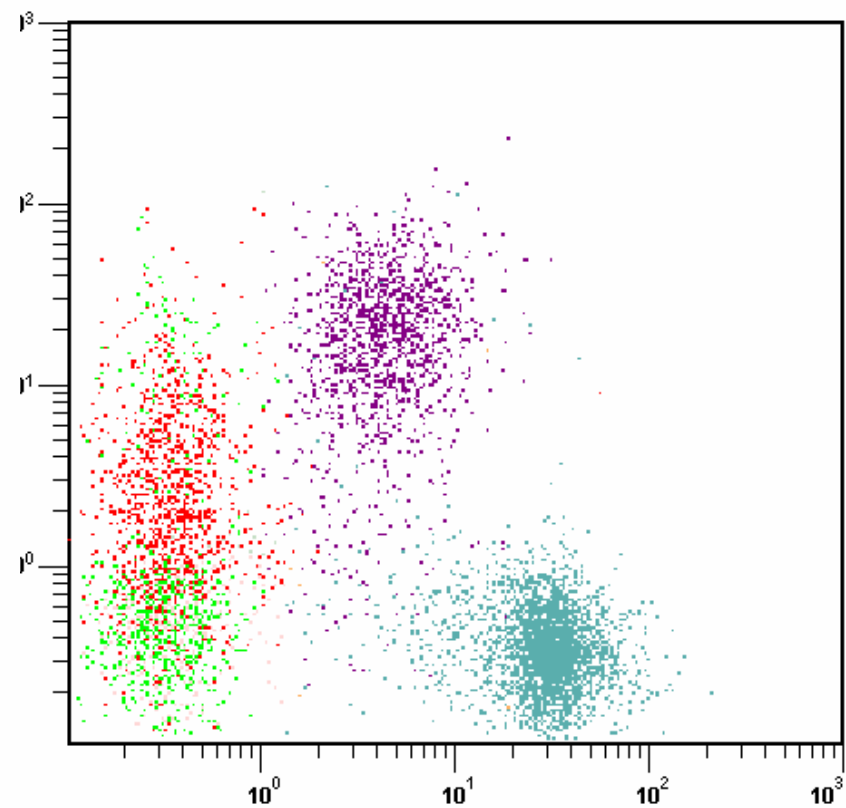
EXEMPLE DOUBLE LYMPHOPATHIE

Lambda



Kappa

CD19



CD5



B. Drénou 2007



Panel Orientation : tube T/NK

PANEL ORIENTATION HYPERLYMPHOCYTOSE				
N°				
1	3	16+56	19	45
2	Kappa	Lambda	19	5
3	3	7	8	4

- Etude des sous-populations T
- Vérification des populations T et T-NK
- Mise en évidence des populations CD3+ CD7(-);
CD4+ CD3(-)



Panel 6C

Suspicion
lymphome T
et/ou
dermatologie

Tubes	FITC	PE	Percp.C y5.5 PE.cy5	PE.cy7	APC	APC.cy 7
1	Kappa	Lambda	19	10	5	20
2	3	16+56	45	4	19	8
(3)	2	7	-	56	3	-

- Prolifération d'une population ?
- Clonalité B ?
- Caractère anormal (trou phénotypique, marqueurs aberrants) ?



B. Drénou 2007



Stratégie dans lymphopathies

Panel B CD5+

+

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	FMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG

et intra



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Stratégie dans lymphopathies

Panel B CD5+

Lymphome du Manteau
LLC

Autre lymphopathie B CD5+

-LNH B CD5+ de novo

-LNH de bas grade proche de la LLC

Exceptions: LNH Folliculaire
 LNH Villeux



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Panels B CD5+

LLC: score de Matutes

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	FMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG



Panels B CD5+

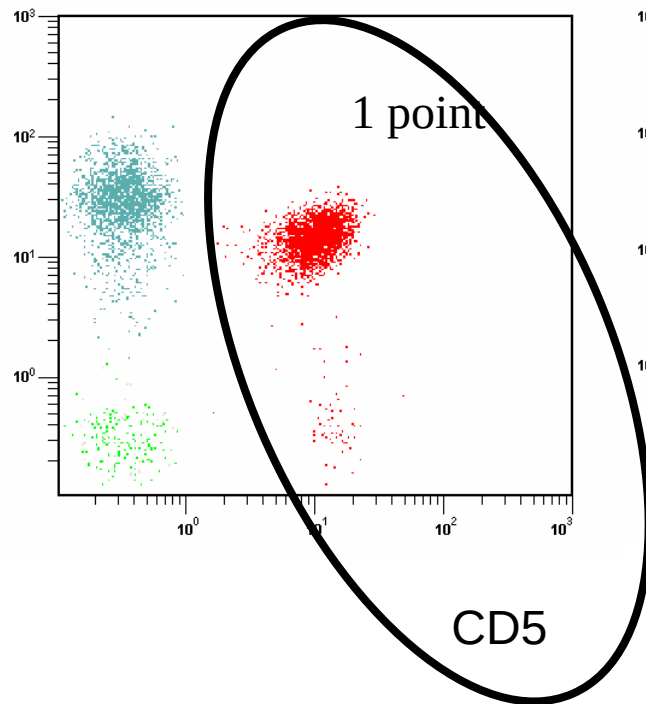
LLC: autres marqueurs

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	EMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG

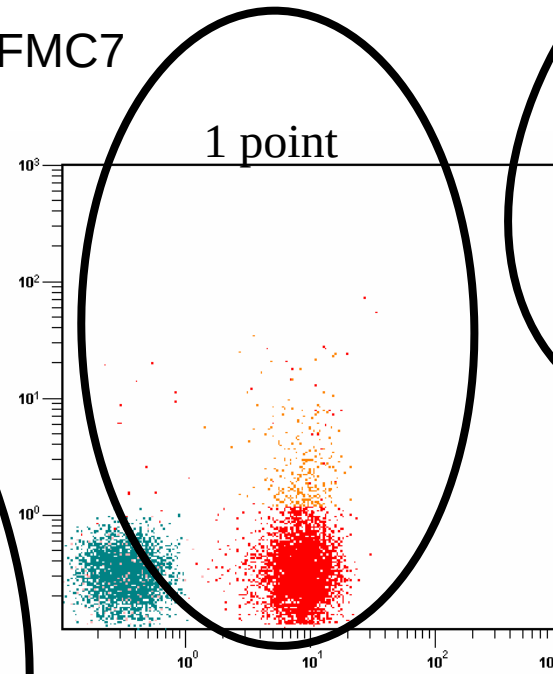


Matutes

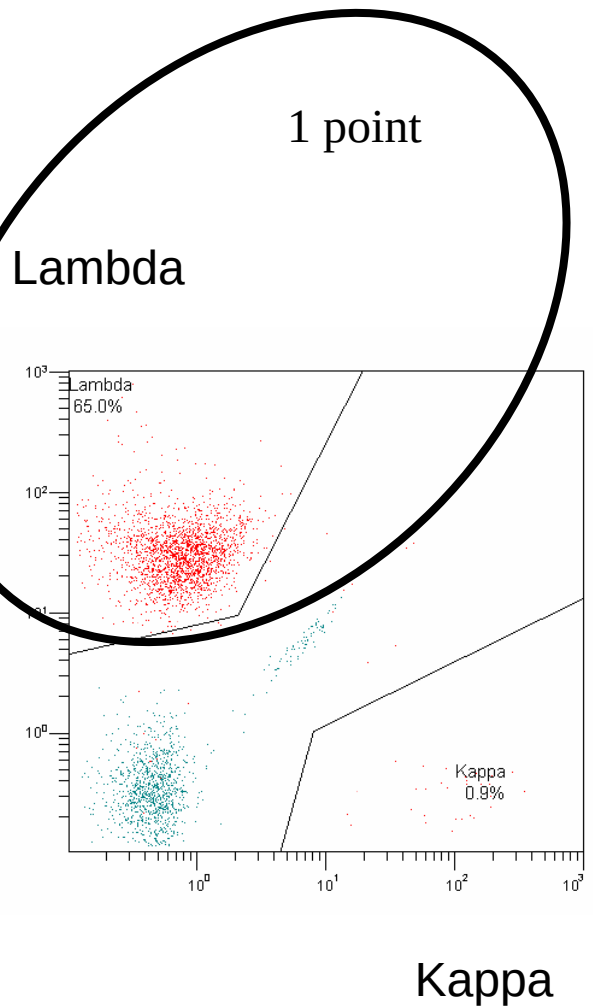
CD19



FMC7



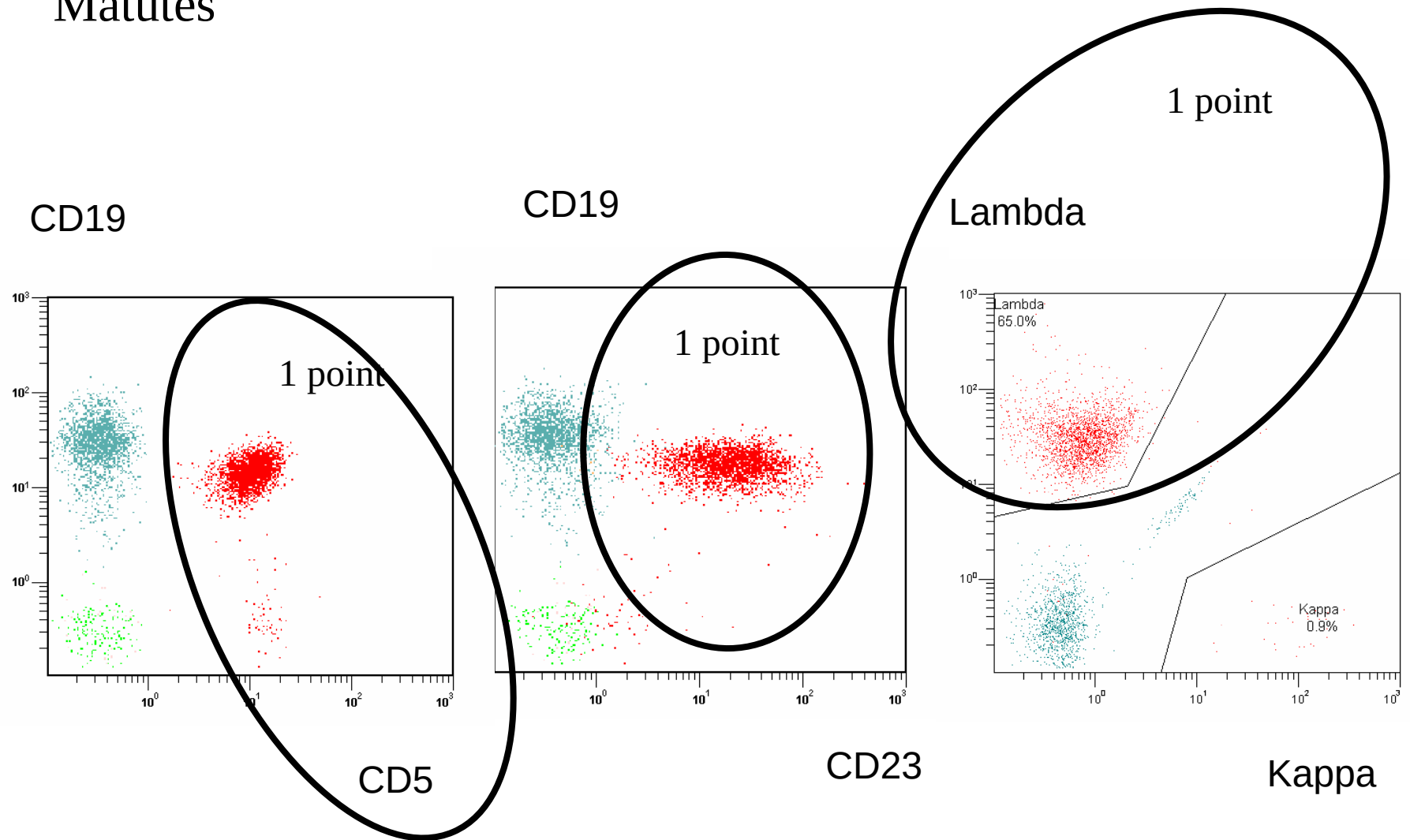
Lambda



B. Drénou 2007



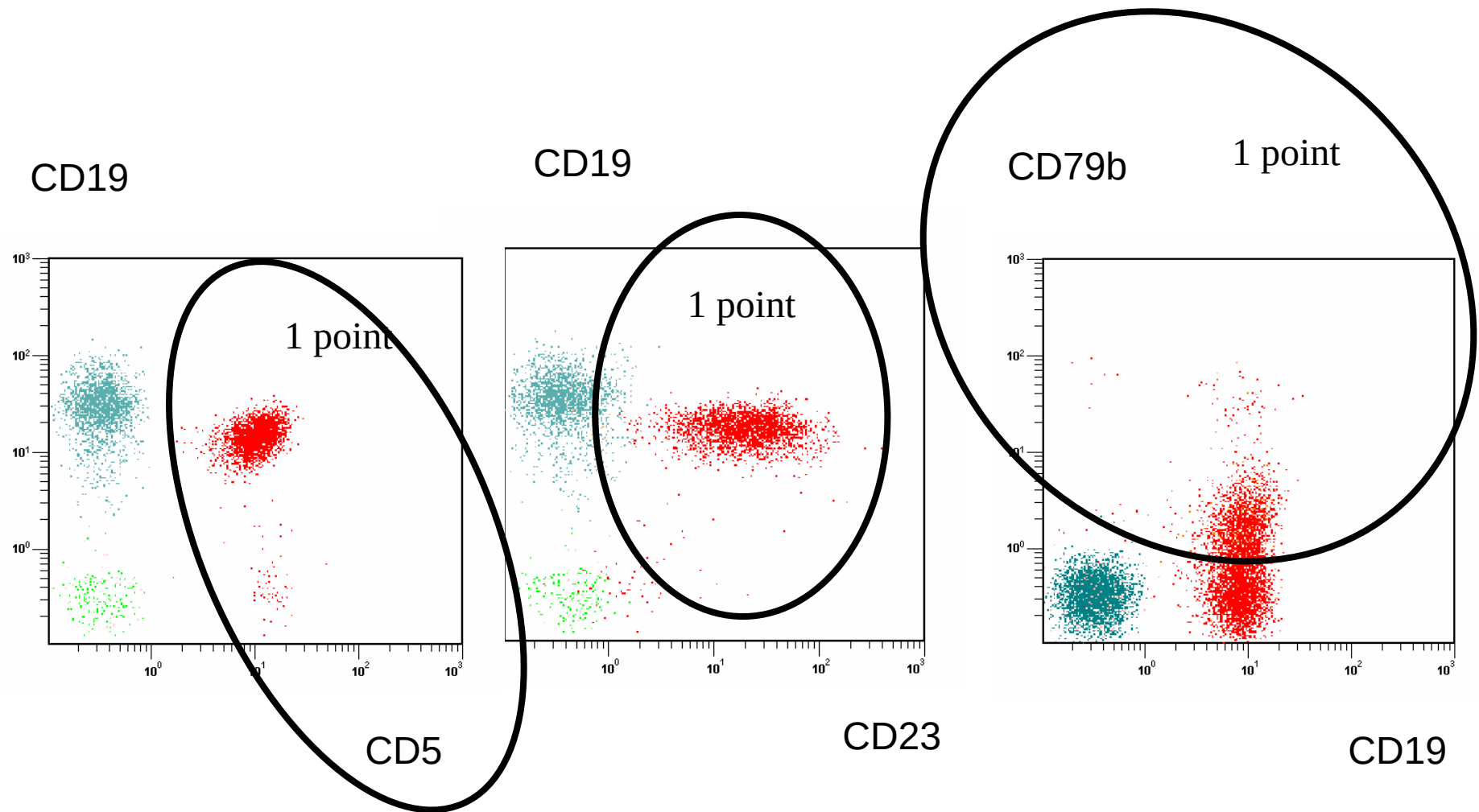
Matutes



B. Drénou 2007



Matutes



B. Drénou 2007



Panels B CD5+

LLC: autres marqueurs

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	FMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG

-Expression de CD38 sur B CD5+
(facteur pronostique des LLC)



B. Drénou 2007



Panels B CD5+

Diagnostic différentiel

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	FMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG

-CD10 marqueur centro-germinal
excluant LLC et Manteau
(diagnostic différentiel manteau
/folliculaire)



Panels B CD5+

Diagnostic différentiel

PANEL COMPLEMENT LYMPHOS B CD5+				
Tube n°				
1	FMC7	38 *	19	5*
2	20	22 *	19	23
3	43	10 *	19	79b *
4 si Lymphome et/ou Ganglion	IgD	IgM	19	IgG

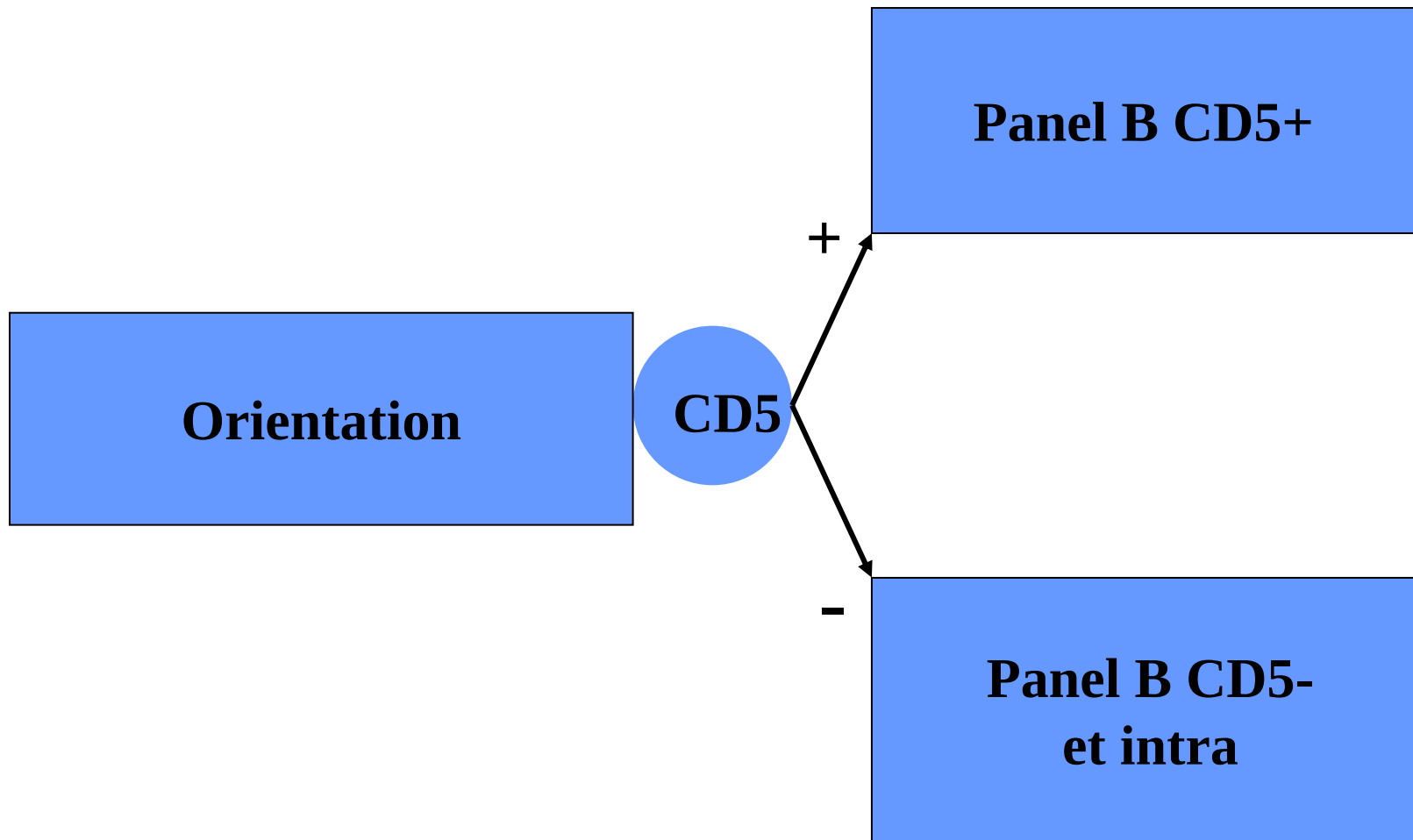
-Principales chaines lourdes utiles pour
diagnostic de LNH du manteau



B. Drénou 2007



Stratégie Lymphome



Panel B CD5-

PANEL COMPLEMENT LYMPHOS B CD5-				
Tube n°				
1	20	22 *	19	23
2	44	10	19	45
3 intra contrôles	Crtl _c	Crtl _c	19	44
4 intra	Ki67 _c	Bcl2 _c	19	44

- Tube n°2 du complément B CD5+
- Mise en évidence des cellules B immatures CD19+ CD20(-)
- Mise en évidence d'une double population B (basée sur intensité de CD20/22 voire CD23)

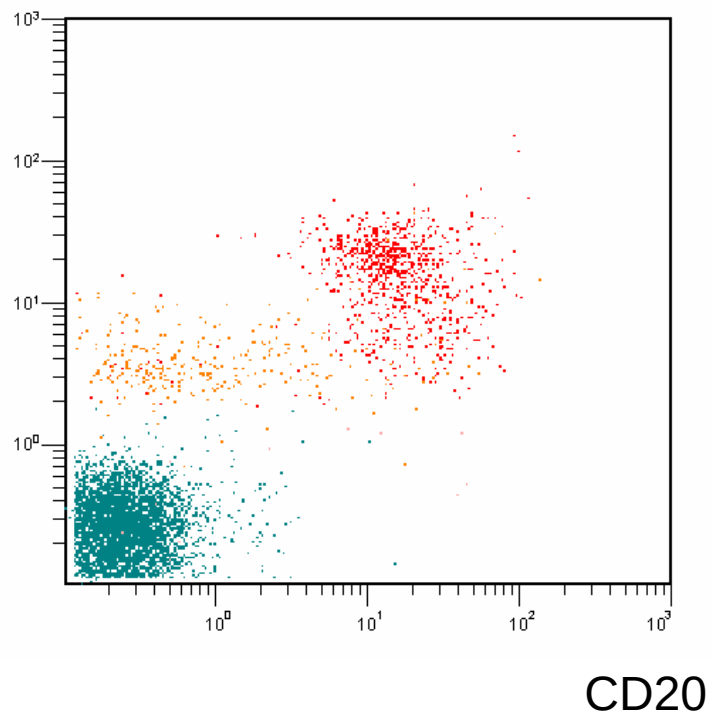


B. Drénou 2007

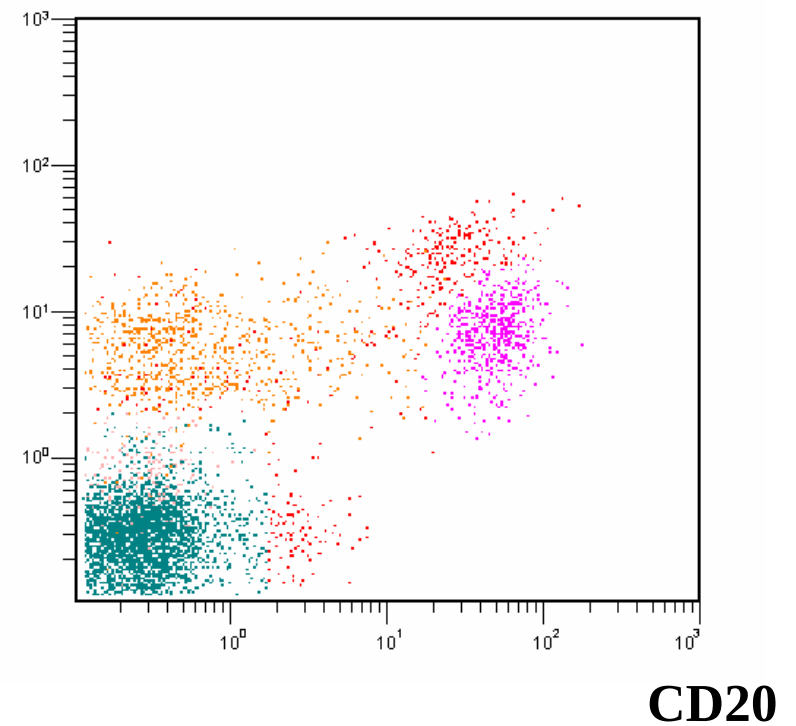


CD20 / CD22

CD22



CD22



B. Drénou 2007



Panels B CD5(-) : tube centrogeminal

PANEL COMPLEMENT LYMPHOS B CD5-				
Tube n°				
1	20	22 *	19	23
2	44	10	19	45
3 intra contrôles	Critl c	Critl c	19	44
4 intra	Ki67 c	Bcl2 c	19	44

- Les cellules CD19+ CD44 « faible » sont repérées
- Le comarquage avec CD45 permet de séparer pré-B et GC
- Il y a corrélation avec l'expression de CD10

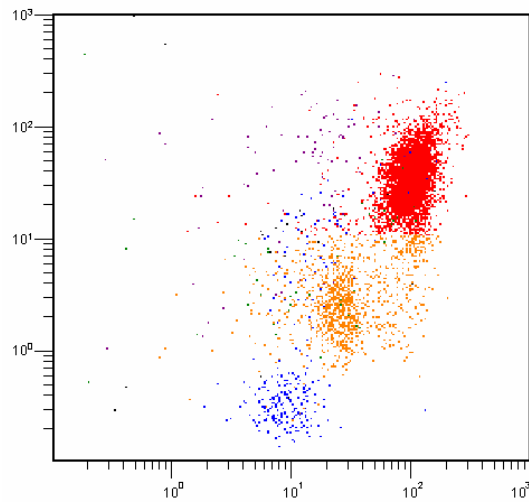


MO normale

MO sous anti CD20

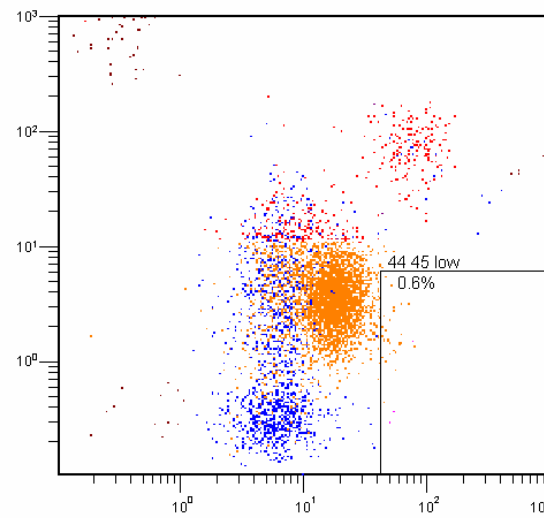
MO LNH folliculaire

CD44



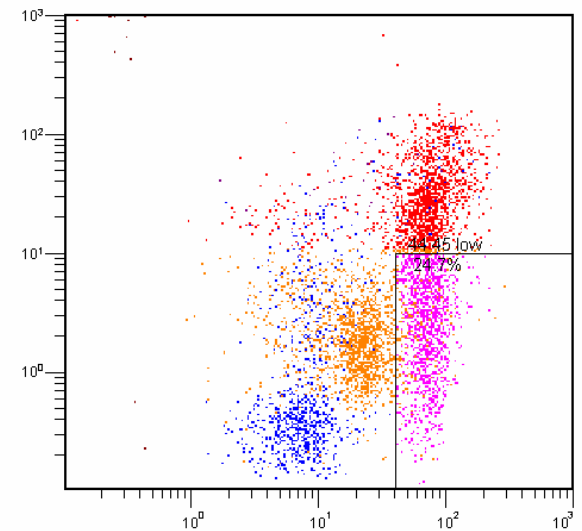
CD45

CD44



CD45

CD44



CD45



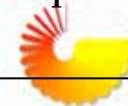
B. Drénou 2007



Panels B CD5(-) : tube intra

PANEL COMPLEMENT LYMPHOS B CD5-				
Tube n°				
1	20	22 *	19	23
2	44	10	19	45
3 intra contrôles	Crtl _c	Crtl _c	19	44
4 intra	Ki67 _c	Bcl2 _c	19	44

- Les cellules lymphomateuses (GC) CD19+ CD44 « faible » sont repérées
- Le comarquage avec Ki67 permet de repérer le degré de prolifération
- Le comarquage avec Bcl2 permet de diagnostiquer les LNH de Burkitt



B. Drénou 2007



Panels B CD5(-) : tube intra

PANEL COMPLEMENT LYMPHOS B CD5-				
Tube n°				
1	20	22 *	19	23
2	44	10	19	45
3 intra contrôles	Crtl _c	Crtl _c	19	44
4 intra	Ki67 _c	Bcl2 _c	19	44

-Le contrôle isotypique est nécessaire en intra cytoplasmique



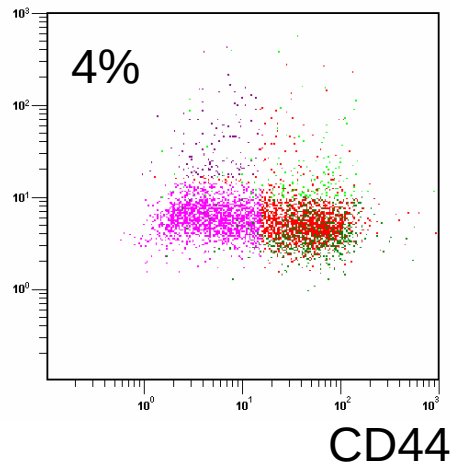
B. Drénou 2007



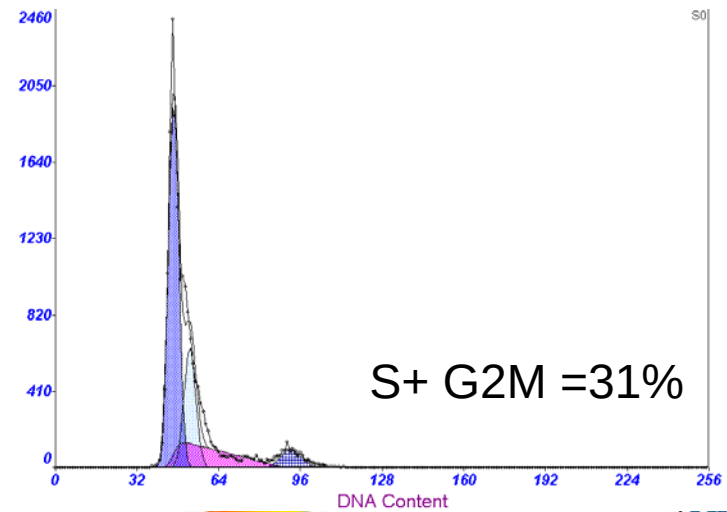
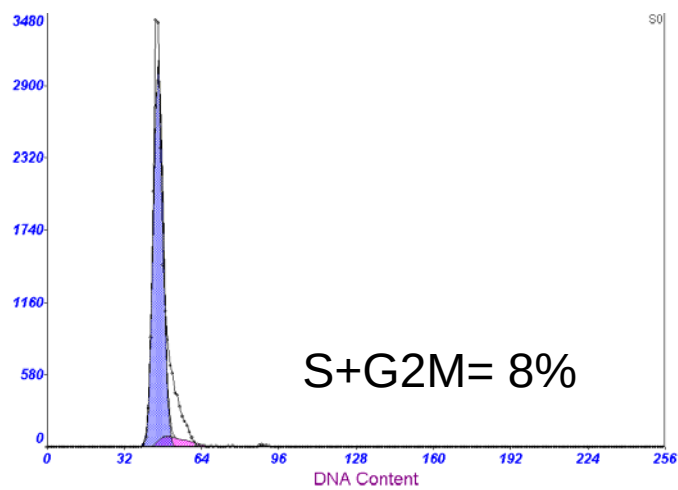
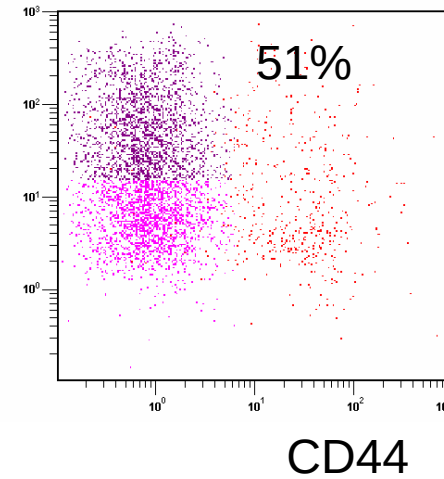
Panels B CD5(-) : tube intra

Ki67

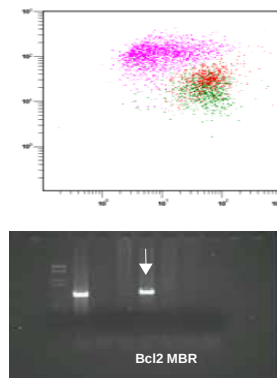
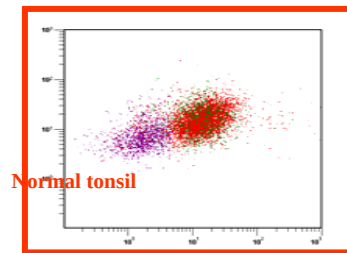
Ki67



Ki67

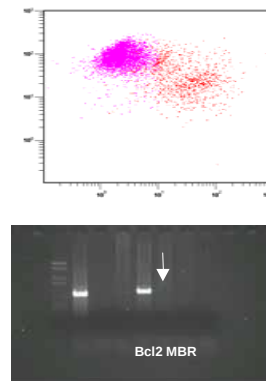


Panels B CD5(-) : tube intra Bcl2



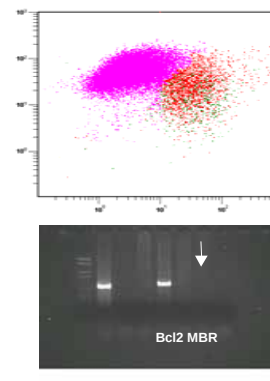
t(14;18)

Bcl2 MBR

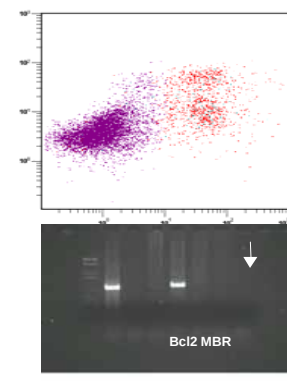
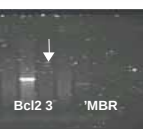


t(14;18)

Bcl2 3' MBR



t(14;18)



Pas de t(14;18)



Bcl2 ↑
CD44 →

Absence de detection
de rearrangement



B. Drénou 2007

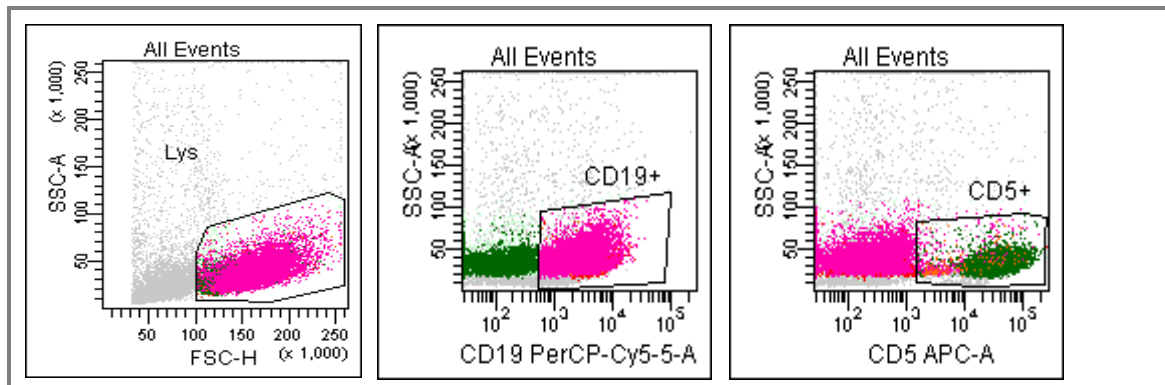


Cas : Ganglion

ORIENTATION Tube 1:

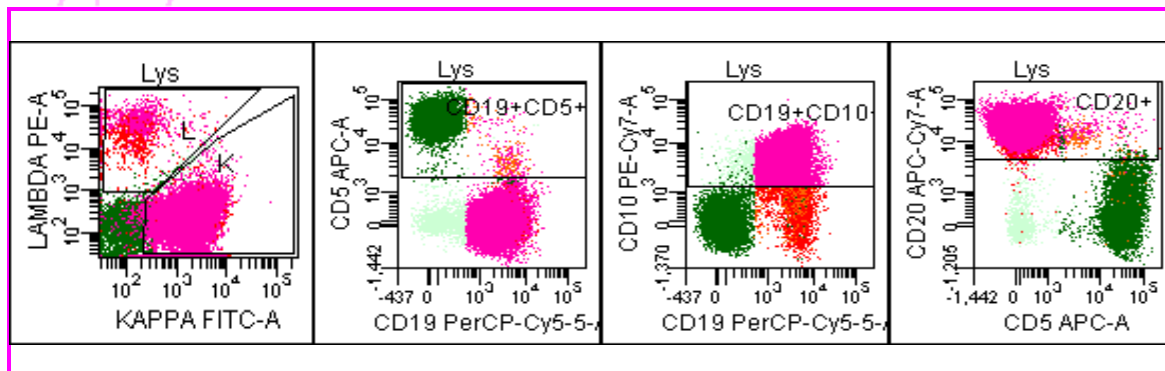
Kappa/Lambda/CD19/CD10/CD5/CD20

Cellules totales



Population	Parent Na...	%Parent
☒ CD20+	Lys	71.0
☒ CD10+	Lys	60.9
☒ K	Lys	64.0
☒ L	Lys	6.1
■ CD5+	Lys	26.7
■ CD19+	Lys	68.9
■ CD19+CD5+	CD19+	2.2
■ CD19+CD10+	CD19+	83.7
☒ CD19+ Kappa	CD19+	90.1
☒ CD19+ Lambda	CD19+	8.6

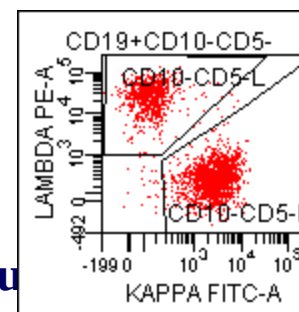
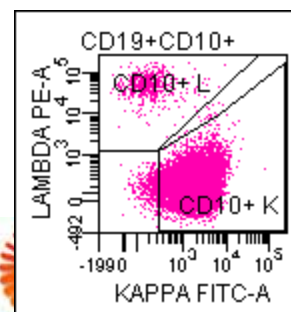
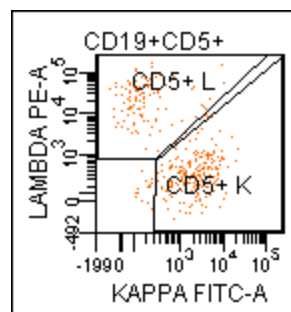
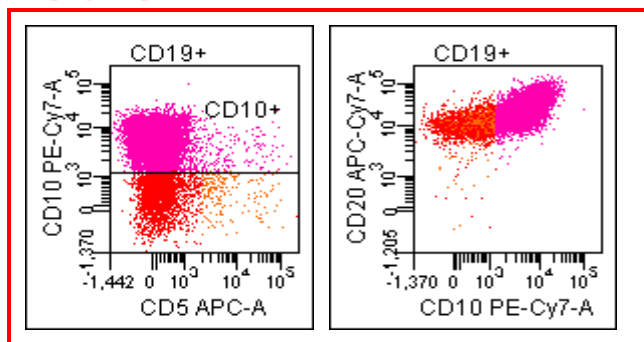
Lymphocytes



+ Sous-populations....

Population	%Parent
☒ CD5+ K	60.9
☒ CD5+ L	32.0
☒ CD10+ K	92.9
☒ CD10+ L	3.7
☒ CD10-CD5-K	64.4
☒ CD10-CD5-L	33.9

Lymphocytes B CD19+

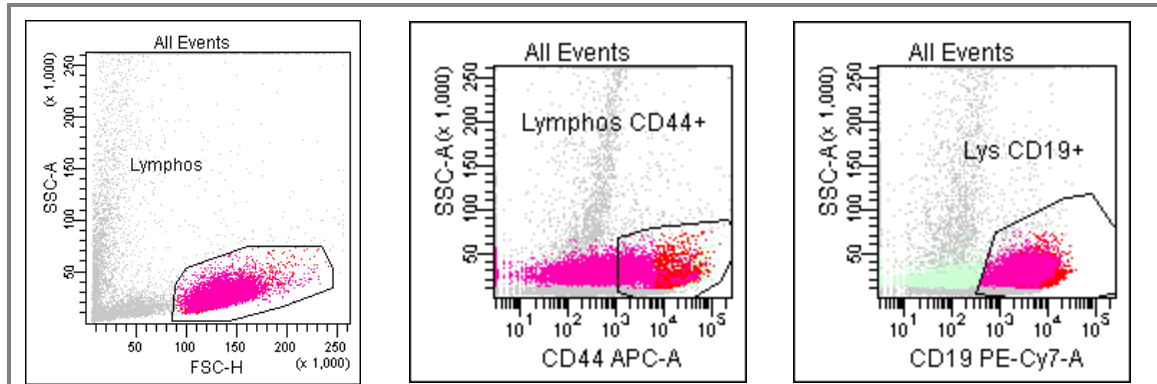


Cas : Ganglion

Panel B CD5- Tube 11:

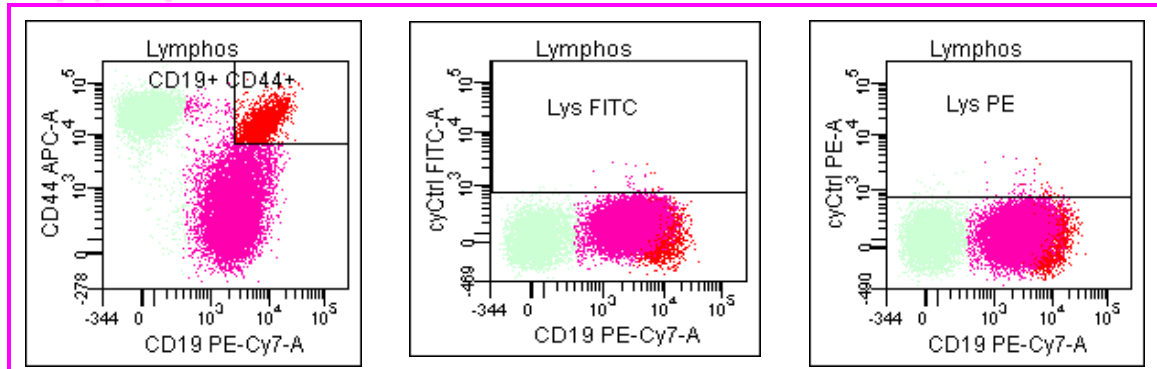
cyCTRL/cyCTRL/-/CD19/CD44/-

Cellules totales



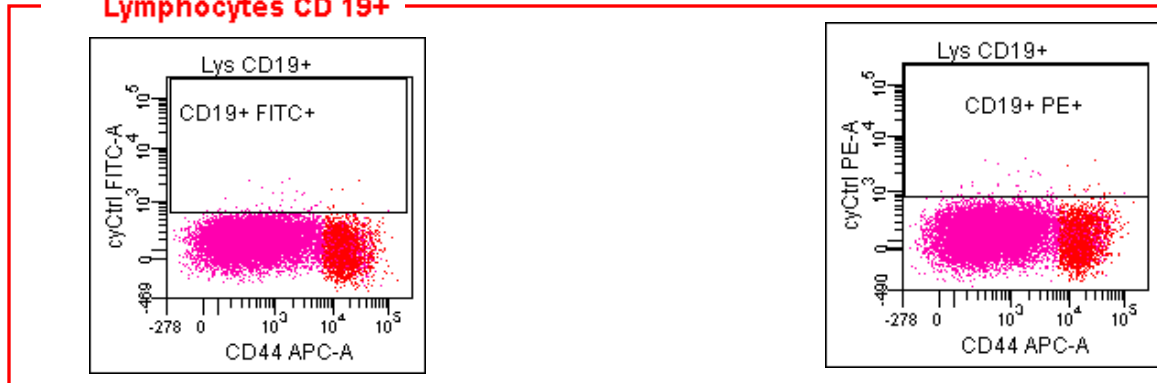
Population	#Events
Lymphos	19,280
Lys CD19+	14,090

Lymphocytes



Population	Parent Name	%Parent
☒ Lymphos CD44+	Lymphos	54.6
☒ Lys FITC	Lymphos	0.2
☒ Lys PE	Lymphos	0.5
☒ Lys CD19+	Lymphos	73.1
☒ CD19+ FITC+	Lys CD19+	0.4
☒ CD19+ PE+	Lys CD19+	0.4
☒ CD19+ CD44+	Lys CD19+	20.2

Lymphocytes CD 19+



B. Drénou 2007

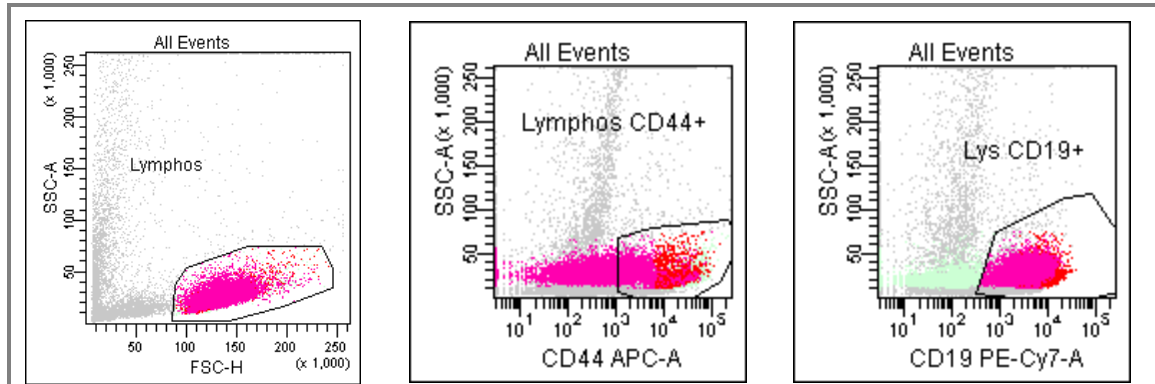


Cas : Ganglion

Panel B CD5- Tube 12:

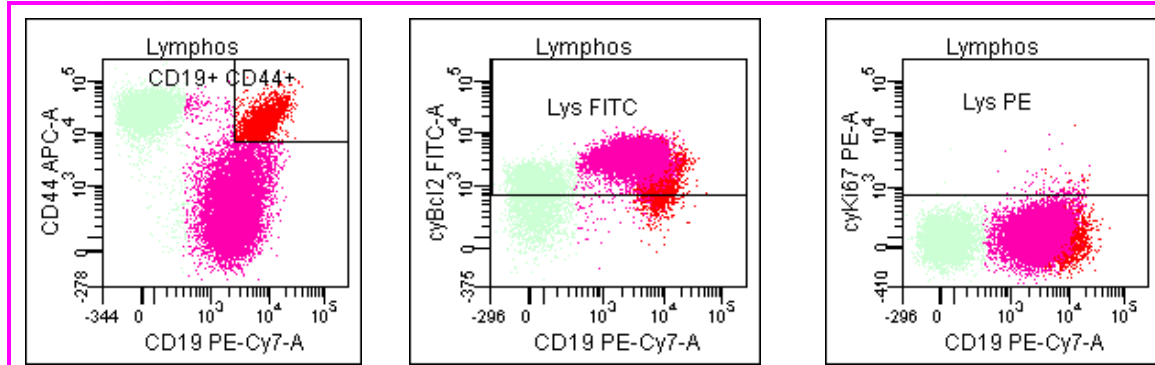
cyBcl-2/cyKi67/-/CD19/CD44/-

Cellules totales



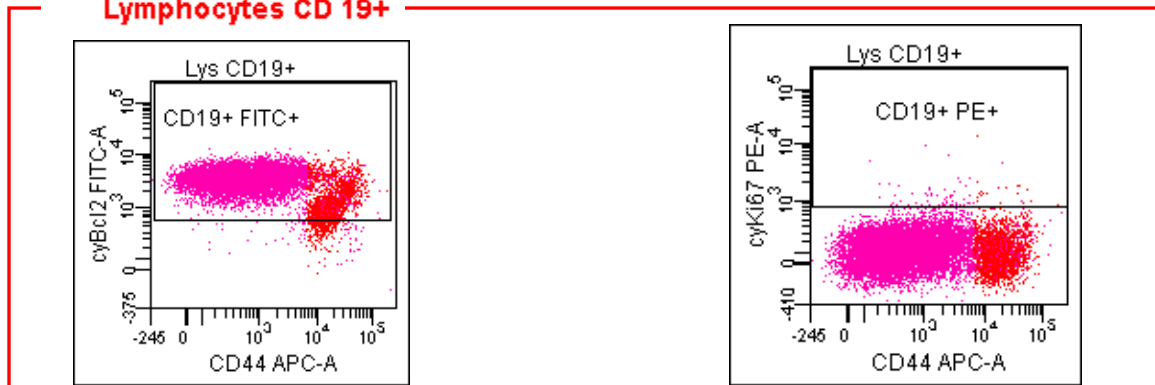
Population	#Events
Lymphos	17,180
Lys CD19+	12,797

Lymphocytes



Population	Parent Name	%Parent
☒ Lymphos CD44+	Lymphos	54.6
☒ Lys FITC	Lymphos	86.2
☒ Lys PE	Lymphos	0.6
☒ Lys CD19+	Lymphos	74.5
☒ CD19+ FITC+	Lys CD19+	97.2
☒ CD19+ PE+	Lys CD19+	0.7
☒ CD19+ CD44+	Lys CD19+	18.2

Lymphocytes CD 19+



B. Drénou 2007



Ex: multi-couleur et diagnostic

60 years old male

Admitted for oral bleeding

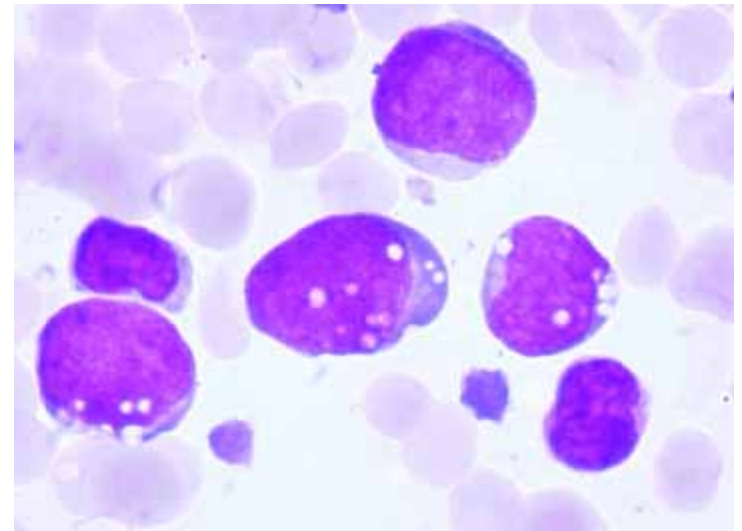
Blood:

- Anaemia Hb 9g/dl
- Thrombopenia 10G/l
- Lymphocytosis (small lymphocytes) 22 G/l

Bonne marrow

- Blast cells 70%

CNS (-)ve



Bone-marrow

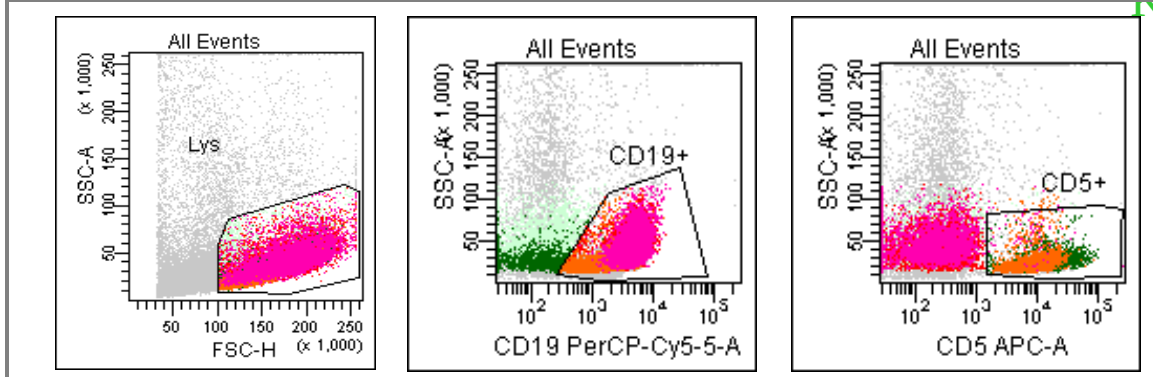


B. Drénou 2007



Bone- marrow analysis using flow-cytometry

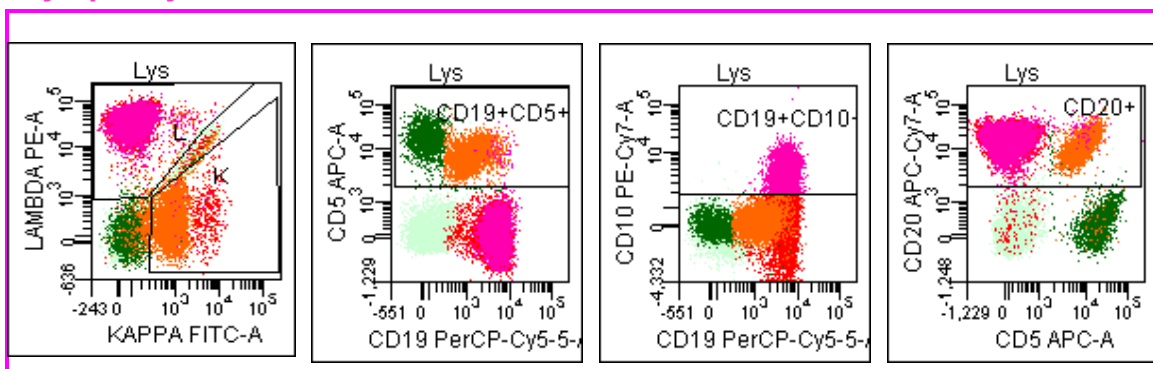
All events



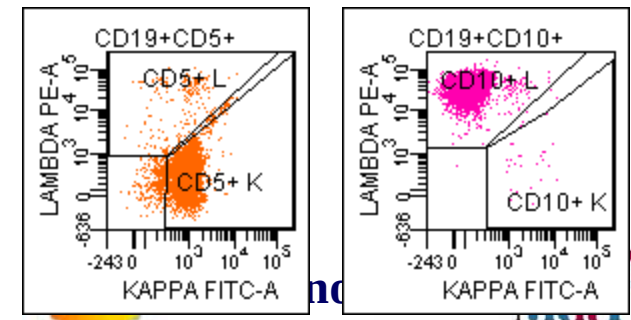
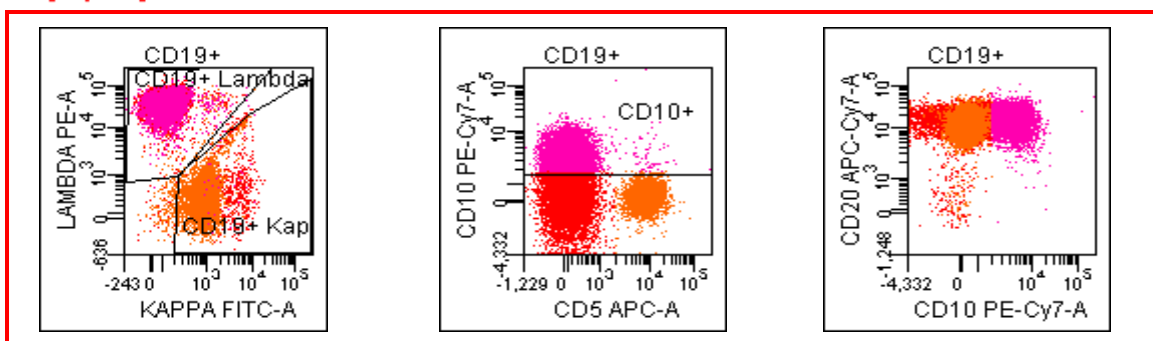
Population	#Events
All Events	30,000
Lys	21,016

Population	Parent Na...	%Parent
CD20+	Lys	73.3
CD10+	Lys	29.1
K	Lys	18.3
L	Lys	55.0
CD5+	Lys	25.0
CD19+	Lys	73.5
CD19+CD5+	CD19+	23.3
CD19+CD10+	CD19+	35.8
CD19+ Kappa	CD19+	24.4
CD19+ Lambda	CD19+	73.8

Lymphocytes

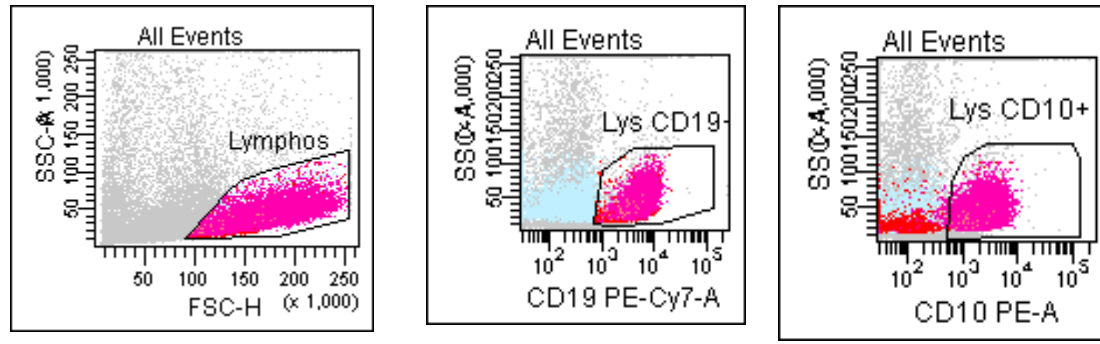


Lymphocytes B CD19+

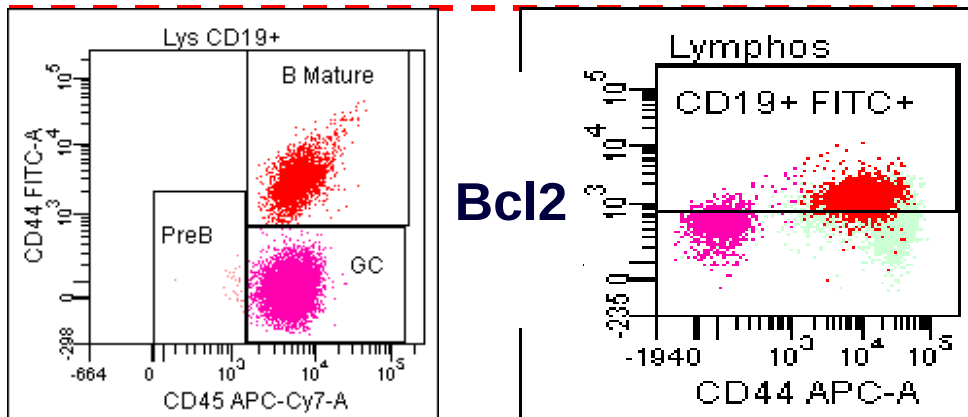
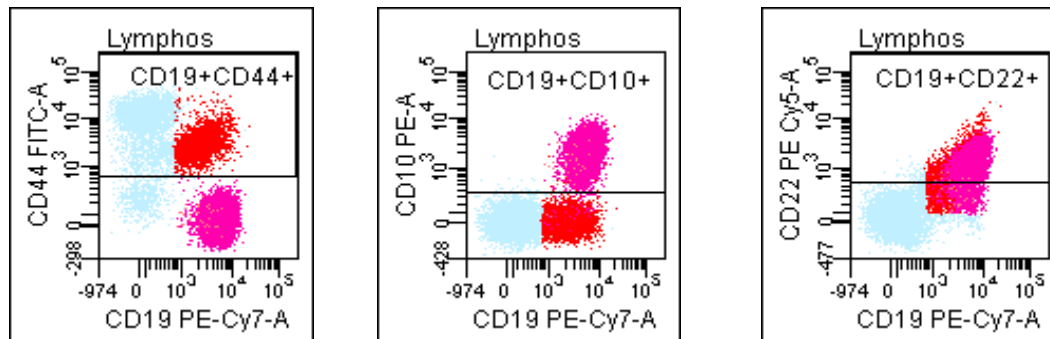


Bone- marrow analysis using flow-cytometry

All events



Population	Parent Na...	%Parent
☒ Lys CD44+	Lymphos	43.7
☒ Lys CD10+	Lymphos	52.2
☒ Lys CD22+	Lymphos	43.1
☐ Lys CD19+	Lymphos	72.8
☒ CD19+CD44+	Lys CD19+	28.0
☒ CD19+CD10+	Lys CD19+	72.3
☒ CD19+CD22+	Lys CD19+	81.9
■ B Mature	Lys CD19+	28.0
■ GC	Lys CD19+	71.7
■ PreB	Lys CD19+	0.3



Bcl2

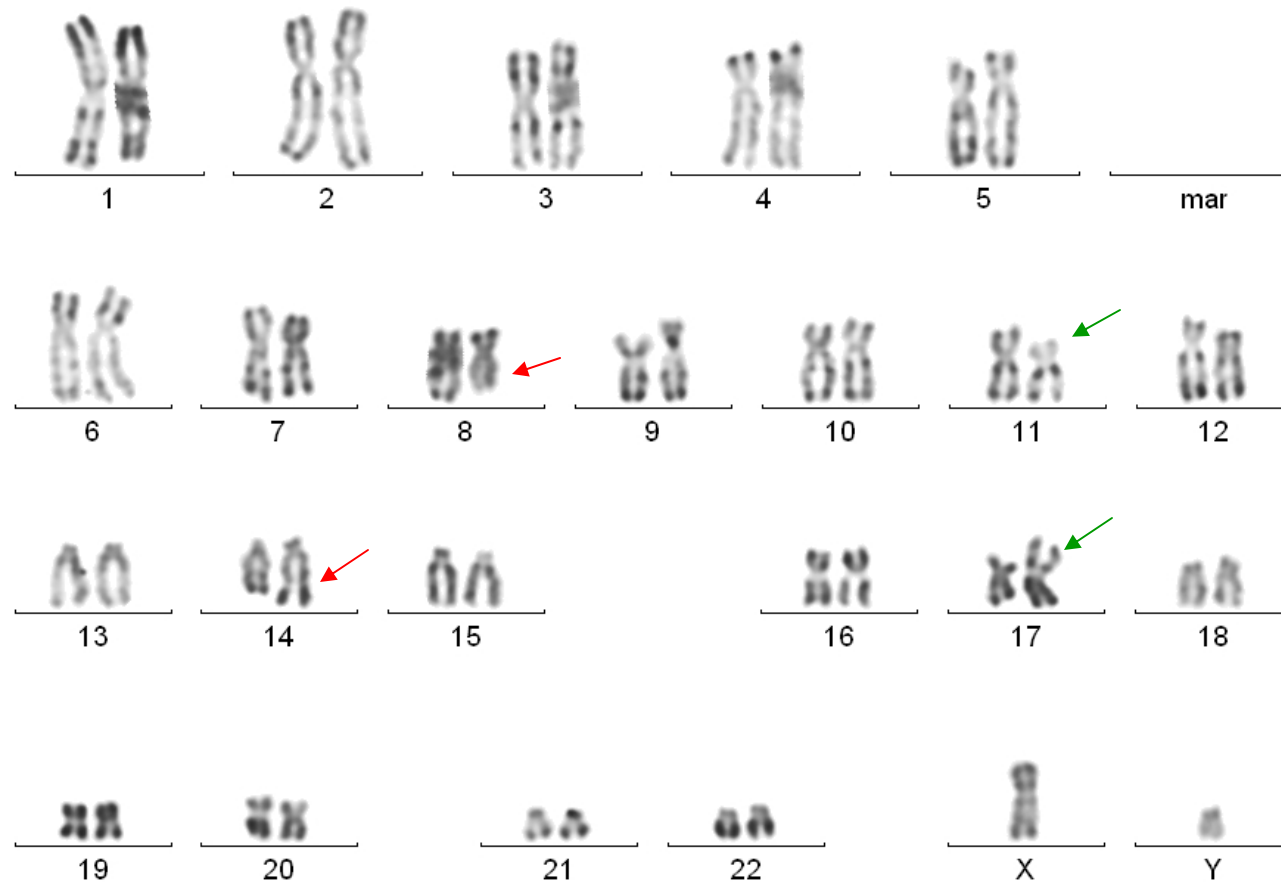
- Two clones
CLL
Atypical Burkitt
- Cytogenetic
t(8;14)
t(11;17)
ATM duplication
Loss of p53



B. Drénou 2007



Cytogénétique conventionnelle...



B. Drénou 2007



Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
- Pronostic
- Thérapeutique

En pratique: marqueurs personnels

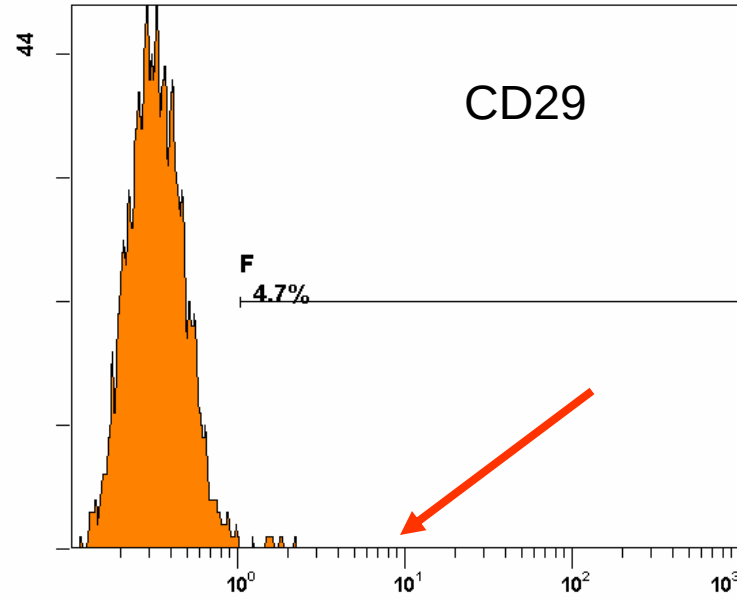
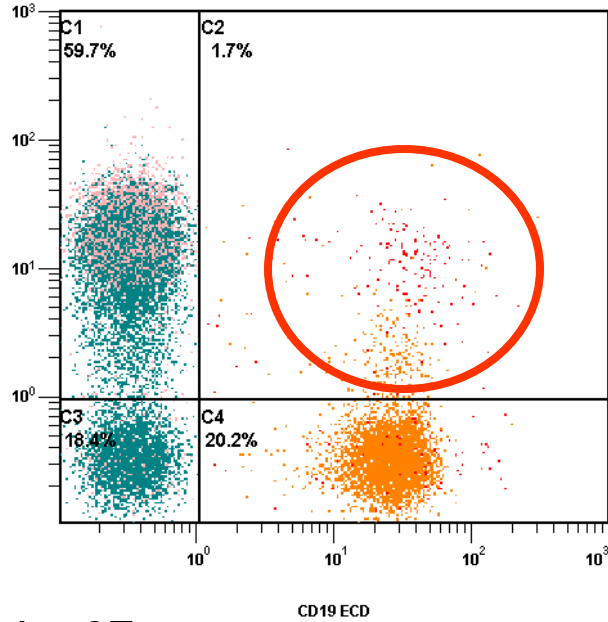
Suivi

– Stratégies multi-couleurs

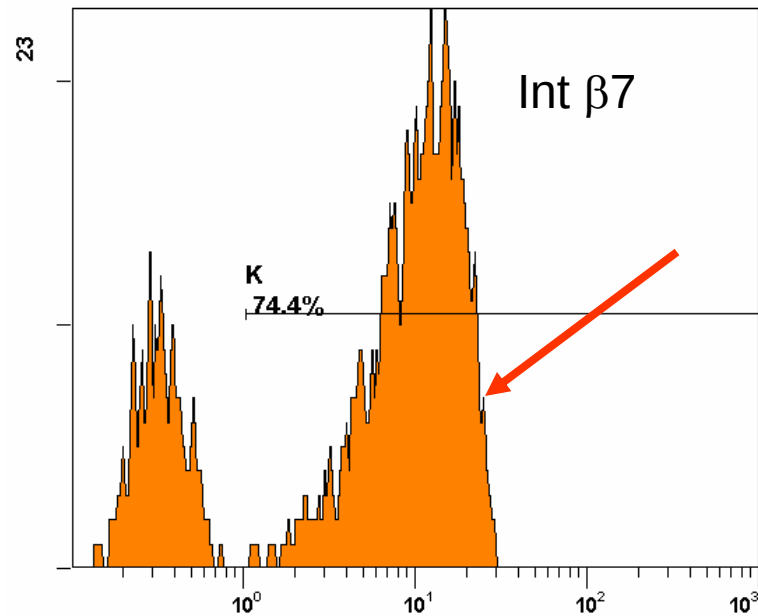
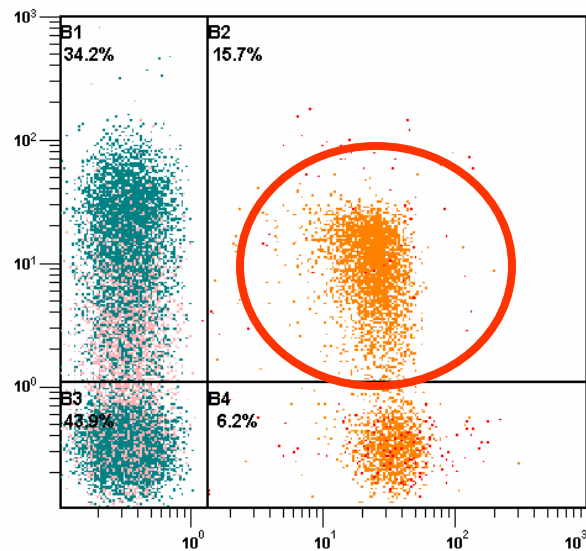


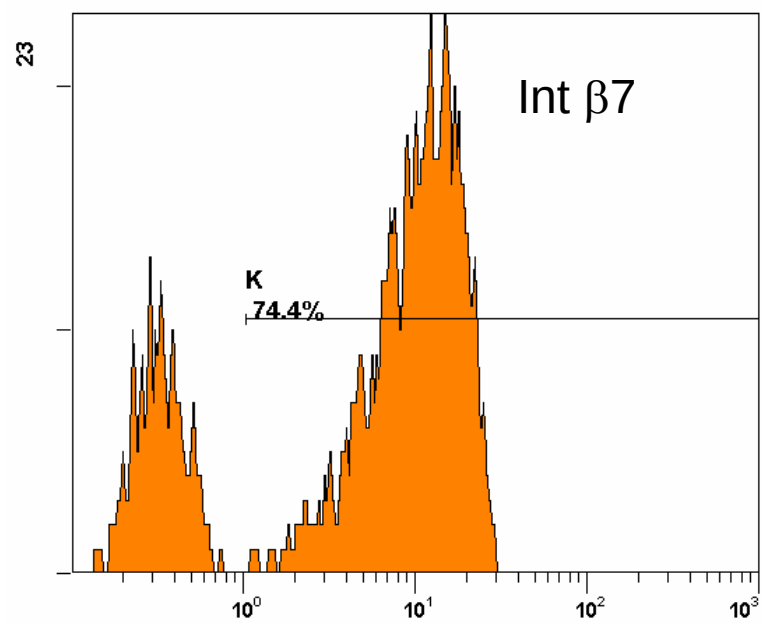
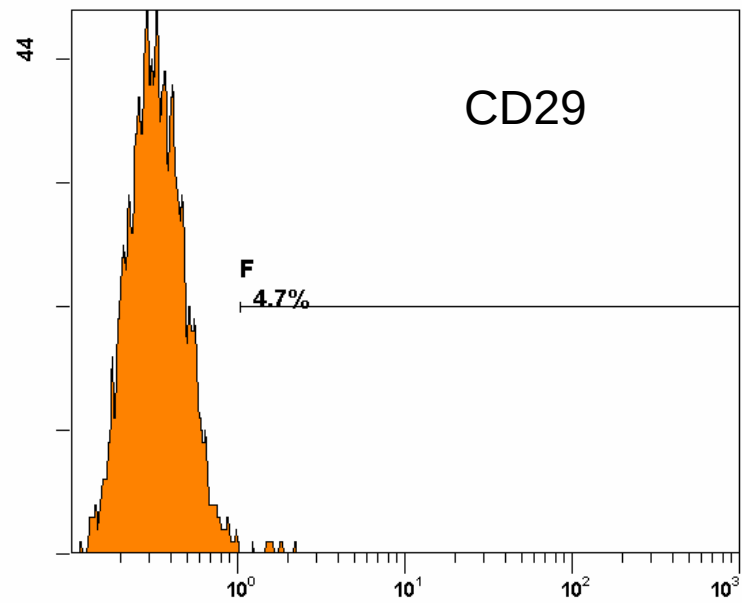
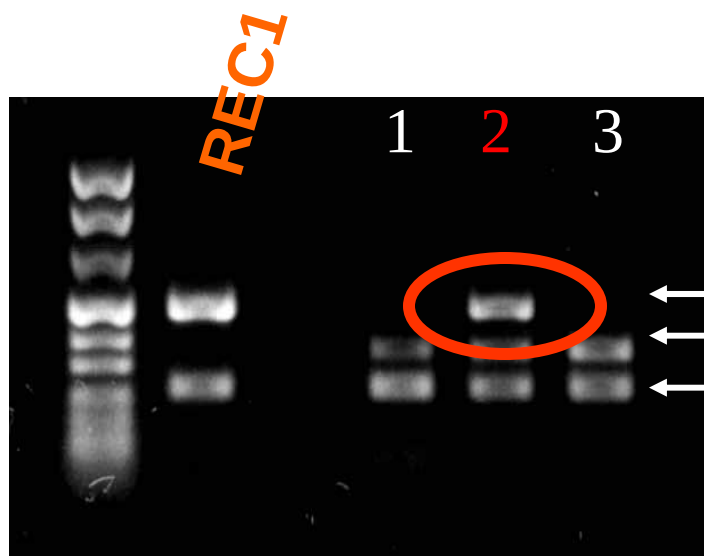
Lymphome du manteau

CD29

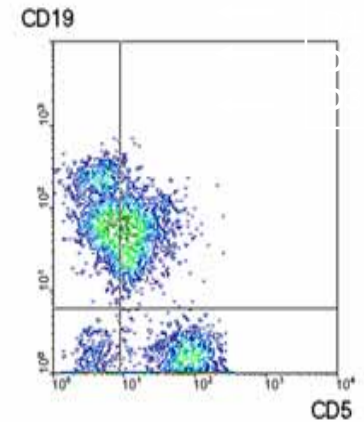
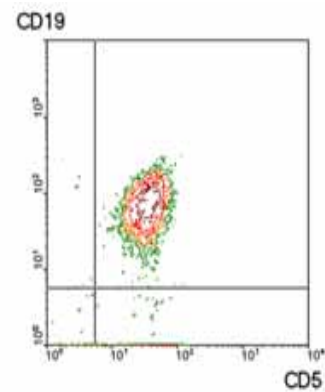
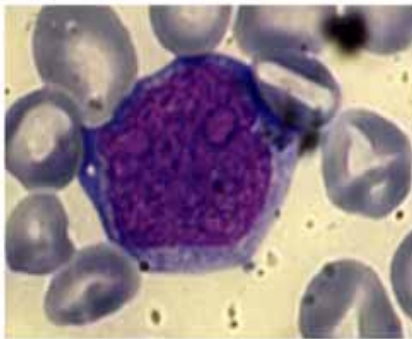
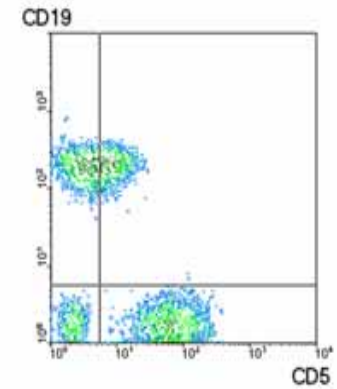
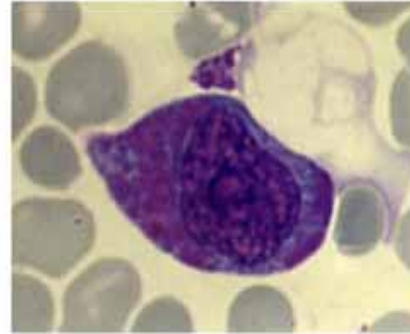
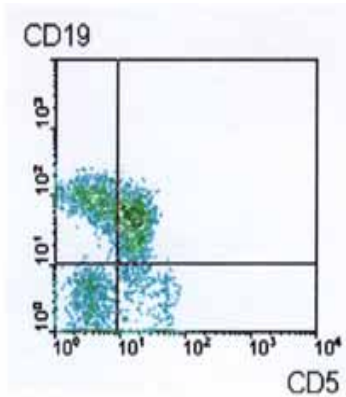
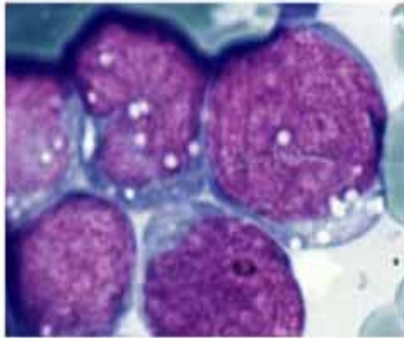


Int β 7





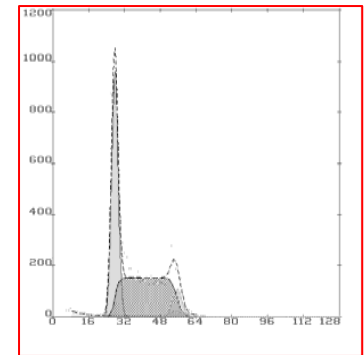
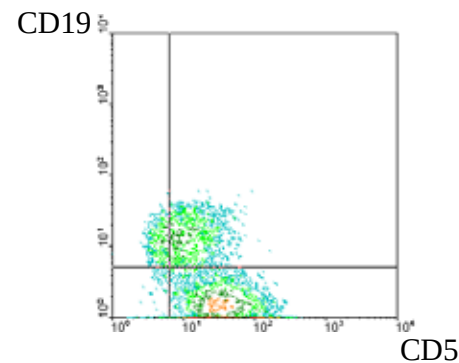
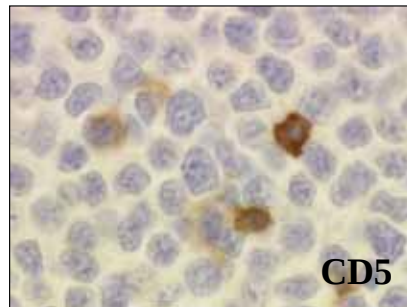
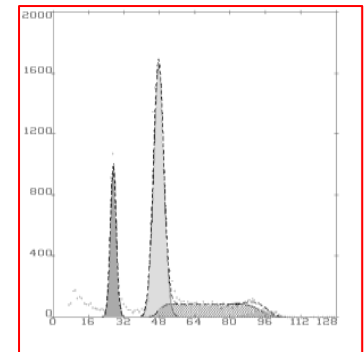
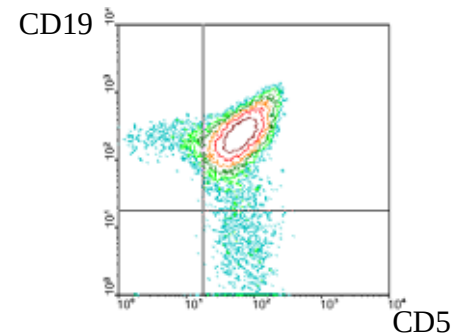
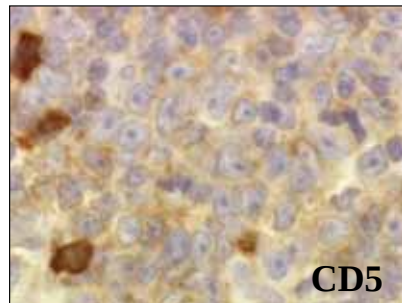
LNH B CD5+ de novo / MCL blastique variant



B. Drénou 2007



Cycle cellulaire : LNH B CD5+ de novo et MCL blastique variant



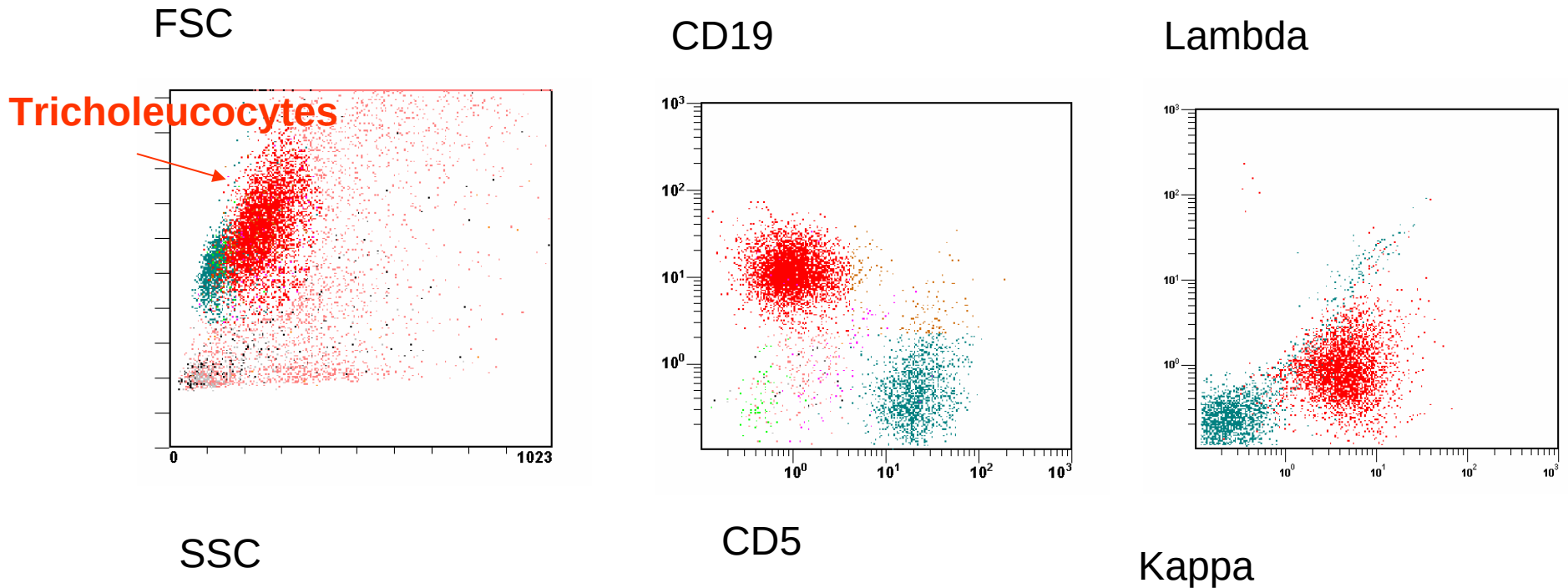
Cycle cellulaire pour mise en évidence de tétraploïdie (MCL)



B. Drénou 2007



Leucémie à tricholeucocytes



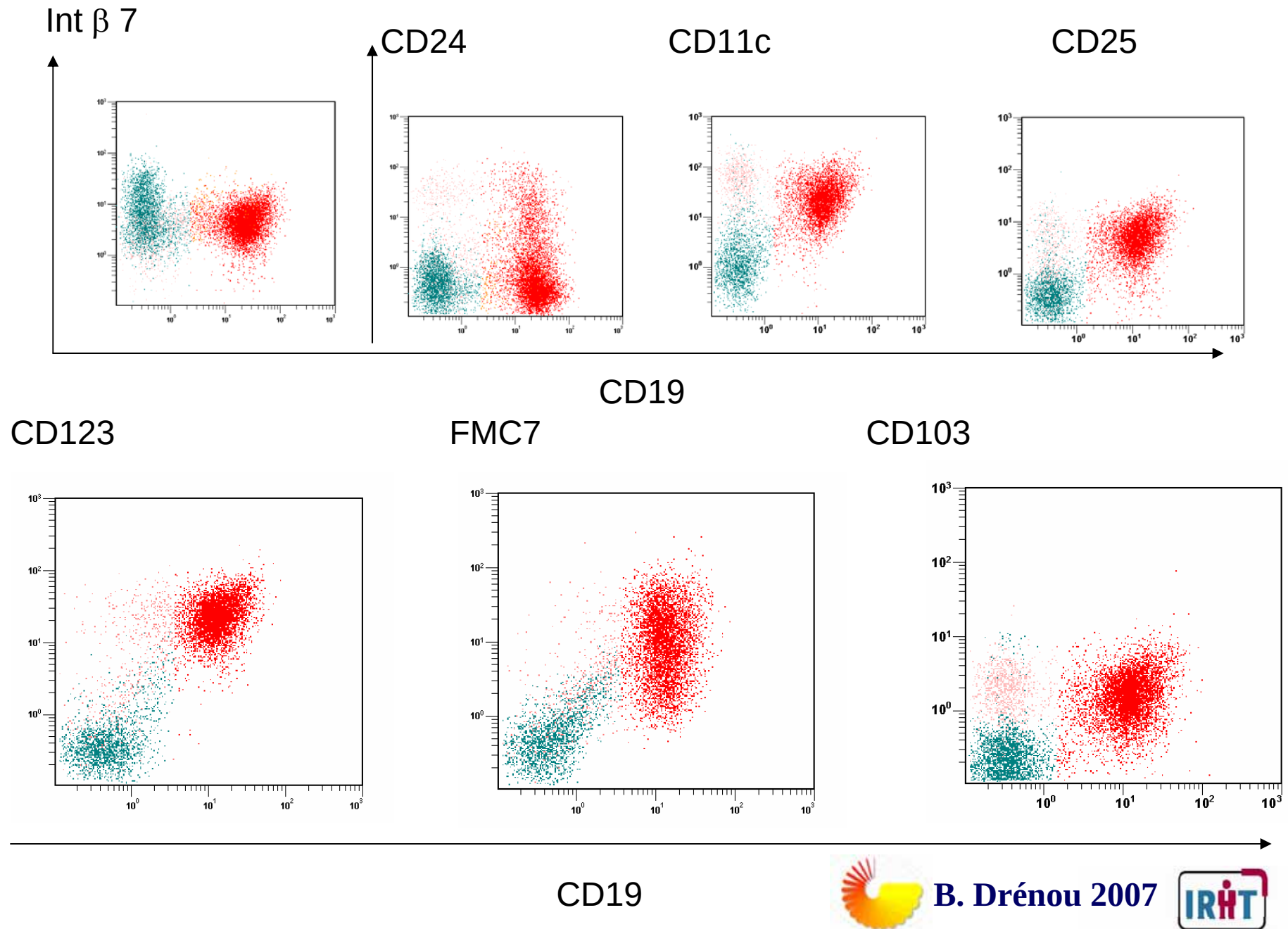
Phénotype: cellules se projetant dans la zone des monocytes

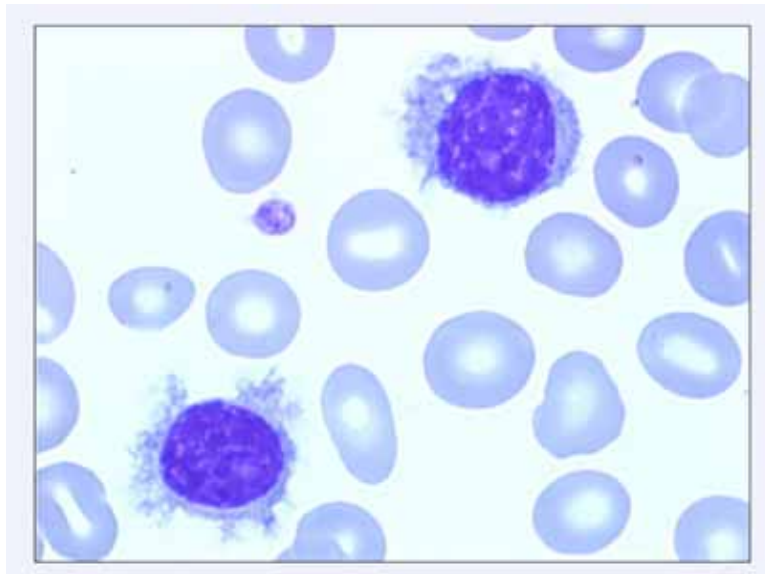


B. Drénou 2007



Leucémie à tricholeucocytes (2)



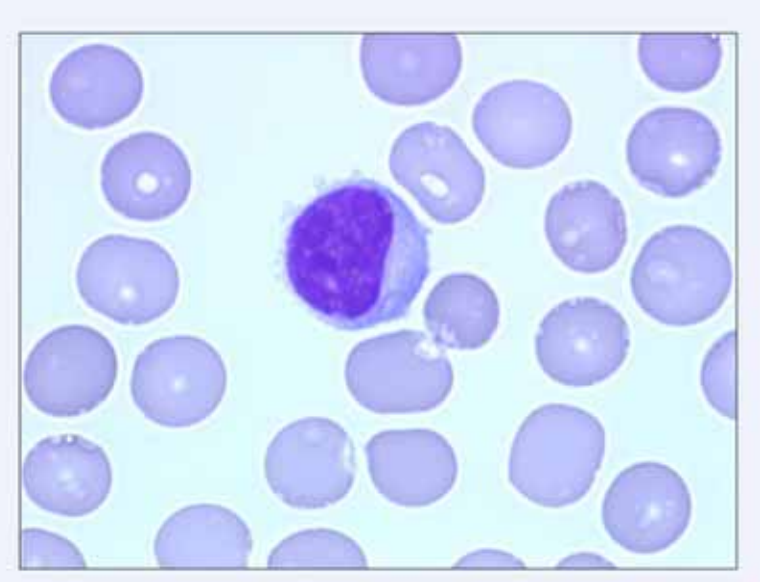


Homme

86 ans

4600/mm³

**Pas de cytopénie
splénomégalie**

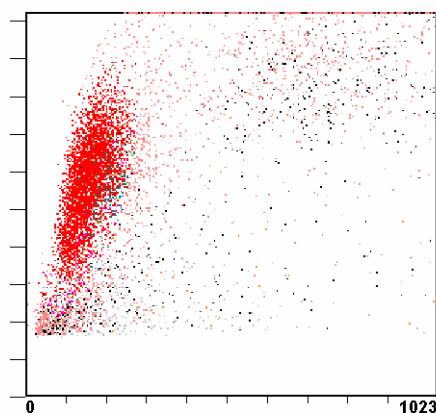


B. Drénou 2007

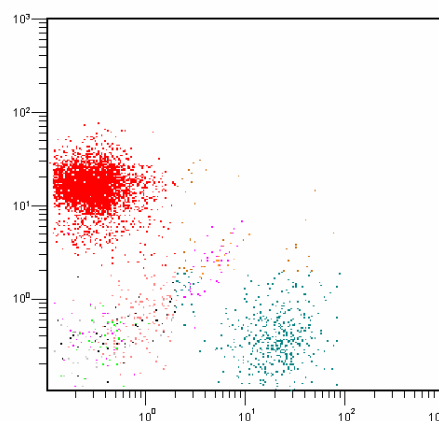


Lymphome splénique à lymphocytes villeux

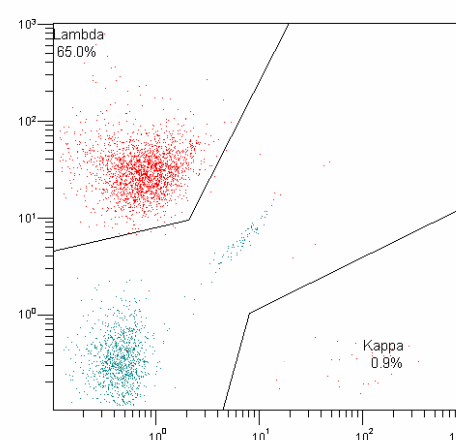
FSC



CD19



Lambda



SSC

CD5

Kappa

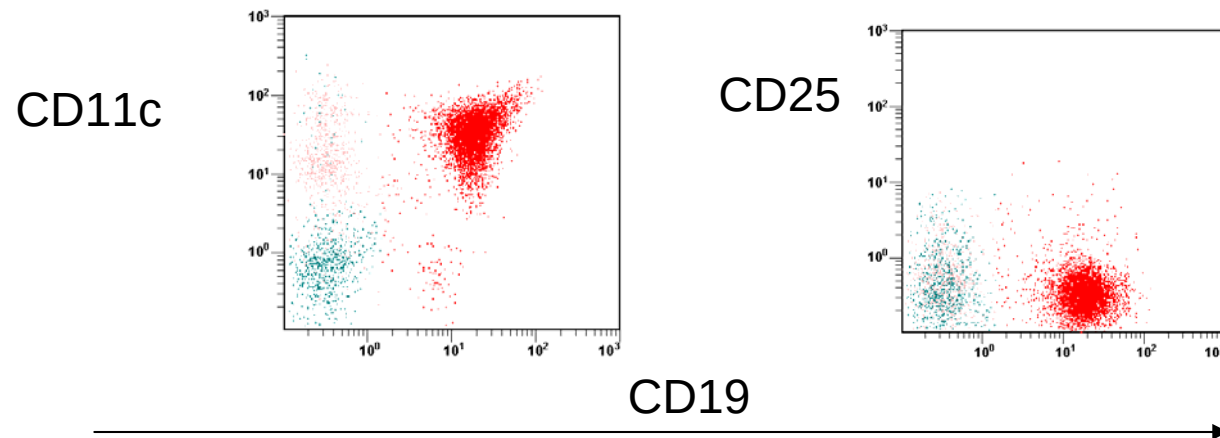
Phénotype: cellules se projetant dans la zone des lymphocytes et non des monocytes



B. Drénou 2007



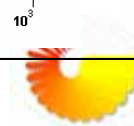
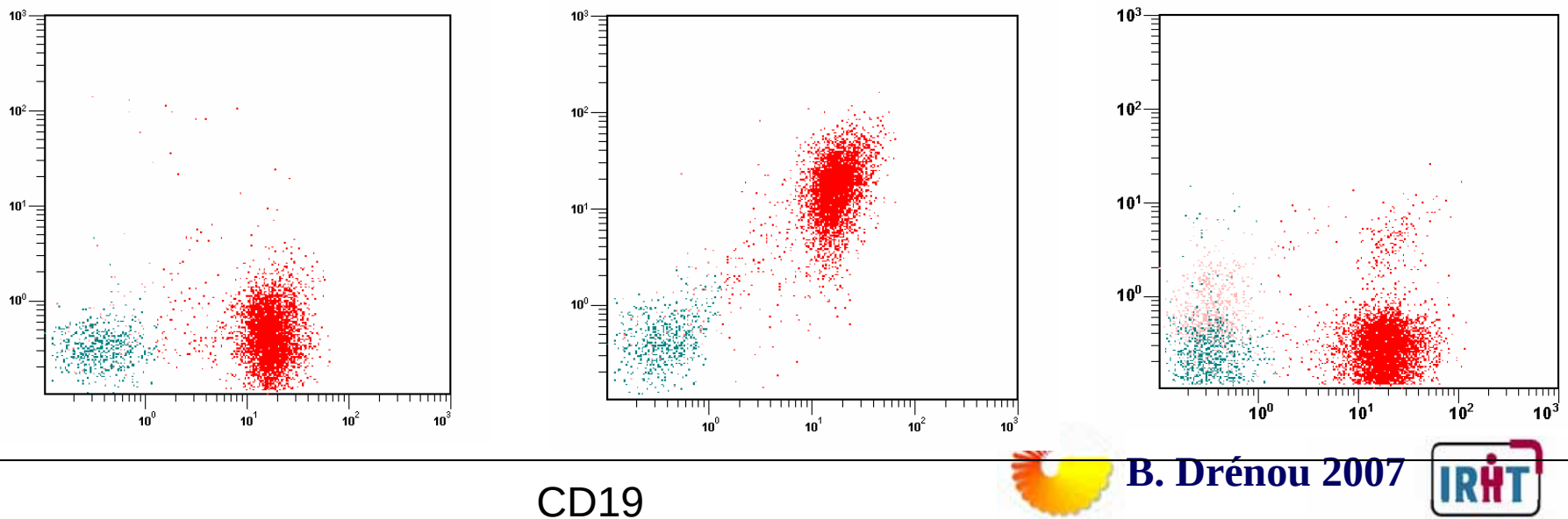
Lymphome splénique à lymphocytes villeux



CD123

FMC7

CD103

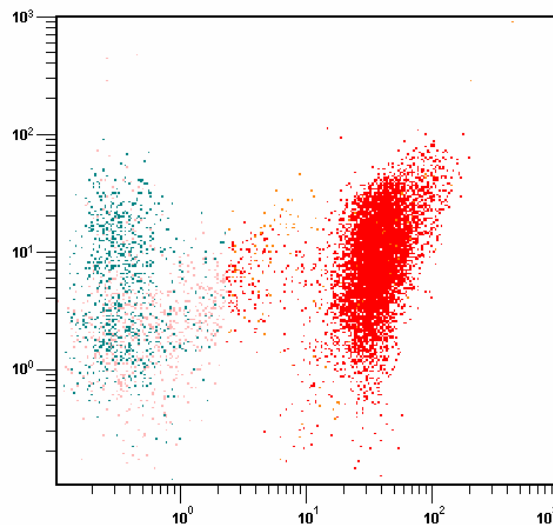


B. Drénou 2007



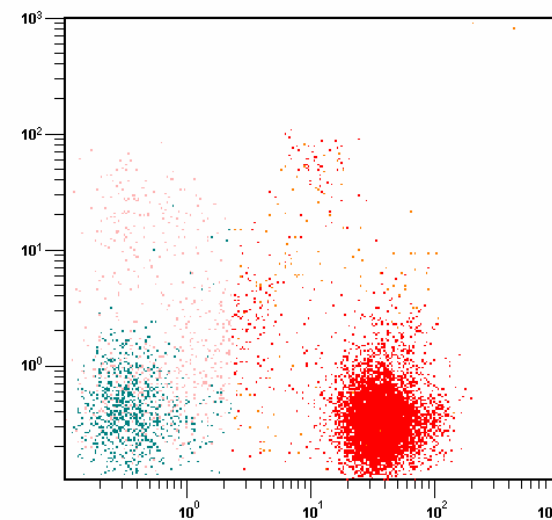
Lymphome splénique à lymphocytes villeux

Int β 7



CD19

CD24



CD19

marqueurs personnels



B. Drénou 2007



Intérêt de la CMF dans les Hémopathies malignes

Diagnostic

- Lignée
- Différenciation dans la lignée
- Clonalité
- Tumorigénicité (trou phéno. Marqueur aberrant)
- Pronostic
- Thérapeutique

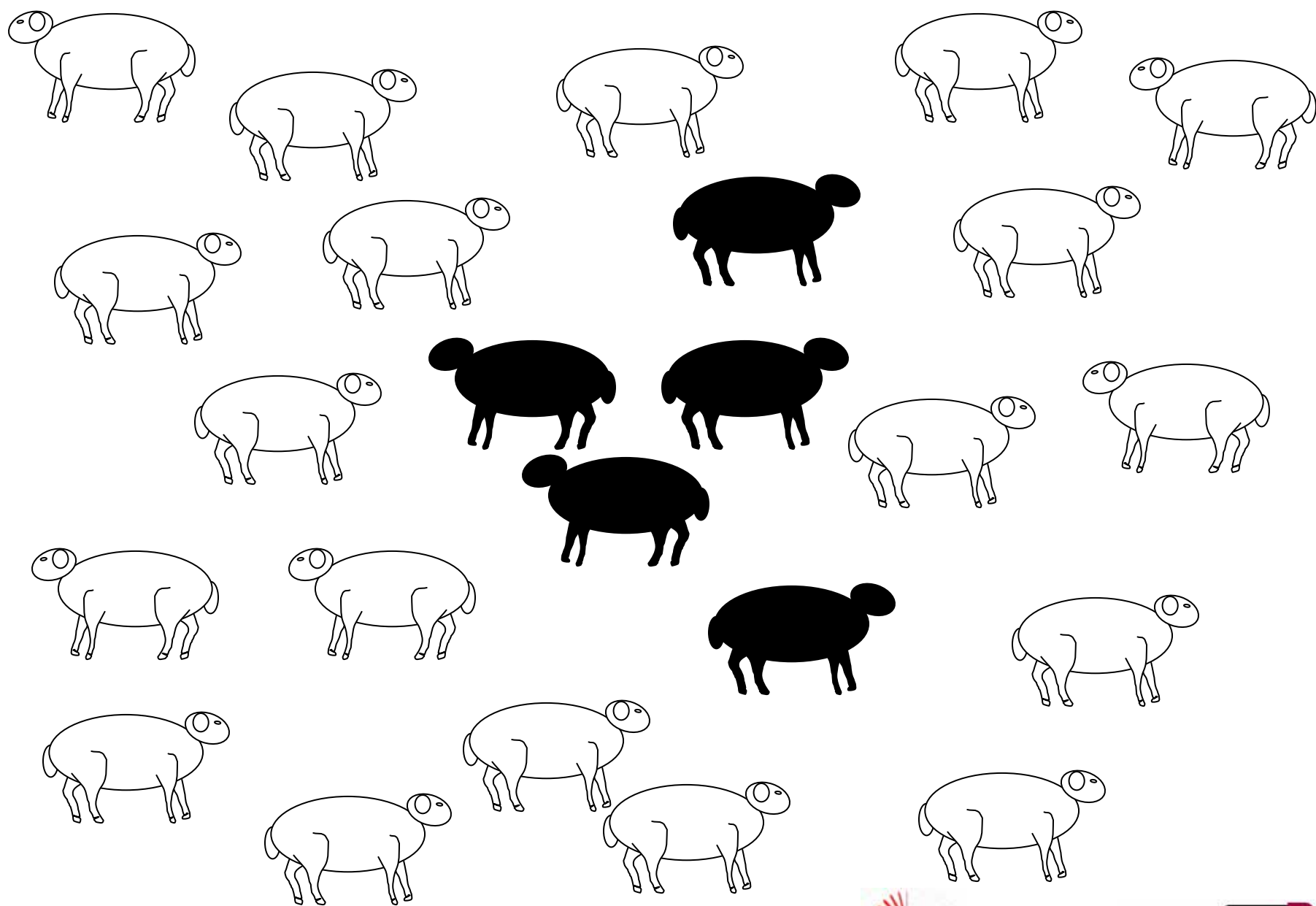
Suivi

– Stratégies multi-couleurs



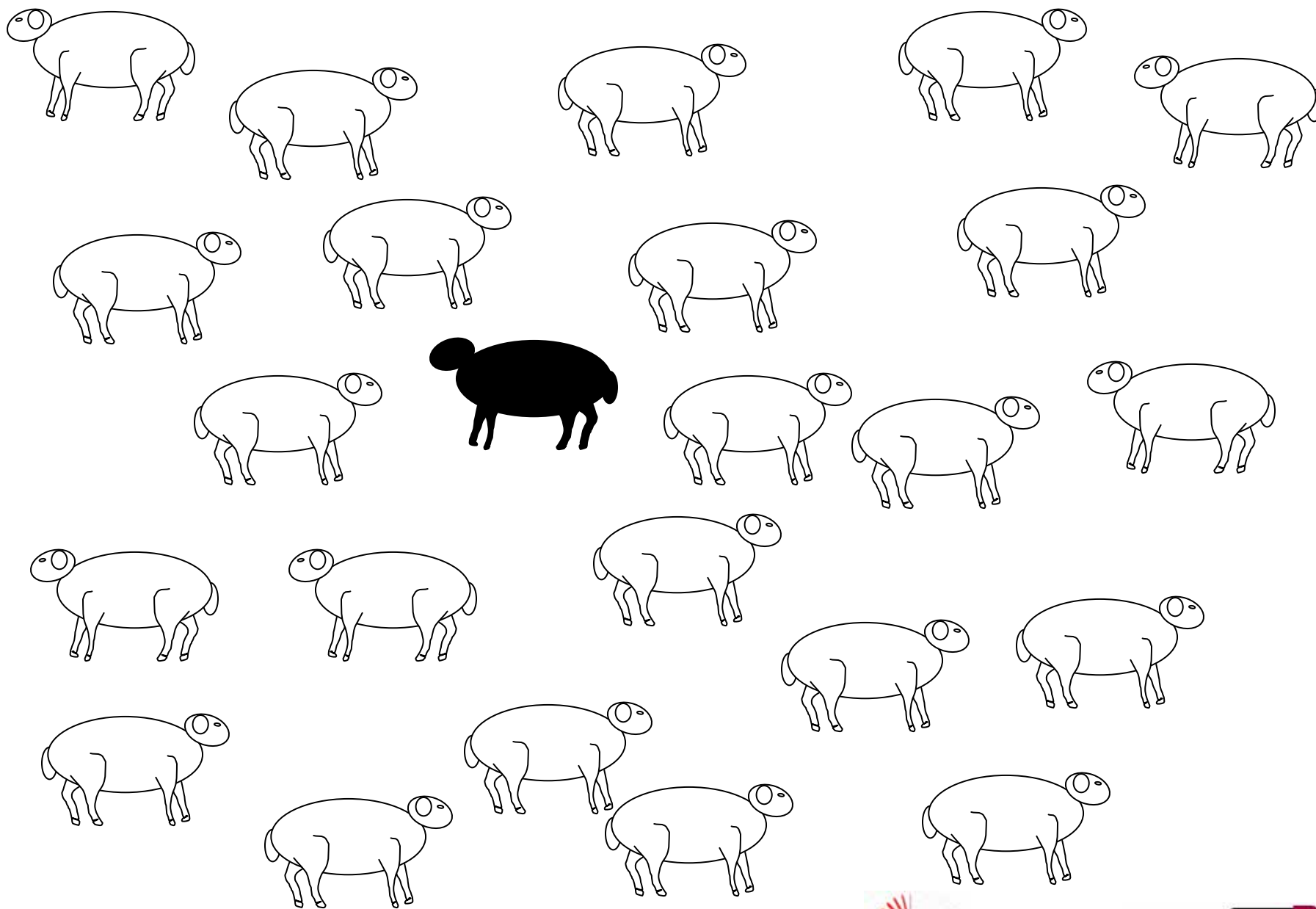
B. Drénou 2007





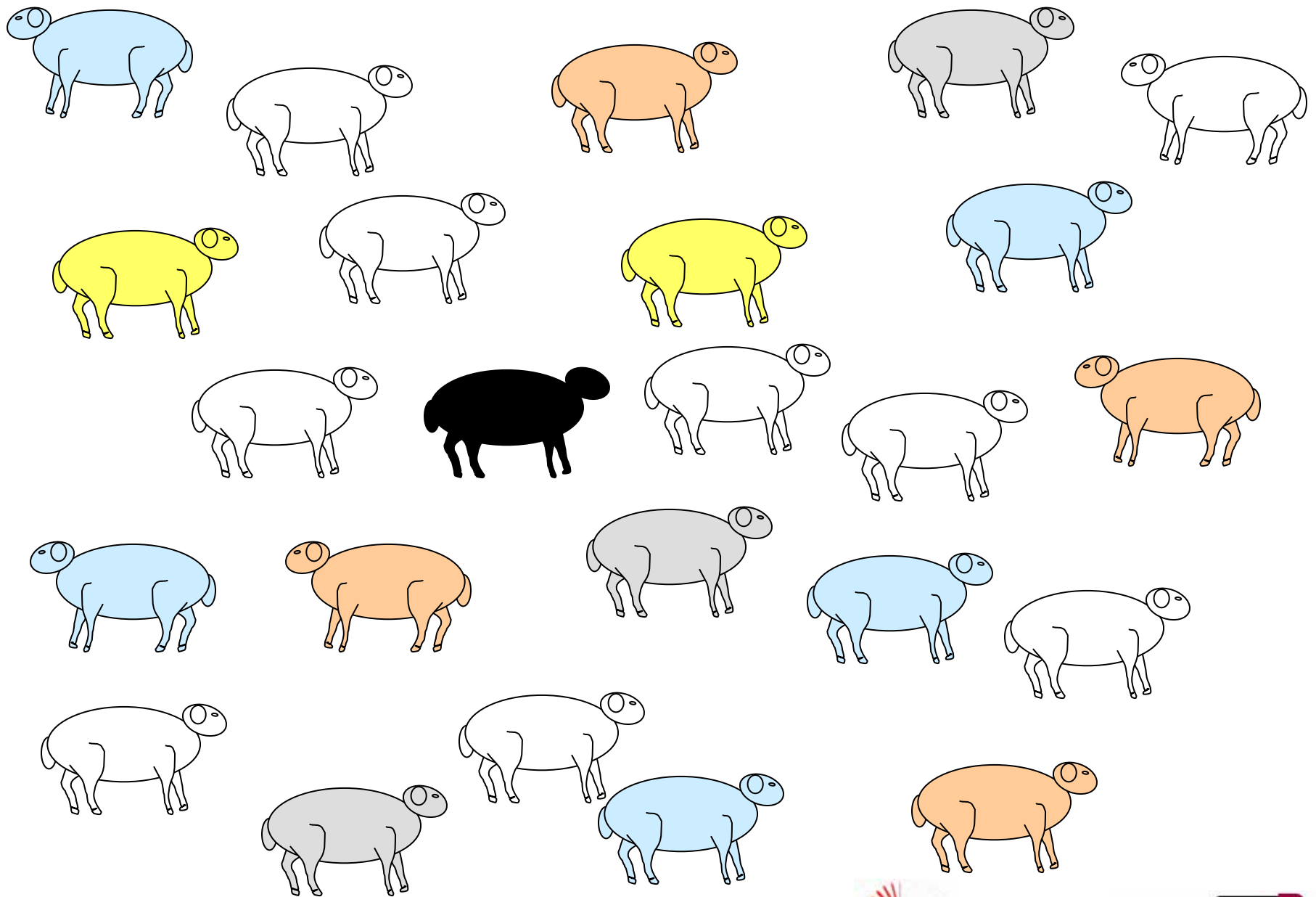
B. Drénou 2007





B. Drénou 2007

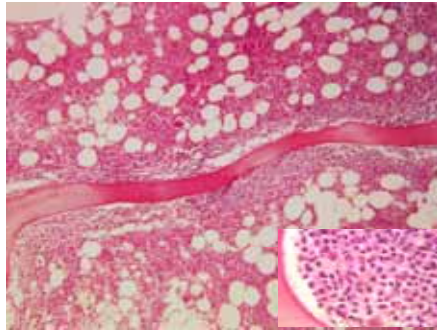




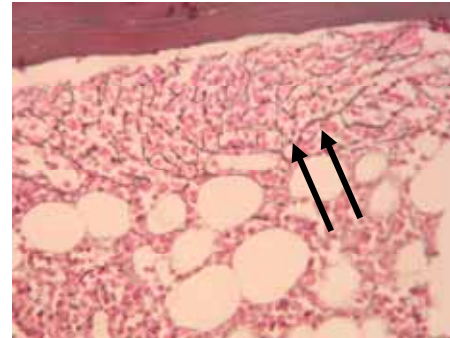
B. Drénou 2007



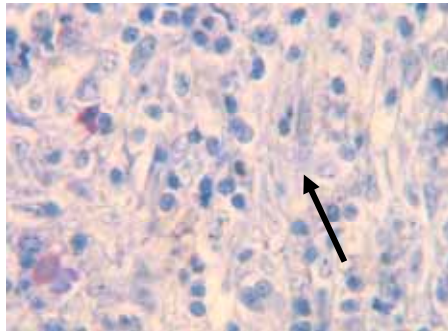
2a



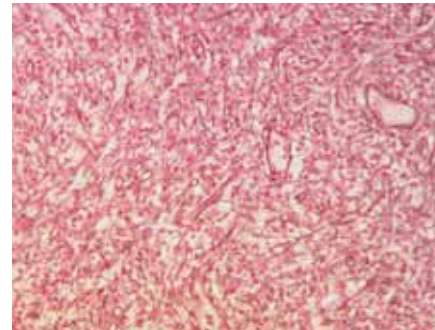
2b



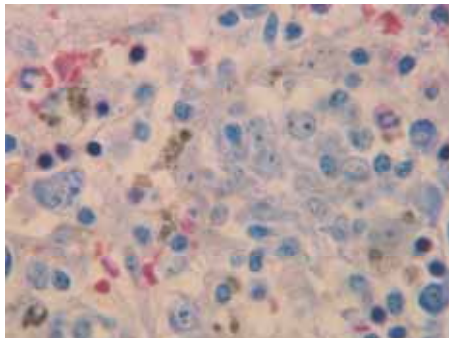
2c



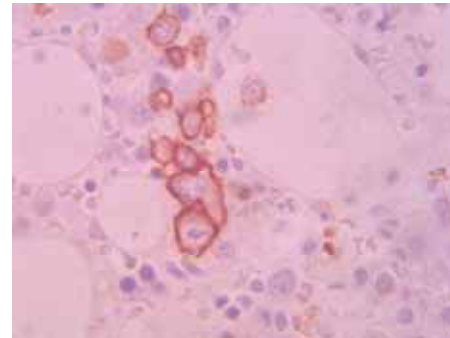
2d



2e



2f



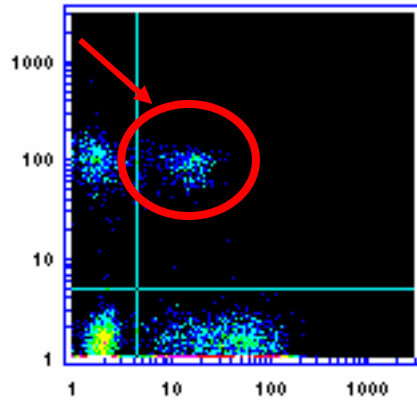
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Minimal residual disease,

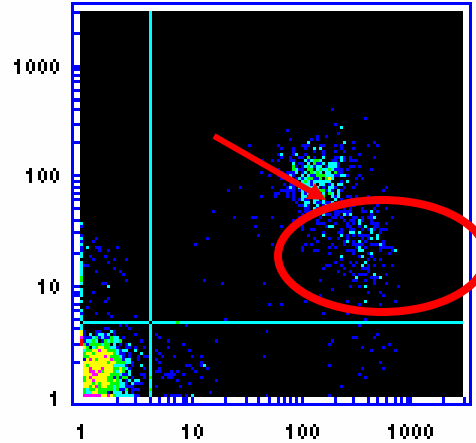
Staging

CD19

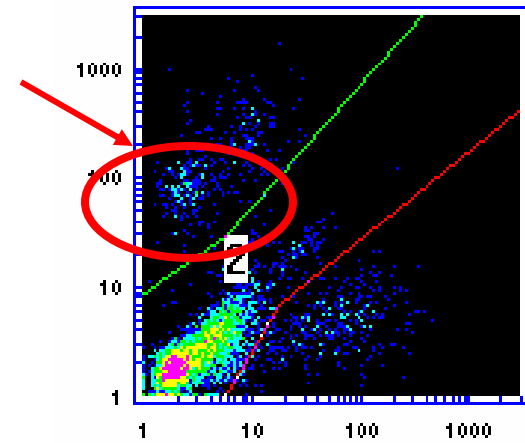


BM

CD22



LAMBDA

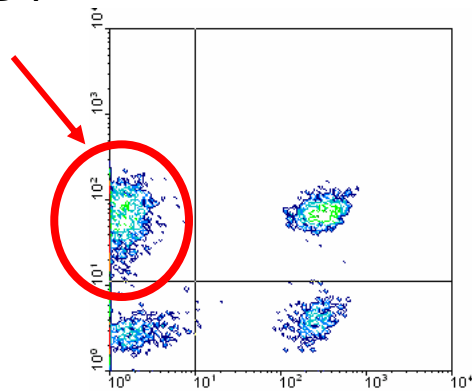


CD5

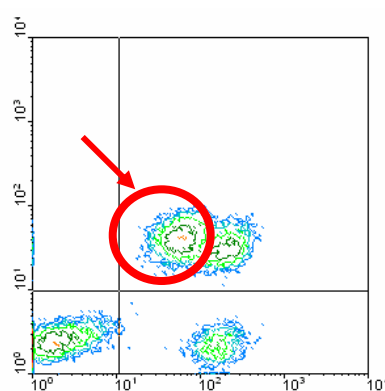
CD20

KAPPA

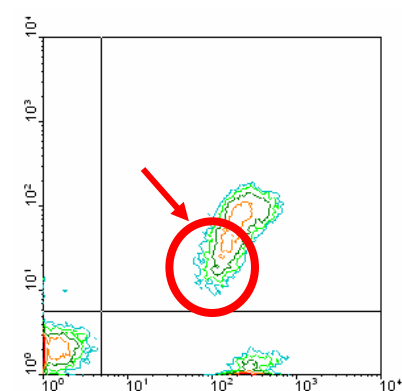
CD4



CD4



CD4



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Bilan d'extension des LNH en CMF "trois couleurs"

Histologie medullaire	+	–	+	–	Total	Valeur predictive	
CMF	+	+	–	–		Histo	CMF
LNH Folliculaire	54 (40%)	5 (4%)	17 (13%)	59 (44%)	135	93%	78%
MCL	53 (87%)	3 (5%)	0	5 (8%)	61	95%	100%
DLBCL	28 (16%)	10 (6%)	11 (6%)	125 (72%)	174	80%	78%
Autres LNH	20 (56%)	7 (19%)	1 (3%)	8 (22%)	36	75%	96%
T-NHL	11(16%)	6 (9%)	2 (3%)	50 (72%)	69	68%	89%
Total	166 (35%)	31 (7%)	31 (7%)	247 (52%)	475	86%	86%

Donnees personnelles

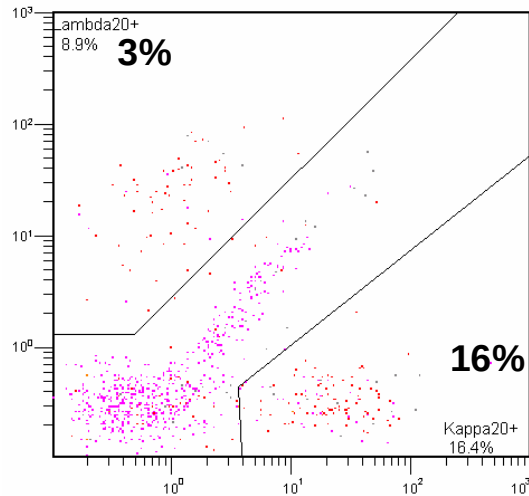


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**FL CD10 – Igs -
BM staging**

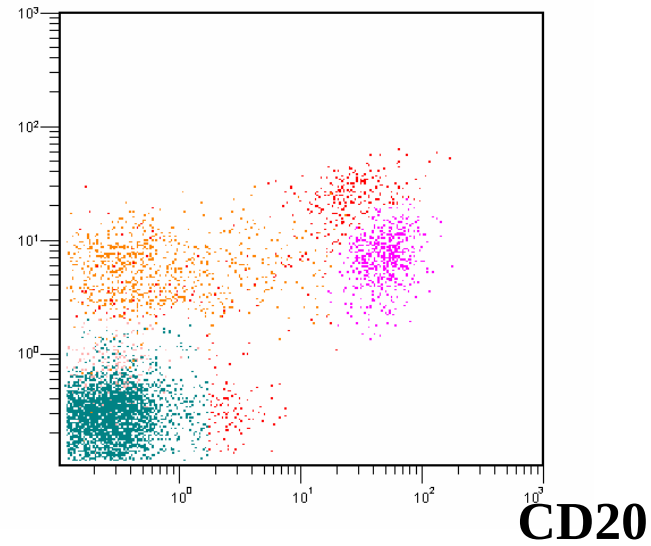
Lambda



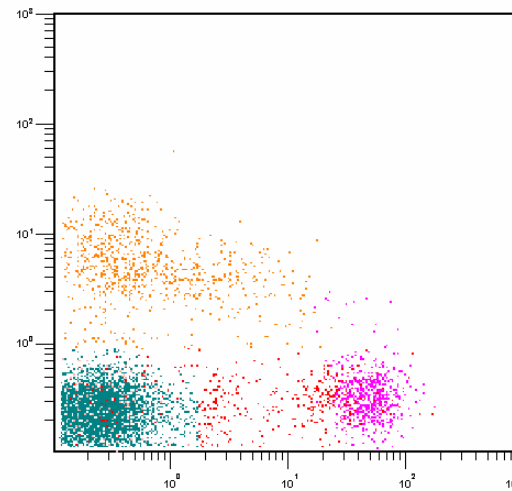
Kappa

gating by CD20

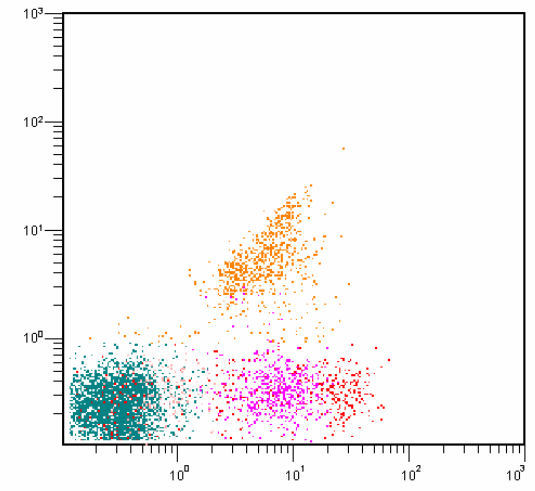
CD22



CD10



CD10

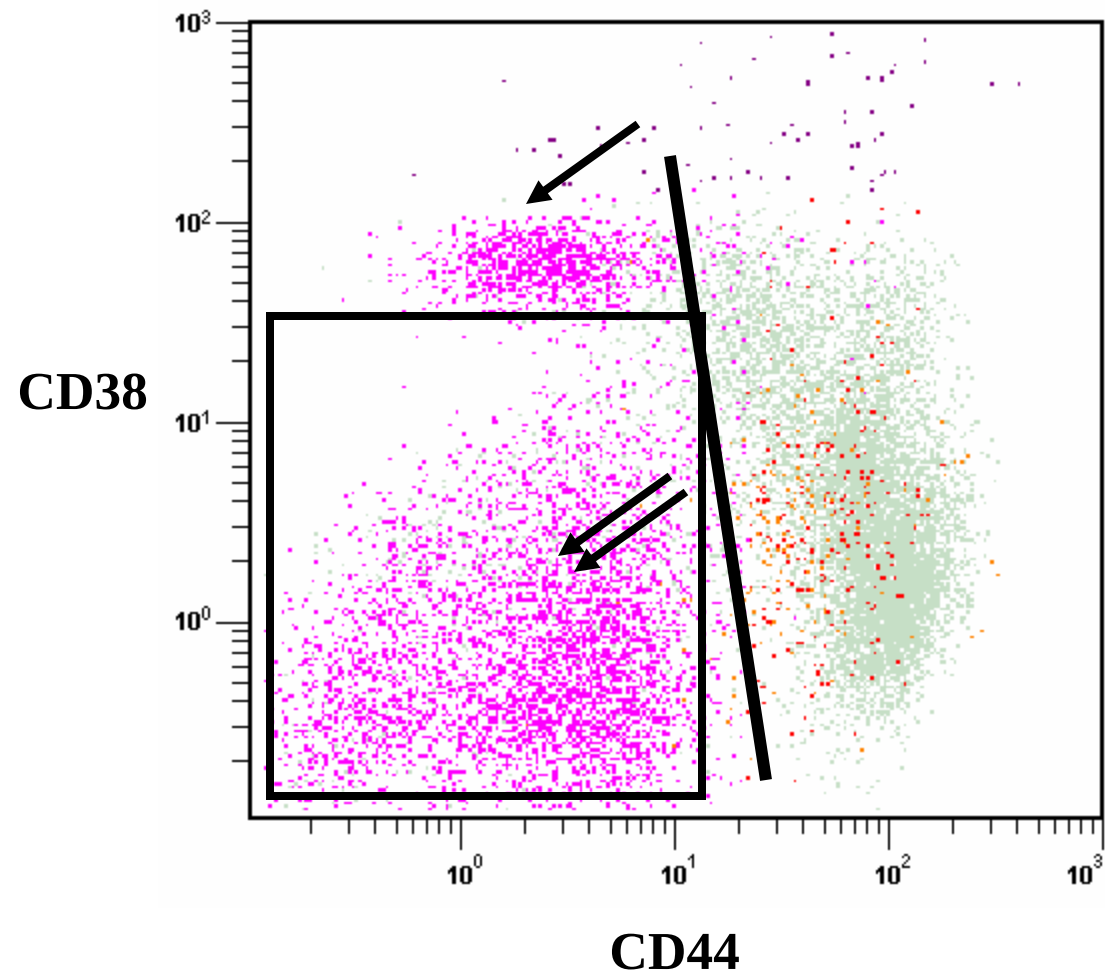


CD20

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CD22

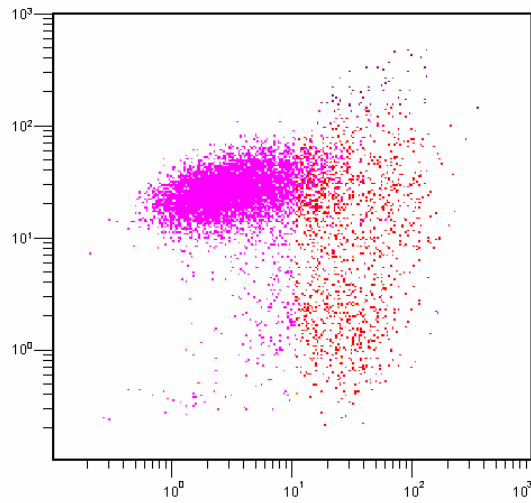




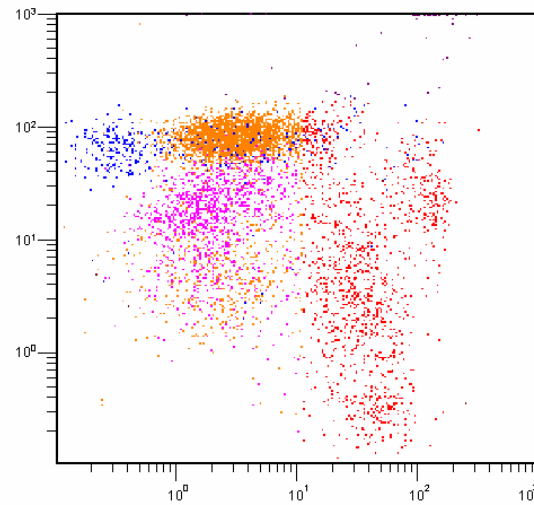
B. Drénou 2007



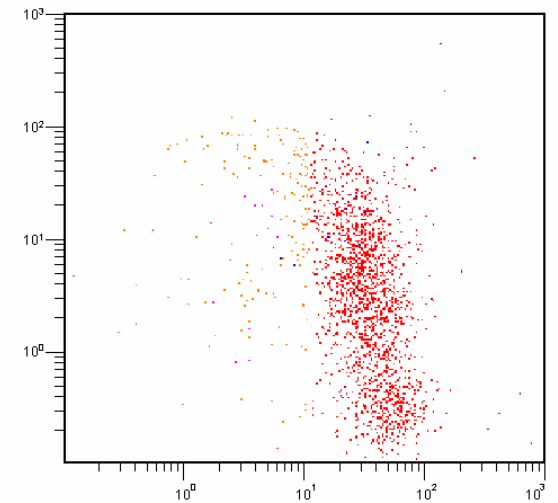
CD38 **Lymph node**



CD38 **Bone-marrow**



CD38 **Blood**



CD44

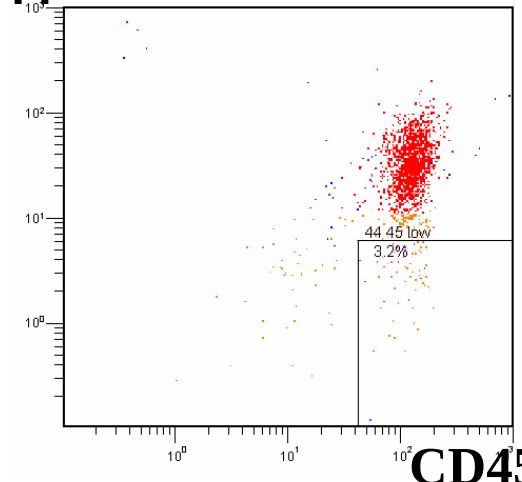
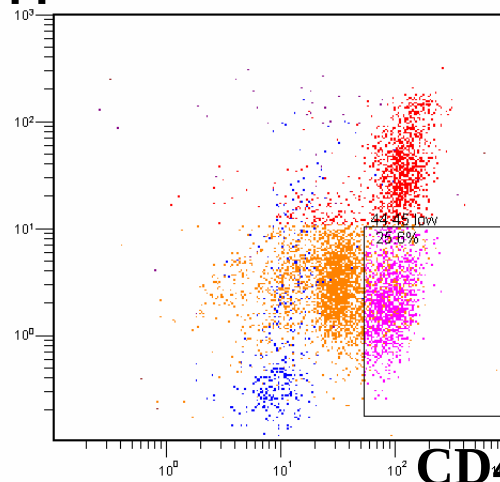
CD44

CD44

CD44

CD44

Follicular Lymphoma



CD45

CD45



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■ Lymphomes folliculaires

■ Etapes pré analytiques

Prélèvement, numération cellules, immunomarquage (CMF).

■ Les dilutions

Obtention de moelles infiltrées en LF à ;

50 %	10 %	5 %	1 %	0,1%	0,01%	0,001%
0,5	10^{-1}	5×10^{-2}	10^{-2}	10^{-3}	10^{-4}	10^{-5}

■ Analyse en cytométrie en flux

Combinaison utilisée :

Cytomètre :

■ CD 19

■ CD 34

■ CD 38

■ CD 44

■ CD 45

FC 500, 5 couleurs

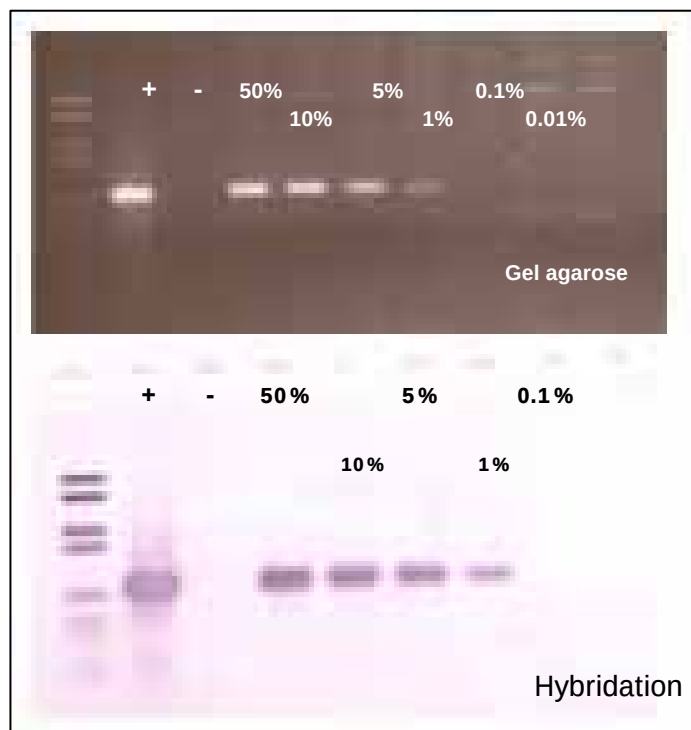
Beckman Coulter®



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Passage de ces mêmes dilutions en biologie moléculaire



RESULTATS

	CD 44	Sensibilité biologie moléculaire		CMF
		Standard (hybridée)	Nested	
Exp 1	limite	1 %	0,1 %	0,1 %
Exp 2	limite	5 %	0,1 %	0,1 %
Exp 3	low	1 %	0,01 %	0,1 %
Exp 4	low	1 %	0,01 %	0,01 %
Exp 5	limite	1 %	0,01 %	0,1 %
Exp 6	limite	1 %	0,01 %	0,1 %
Exp 7	limite	1 %	0,001 %	1 %
Exp 8	low	5 %	1 %	1 %
Exp 9	low	1 %	0,1 %	1 %
Exp 10	low	0,1 %	0,001 %	0,1 %
Moyenne		1,7 %	0,13 %	0,36 %

➔ Sensibilité

10^{-2} pour PCR Standard

10^{-3} pour PCR nested et Cytométrie.



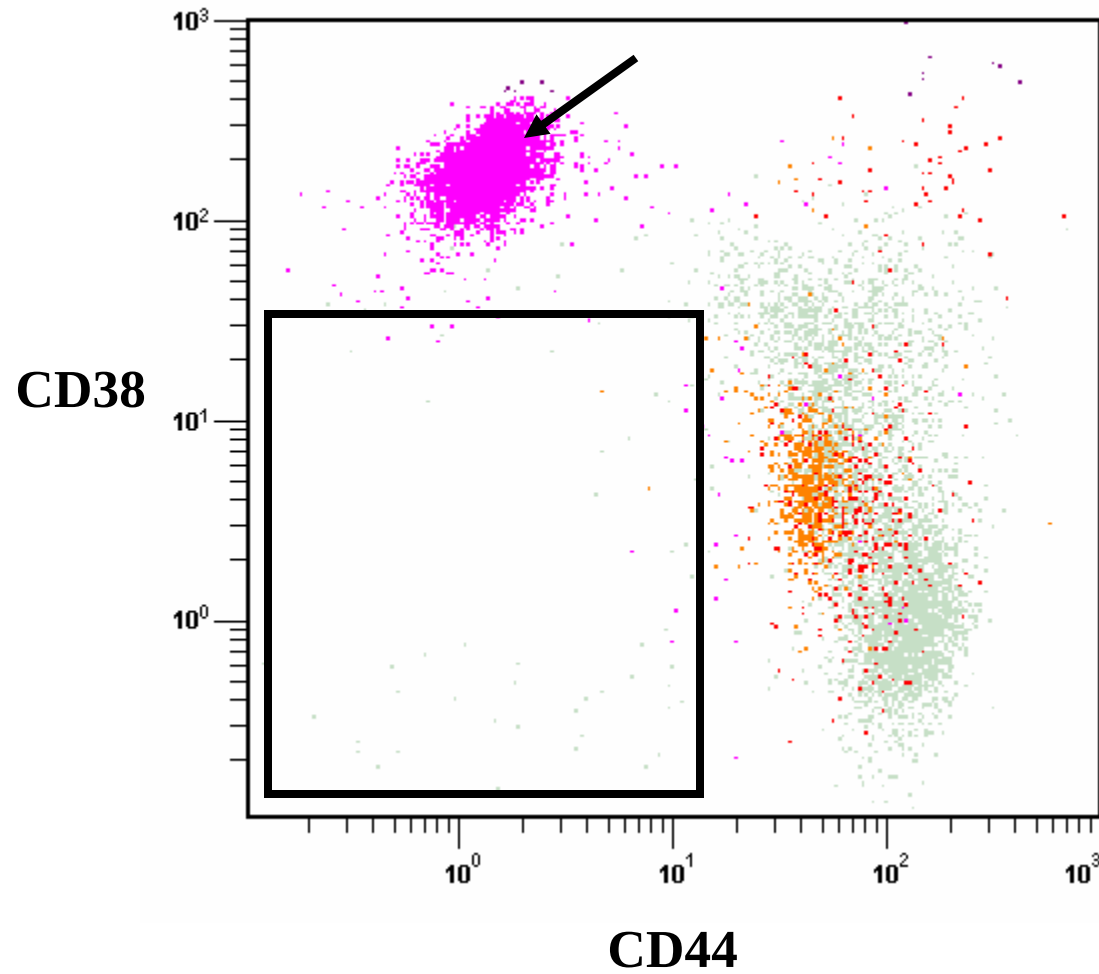
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Burkitt NHL

t(8,14)

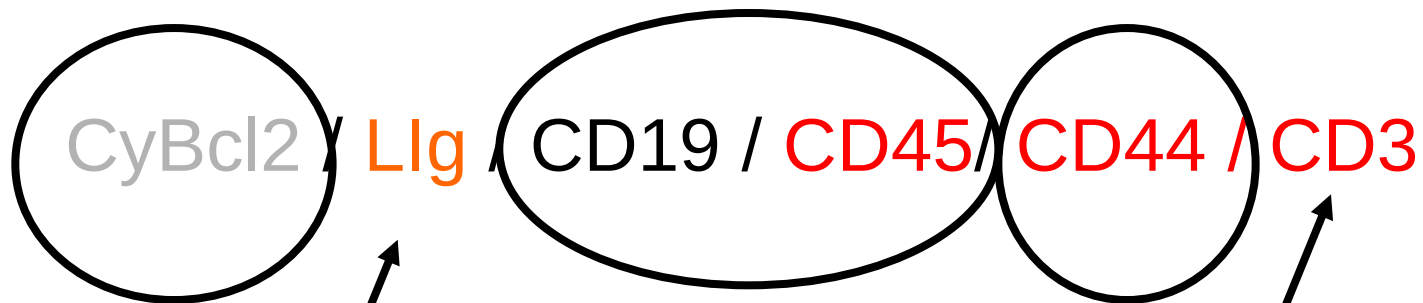
BM staging



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Maladie résiduelle: Tube folliculaire



Passage en 2 étapes
 (manque un peu d'événements: gating -1 million de Lymphos)
 Vérification CD19+ ou arrêt temps

Contrôle interne



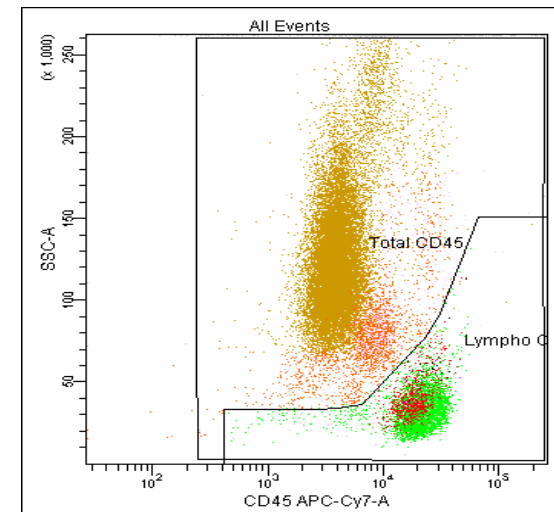
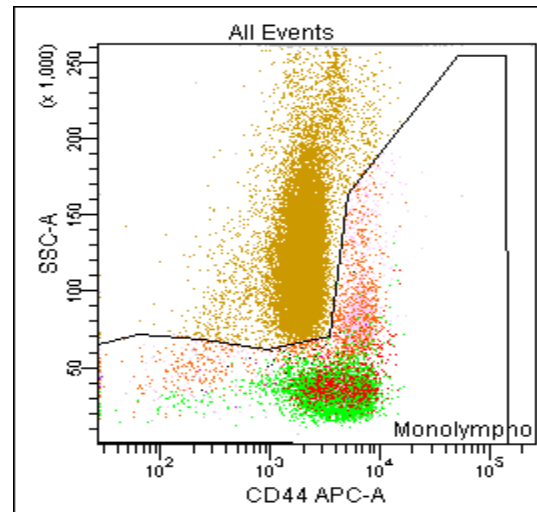
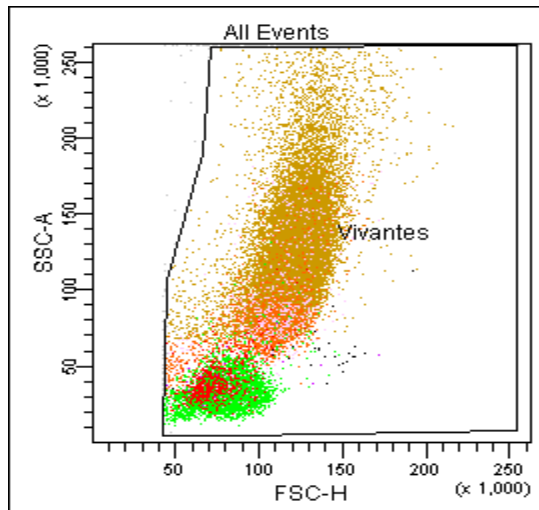
B. Drénou 2007



Méthode d'analyse

Acquisition 1:

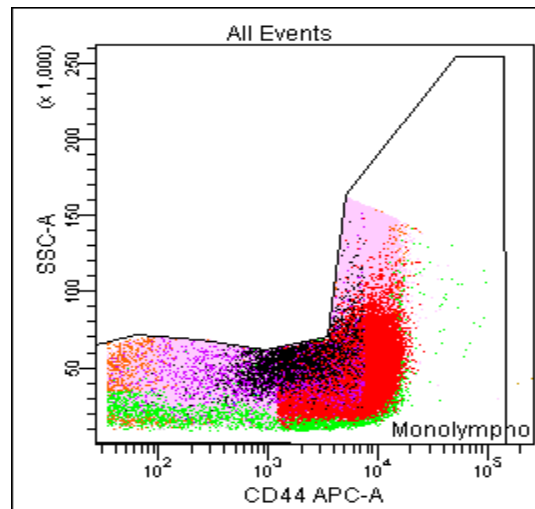
Population	#Events
All Events	30,000
Monolympho	9,117
Lympho CD45+	6,531



➤ Calcul du % de CMN et détermination du gate CMN

Acquisition 2:

Population	#Events
All Events	1,320,147
Monolympho	1,318,287
Lympho CD45+	965,000



➤ Acquisition des CMN jusqu'à 1 million de lymphocytes



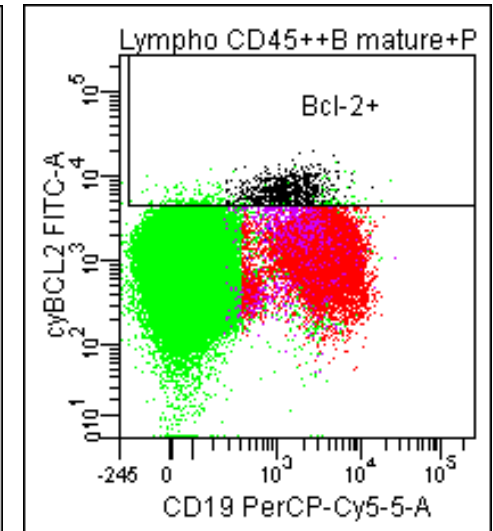
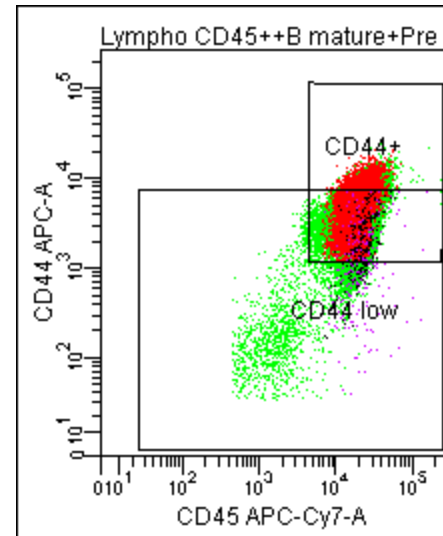
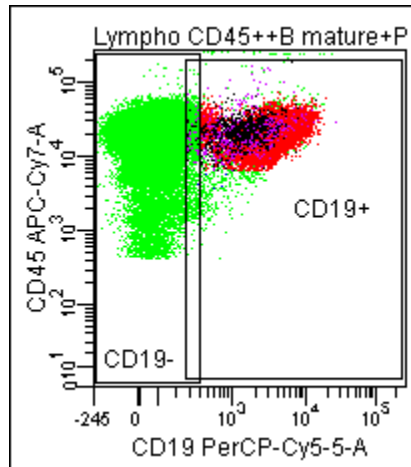
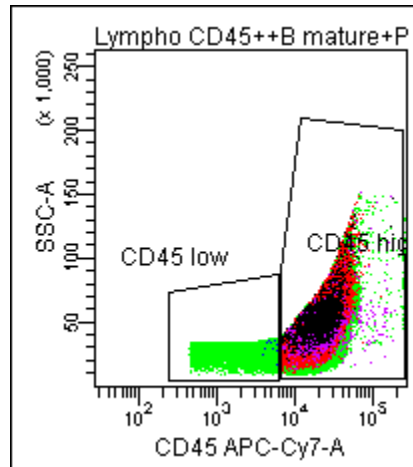
B. Drénou 2007



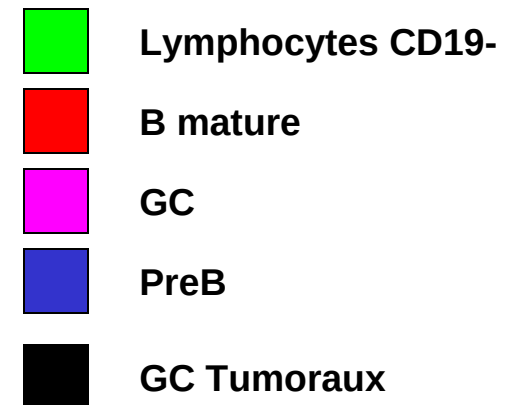
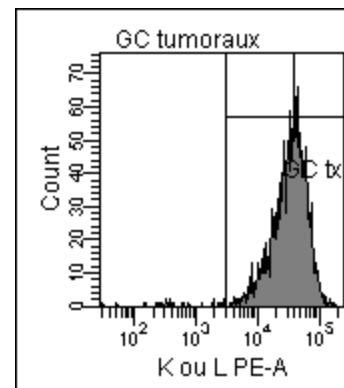
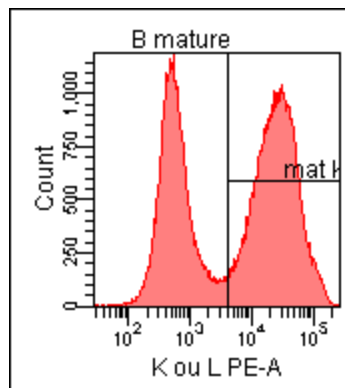
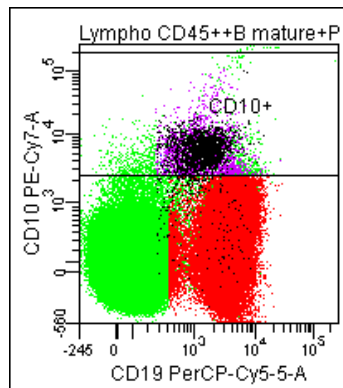
Acquisition 2:

Population	#Events
All Events	1,320,147
Monolympho	1,318,287
Lympho CD45+	965,000

En 4 couleurs...



➤ Détermination cellules tumorales: fenêtre large, difficulté des marqueurs faibles



➤ Vérification de la chaîne légère et du CD10



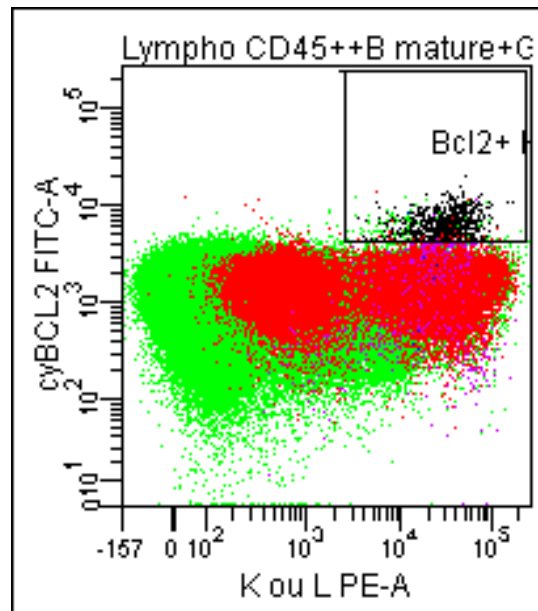
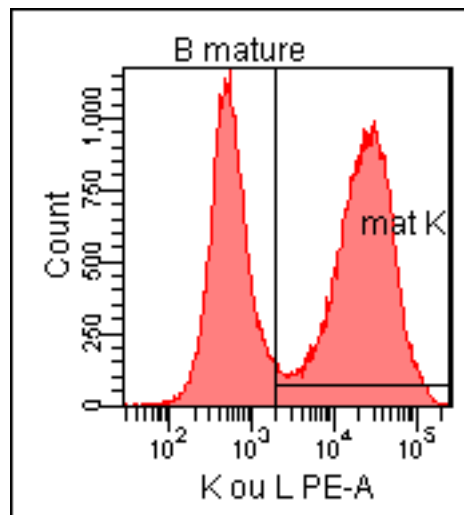
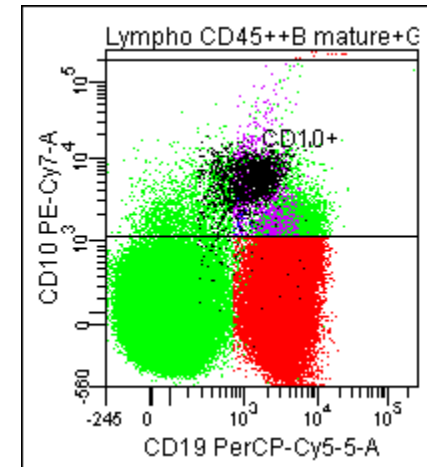
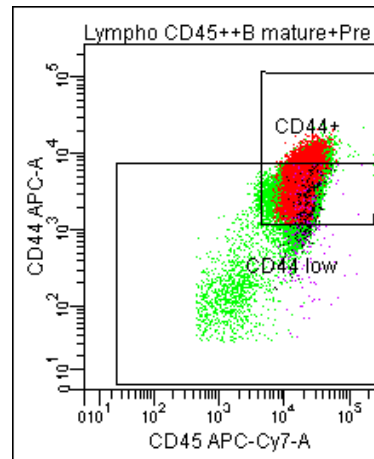
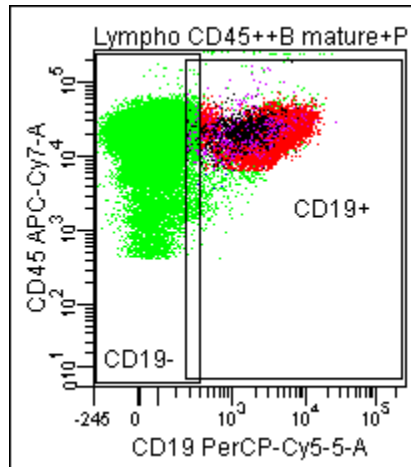
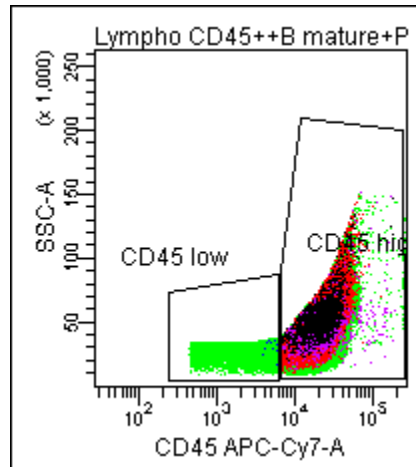
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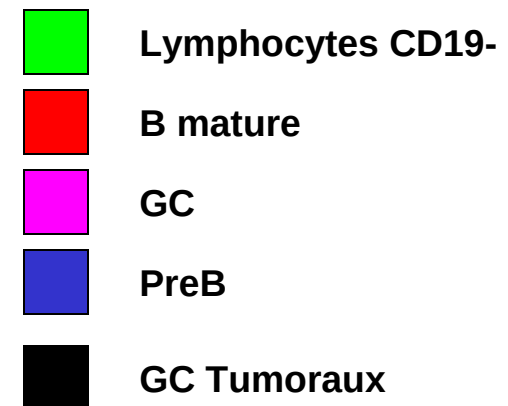
Acquisition 2:

Population	#Events
All Events	1,320,147
Monolympho	1,318,287
Lympho CD45+	965,000

En 5 couleurs...



➤ Vérification du CD10



➤ Utilisation de la chaîne légère pour déterminer les cellules tumorales

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Valeurs seuil

Bruit de fond en 4 couleurs:

- Passage de témoins 5 Kappa et 9 Lambda
- Différence: seuil K = $1 \cdot 10^{-4}$; seuil L = $8 \cdot 10^{-5}$
- Moyenne du seuil = $1 \cdot 10^{-4}$

Bruit de fond en 5 couleurs:

- Passage de témoins 5 Kappa et 9 Lambda
- Pas de différence entre K et L
- Seuil = $1 \cdot 10^{-5}$



Remerciements

- **GRHIM**
 - BD Biosciences
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 - Dr Amiot
- **CH Mulhouse**
 - Dr Debliquis



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