Les Syndromes myélodysplasiques

L'apport de la cytométrie en flux

Corata Belgique 2015, Beaune

JF Classen

Myélodysplasie

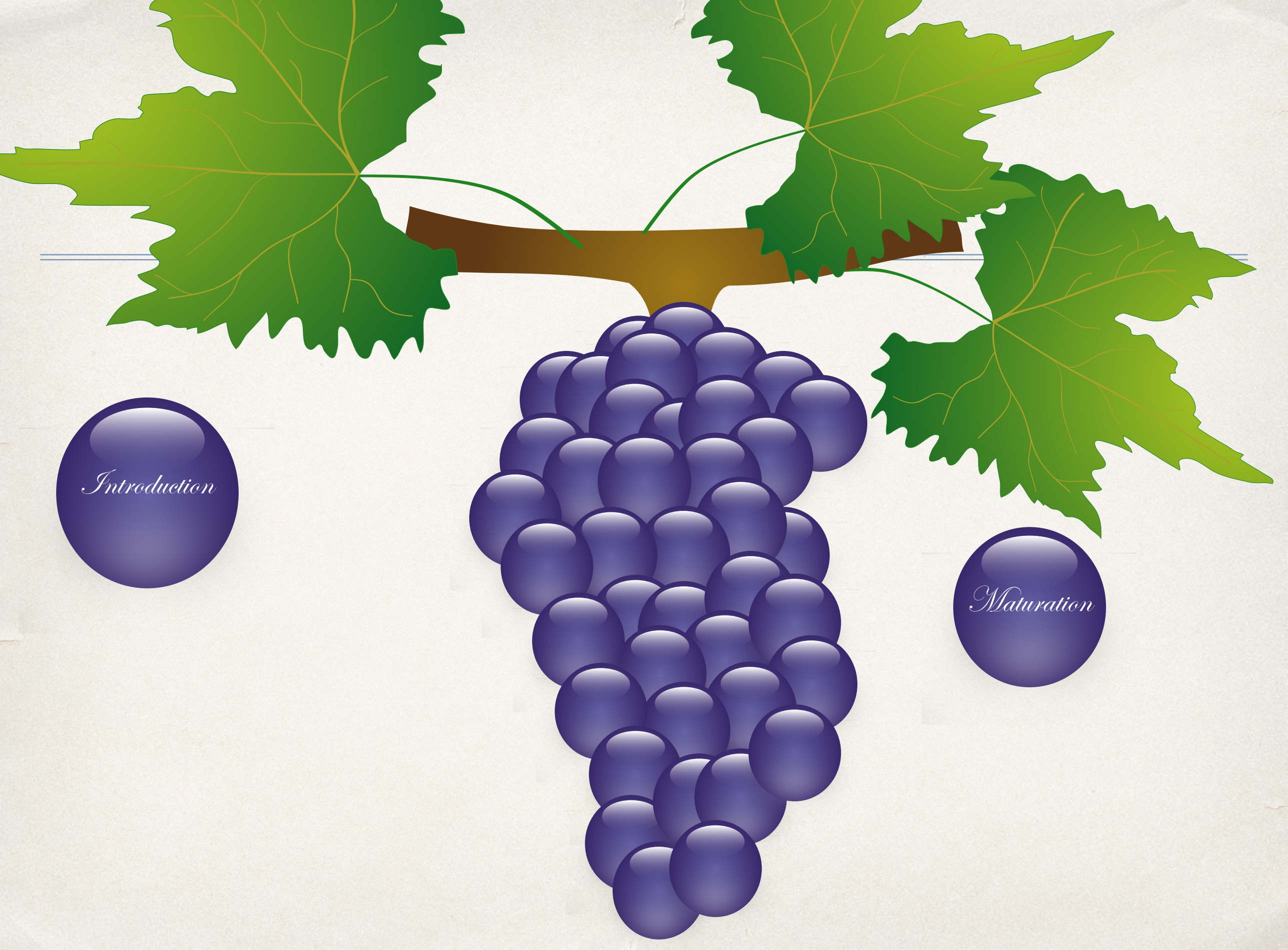
SMD/MDS

2008

«The myelodysplastic syndromes are a group of clonal haematopoietic stem cell diseases characterized by cytopenia(s), dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis, and increased risk of development of acute myeloid leukaemia»

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th Edition, 2008

Introduction


Introduction
Maturation

Introduction

Quid dans
SMD ?

Maturation

Introduction

Quid dans SMD ?

Maturation

Scoring System

Introduction

Quid dans SMD ?

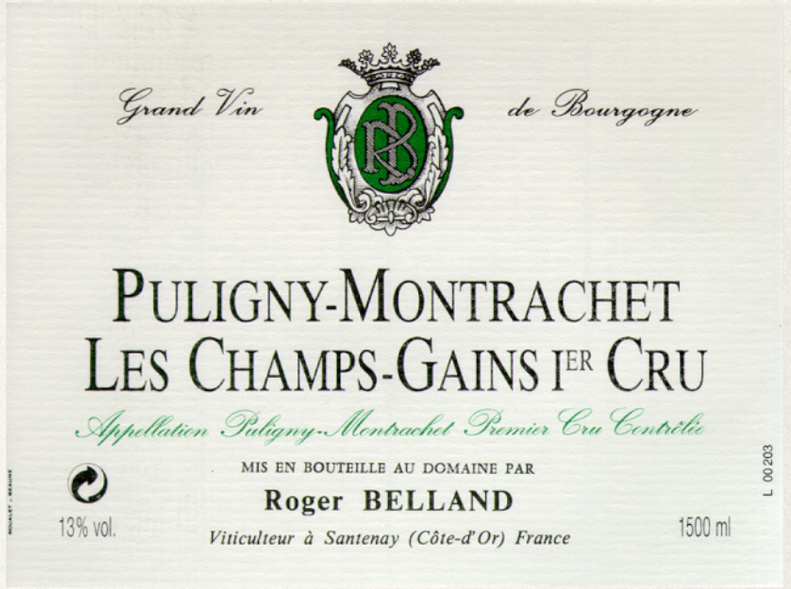
Mise en pratique

Maturation

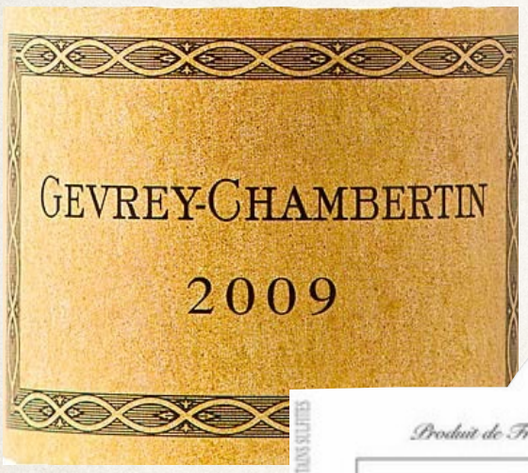
Scoring System

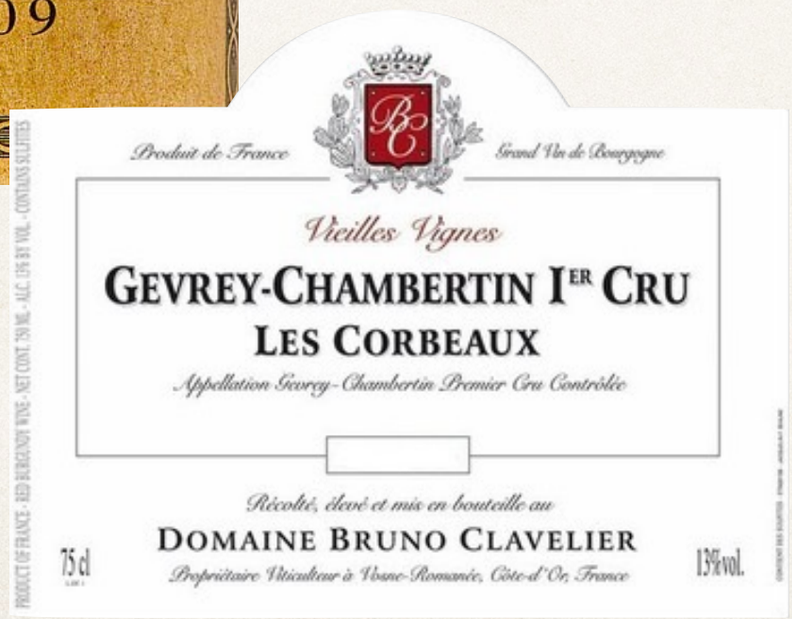
Refractory cytopenia with unilineage dysplasia (RCUD)

Refractory cytopenia with multilineage dysplasia (RCMD)



Refractory anemia with ring sideroblasts (RARS)





Refractory anemia with excess blasts : RAEB-1 or RAEB-2

Myelodysplastic syndrome with isolate 5q-

Myelodysplastic syndrome unclassifiable

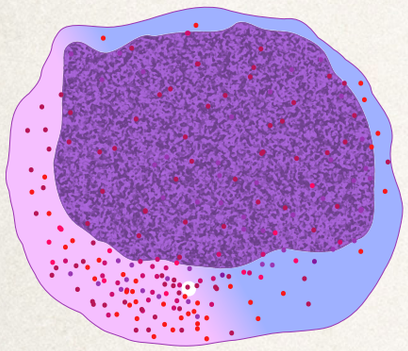
SMD ?

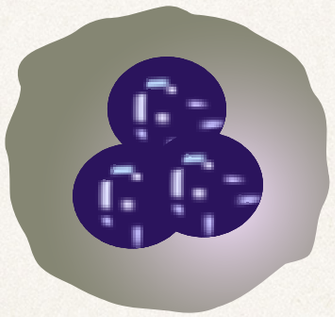
2008

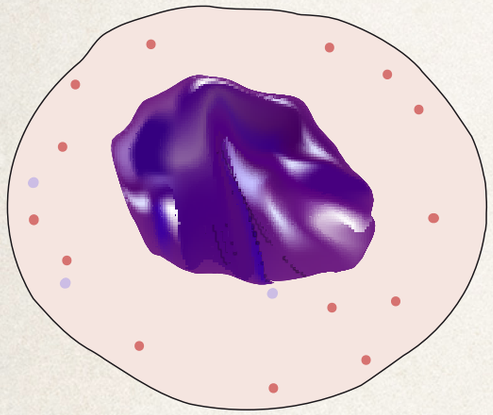
- Etiologie : maladies hétérogènes. Sélection clonal d'une cellule souche hématopoïétique (comment ?), avantage de croissance.
- Physiopathologie : anomalies des voies apoptotiques, instabilités génétiques et génomiques, épigénétique, …
- Epidemiologie
  - 2-10/100.000, augmente avec l'âge, prédominance masculine.
- IPSS (*International Prognostic Scoring System*) : %blastes, cytopénies, cytogénétique
- WPSS (WHO 2008) : WHO categorie, Caryotype, besoins transfusionnels

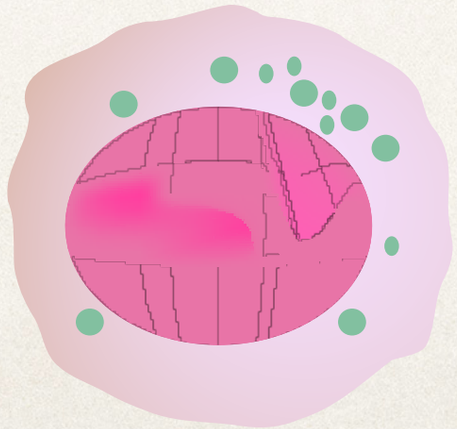
Outils au laboratoire

2008

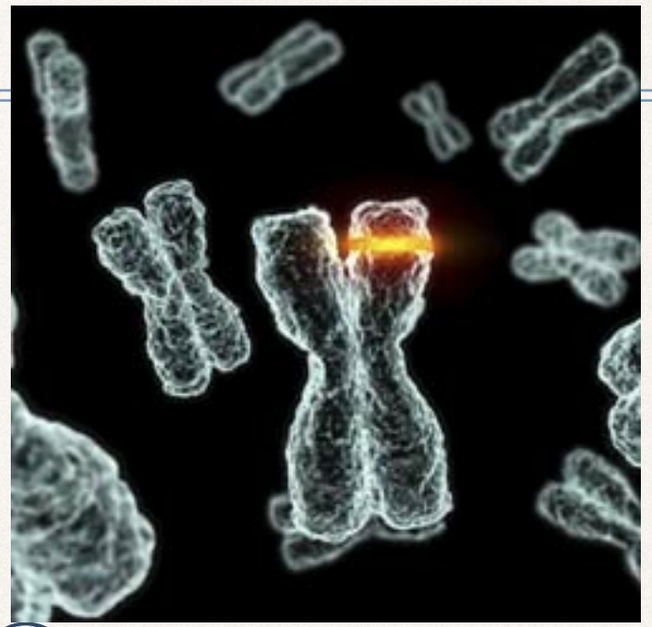




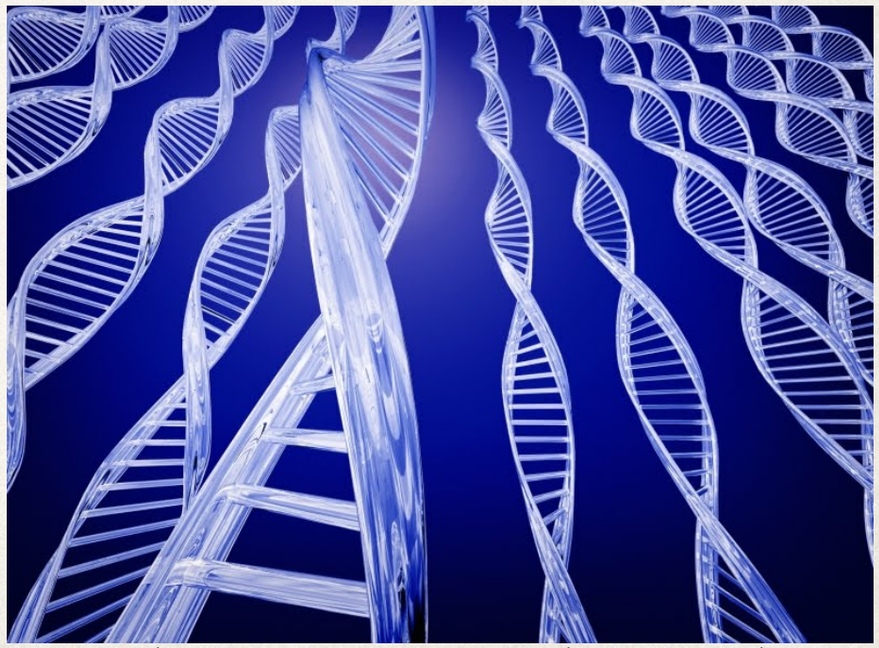




Morphologie



Cytogénétique



Biologie moléculaire

Morphologie

- SMD bas grade sans anomalies morphologiques
- Toxique (alcool, médicaments, ...), infections virales, carences nutritionnelles.
- Certains cas : hypocellularité ou fibrose.

Cytopénie :

- Hémoglobine < 10 g/dL
- Neutrophiles < 1,8 x 10
- Plaquettes < 100 x 10

Dysplasie :

- ≥ 10% de dysplasie dans au moins 1 lignée

Blastes médullaires :

- < 20% (5-9% : AREB-1 et 10-19% : AREB-2)

Cytogénétique

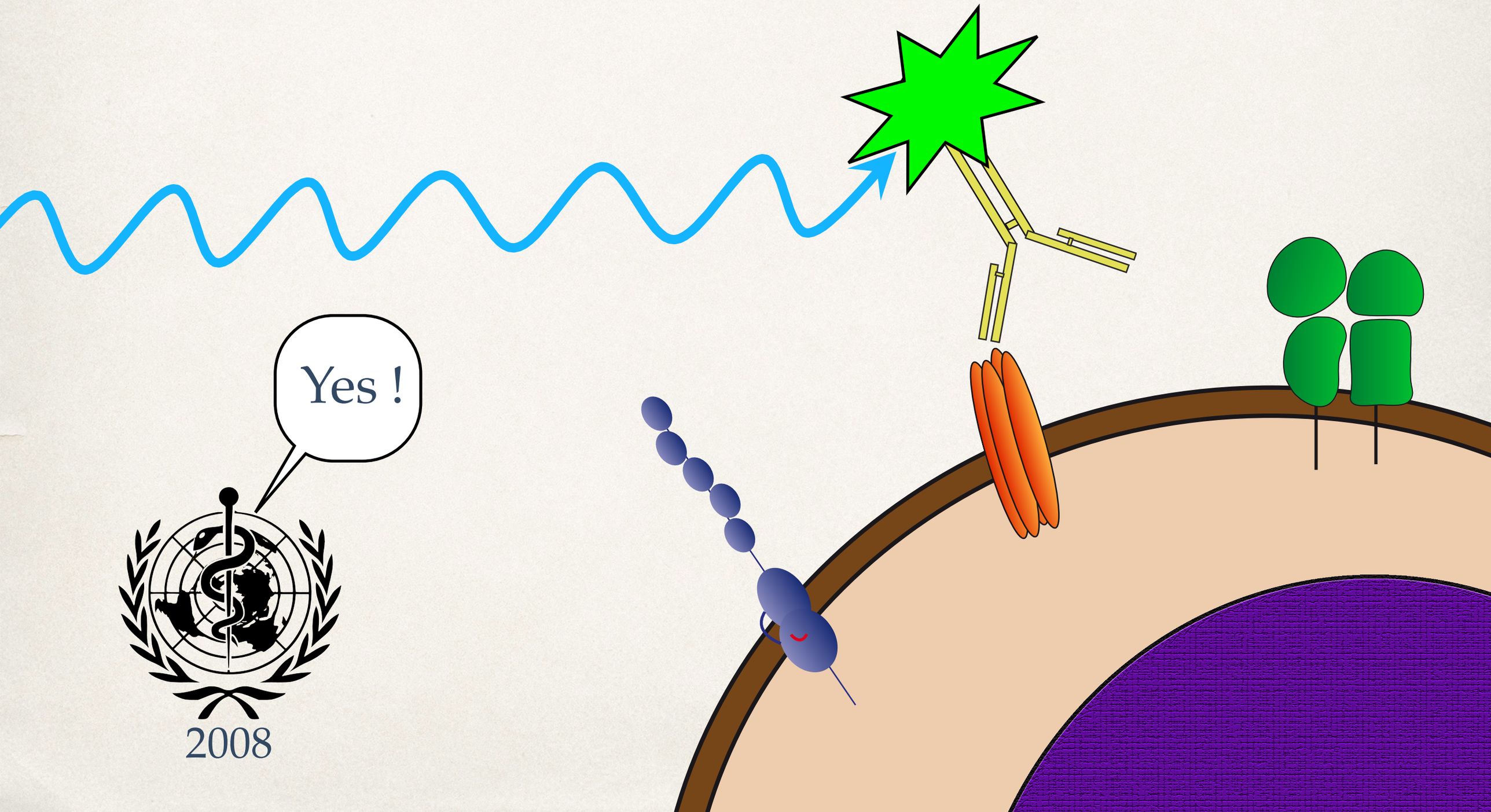
- Anomalies cytogénétiques clonales **~50%** des cas de SMD
- -5/del(5q), -7/del(7q), del(20q), 17p, +8, -Y, caryotypes complexes, ...
- **Hétérogénéité** des anomalies génétiques
- Pronostique !
- Nouveaux outils, études plus larges, ...

Biologie moléculaire

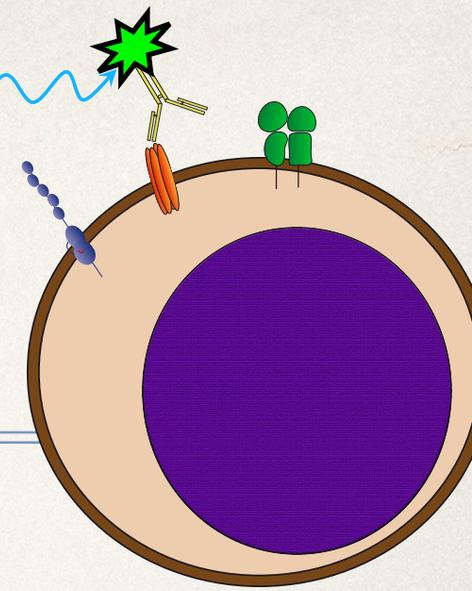
- Anomalies moléculaires dans les SMD sont moins bien caractérisées
- Ras, AML1, TP53, ...
- Intérêt pour les anomalies épigénétiques (méthylation des promoteurs, …)

Pas toujours suffisant… particulièrement dans les SMD de bas grade !

Est-ce que la CMF peut aider ?



Cytométrie en flux dans les SMD



- Altération de l'expression des antigènes
- Evaluation du nombre de blastes et/ou détection de blastes à l'immunophénotype aberrant
- Identification de caractéristiques de dysmaturation sur les cellules de la lignée myéloïde.
- Exclusion d'autres pathologies
- Suivi, émergence de nouvelles populations pathologiques --> evolution ?

WHO 2008 : ≥ 3 aberrant features by FC and borderline dysplasia by morphology and no cytogenetic abnormalities --> highly suggestive for MDS (--> revaluation over several months). A single aberrant feature by FC is not enought.

Implementation of flow cytometry in the diagnostic work-up of myelodysplastic syndromes in a multicenter approach: Report from the Dutch Working Party on Flow Cytometry in MDS

Theresia M. Westers[a,*], Vincent H.J. van der Velde
Rik A. Brooimans[d], Claudia Cali[a], Angelika M. Dräg
Anja de Jong[a], P. (Ellen) A. Kuiper-Kramer[g], Marije
Jeroen G. te Marvelde[b], Joke K. van der Molen-Sinl
Frank W.M.B. Preijers[h], Roger K. Schindhelm[g], Alit
August H. Westra[a], Arjan A. van de Loosdrecht[a], c
MDS of the Dutch Society of Cytometry (NVC)

Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study

Kiyoyuki Ogata,[1] Matteo G. Della Porta,[2] Luca Malcovati,[2] Cristina Picone,[2] Norio Yokose,[3] Akira Matsuda,[4] Taishi Yamashita,[1,5] Hideto Tamura,[1] Junichi Tsukada,[6] and Kazuo Dan[1]

Myelodysplastic syndromes: the role of flow cytometry in diagnosis and prognosis

M. STETLER-STEVENSON, C. M. YUAN

Validation of a flow cytometric scoring system as a prognostic indicator for posttransplantation outcome in patients with myelodysplastic syndrome

Bart L. Scott,[1,2] Denise A. Wells,[3] Michael R. Loken,[3] David Myerson,[1,2] Wendy M. Leisenring,[1,2] and H. Joachim Deeg[1,2]

Diagnostic flow cytometry for low-grade myelodysplastic syndromes

Kiyoyuki Ogata*
Division of Hematology, Department of Medicine, Nippon Medical School, Tokyo, Japan

Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes

Arjan A. van de Loosdrecht,[1] Canan Alhan,[1] Marie Christine Béné,[2] Matteo G. Della Porta,[3] Ange
Jean Feuillard,[4] Patricia Font,[5] Ulrich Germing,[6] Detlef Haase,[7] Christa H. Homburg,[8] Robin Irela
vati,[3] Jeroen G. te Marvelde,[12] Ghulam J. Mufti,[9] Kiyoyuki Ogata,[13] A
Porwit,[15] Frank W. Preijers,[10] Stephen J. Richards,[16] Gerrit Jan Schu
t,[18] Vincent H.J. van der Velden,[12] Paresh Vyas,[19] August H. Westra,[1
Loken,[20] and Theresia M. Westers[1]

Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation

Denise A. Wells, Martin Benesch, Michael R. Loken, Carlos Vallejo, David Myerson, Wendy M. Leisenring, and H. Joachim Deeg

Bone Marrow Cells from Myelodysplastic Syndromes Show Altered Immunophenotypic Profiles That May Contribute to the Diagnosis and Prognostic Stratification of the Disease: a Pilot Study on A Series of 56 Patients

Sergio Matarraz,[1] Antonio López,[1] Susana Barrena,[1] Carlos Fernandez,[1] Evan Jensen,[1] Juan Flores-Montero,[1] Ana Rasillo,[1] José María Sayagues,[1] Maria Luz Sánchez,[1] Paloma Bárcena,[1] Jesús María Hernandez-Rivas,[2] Carlos Salvador,[3] Nuria Fernandez-Mosteirín,[3] Manuel Giralt,[3] Luis Perdiguer,[4] Paula Laranjeira,[5] Artur Paiva,[5] and Alberto Orfao[1*]

[1]Centro de Investigación del Cáncer (Instituto de Biología Molecular y Celular del Cáncer; CSIC-USAL), Servicio General de Citometría and Departamento de Medicina, Universidad de Salamanca, Salamanca, Spain
[2]Servicio de Hematología, Hospital Universitario de Salamanca, Salamanca, Spain
[3]Servicio de Hematologia. Hospital Miguel Servet, Zaragoza, Spain
[4]Servicio de Hematologia, Hospital de Alcañiz, Teruel, Spain
[5]Unidade de Citometria, Centro de Histocompatibilidade do Centro-Coimbra, Coimbra, Portugal

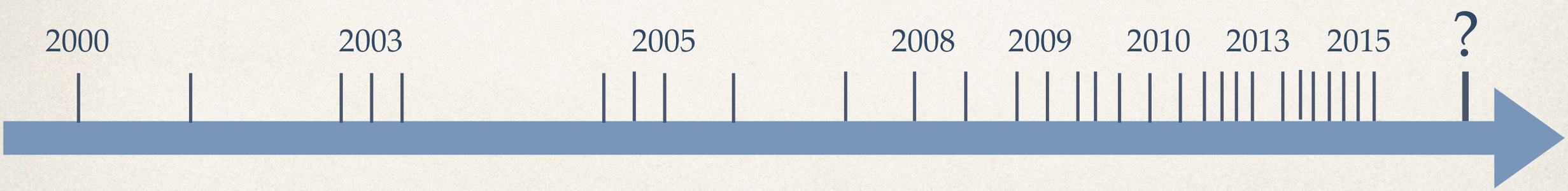
Identification of distinct prognostic subgroups in low- and intermediate-1–risk myelodysplastic syndromes by flow cytometry

Arjan A. van de Loosdrecht,[1] Theresia M. Westers,[1] August H. Westra,[1] Angelika M. Dräger,[1] Vincent H. J. van der Velden,[2] and Gert J. Ossenkoppele[1]

Flow cytometric scoring system as a diagnostic and prognostic tool in myelodysplastic syndromes

Sung-Chao Chu[a,b], Tso-Fu Wang[a], Chi-Cheng Li[d], Ruey-Ho Kao[a,c], Dian-Kun Li[c,f], Yu-Chieh Su[c,f], Denise A. Wells[e], Michael R. Loken[e,*]

Comités internationaux
Standardisation
Guidelines



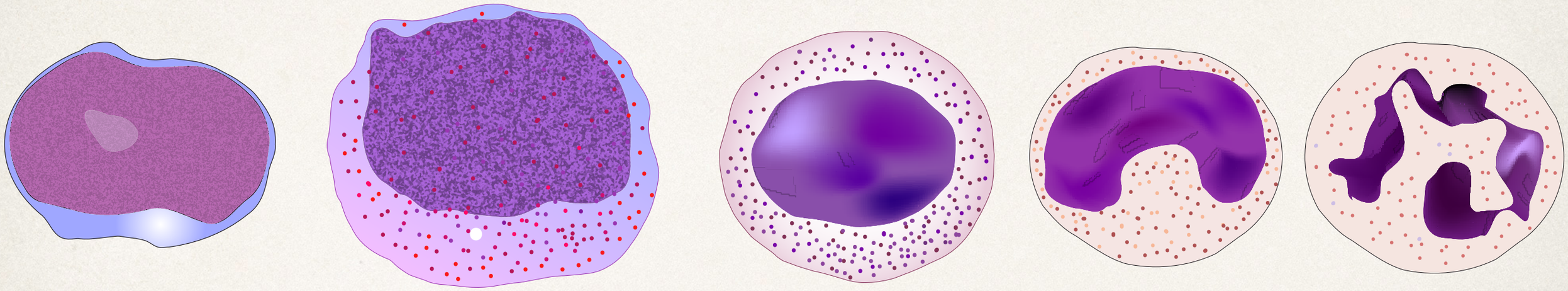
Hématopoïèse normale dans la moelle

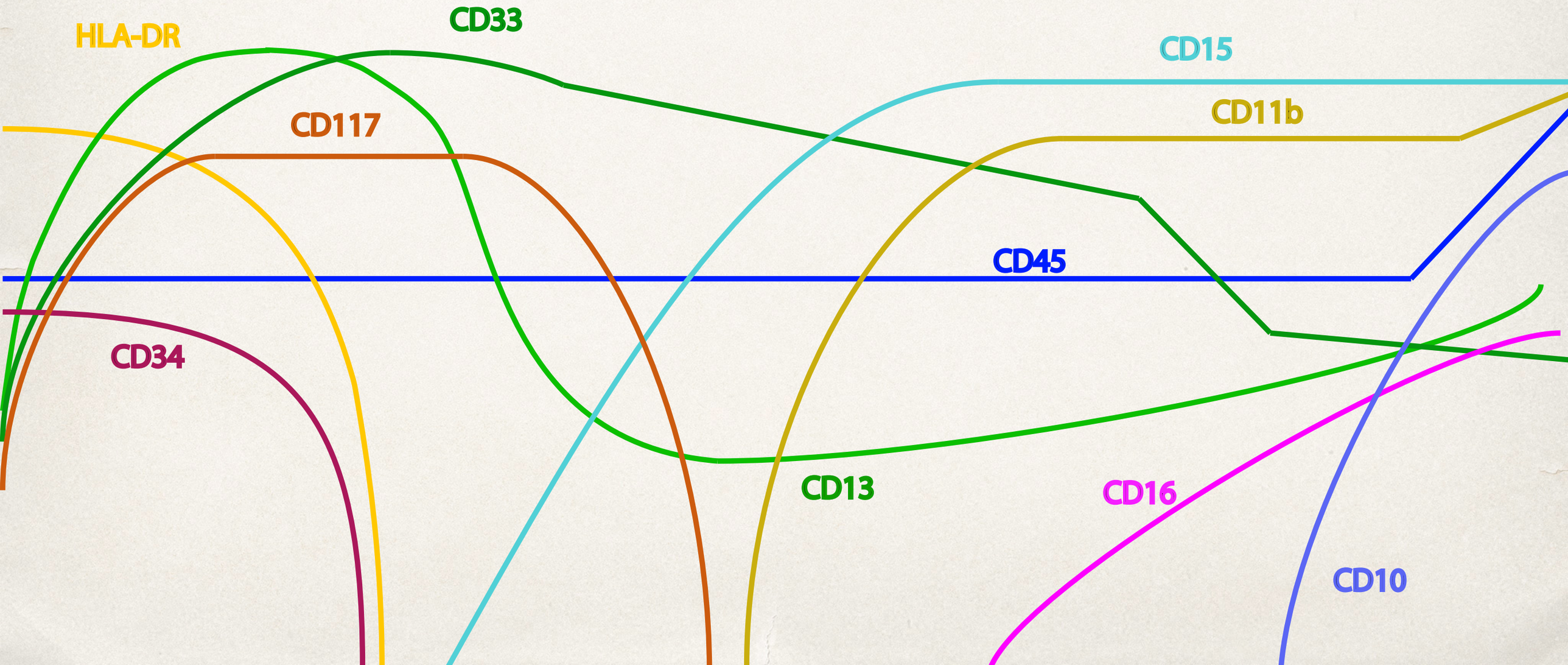
Immunophenotypic Differentiation Patterns of Normal Hematopoiesis in Human Bone Marrow : Reference Patterns for Age-Related Changes and Disease-Induced Shifts, EG van Lochem et al, Cytometry Part B (Clinical Cytometry) 60B:1-13 (2004)

- **Différenciation des granulocytes** CD34/CD117/CD45/CD13-33 CD16/CD13/CD45/CD11b
- Différenciation monocytaire CD14/CD33/CD45/CD34
- Différenciation cellules B TdT/CD20/CD19/CD10 CD45/CD34/CD19/CD22
- Différenciation érythroïde CD71/CD235a/CD45/CD34

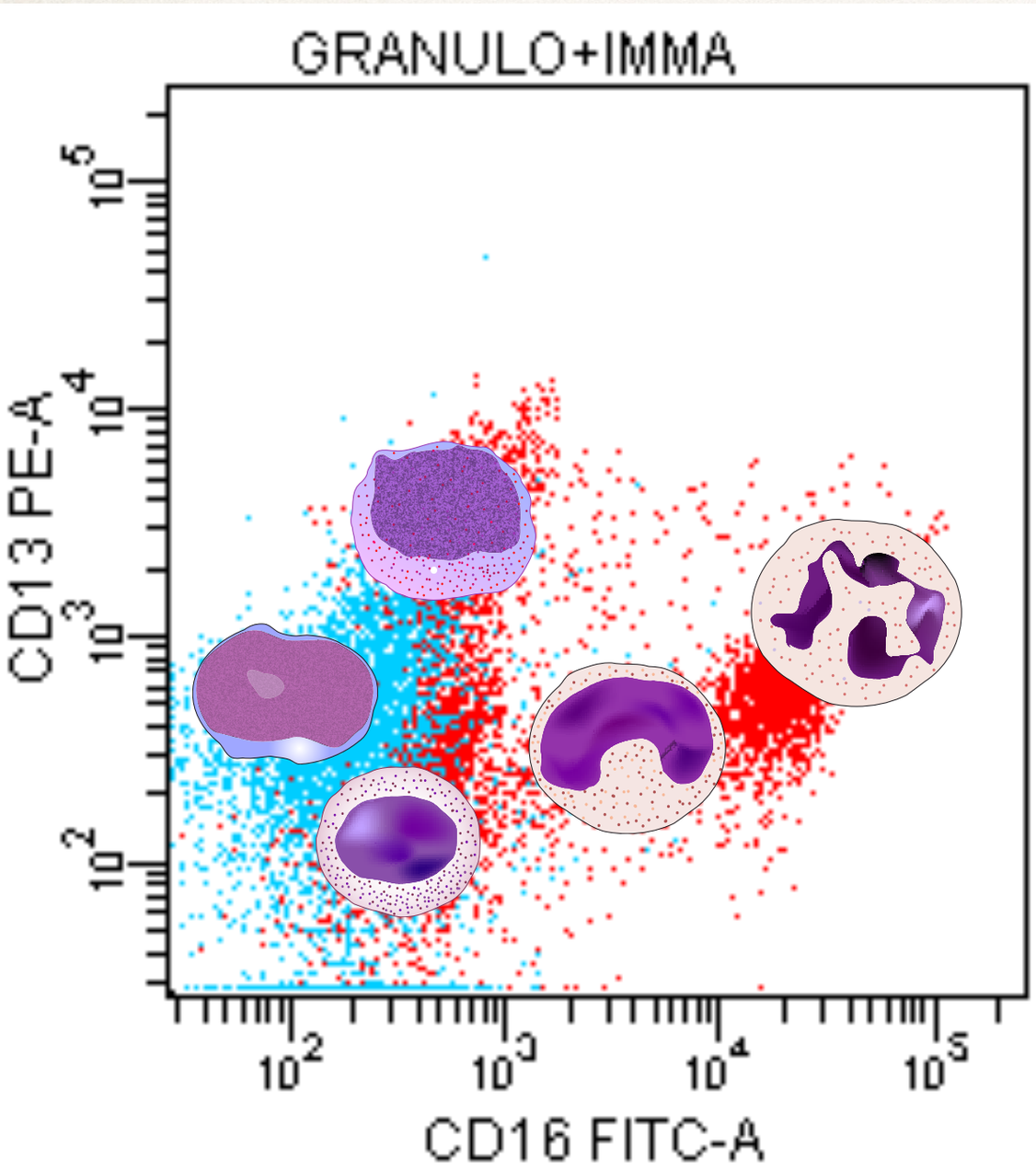
Basé sur «Flow Cytometry in Neoplastic Haematology»
Gorczyca 2010

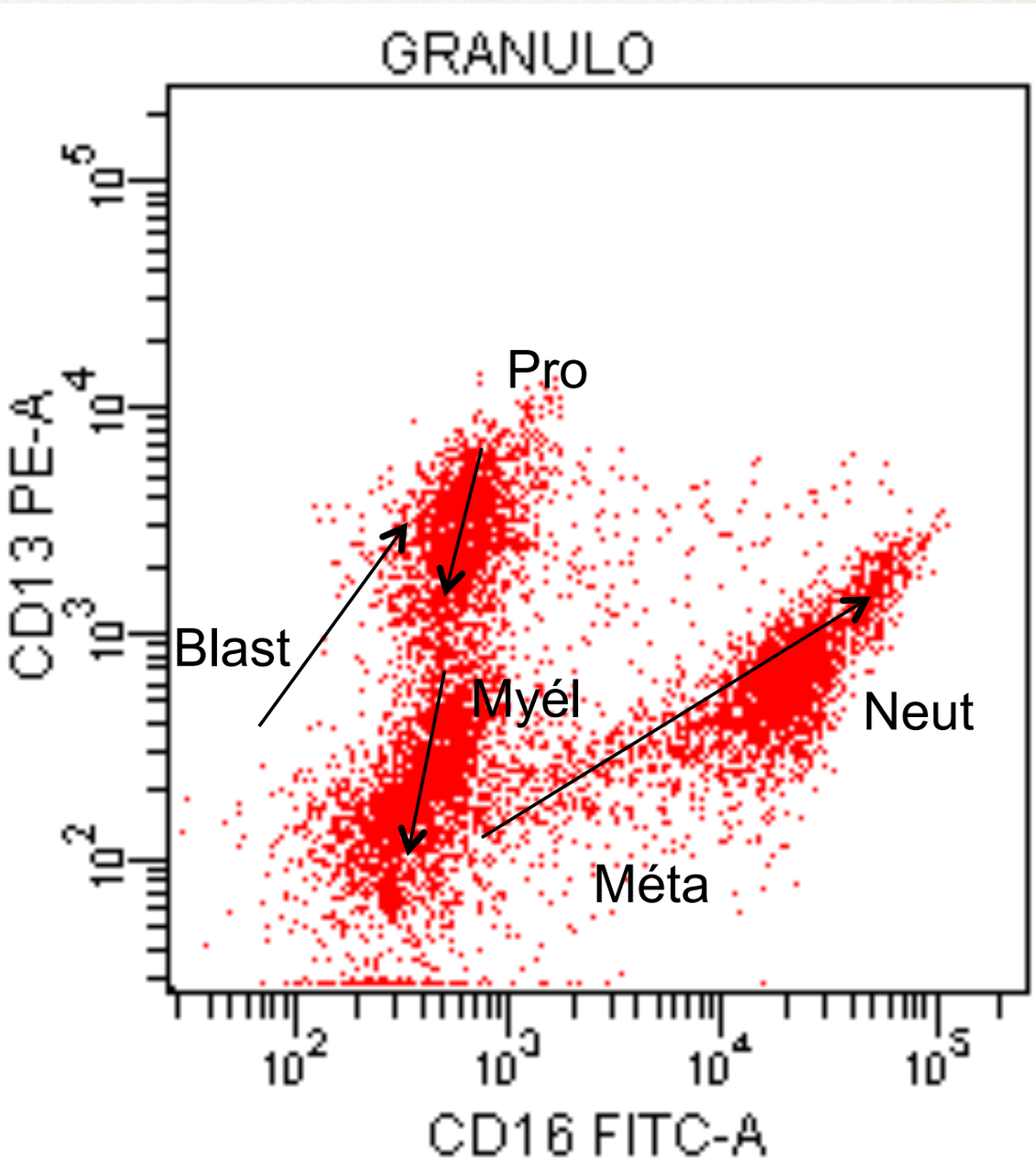
Maturation myéloïde





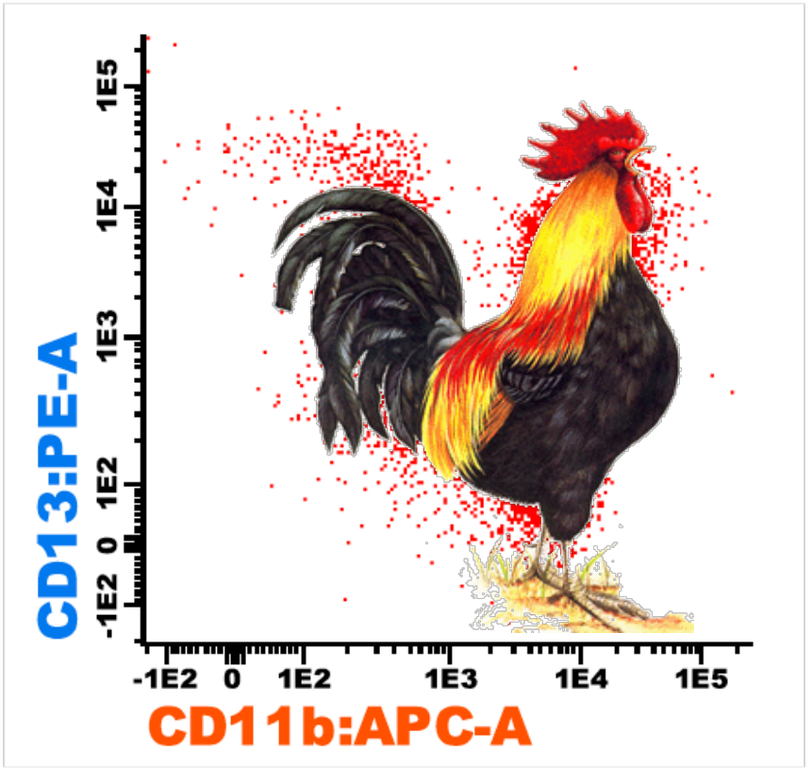
Différenciation myéloïde normale

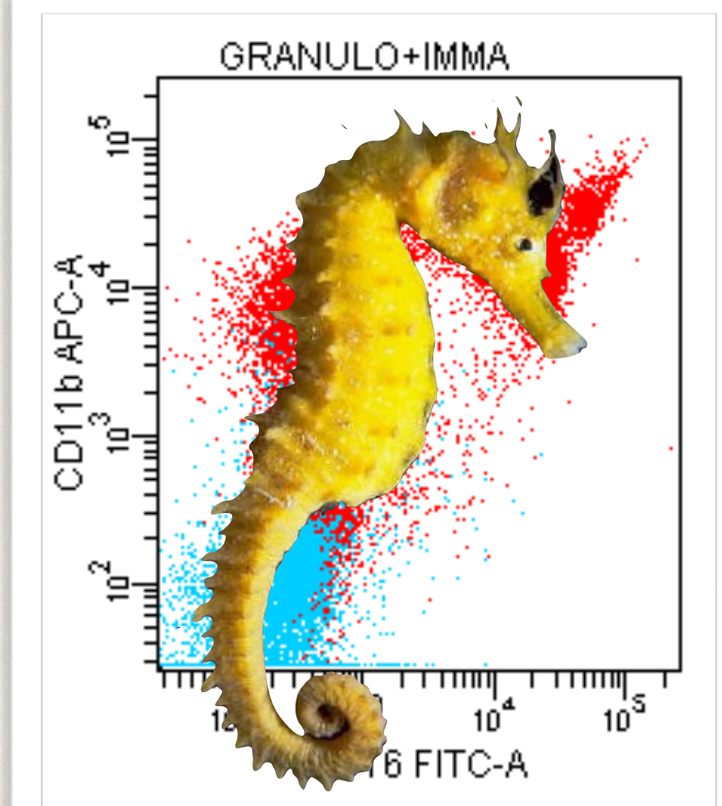


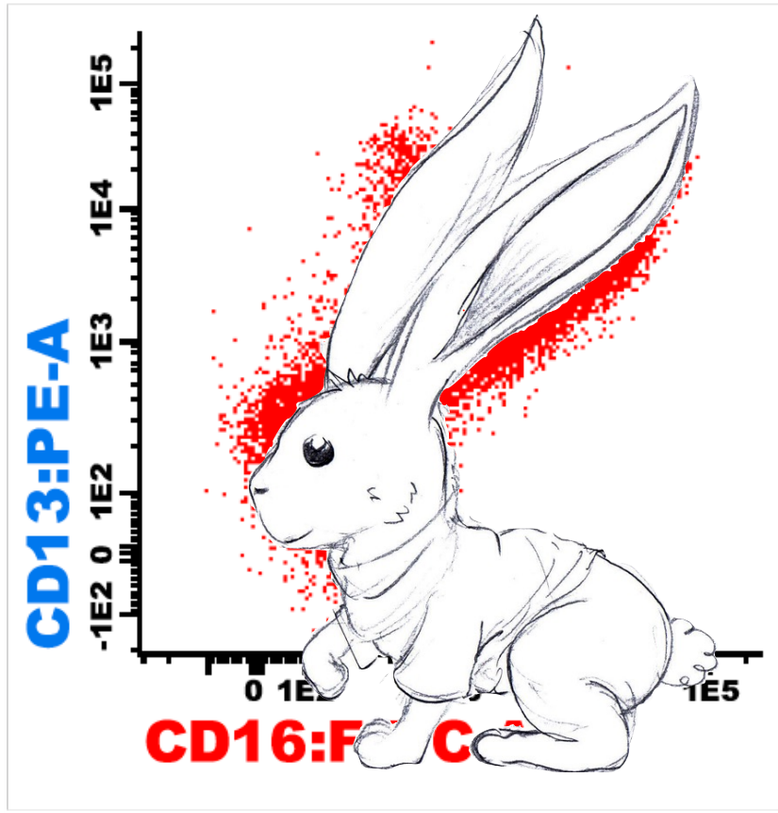


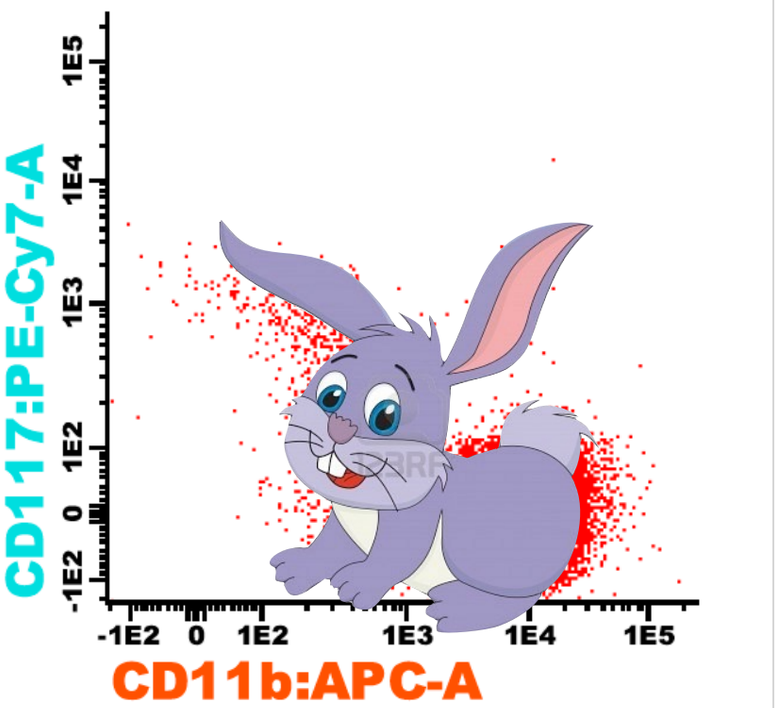
Différenciation myéloïde normale

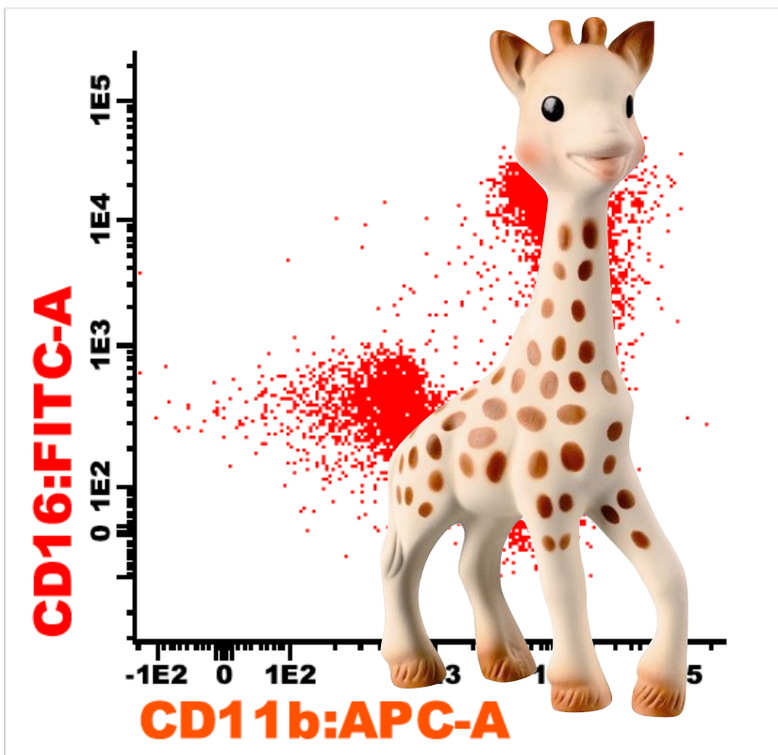
CD13/CD16/CD11b/CD117



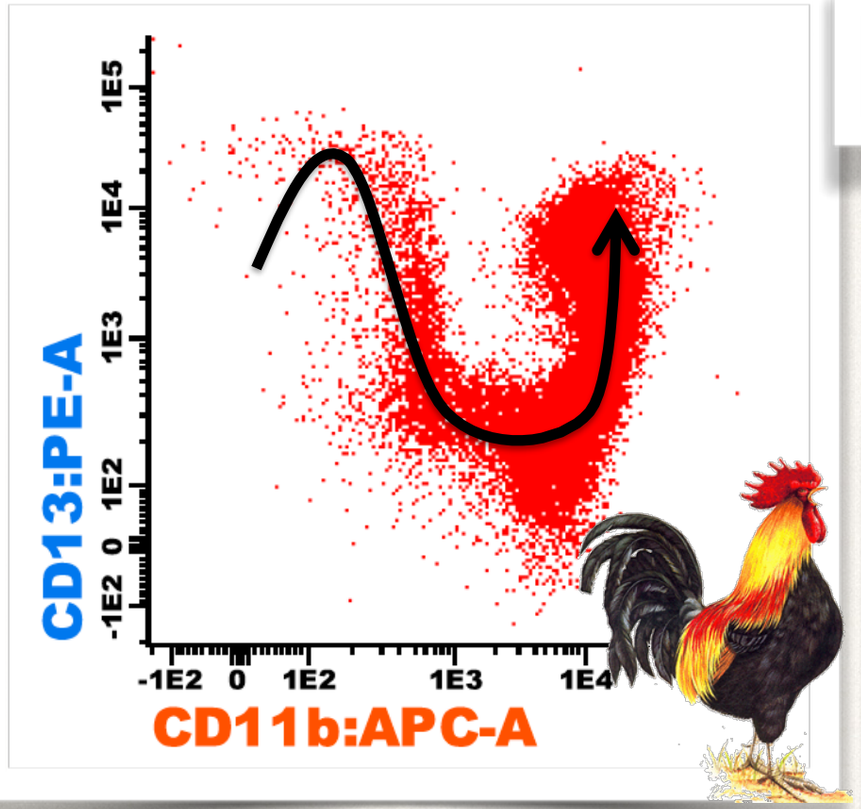


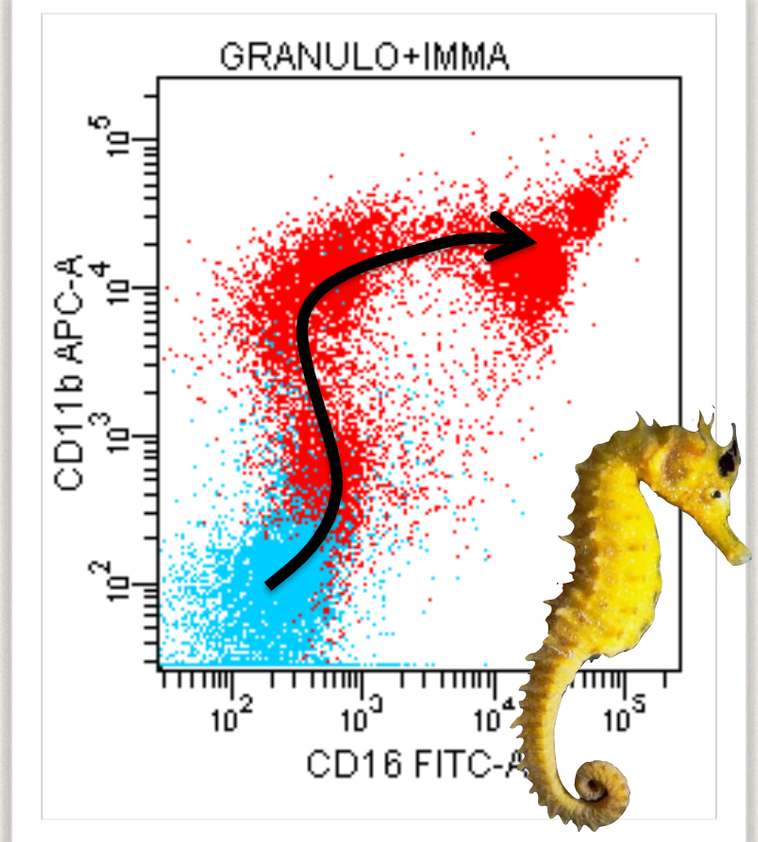


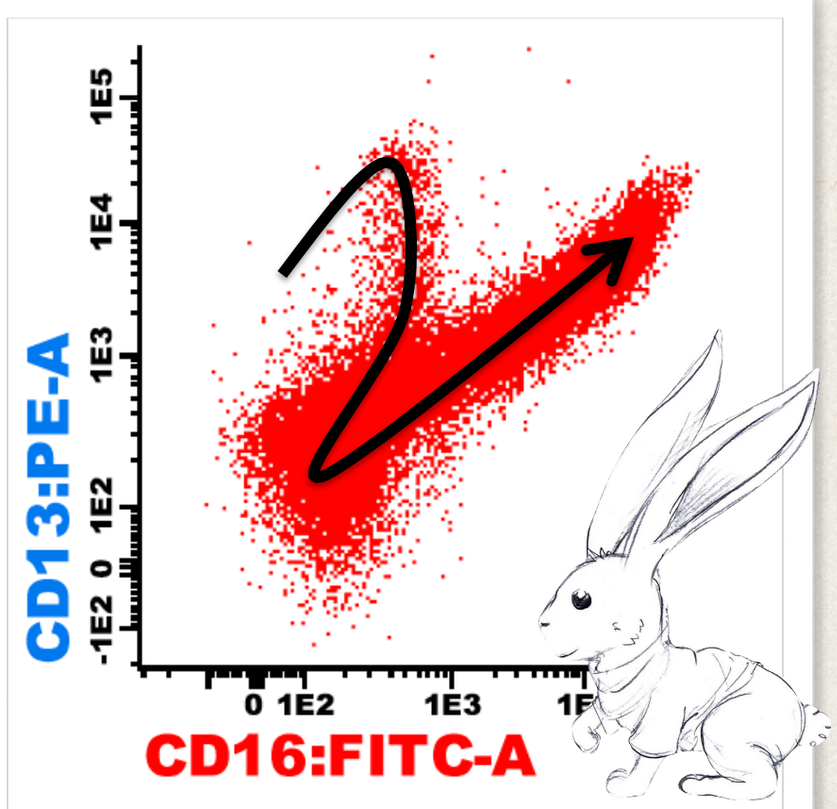


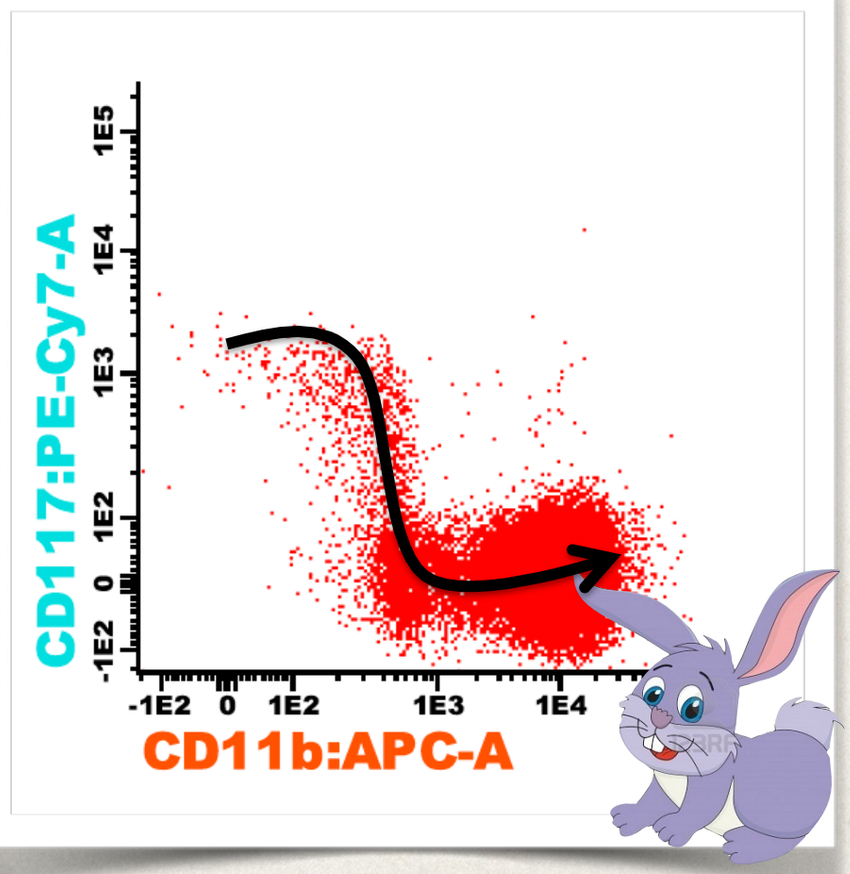


CD13/CD16/CD11b/CD117

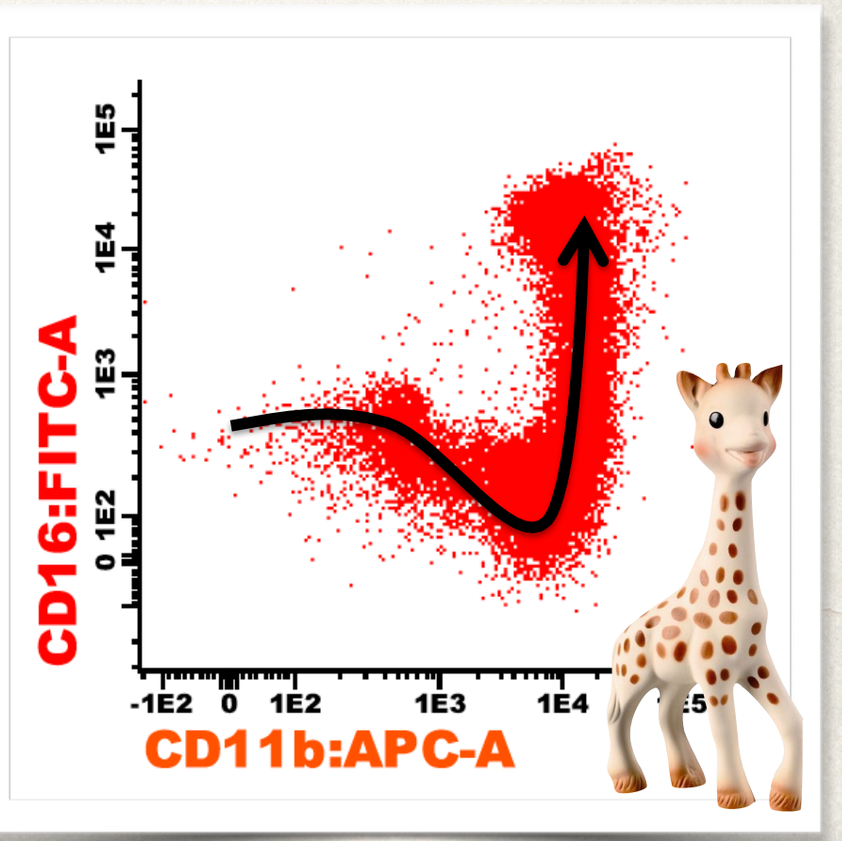








Connaître ces arcs permet de traquer les anomalies !



La maturation a du bon…

↘ SSC des neutrophiles

↗ % CD34+

Niveau d'expression anormal de marqueurs

Que se passe-t-il dans un SMD ?

Expression d'un marqueur aberrant

↘ Progéniteurs B (parmi les CD34+)

Expression asynchrone de marqueurs de maturation

...

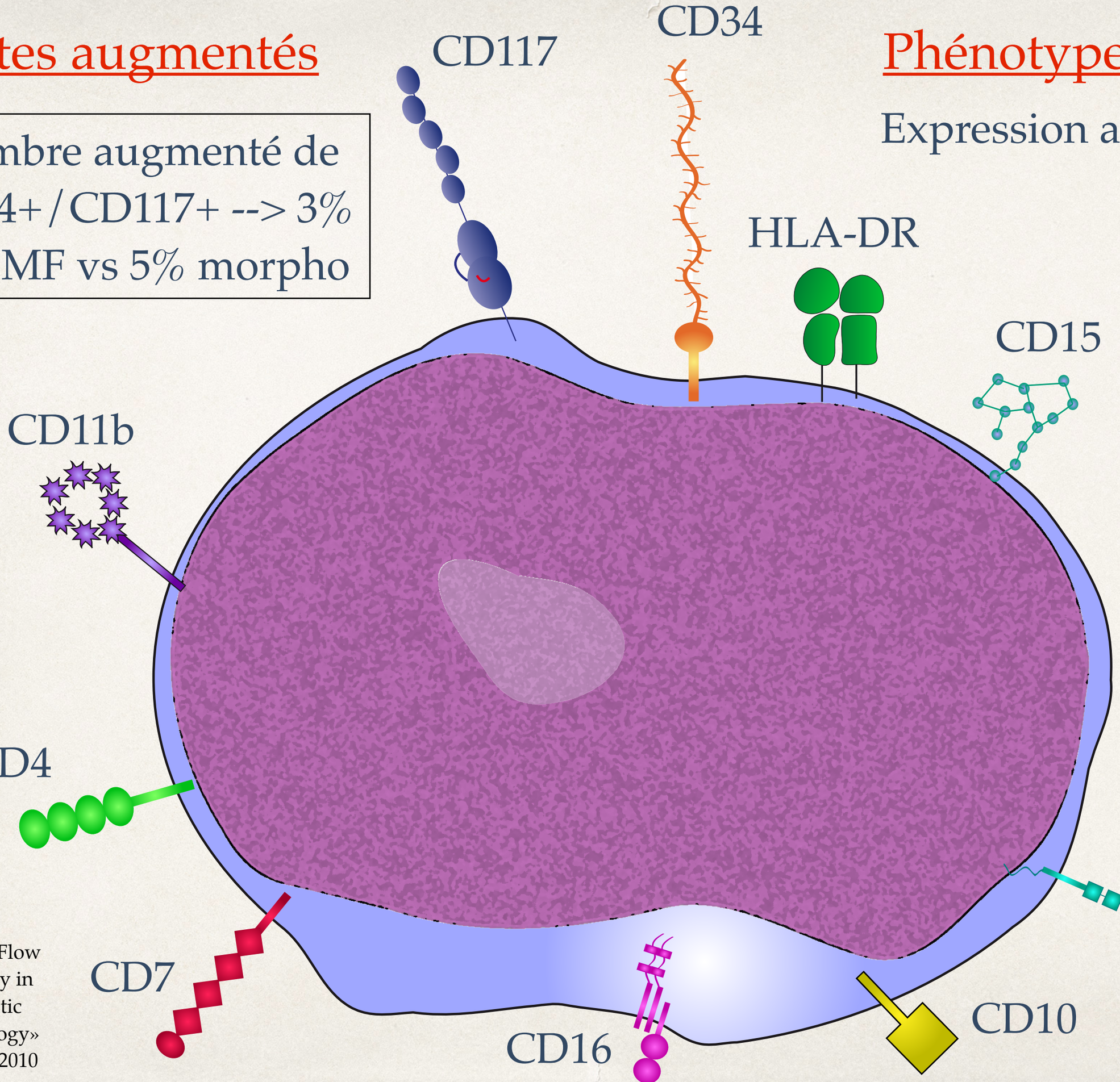
Blastes augmentés

Nombre augmenté de CD34+/CD117+ --> 3% en CMF vs 5% morpho

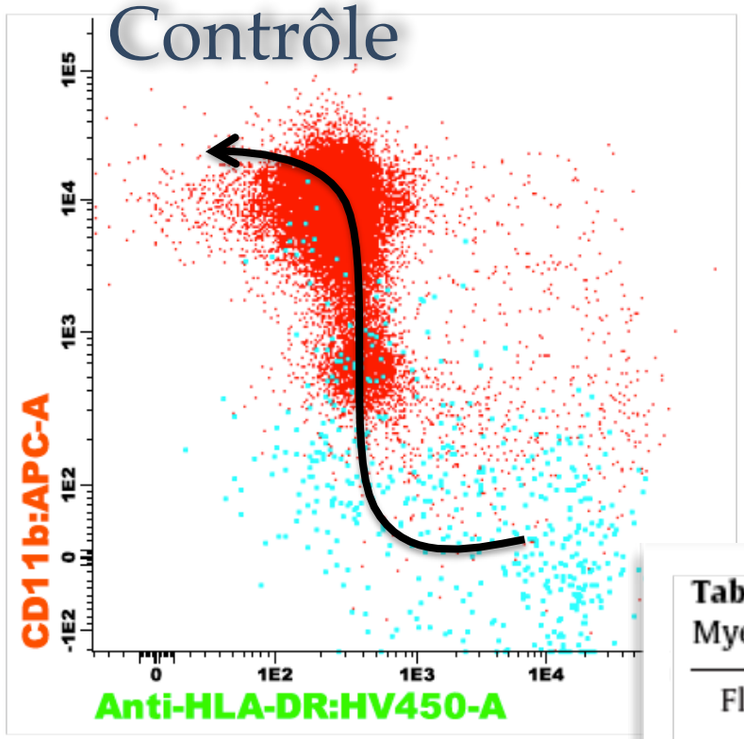
Phénotype aberrant

Expression aberrante de

- CD11b
- CD15
- CD4
- **CD7**
- CD10
- CD56
- CD16
- ...
- DR-
- CD38-

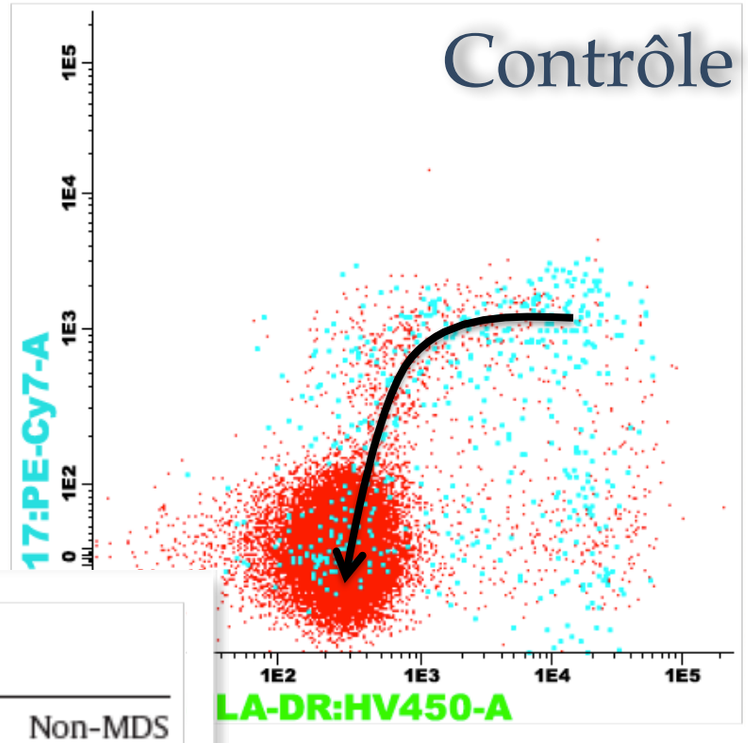


Basé sur «Flow Cytometry in Neoplastic Haematology» Gorczyca 2010



HLA-DR/CD11b

Gate : IMMA + granulo

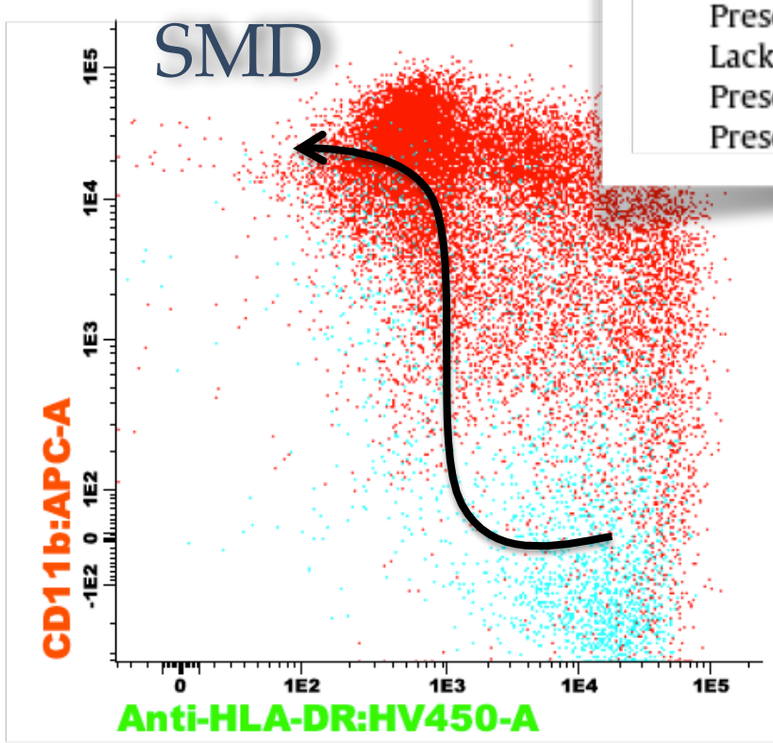


HLA-DR/CD117

Table 3
Myeloid and monocytic abnormalities detected by flow cytometry.

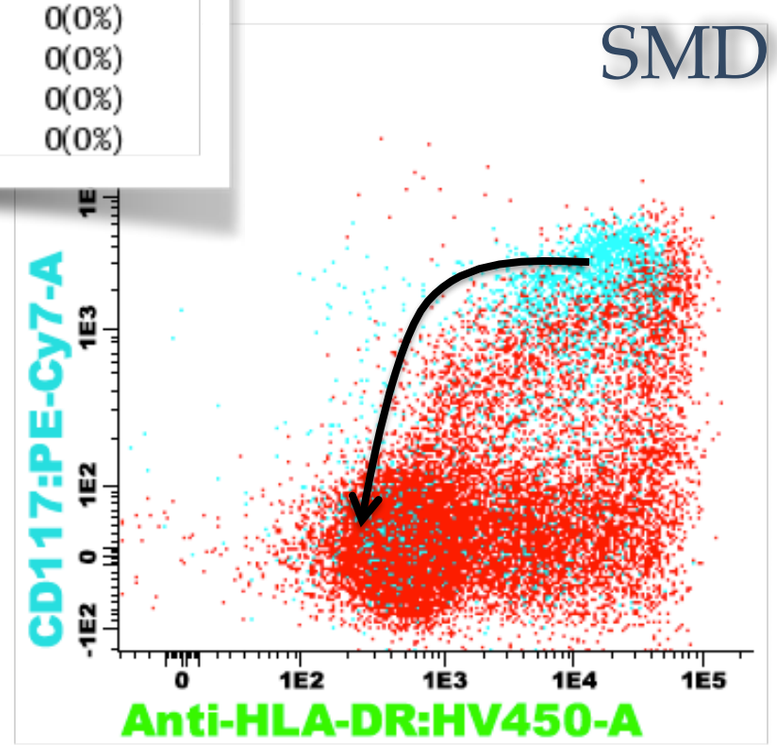
Flow cytometric abnormality	MDS (n = 56)	Non-MDS (n = 27)
Abnormal maturing myeloid, no. (%)[a]		
Abnormal granularity	9(16%)	0(0%)
Abnormal decrease CD45	3(5%)	0(0%)
Abnormal relationship CD13/CD16	18(32%)	2(7%)
Abnormal relationships HLA-DR/CD11b	14(25%)	0(0%)
Asynchronous shift to the left	11(20%)	0(0%)
Presence of CD56	13(23%)	0(0%)
Lack of CD33	0(0%)	0(0%)
Presence of CD34	2(4%)	0(0%)
Presence of lymphoid antigens	0(0%)	0(0%)

SMD

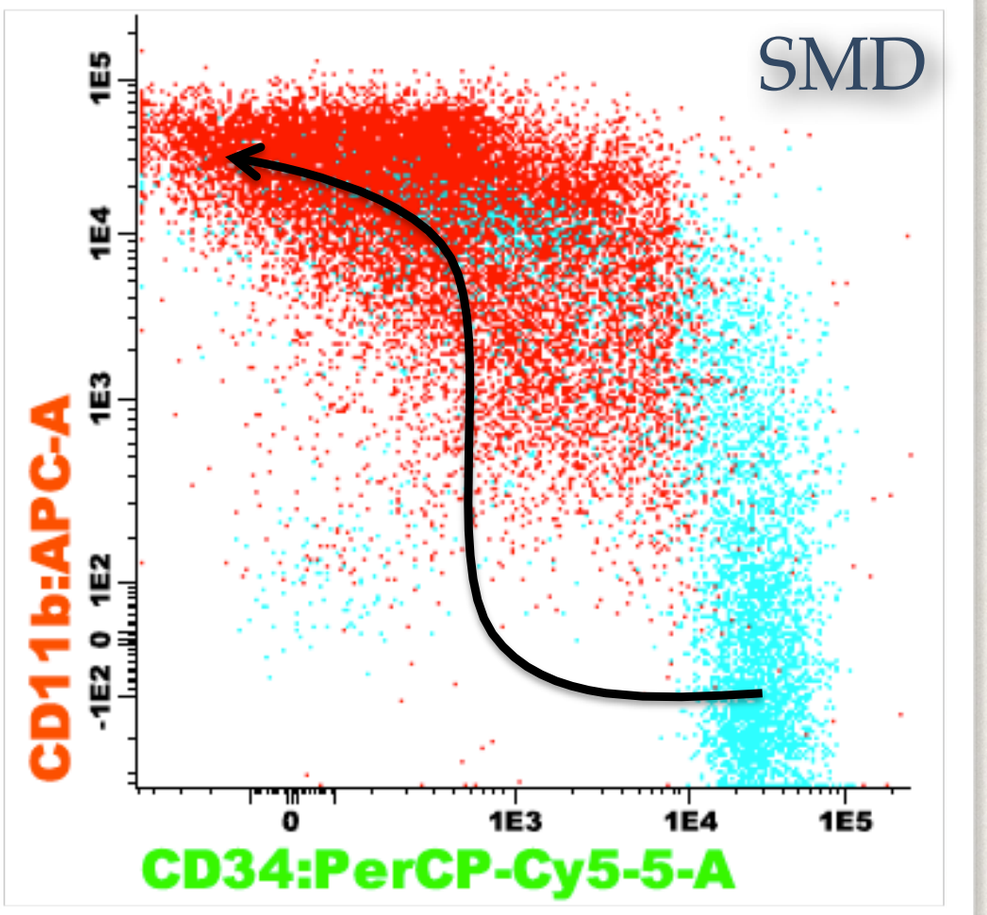


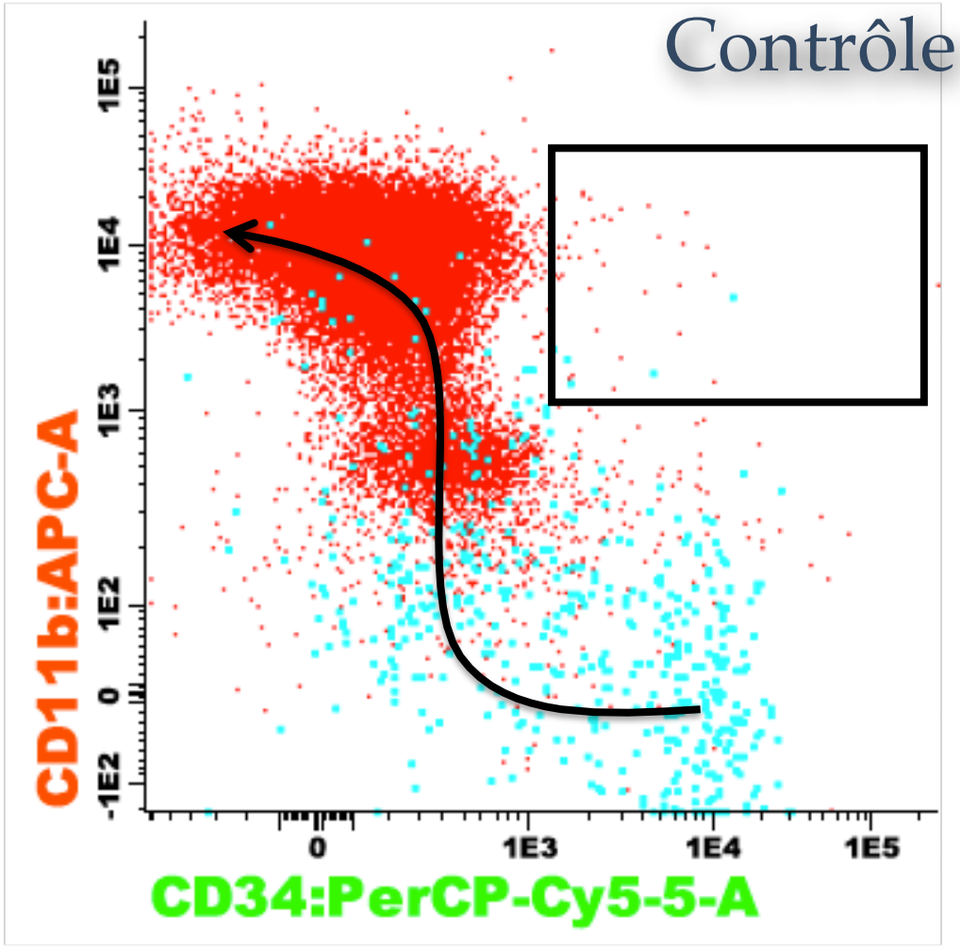
Flow Cytometric scoring system as a diagnostic and prognostic tool in myelodysplastic syndromes, Chu et al, Leukemia Research 35 (2011) 868-873

SMD

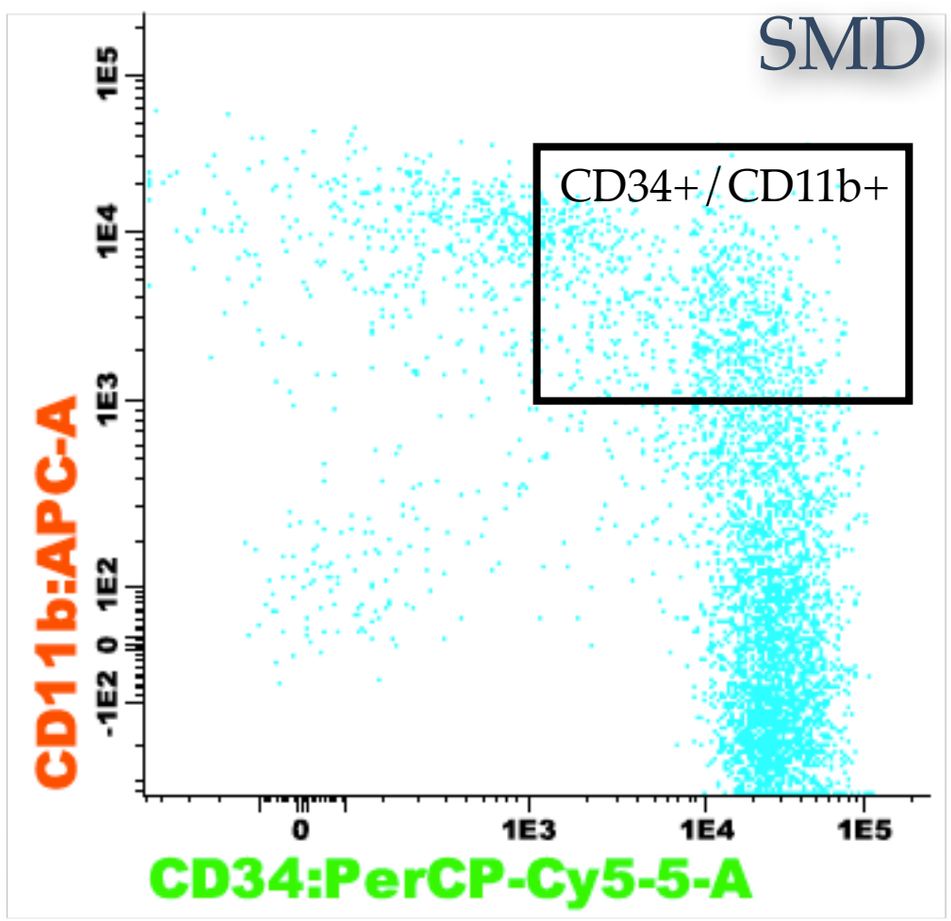


CD34/CD11b





Pas toujours aussi clair !
En particulier dans les formes intermédiaires…



Phénotype aberrant des blastes
ex : CD33 - CD7

«min. 10%»

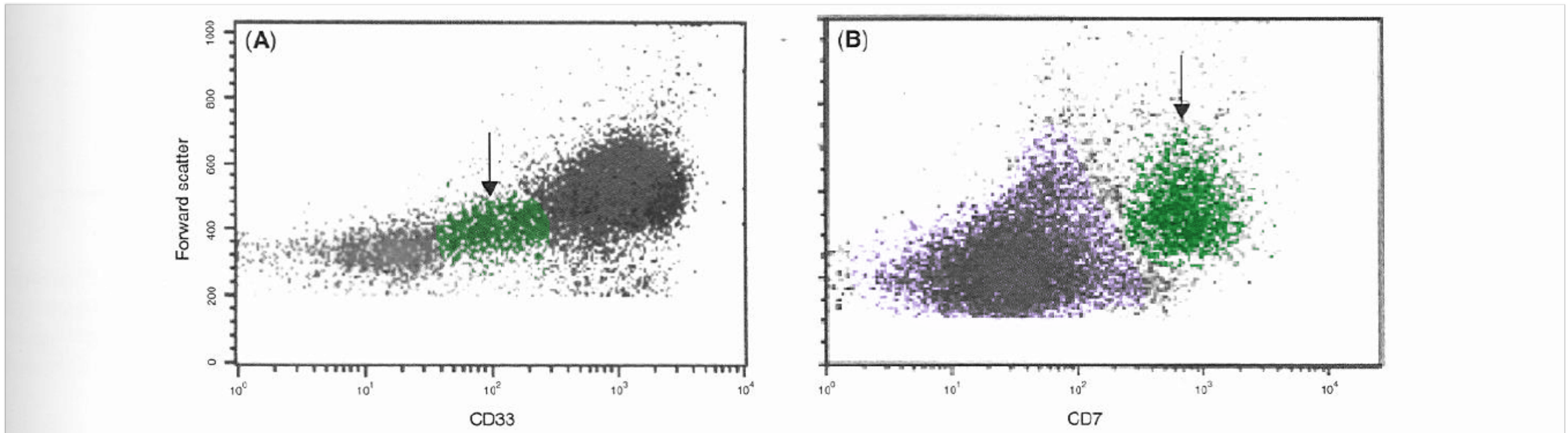
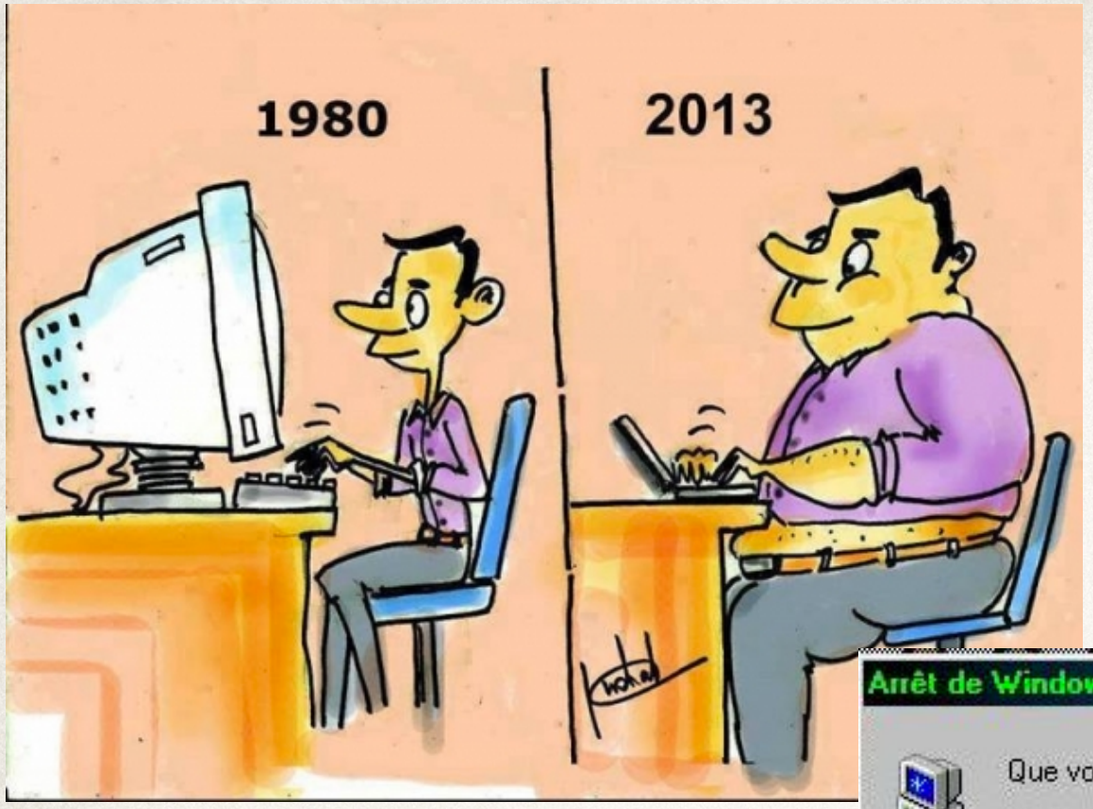


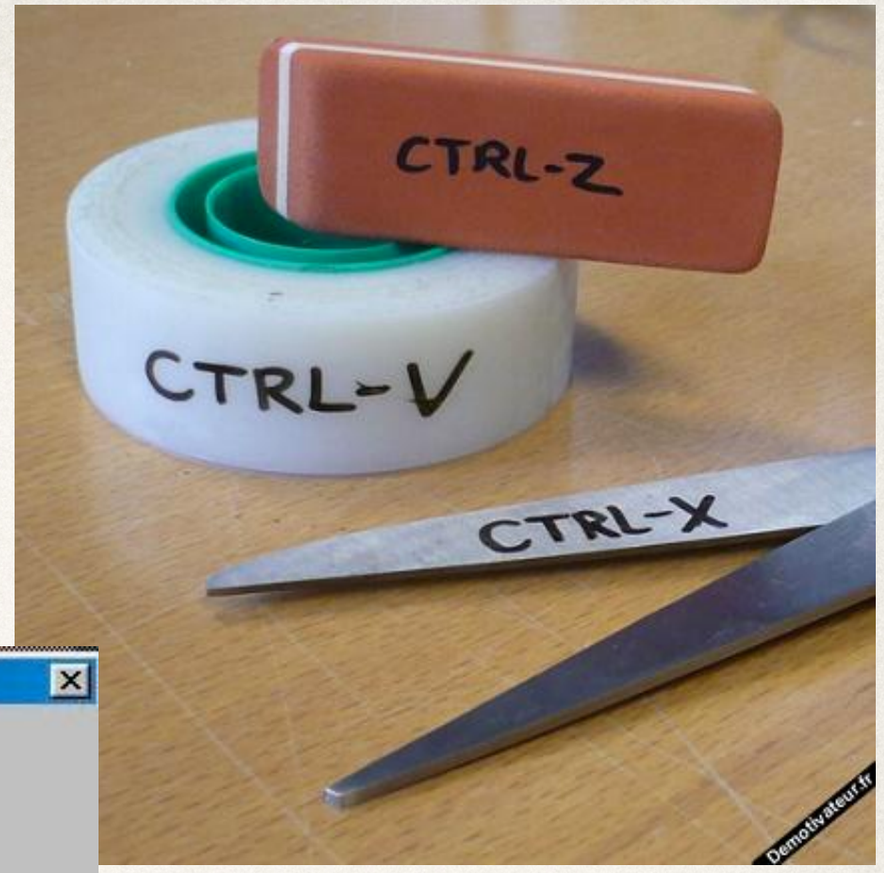
Figure 6.13 Aberrant phenotype of blasts in myelodysplastic syndrome (two cases). **A** shows blasts with aberrant (dim) expression of CD33 (arrow) and **B** shows blasts with aberrant expression of CD7 (arrow).

Marqueurs d'infidélité de lignées : CD5, CD7, CD19, CD56...

! CD7 est exprimé sur un petit contingent de blastes précoces et normaux, en particulier dans une hématopoïèse régénérative

Flow Cytometry in Neoplastic Haematology, Gorczyca 2010, second edition


1980
2013


CTRL-Z
CTRL-V
CTRL-X
Demotivateur.fr

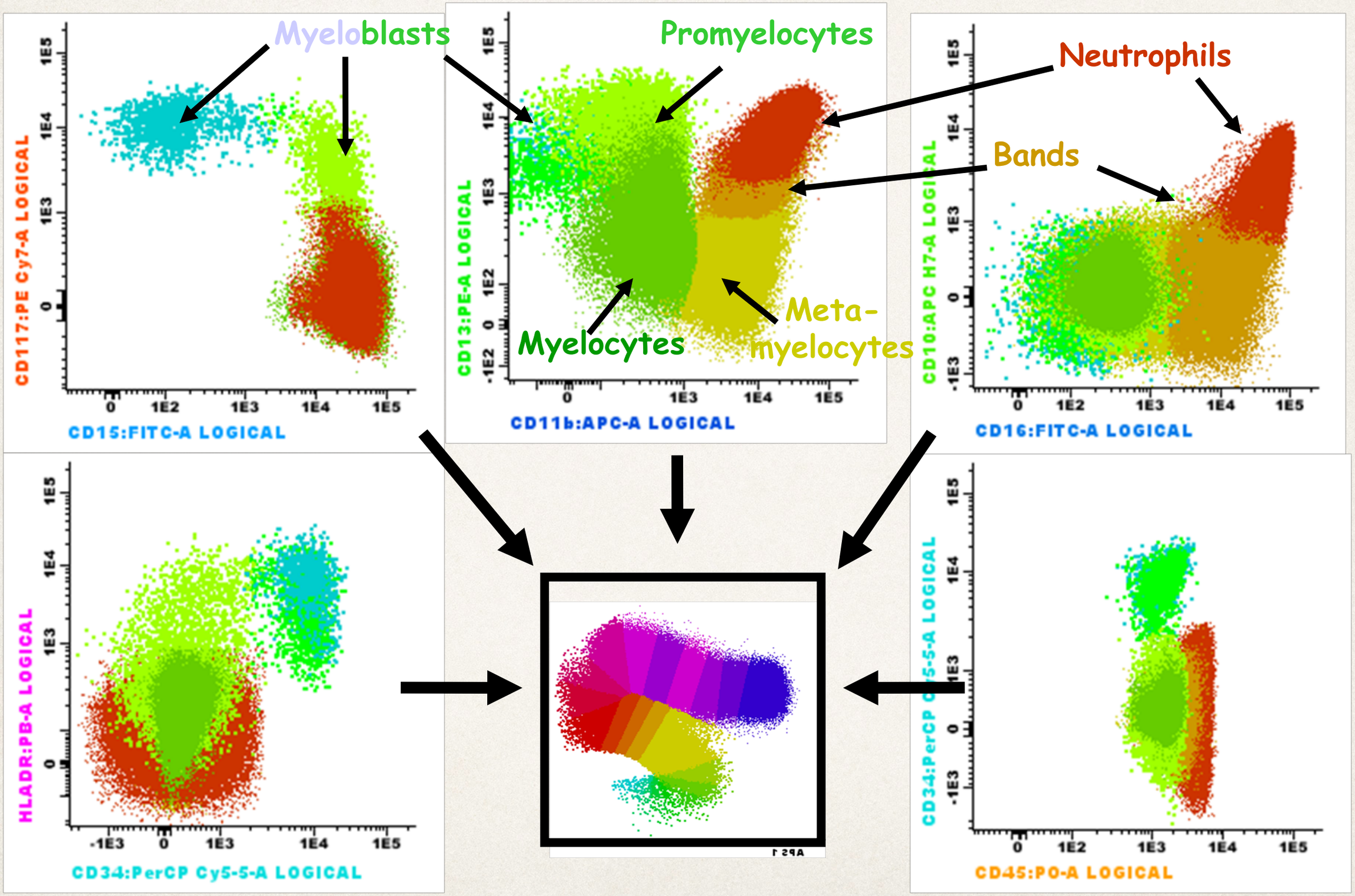

Arrêt de Windows
Que voulez-vous faire ?
Mettre en veille
Arrêter
Redémarrer
Redémarrer en mode MS-DOS
Aller promener le chien
Aller au bistro du coin
Aller se coucher
Aller faire les courses
Décider que cela ira pour aujourd'hui !
Autres urgences
Ne plus poser cette question !
OK
Annuler
Aide


POUR NE PAS USER MON DISQUE DUR
JE CORRIGE LES FAUTES AU TIPP-EX DIRECTEMENT SUR L'ÉCRAN


1
ENTER

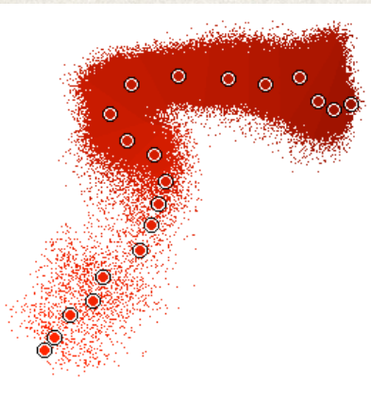
N-DIMENSIONAL NEUTROPHIL MATURATION IN NORMAL BM

EuroFlow

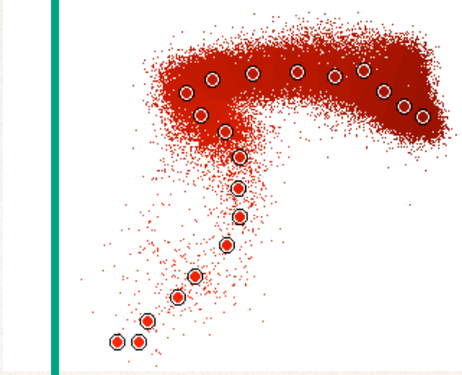


Orfao et al

BM 1



BM2



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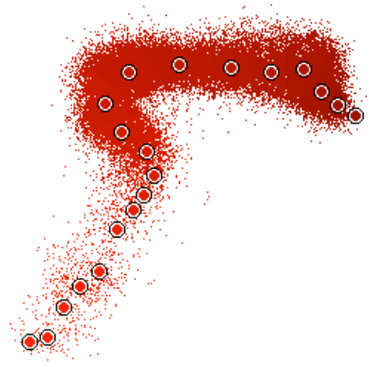
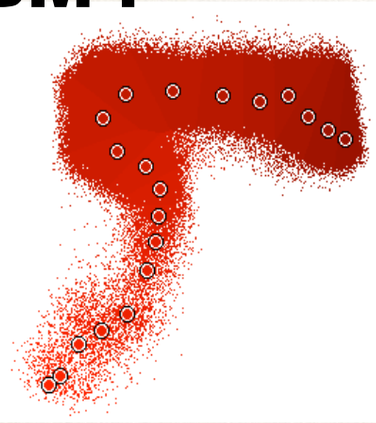
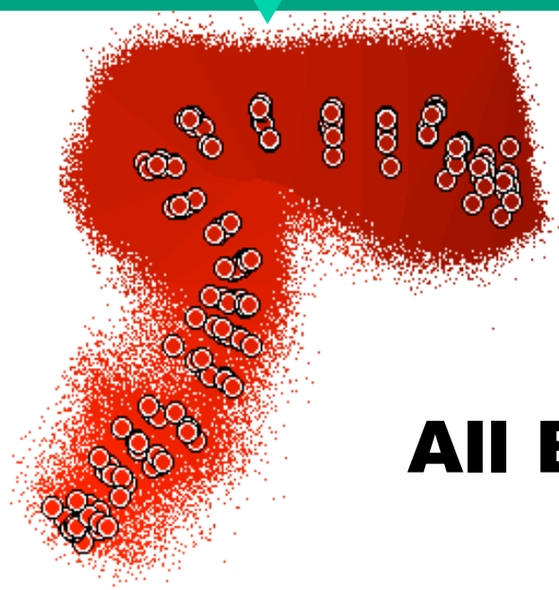


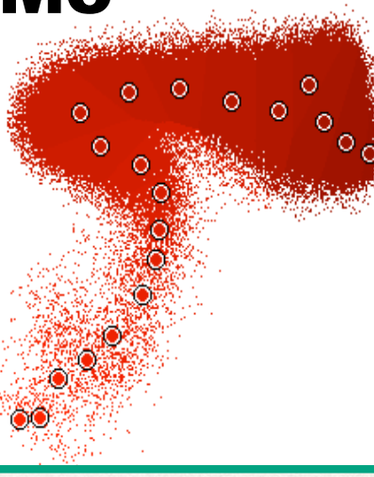
BM4



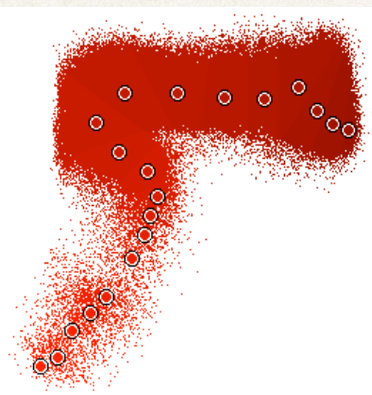
All BM



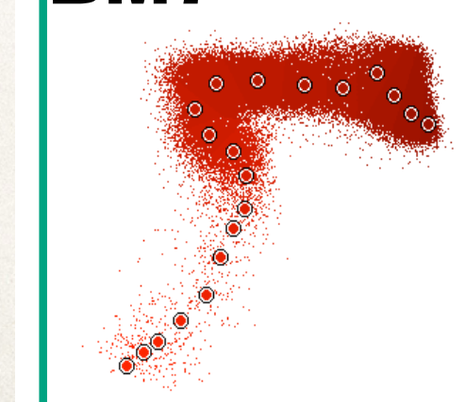
BM5



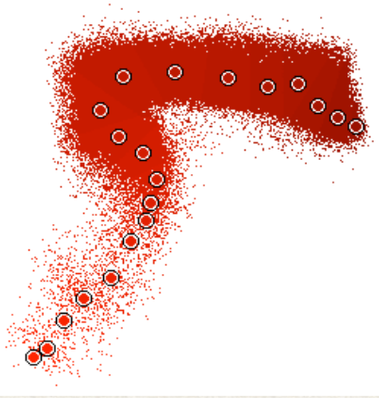
BM6



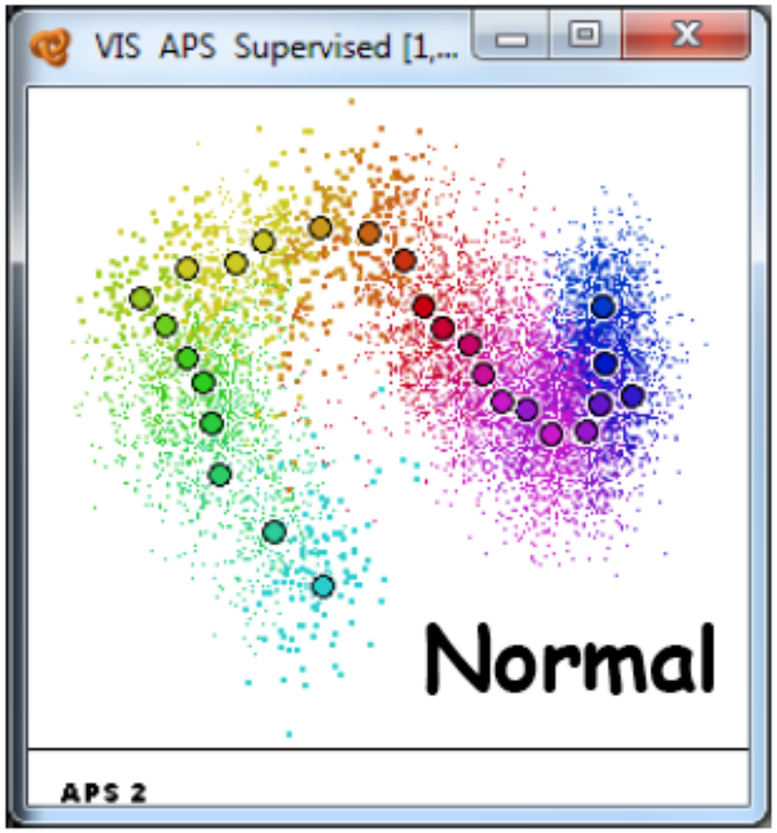
BM7

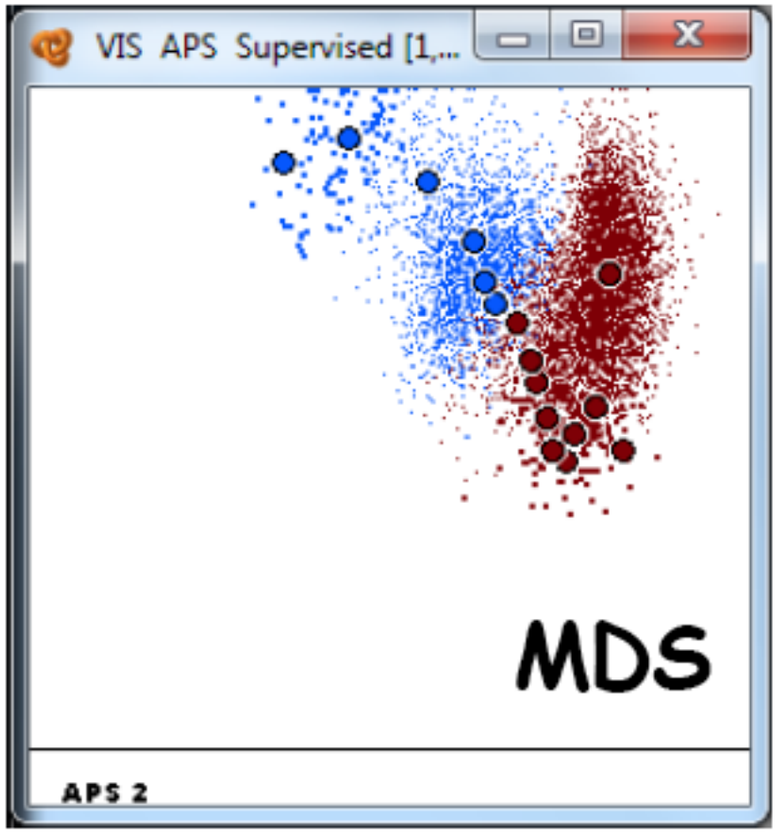


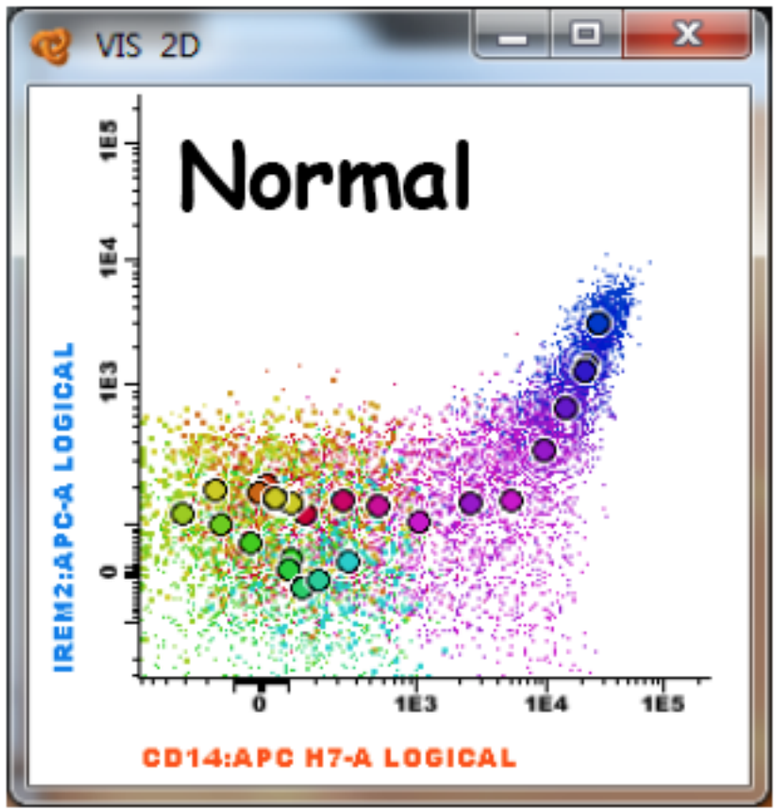
BM8



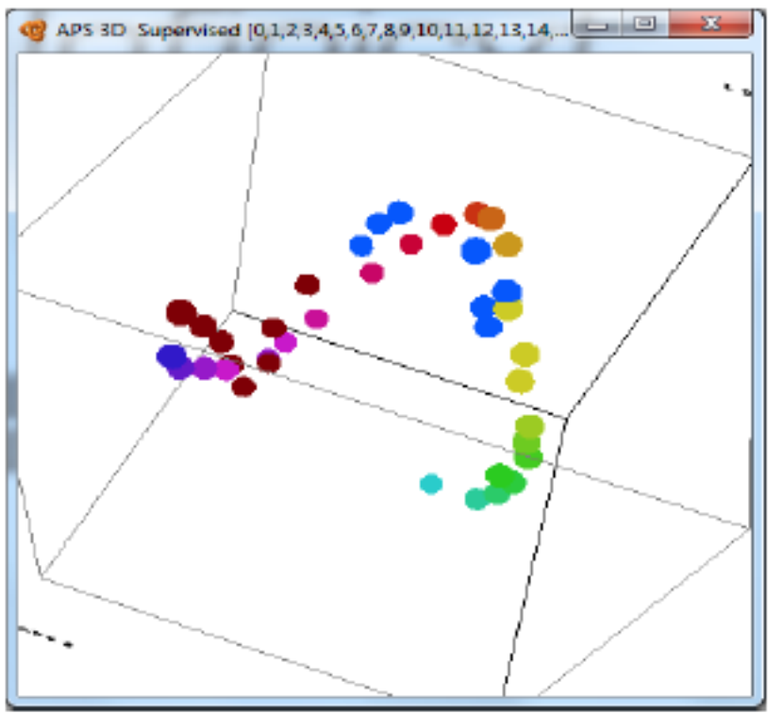
Orfao et al

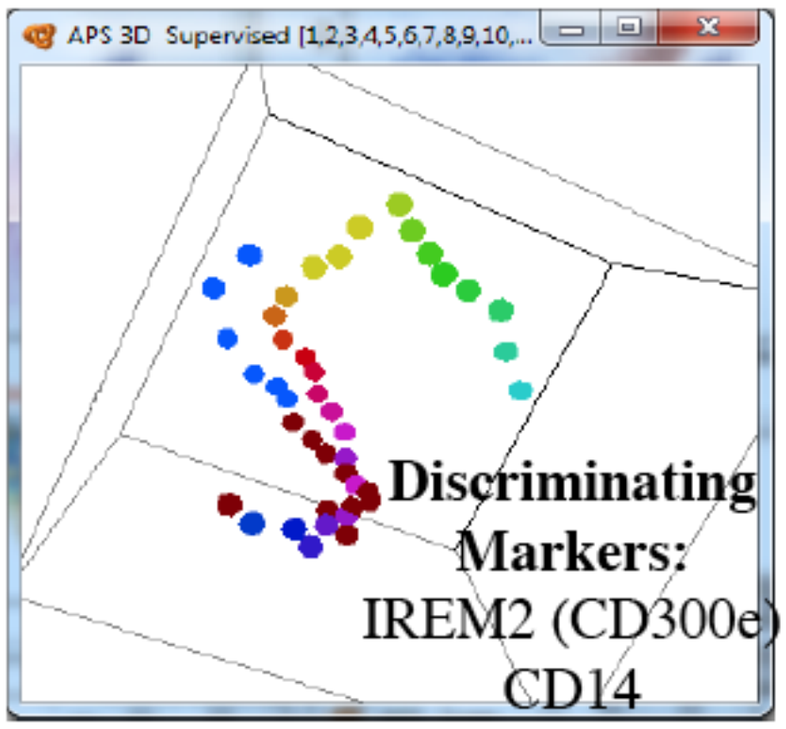


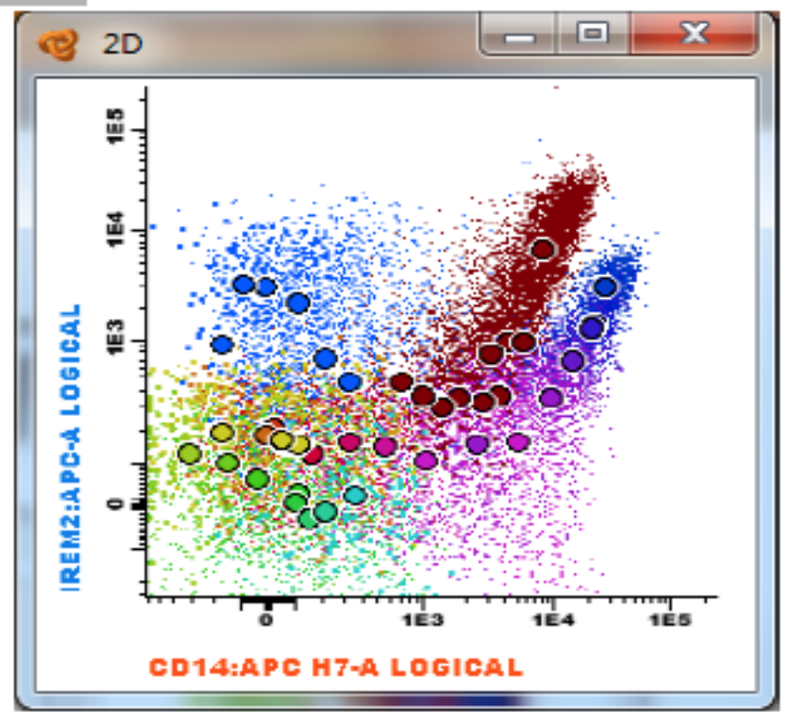




NORMAL + MDS OVERLAY



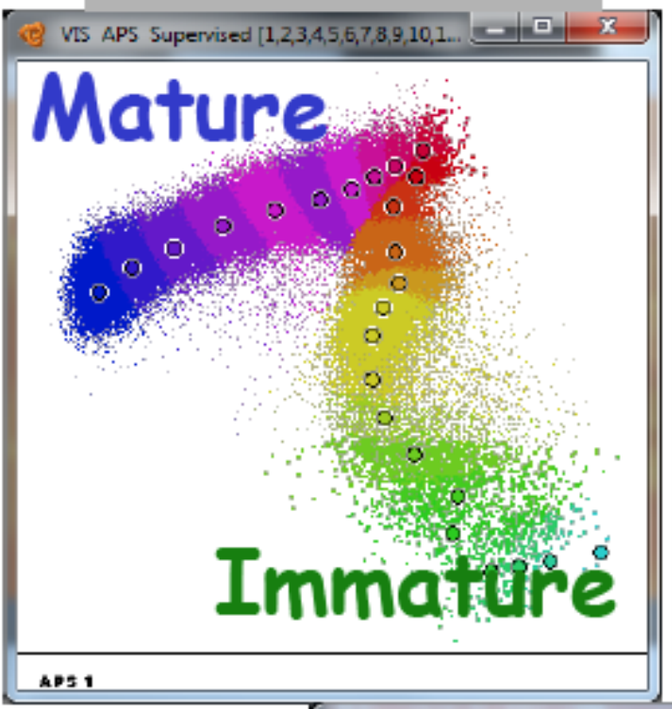


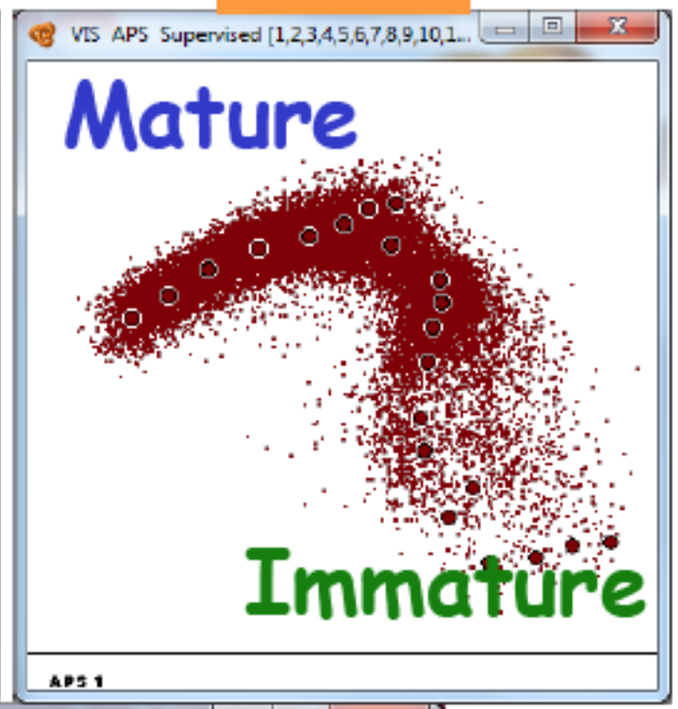


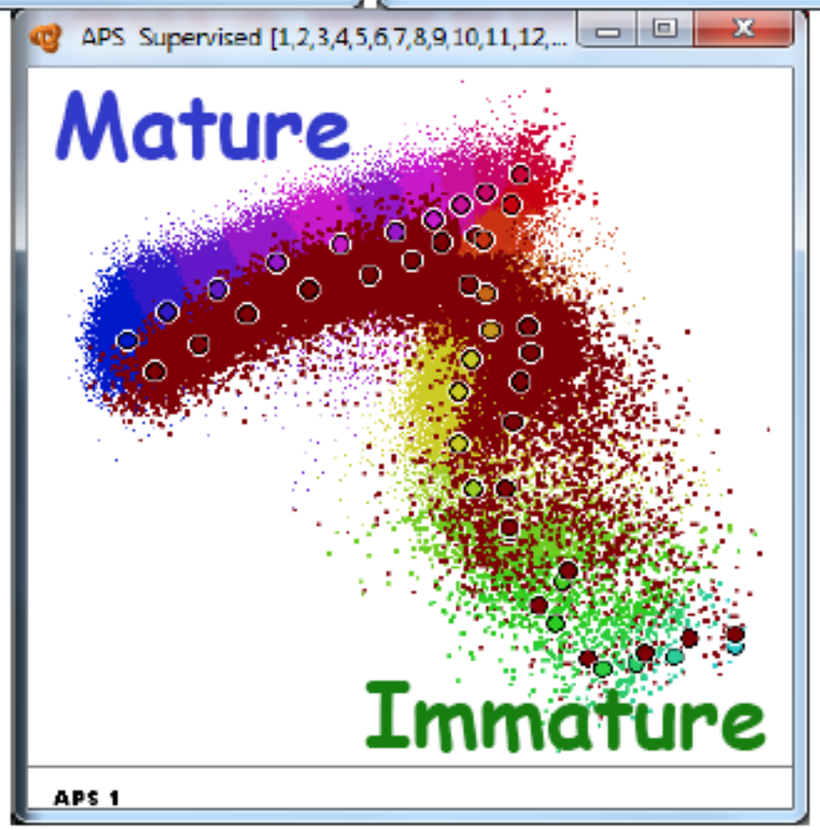
Orfao et al

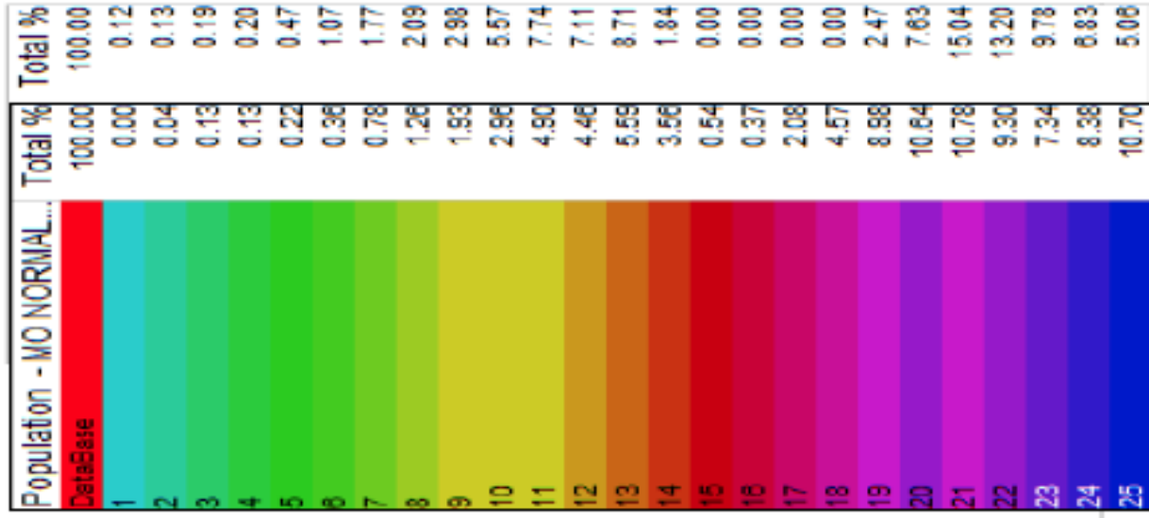
Normal BM

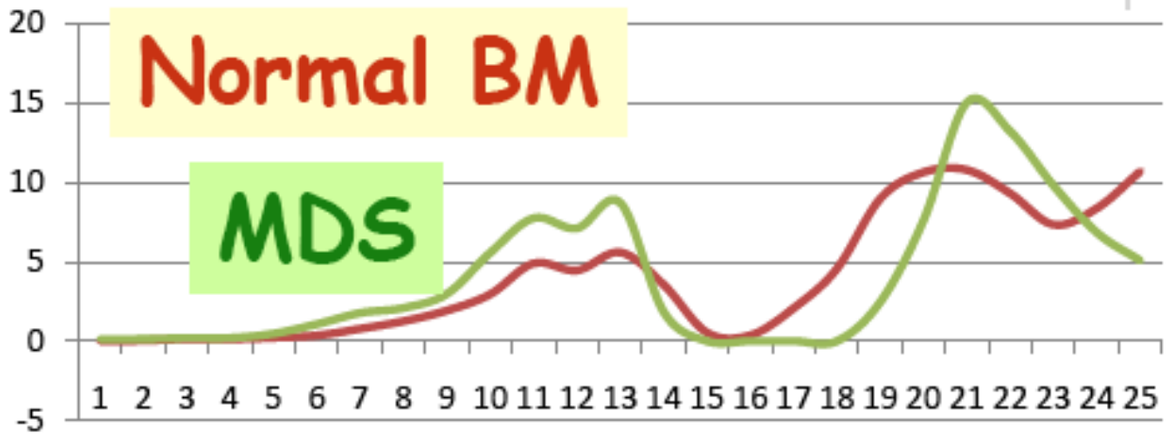
MDS

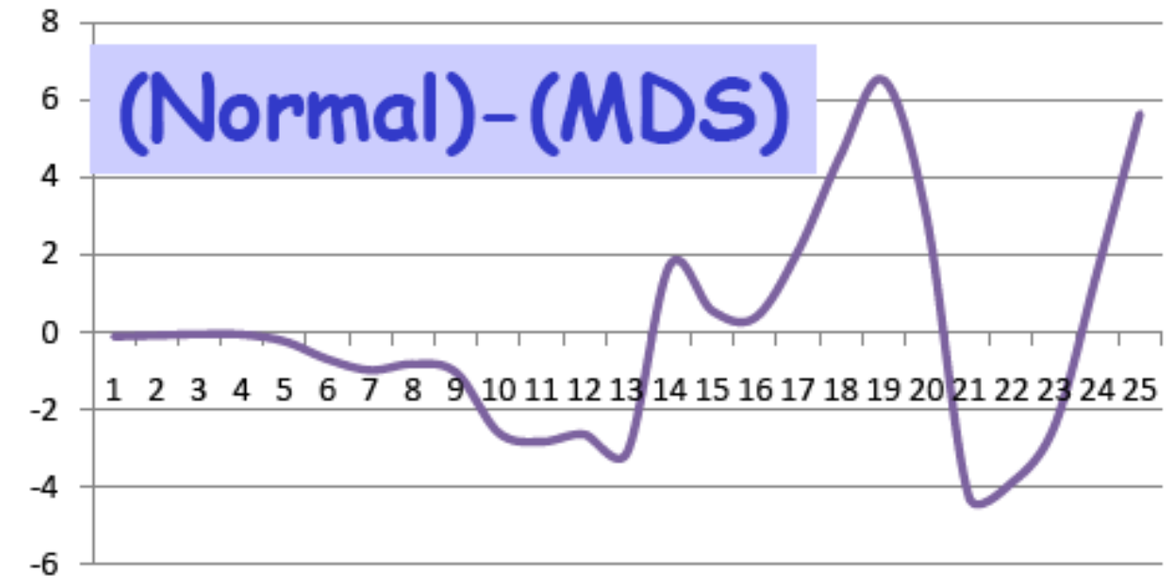












Orfao et al

Outils informatiques performants…

Multiples stratégies
Identification des précurseurs myéloïdes

- $CD45^{dim}\ SSC^{low/int}$
- $CD45^{dim}\ SSC^{low/int}$ CD34+
- $CD45^{dim}\ SSC^{low/int}$ HLA-DR+ CD11b-
- $CD45^{dim}\ SSC^{low/int}$ HLA-DR+ CD117+

! Expressions aberrantes
Importance de combinaisons redondantes !

Loken et al, Leuk Res 2008 Jan;32(1):5-17

↘ SSC des neutrophiles

↗ % CD34+

Niveau d'expression anormal de marqueurs

Que se passe-t-il dans un SMD ?

Expression d'un marqueur aberrant

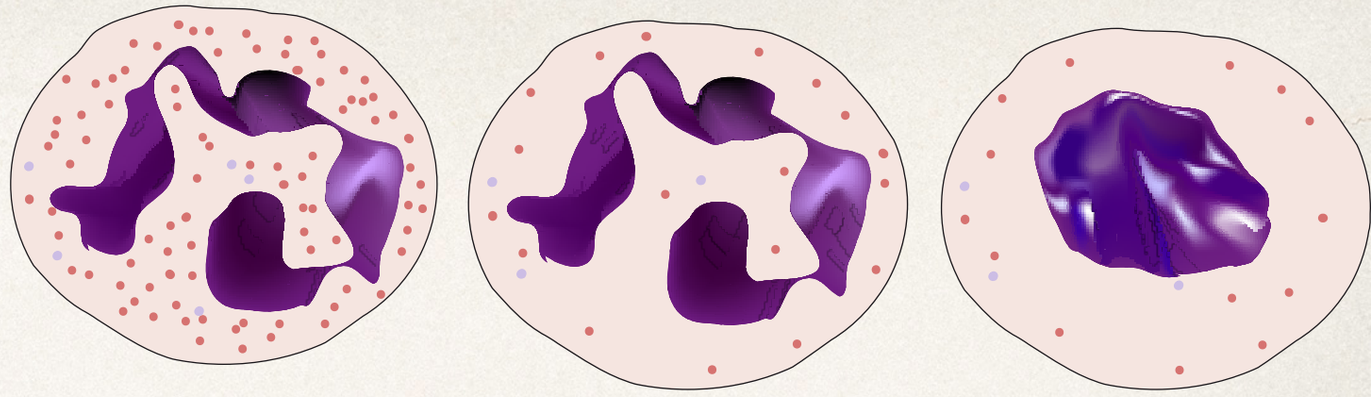
↘ Progéniteurs B (parmi les CD34+)

Expression asynchrone de marqueurs de maturation

...

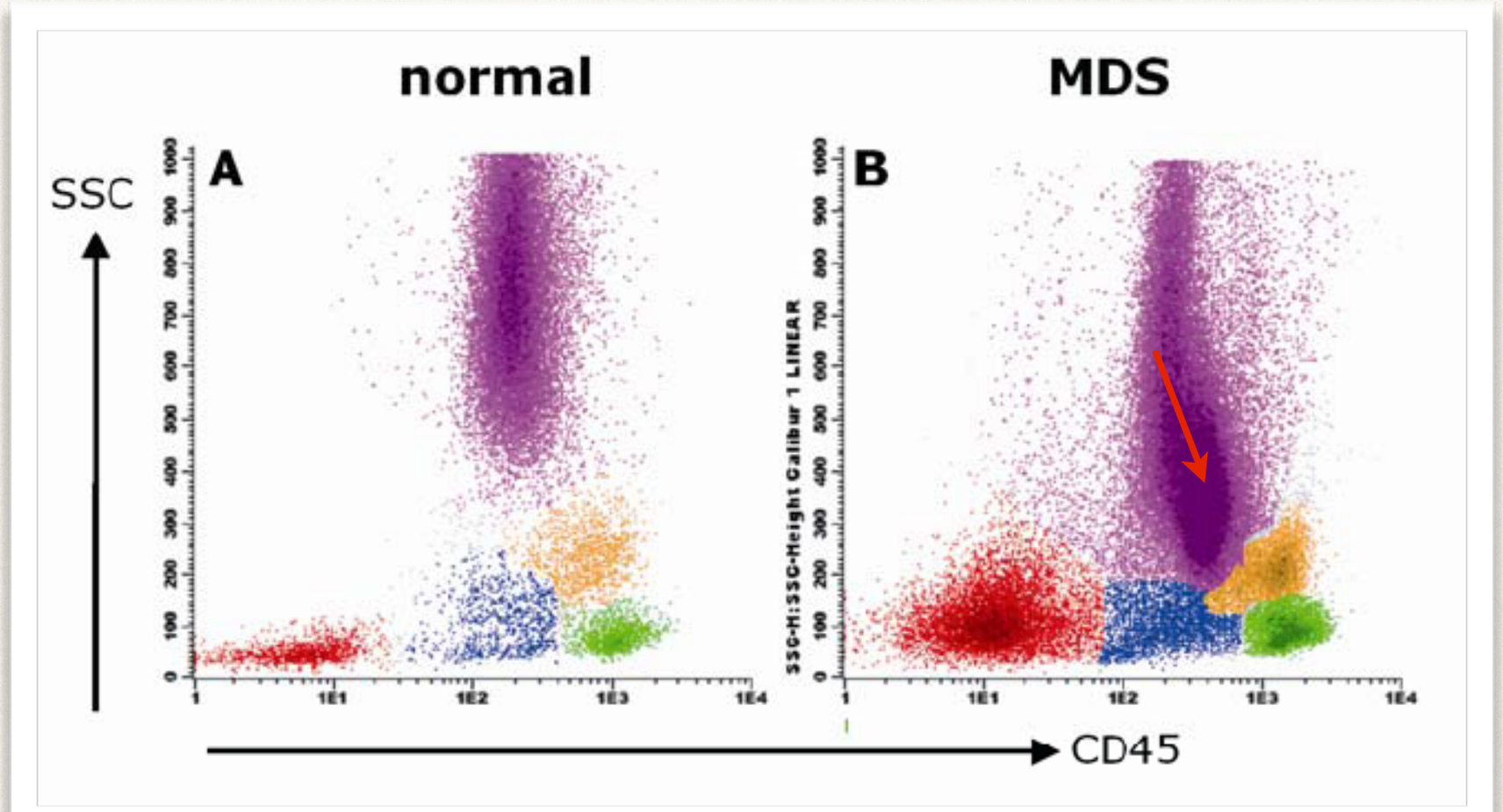
Pseudo Pelger-Huët

SSC diminué



- SSC diminué dû à l'hypogranularité
- Possible manque de CD10 sur les neutrophiles matures
- **Le plus fréquent** : relations aberrantes entre CD13/CD11b et/ou CD13/CD16.
- Autres marqueurs :
  - Expression altérée de CD45 ou CD33 (! polymorphismes !)
  - Expression asynchrone de CD34
  - Expression de CD56, ...

SSC abaissé (granulocytes)



Standardization of flow cytometry in myelodysplastic syndromes : a report from an international consortium and the European leukemianet working group, TM Westers et al, Leukemia 2012

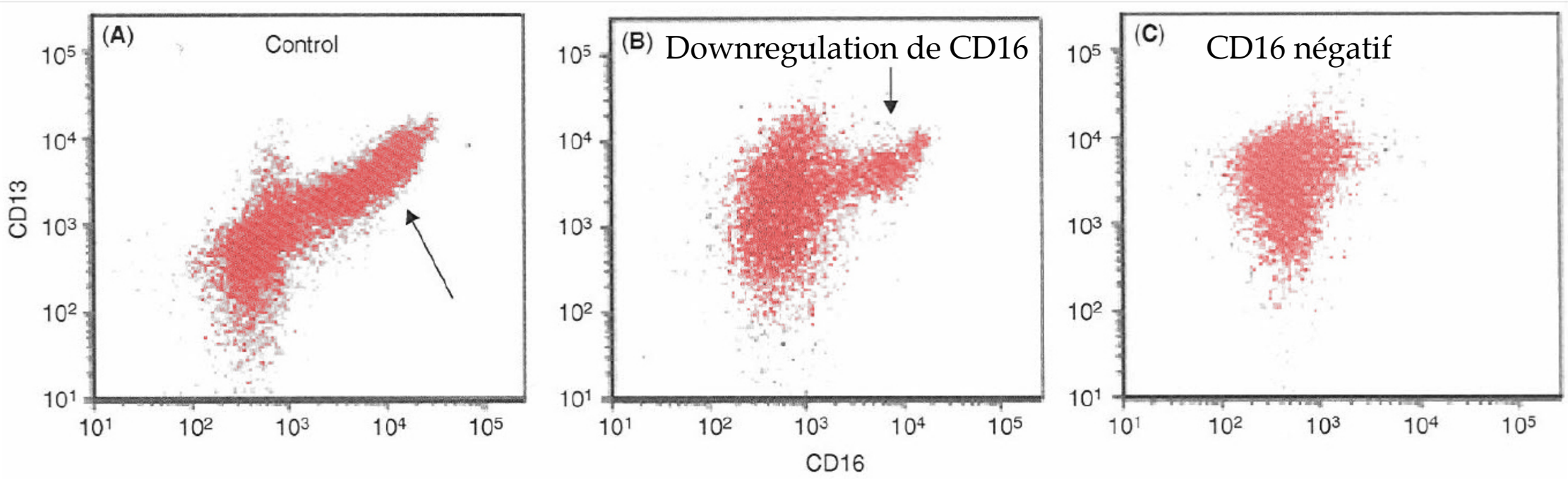


Figure 6.7 Flow cytometric feature of myelodysplastic syndrome—aberrant expression of CD16. (A) Normal (control) sample with variable expression of CD16 [mature elements are positive (arrow)]. Panel B shows prominent downregulation of CD16. Only minute subset of granulocytes is positive (arrow). Panel C shows negative CD16 in all granulocytes.

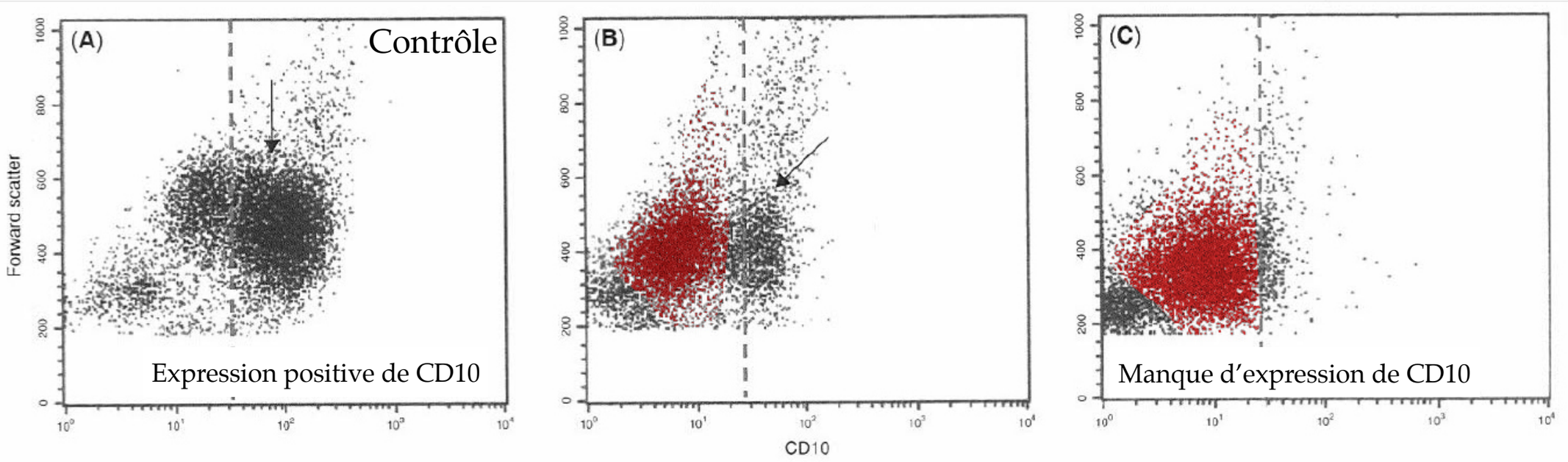


Figure 6.4 Flow cytometric feature of myelodysplastic syndrome (MDS)—aberrant expression of CD10 by granulocytes. (**A**) Normal (control) sample with positive CD10 expression (arrow). (**B**) MDS with partial loss of CD10 expression; only minute subset of granulocytes (arrow) is $CD10^+$. (**C**) MDS with complete lack of CD10 expression by granulocytes.

Flow Cytometry in Neoplastic Haematology, Gorczyca 2010, second edition

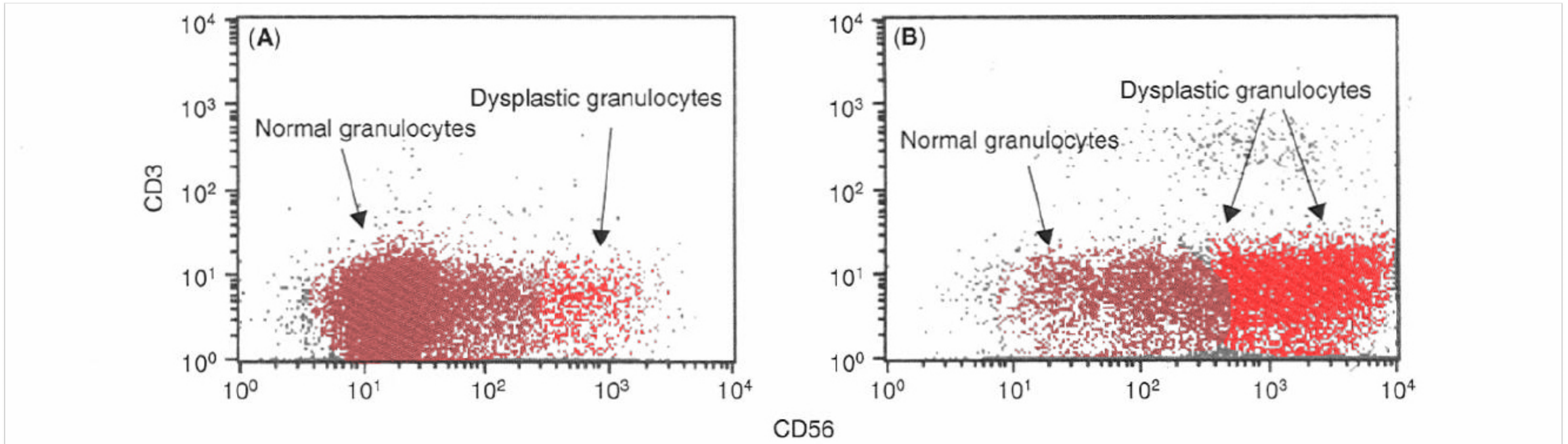
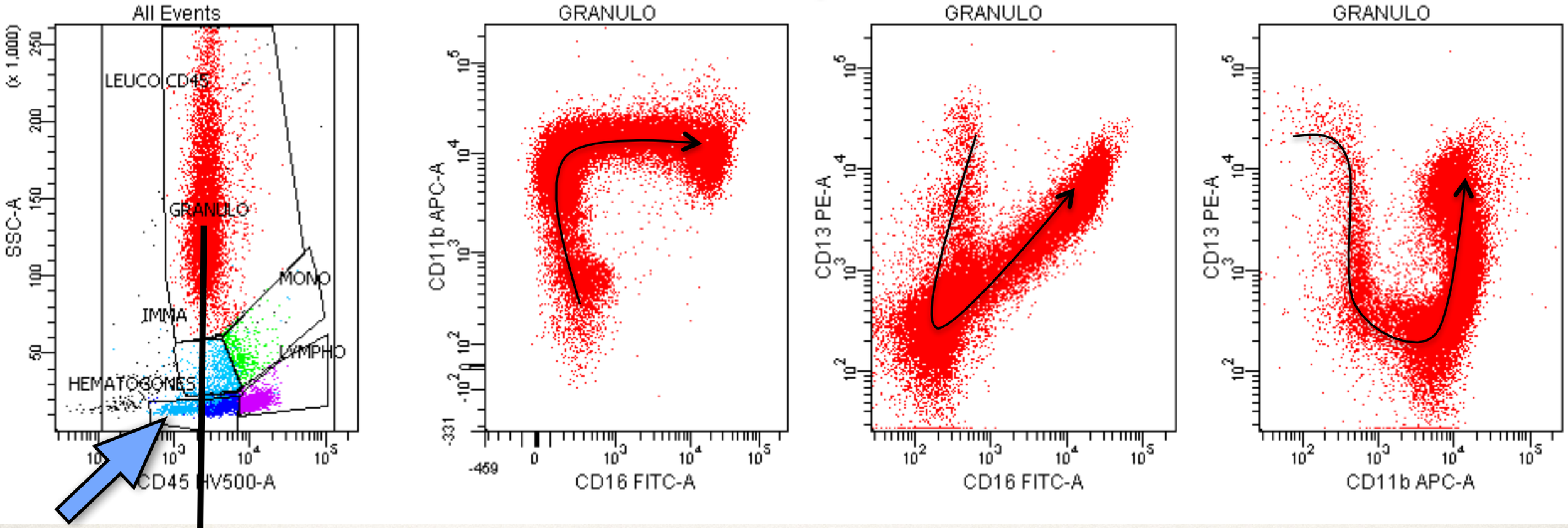


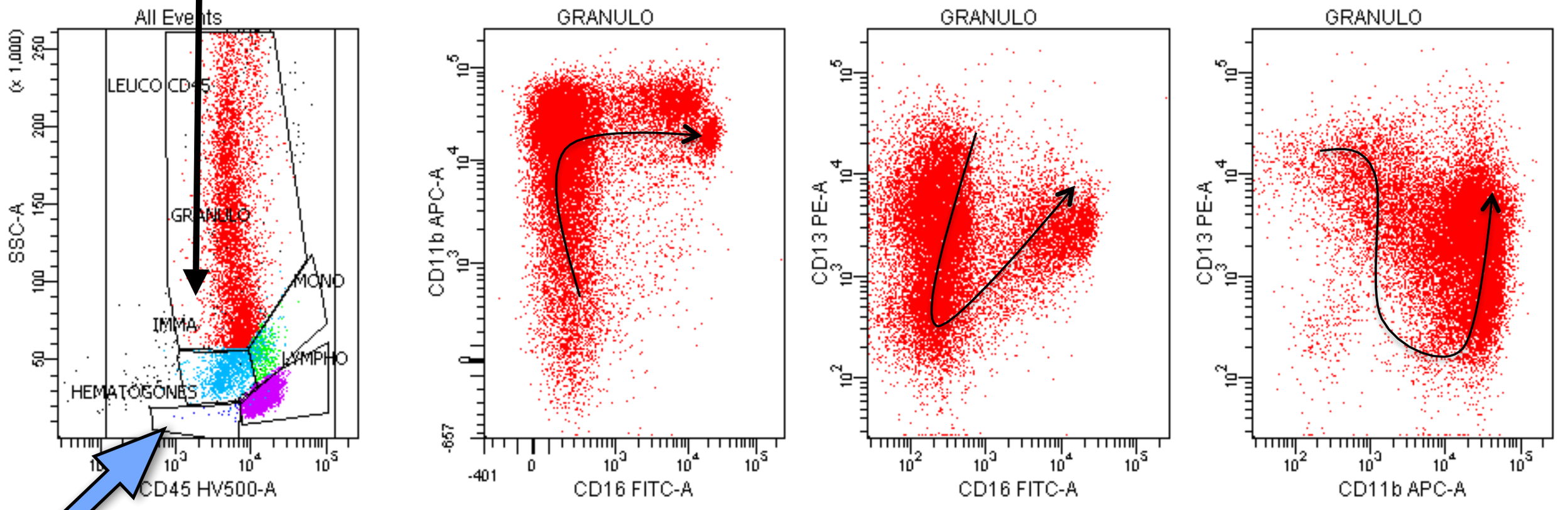
Figure 6.8 Flow cytometric feature of myelodysplastic syndrome—aberrant expression of CD56 by granulocytes (**A**, mild upregulation; **B**, marked upregulation).

Flow Cytometry in Neoplastic Haematology, Gorczyca 2010, second edition

Normal pattern



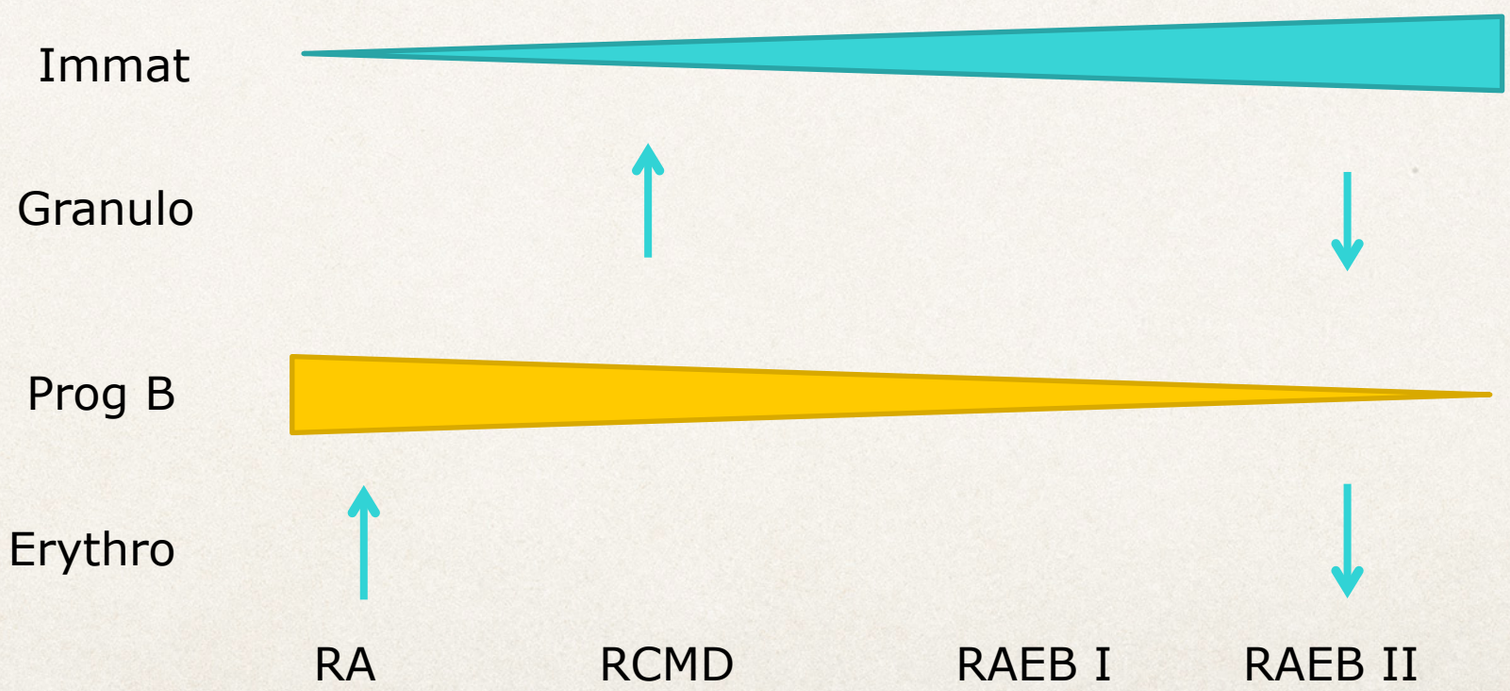
SMD : motifs anormaux de maturation



Diminution des progéniteurs lymphoïdes B (hematogones)

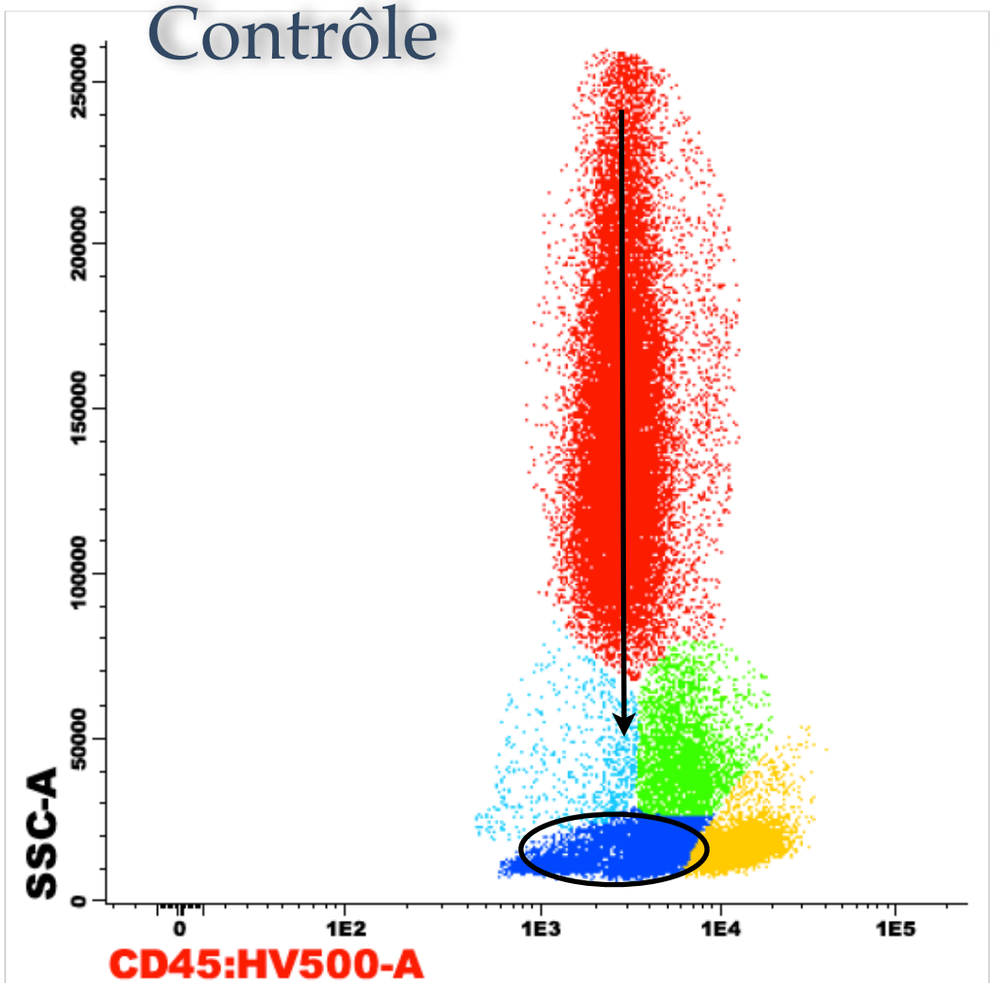
Matarraz et al, Leukemia, 2008

- CD34/TdT/CD10 et habituellement CD19 et CD22.
- Particulièrement informatif dans les SMD de bas grade
- $CD45^{dim/low}SCC^{low}$ et CD34+/CD19+ ou **CD34+/CD10+** (*Ogata et al*) --> ProgB/CD34tot
- Fraction des CD34+ :

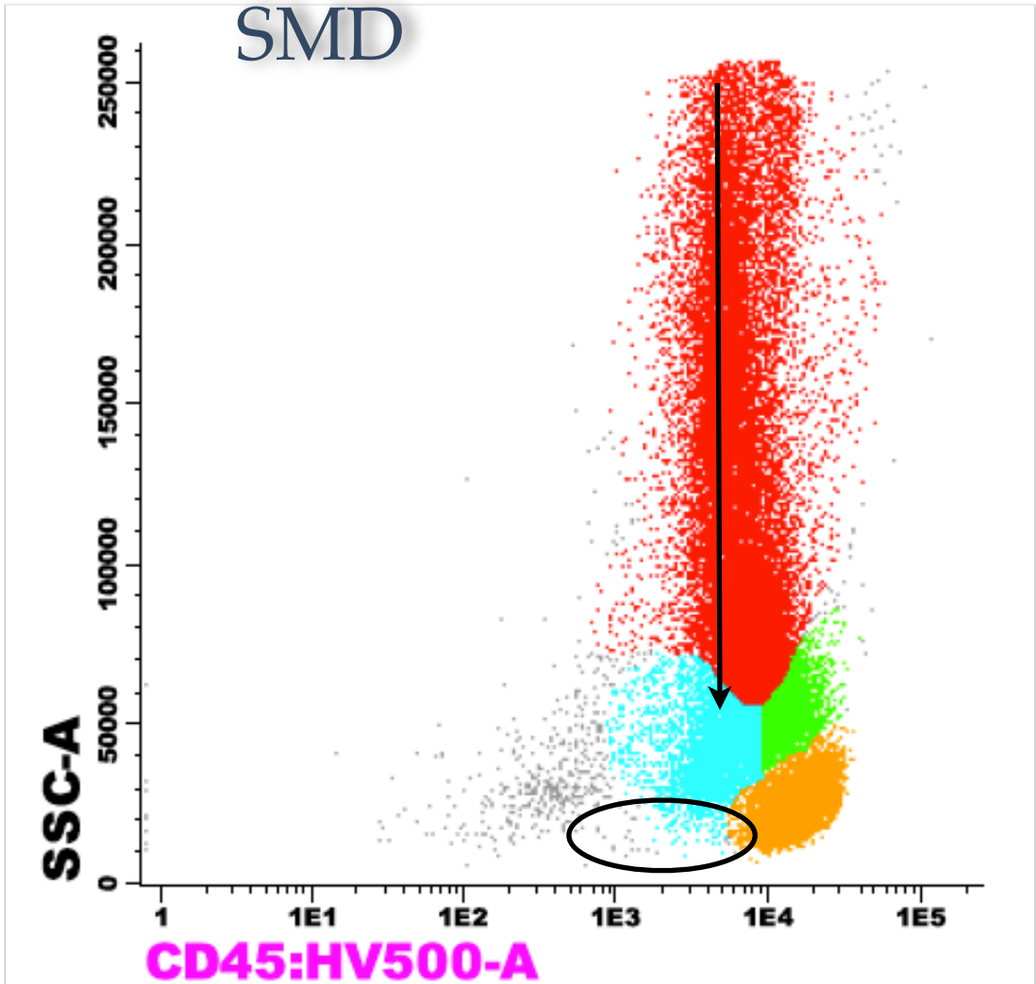


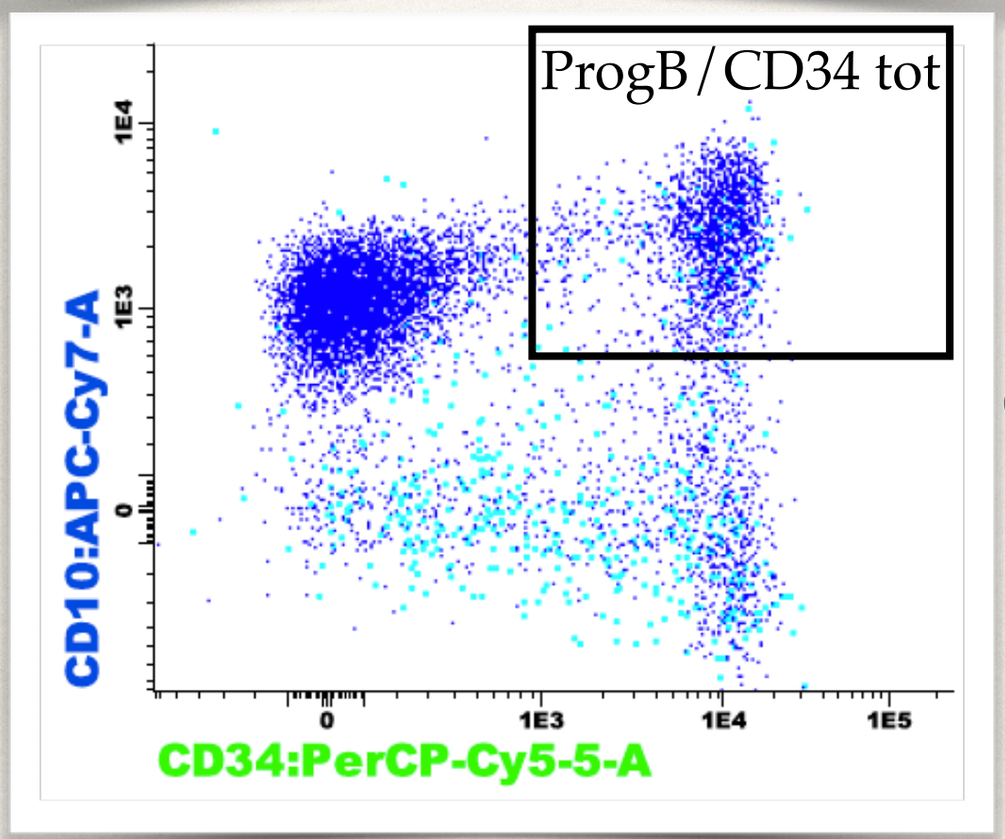
! Diminution non spécifique des SMD : age, LMC, immunodéficiences, ...

Contrôle

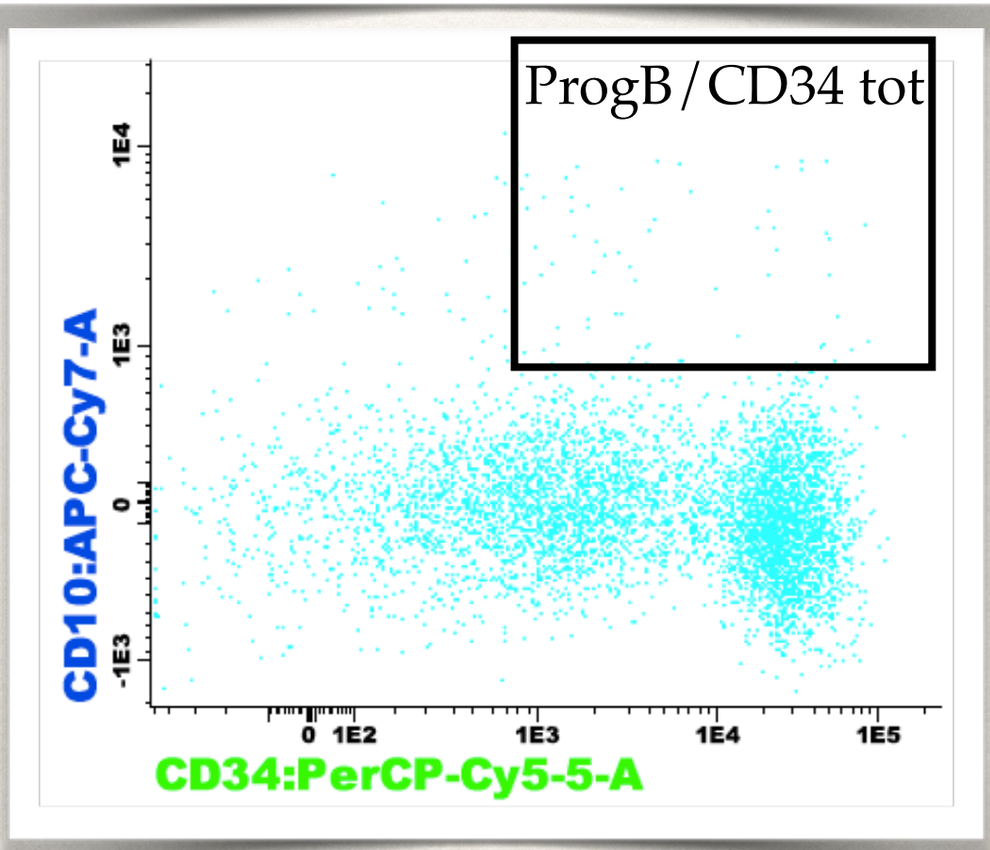


SMD





Gate sur CD34+



Que peut-on faire au laboratoire ?

- Le diagnostic des SMD n'est pas si évident lorsque les patients manquent de marqueurs spécifiques (excès de blastes, caryotype, sidéroblastes en couronne).

- C'est parfois un challenge pour les SMD de bas grade qui ne possèdent pas ces spécificités.

- Monitorer le traitement et la progression de la pathologie.

- Pattern recognition analysis --> déviation de l'expression normale des antigènes… Bon outil pour les « opérateurs experts »… Mais reproductibilité ? Besoin de paramètres hautement reproductibles !

- Etude des caractéristiques cytométriques —> scores (pour discriminer les SMD de bas grade des cytopénies non-clonales.

FCSS (*Flow Cytometry Scoring System*)

2012

Standardisation

Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European leukemianet working group

TM Westers, R Ireland, W Kern, C Alhan, JS Balleisen, P Bettelheim, K Burbury, M Cullen, JA Cutler, MG Della Porta , AM Dräger, J Feuillard, P Font, U Germing, D Haase, U Johansson, S Kordasti, MR Loken, L Malcovati, JG te Marvelde, S Matarraz, T Milne, B Moshaver, G J Mufti, K Ogata, A Orfao, A Porwit, K Psarra, SJ Richards, D Subirá, V Tindell, T Vallespi, P Valent, VHJ van der Velden, TM de Witte, DA Wells, F Zettl, MC Béné and AA van de Loosdrecht

Score

Multicentre validation of a reproducible flow cytometric score for the diagnosis of low-risk myelodysplastic syndromes: results of a European LeukemiaNET study

by Matteo G. Della Porta, Cristina Picone, Cristiana Pascutto, Luca Malcovati, Hideto Tamura, Hiroshi Handa, Magdalena Czader, Sylvie Freeman, Paresh Vyas, Anna Porwit, Leonie Saft, Theresia M. Westers, Canan Alhan, Caludia Cali, Arjan A. Van de Loosdrecht, and Kiyoyuki Ogata

Haematologica 2012 [Epub ahead of print] *étude multi-centrique*

Standardisation ?

- CMF est de plus en plus reconnue pour jouer un rôle important dans diagnostic et le pronostic des SMD.

- European LeukemiaNet (ELN) MDS workshops.

- **<u>4 paramètres hautement reproductibles :</u>**

  - Pourcentage augmenté des progéniteurs CD34+ (cellules nucléées)
  - Nombre diminué de progéniteurs B parmi les CD34+
  - Diminution ou augmentation de l'expression du CD45 sur les cellules myéloïdes progénitrices
  - Dimution du SSC des neutrophiles

Scoring system

Multicentre validation of a reproducible flow cytometric score for the diagnosis of low-risk myelodysplastic syndromes: results of a European LeukemiaNET study

by Matteo G. Della Porta, Cristina Picone, Cristiana Pascutto, Luca Malcovati, Hideto Tamura, Hiroshi Handa, Magdalena Czader, Sylvie Freeman, Paresh Vyas, Anna Porwit, Leonie Saft, Theresia M. Westers, Canan Alhan, Caludia Cali, Arjan A. Van de Loosdrecht, and Kiyoyuki Ogata

Haematologica 2012 [Epub ahead of print]

797 patients

- Devrait être reproductible entre les différents opérateurs
- Les résultats doivent être facilement compris par les cliniciens

Score SMD	Valeurs cut-off (normalité)
Myeloblast-related cluster size (%)*	< 2 %
B-progenitor-related cluster size (%)**	> 5 %
Lymphocytes to myeloblasts CD45 ratio	4 - 7,5
Granulocytes to lymphocytes SSC ratio	> 6

* in all nucleated cells
** in all CD34+

Diagnostic des SMD bas grade

Multicentre validation of a reproducible flow cytometric score for the diagnosis of low-risk myelodysplastic syndromes: results of a European LeukemiaNET study

by Matteo G. Della Porta, Cristina Picone, Cristiana Pascutto, Luca Malcovati, Hideto Tamura, Hiroshi Handa, Magdalena Czader, Sylvie Freeman, Paresh Vyas, Anna Porwit, Leonie Saft, Theresia M. Westers, Canan Alhan, Caludia Cali, Arjan A. Van de Loosdrecht, and Kiyoyuki Ogata

Haematologica 2012 [Epub ahead of print]

Positifs n=281

Negatifs n=257

Learning cohort	**FCM-score**							
	0	*1*	*2*	*3*	*4*	*positive cases*	*sensitivity (%)*	*specificity (%)*
Low-risk MDS	33	50	107	67	24	198/281	70%	
*Specific markers of dysplasia**								
MDS with specific markers	17	26	58	49	16	123/166	74%	
MDS without specific markers	16	24	49	18	8	75/115	65%	
WHO category								
RCUD	8	12	26	6	3	35/55	64%	
RARS	7	5	13	5	0	18/30	60%	
RCMD/RS	15	31	61	51	20	132/178	74%	
MDS with del5q	1	1	6	5	1	12/14	86%	
MDS-U	2	1	1	0	0	1/4	-	
Controls	132	107	16	2	0	18/257		93%
Idiopathic or iatrogenic hypoplasia**	6	7	7	0	0	7/20		35%
Anemia***	42	57	5	0	0	5/104		95%
Cytopenia associated with BM infiltration	6	5	1	2	0	2/14		86%
Cytopenia in transplant recipients	0	3	0	0	0	0/3		-
Infective cytopenia	2	4	0	0	0	0/6		-
Immune cytopenia****	76	31	3	0	0	3/110		97%

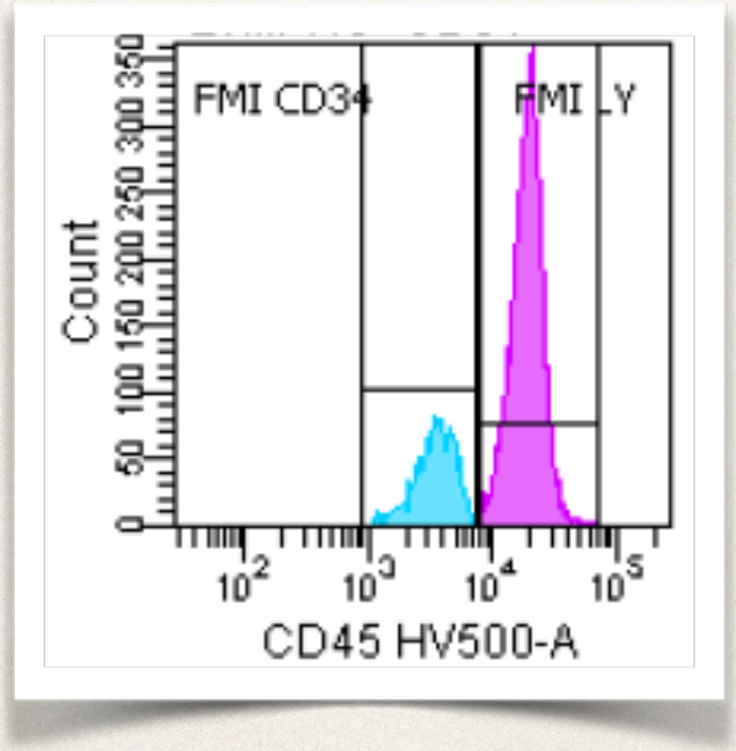
% **Myeloblasts** < 2 %

FSC/SSC --> SSC^{low}
CD45/CD34 --> CD34+
CD45/SSC --> Myelobl.

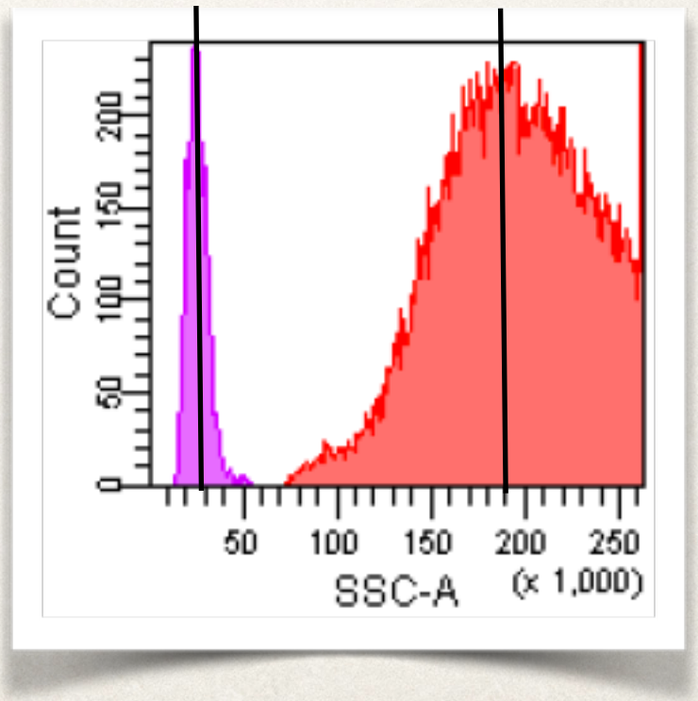
% **ProgB/CD34$_{tot}$** > 5 %

FSC/SSC --> SSC^{low}
CD45/CD34 --> CD34+
CD45/SSC --> ProgB

+1 si hors range



Score positif ≥ 2



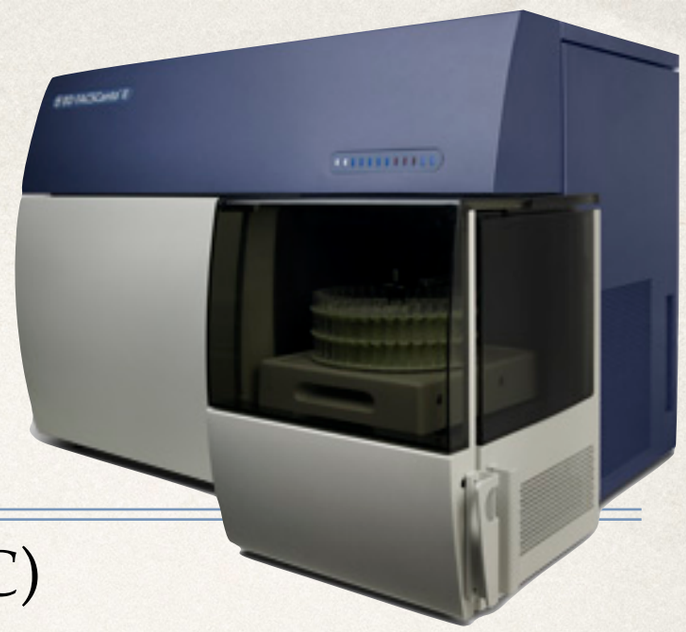
Mean CD45 FMI Ly/FMI CD34 4 - 7,5

SSC Granulocytes/Lympho > 6

Arlon

Les Cliniques du Sud Luxembourg

Notre expérience

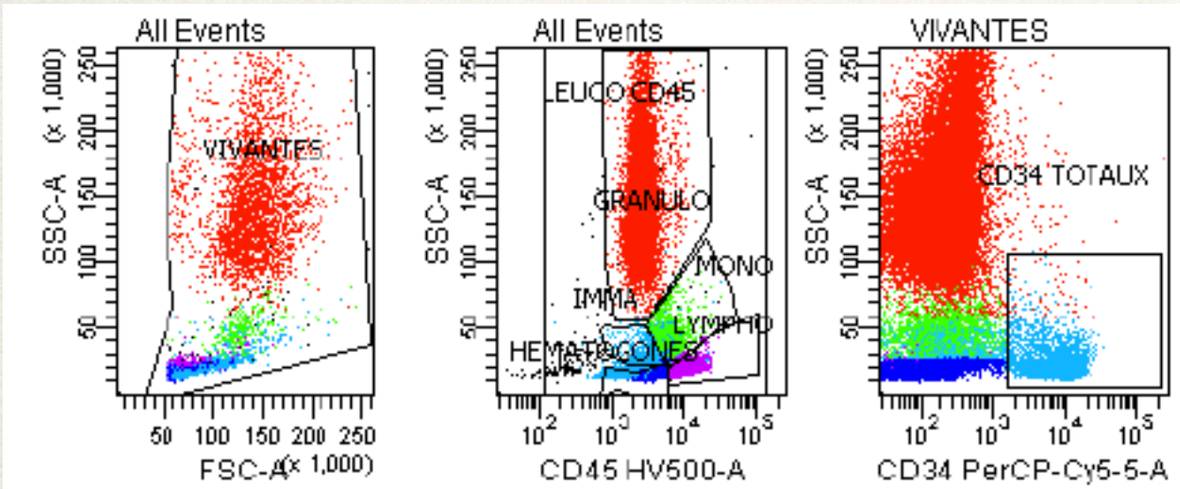


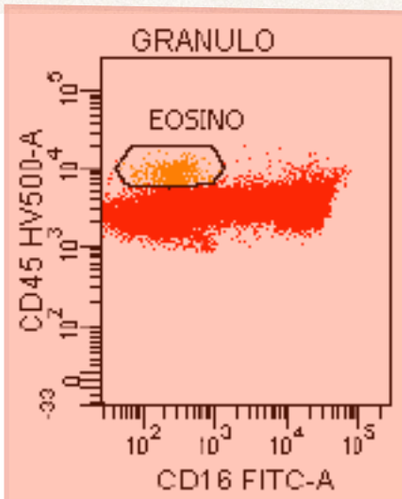
- **433 patients** --> Analyse médullaire (Aspiration, Biopsie, FC)
- 8 couleurs... AML-1 tube EuroFlow :
  - **HLA-DR / CD45 / CD16 / CD13 / CD34 / CD117 / CD11b / CD10**
- Scoring System :

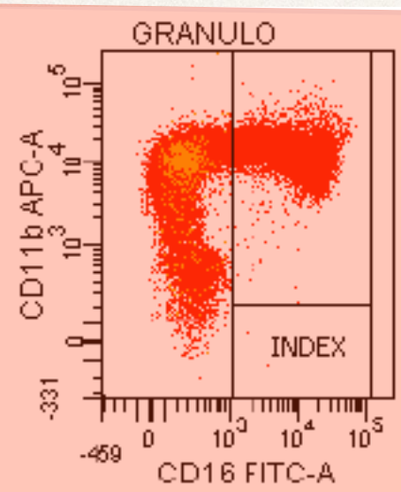
Score SMD	Valeurs cut-off (normal)
% Myeloblasts	< 2 %
% ProgB/CD34$_{tot}$	> 5 %
Mean CD45 FMI Ly/FMI CD34	4 - 7,5
SSC Mode CD10-/LYMPHO	> 6

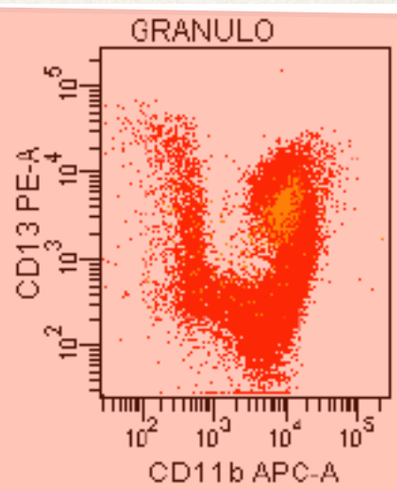
Multicentre validation of a reproducible flow cytometric score for the diagnosis of low-risk myelodysplastic syndromes : results of a European LeukemiaNET study, **Haematologica 2012**

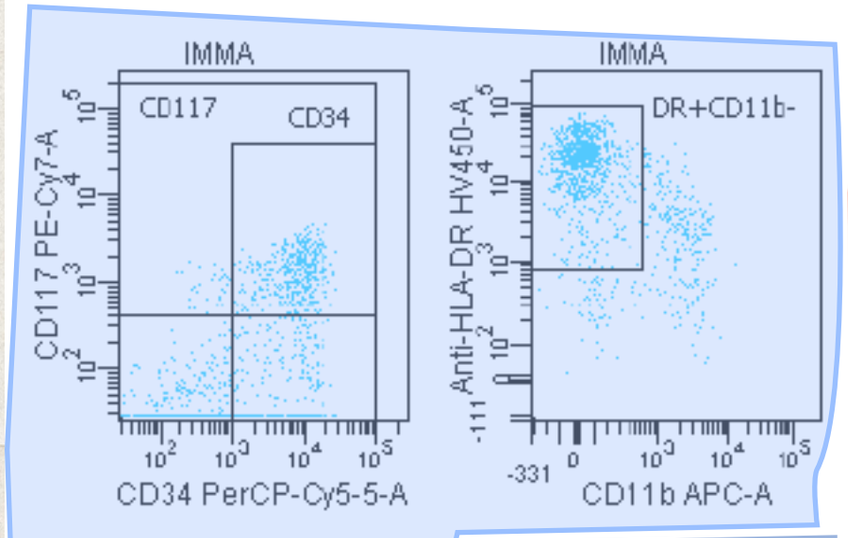
Notre pattern - tube AML1

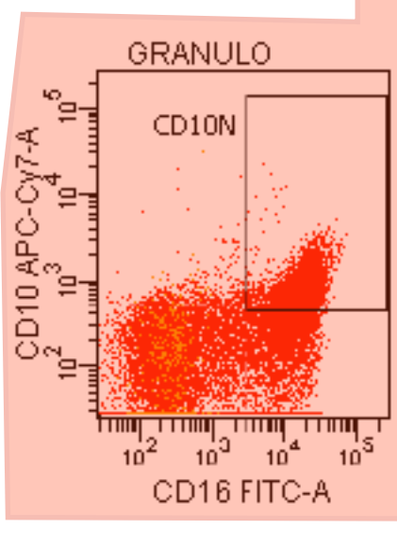


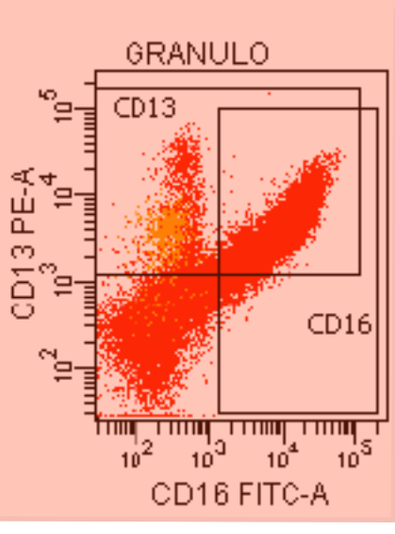


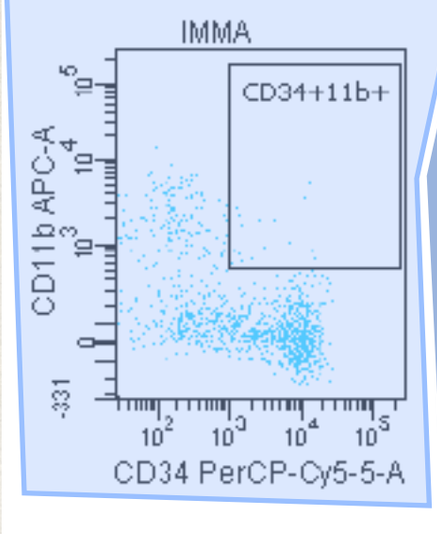


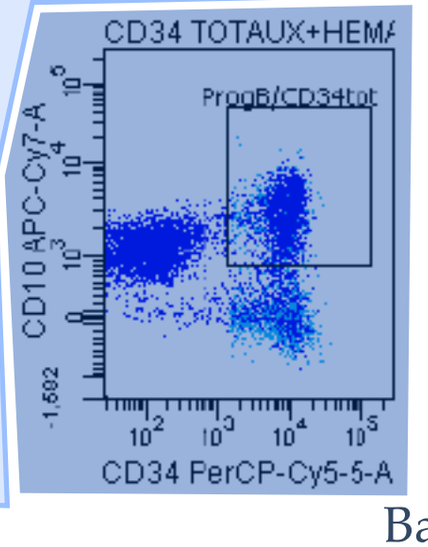


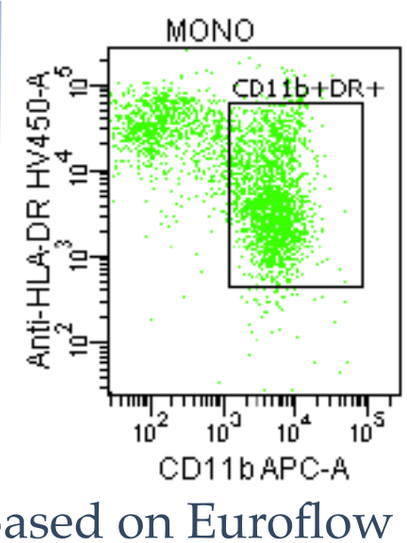


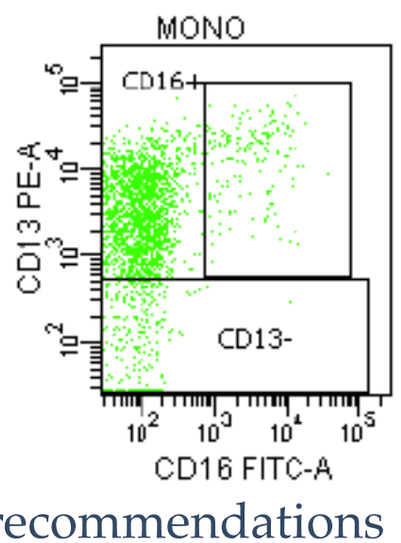












Specimen Name: M3213 1481 AML1

Population	#Events	%Parent	%Grand ...
VIVANTES	49,878	99.8	####
GRANULO	27,898	55.9	55.8
EOSINO	485	1.7	1.0
INDEX	10,962	40.0	39.3
CD10N	3,887	13.9	7.8
MONO	3,510	7.0	7.0
CD11b+DR+	2,258	64.3	4.5
CD16+	179	5.1	0.4
CD13-	569	16.2	1.1
LYMPHO	8,056	16.2	16.1
IMMA	1,109	2.2	2.2
CD117	410	37.0	0.8
CD34	631	56.9	1.3
CD16	19	1.7	0.0
CD13	465	41.9	0.9
DR+CD11b-	816	73.6	1.6
CD34+11b+	19	1.7	0.0
LEUCO CD45	49,787	99.8	99.6
HEMATOGONES	6,798	13.6	13.6
CD34 TOTAUX	3,202	6.4	6.4
ProgB/CD34tot	1,821	56.9	3.7
GRANULO CD10-	####	48.1	48.0

Based on Euroflow recommendations

Résultats patients (exclu : LMA de novo, …)

	Score = 4	Score = 3	Score = 2	Score = 1	Score = 0
n = 433	10	32	44	105	242

- **Score = 4 :**
  - AREB-I, AREB-II, AREB/LMA
- **Score = 3 :**
  - AREB, RCMD, LMMC, MDS del(7), AREB/LMA, …
- **Score = 2 :**
  - (AREB), RA, 5q-, aplasie toxique, moelle régénérative (en particulier post-LLA), déficience B12, SMP (peu hématog)
- **Score = 1 or 0 :**
  - Moelle normale ou régénérative, MGUS, Myelome, Vaquez, Hypoplasie, LLC, LNH, LMC, ARSI, MK, ...

Résultats patients

	Score = 4	Score = 3	Score = 2	Score = 1	Score = 0
n = 433	10	32	44	105	242
SMD	10	32	28	9	6
non-SMD	0	0	16	96	236

Sensibilité : 77%
Spécificité : 97%
VPP : 87%
VPN : 94 %

- Score of 4 ou 3 : spécificité de 100% ! chez nos patients.
- Score of 1 ou 0 : argument contre une SMD, mais pas exclu…
- Score of 2 : attention ! Suivre le patient.

Un exemple…

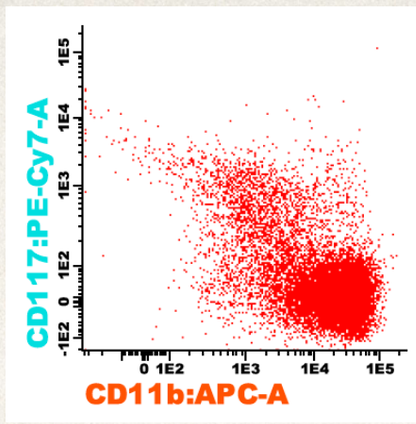
- Homme de 55 ans, pancytopenie.

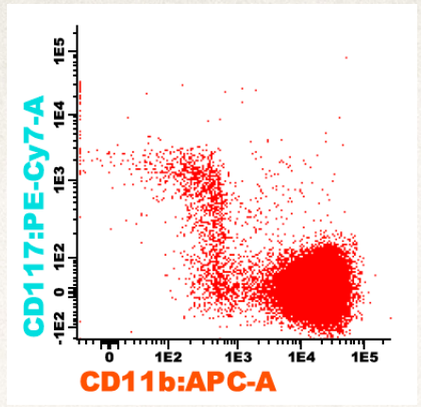
- **Hb** : 9,1 g/dL
- **GR** : 2,47 millions/µL
  - *MCV* : 105,7 fl
  - *Retic* : 20.300/µL (0,8%)
- **GB** : 3.640/µL
  - *Neutro* : 19,3%(700/µL)
  - *Blastes* : 0,8%

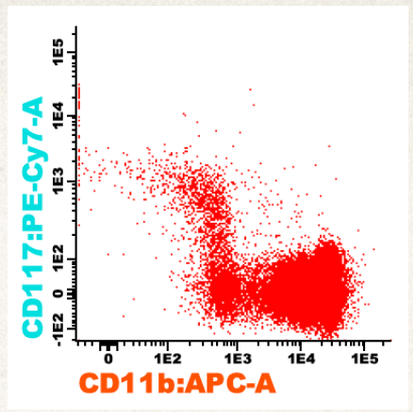
- Moelle : dysplasie multi-lignée, excès de blastes --> **AREB II**
- Cytogénétique : mauvais pronostic
  - clone 1 : 44XY, ADD(2)(P21), **-7**, DEL(12)(P11)
  - clone 2 : 44, SL, DER(5) T(2;5) (P12;Q24)

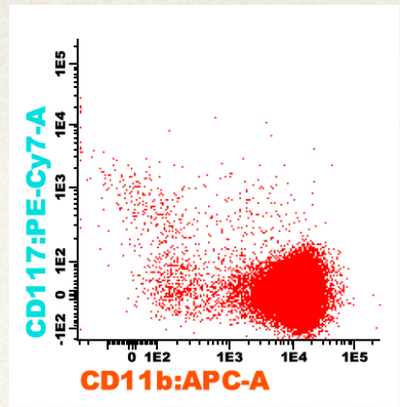
Cytométrie en flux ?

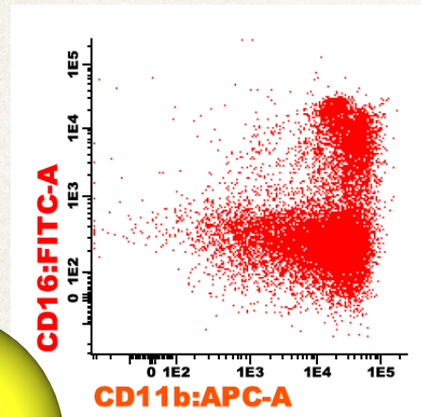
11/2012 02/2012 03/2012 04/2012

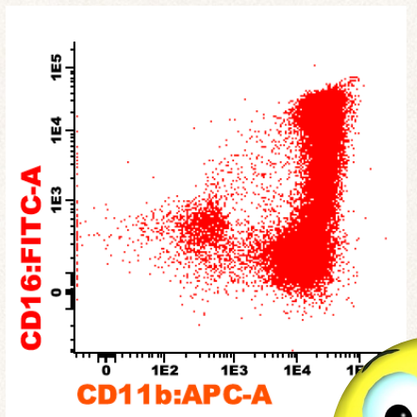


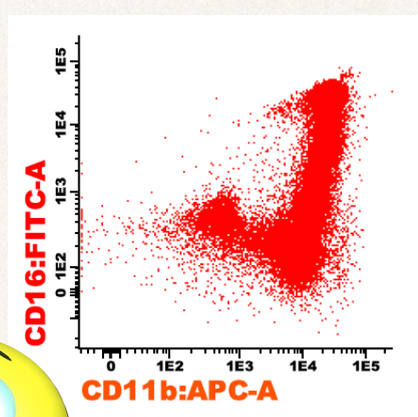


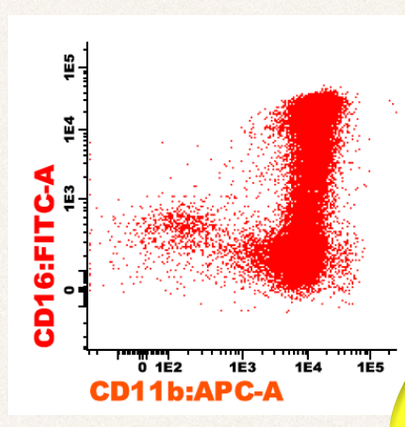


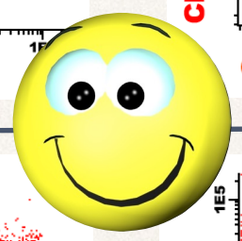


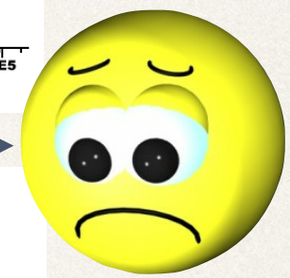


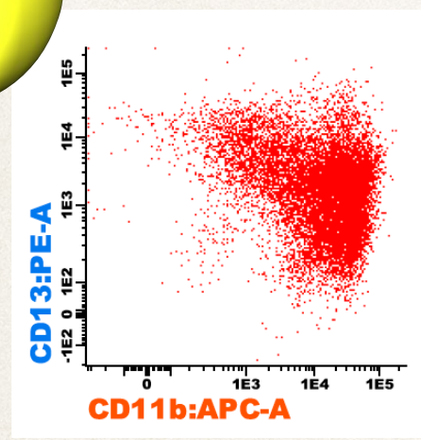


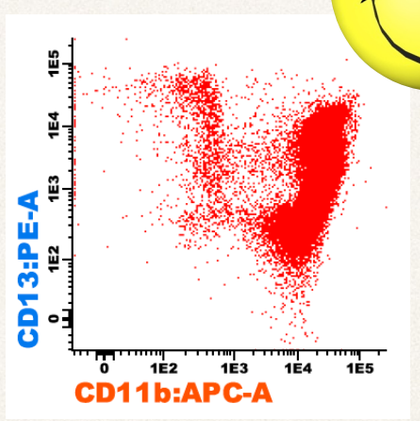


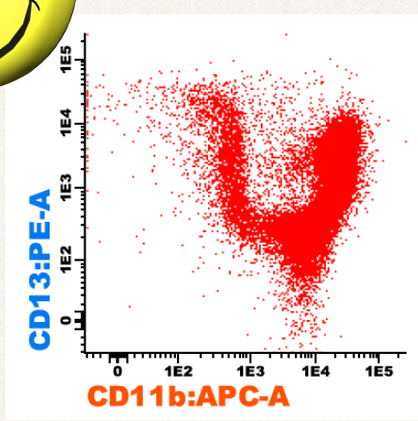


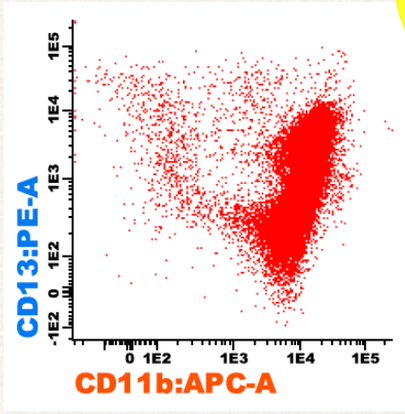


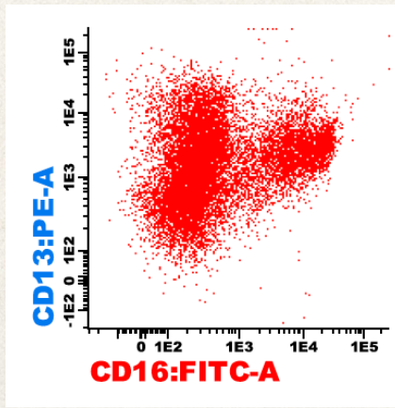


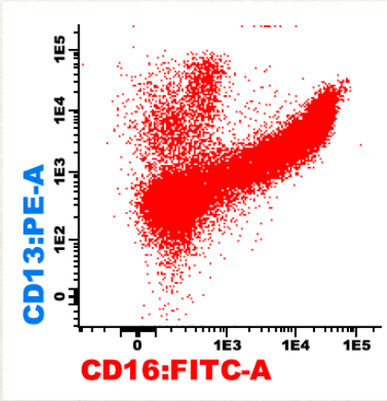


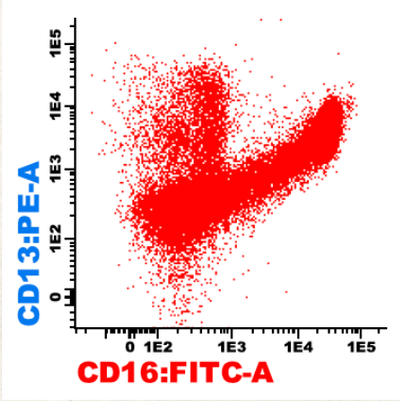


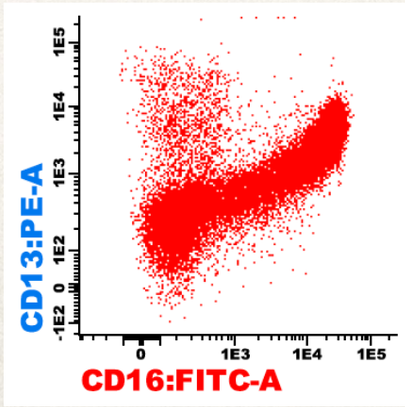












Traitement

FCSS	11/2011 * Score = 4	02/2012 Score = 0	03/2012 Score = 0	04/2012 Score = 2
% Myeloblasts (< 2%)	8	1,1	1,9	3,7
% ProgB/ $CD34_{tot}$ (> 5%)	3,5	10,5	7,5	5,3
Mean CD45 FMI Ly/FMI CD34 (4-7,5)	2,9	5,6	5	5,5
SSC Mode CD10-/ LYMPHO (> 6)	4	7	8	6

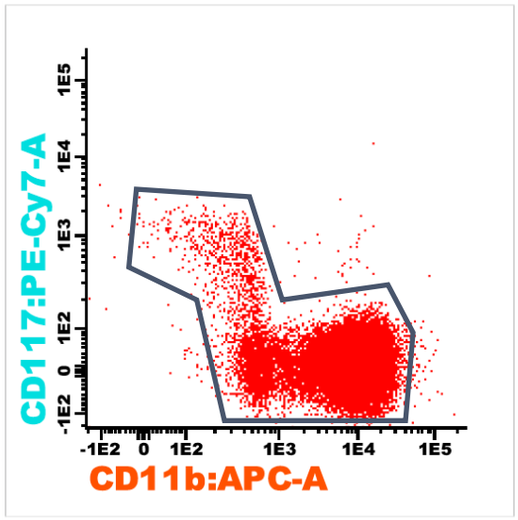
* Traitement

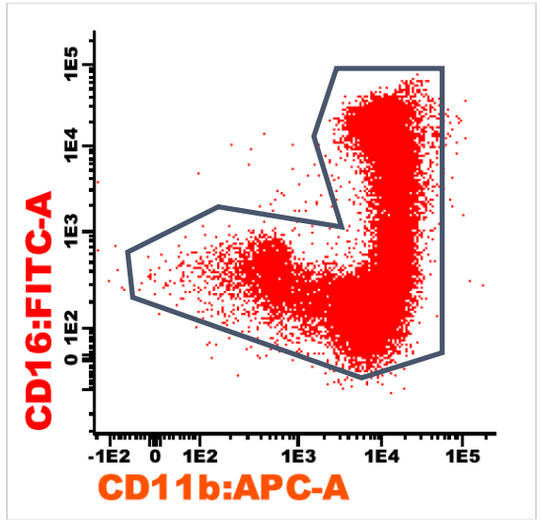
Que peut-on observer ?

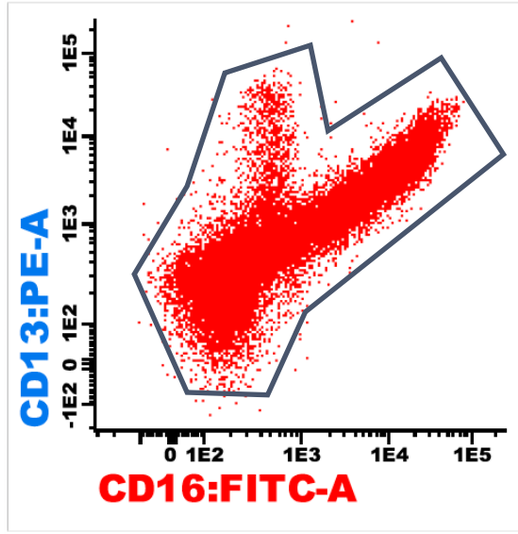
- ! Il s'agit d'un patient !
- Score = 4 au diagnostic --> traitement --> score = 0
- 5 mois après le diagnostic: rechute --> score = 2
- Le FCSS semble suivre l'évolution des patients ?

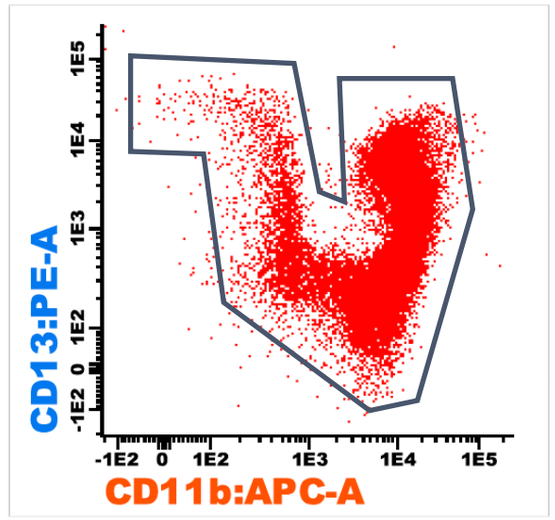
Et maintenant ?

Normal



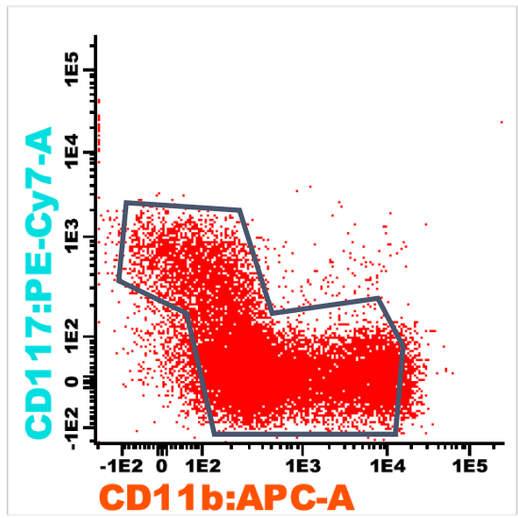


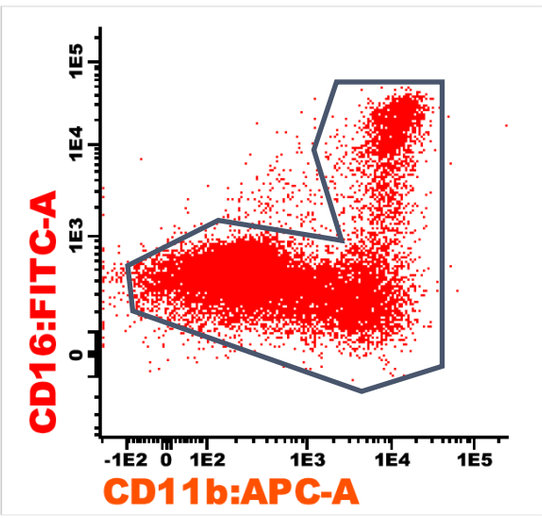


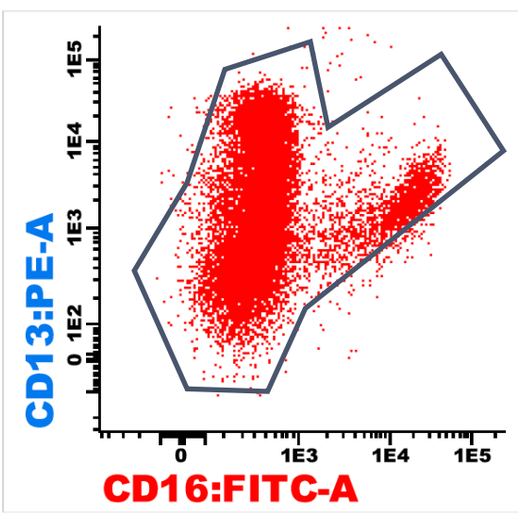


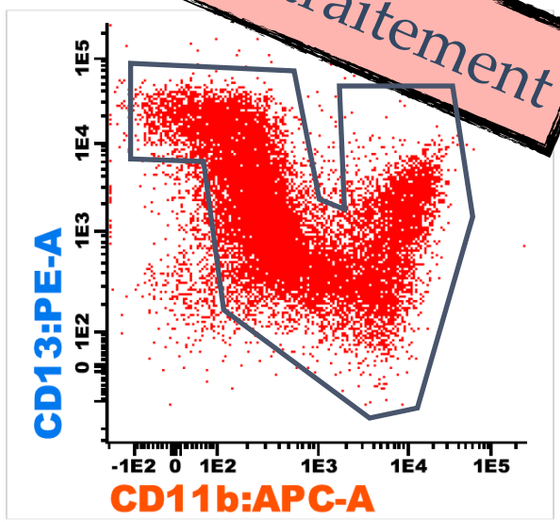
Blocage maturation

LLC post-traitement

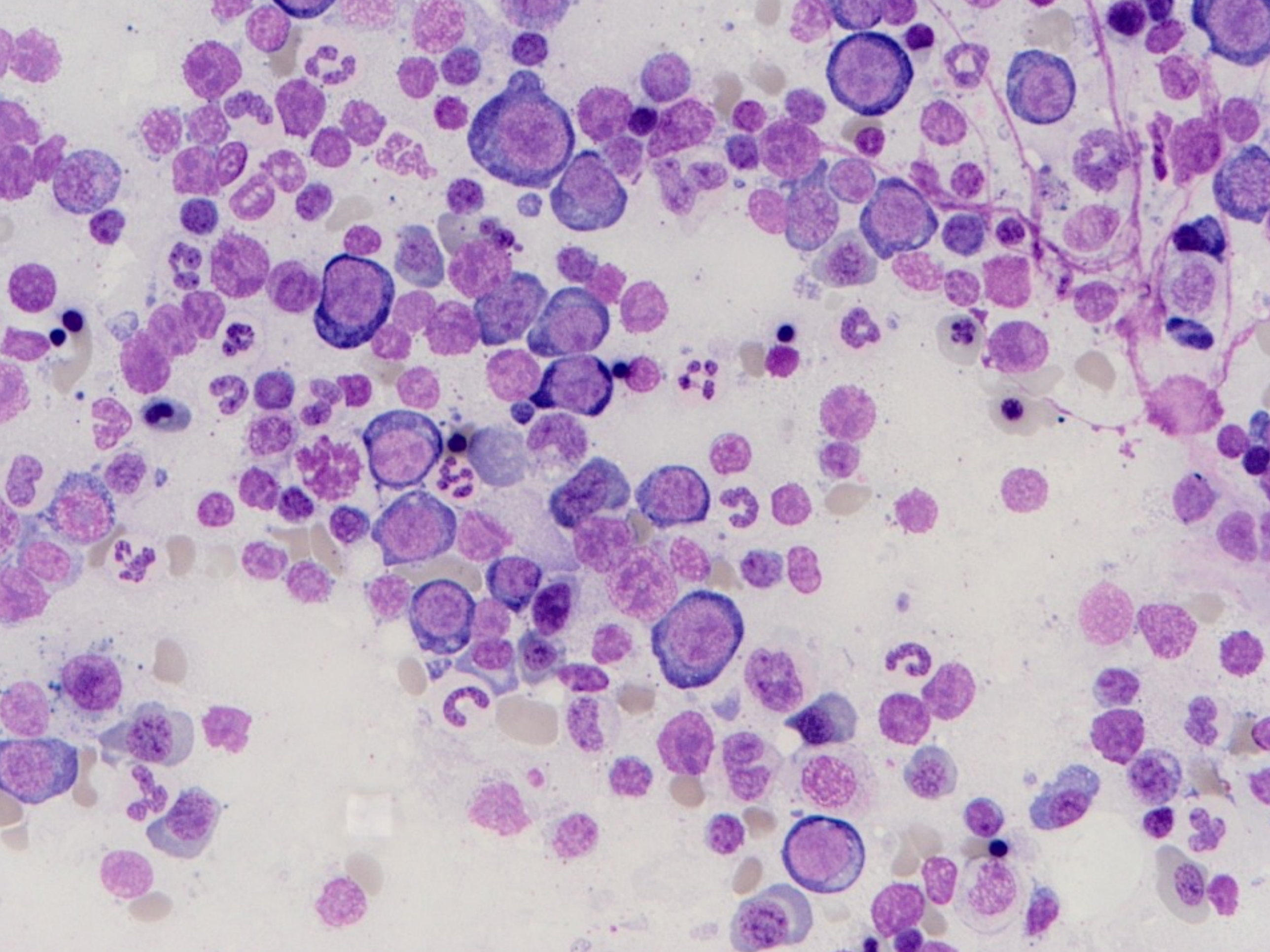


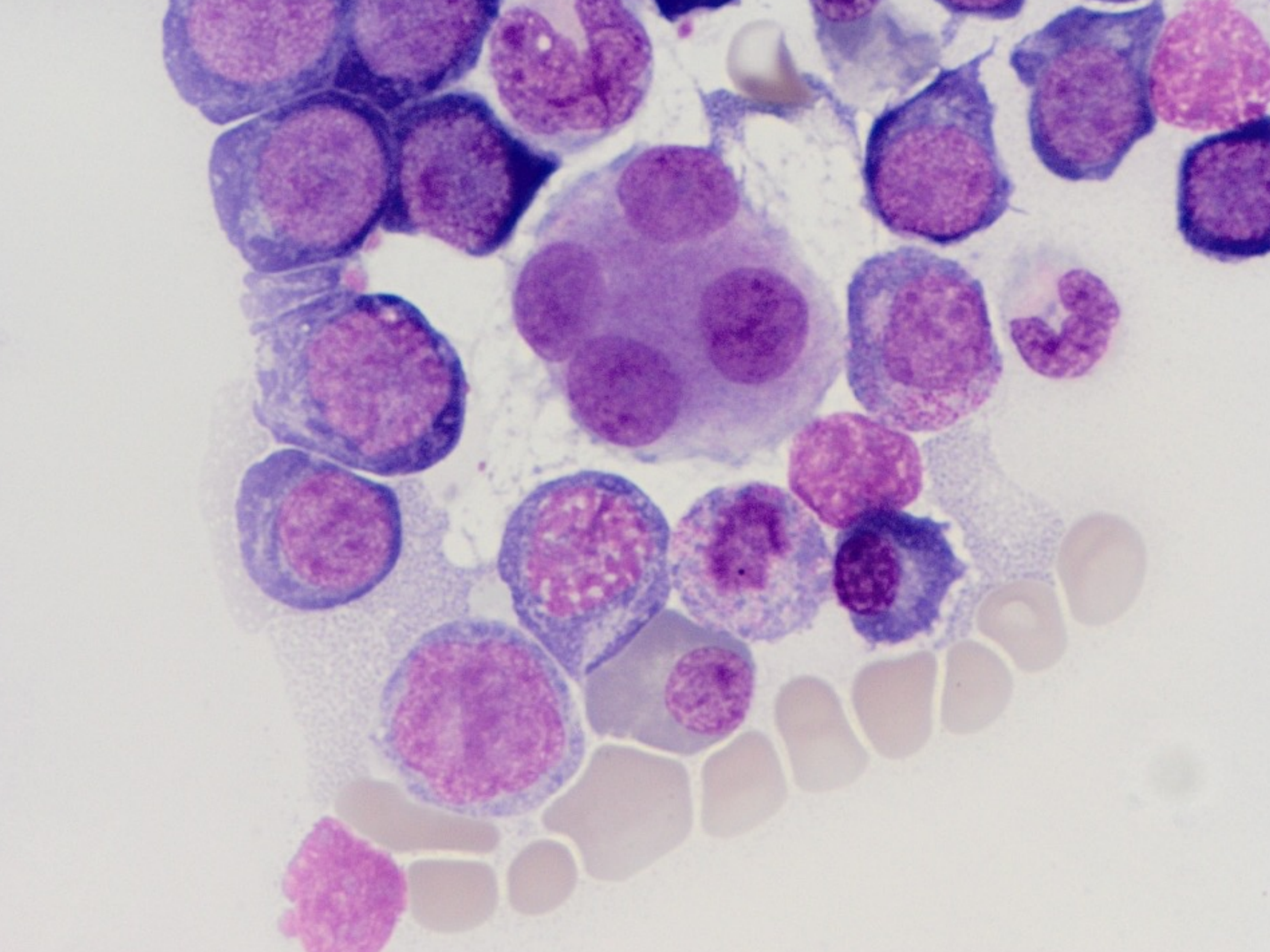


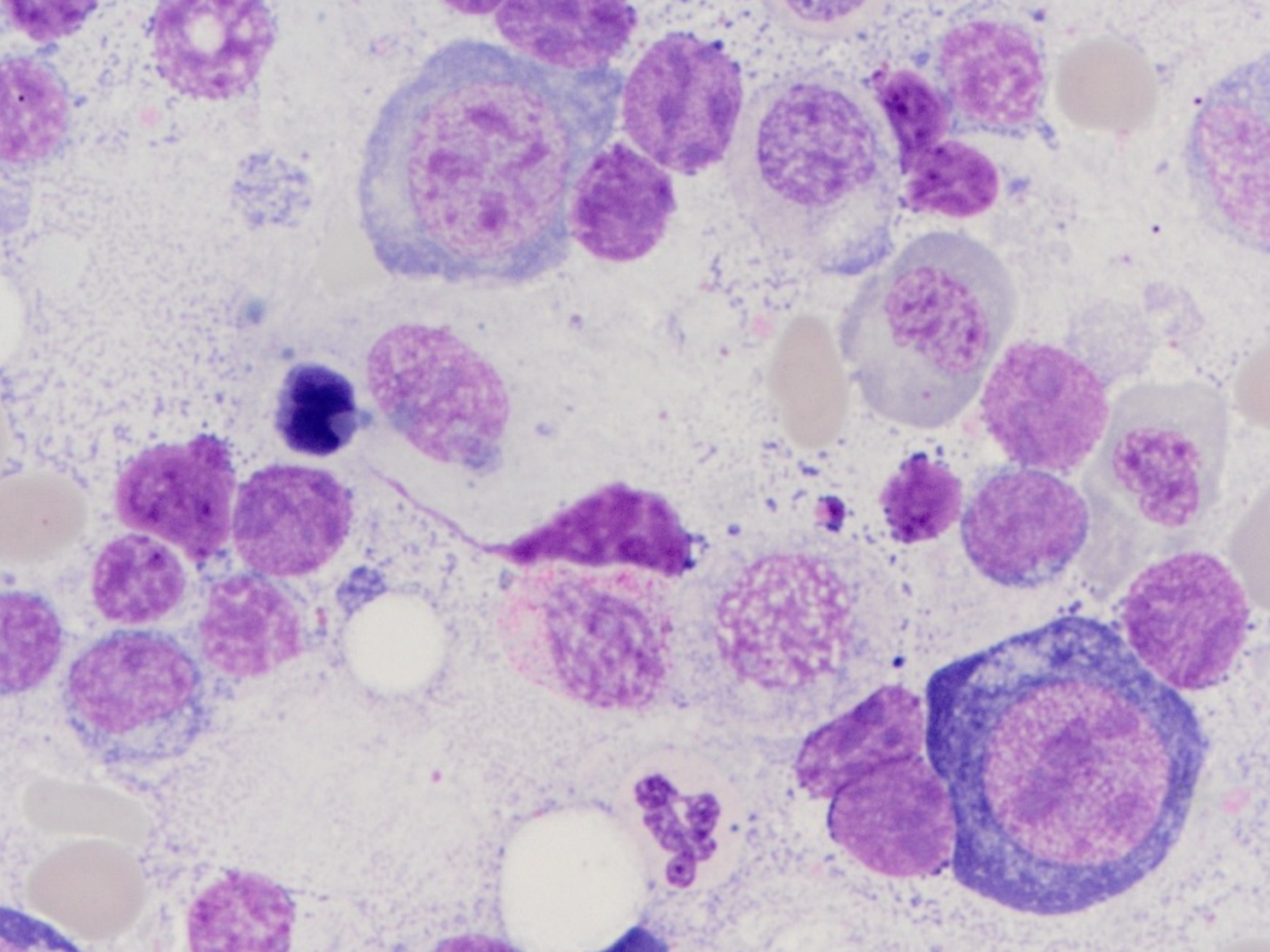


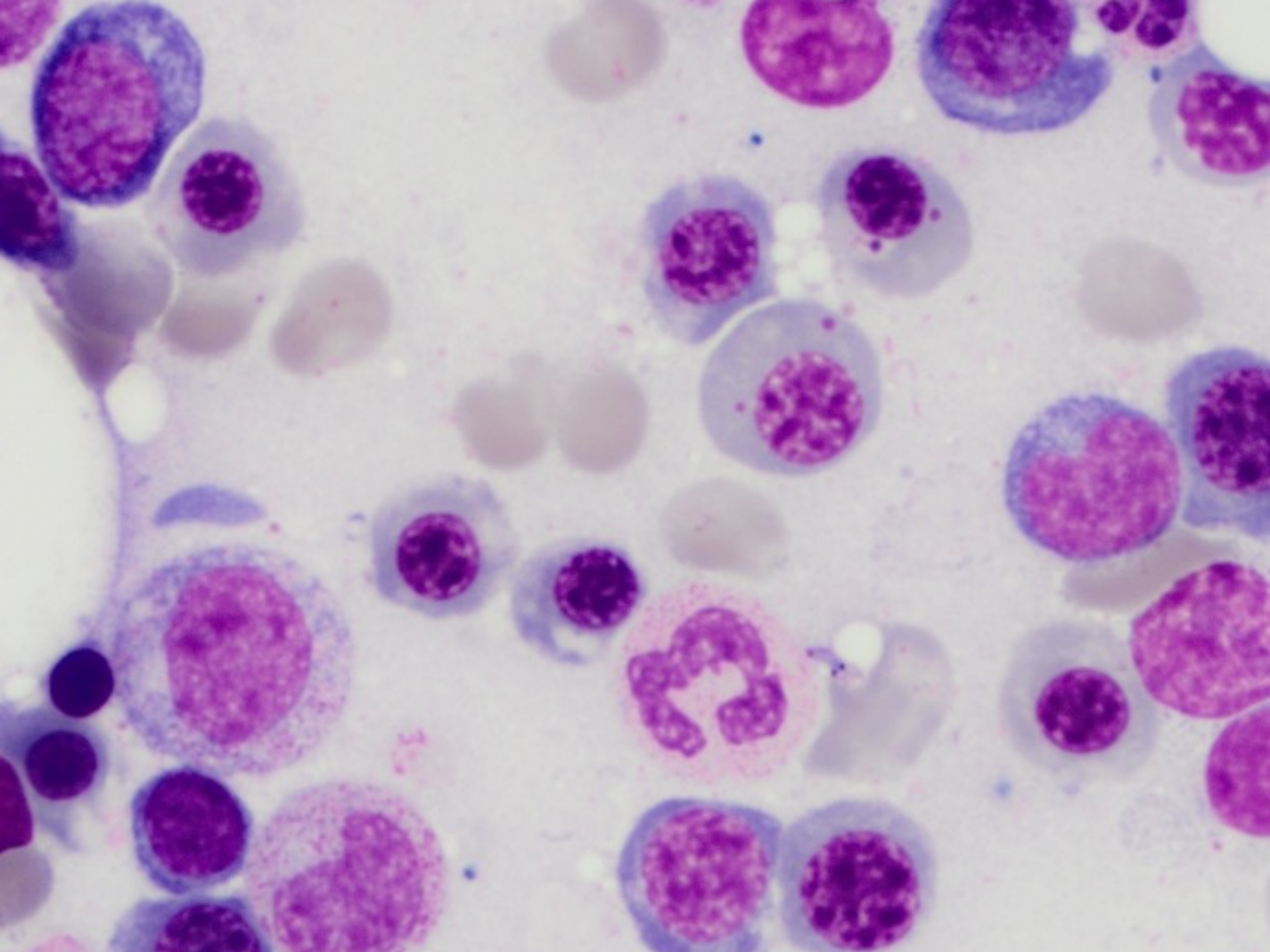


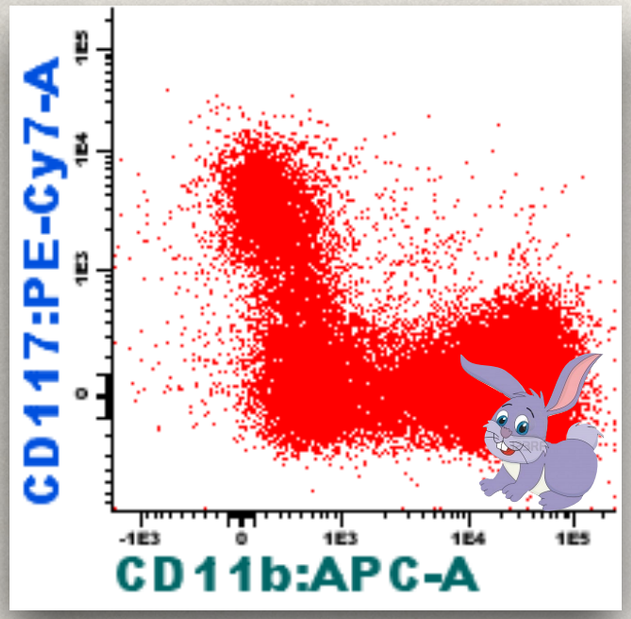
Score = 1

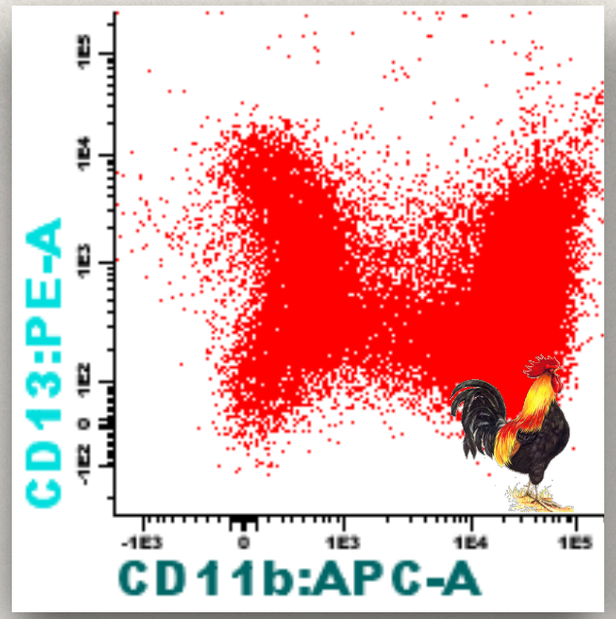


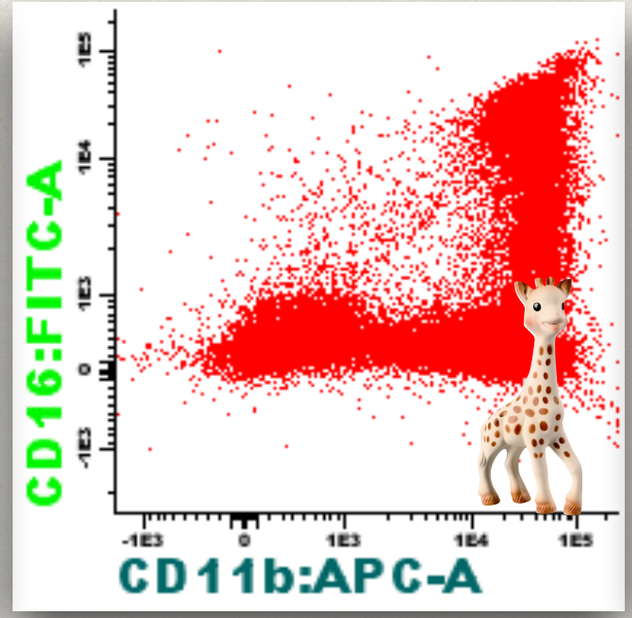


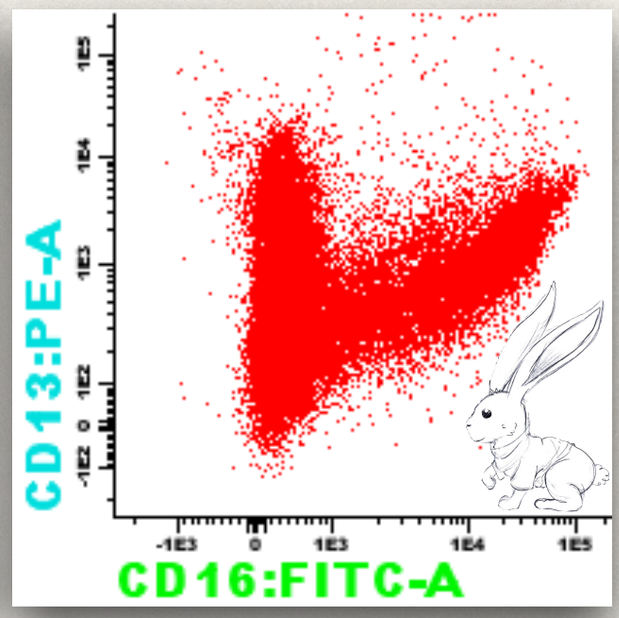


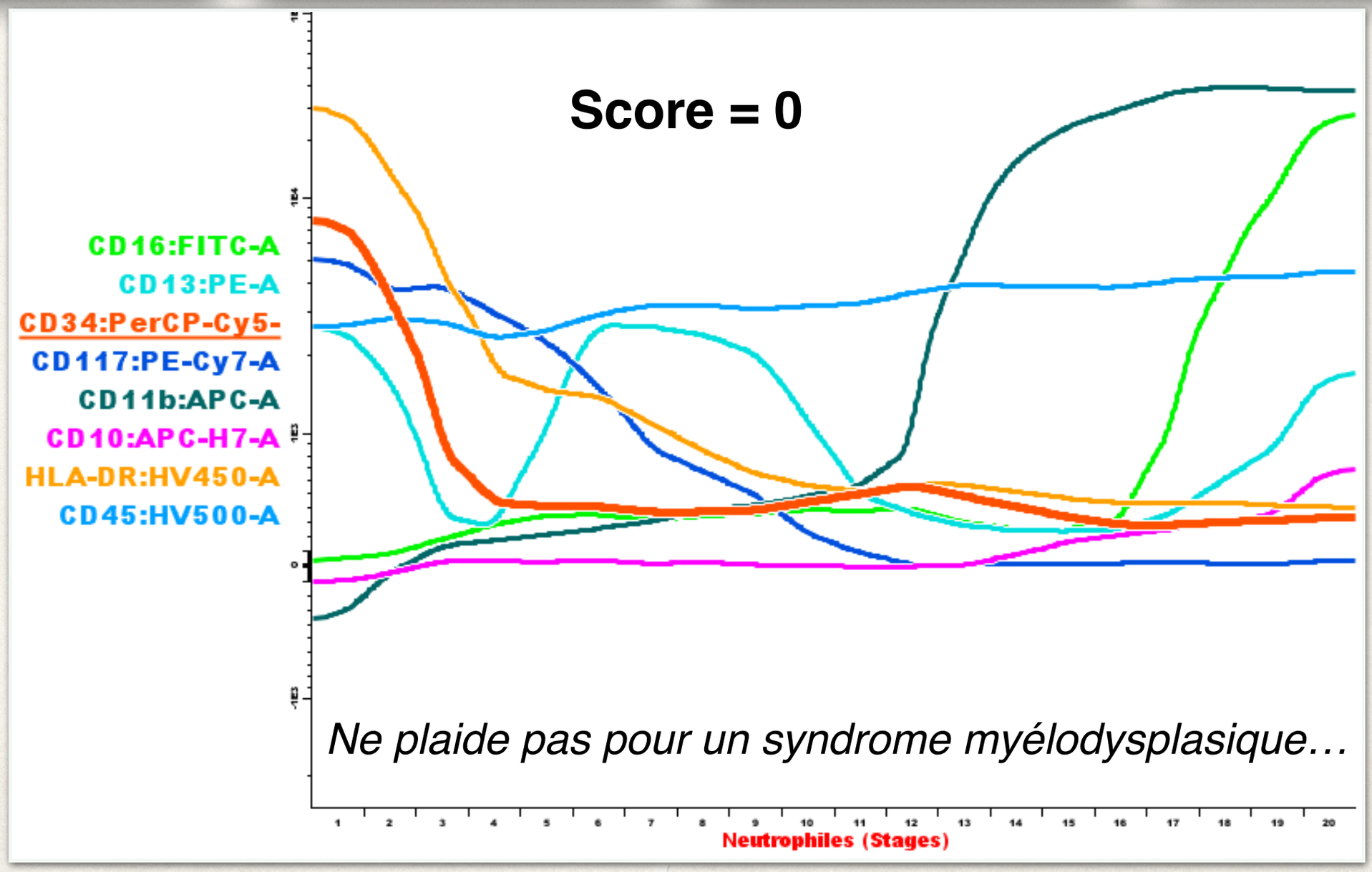












Ne plaide pas pour un syndrome myélodysplasique…

Carence vitamine B12 et folates

Cytogénétique ?
50% …

Morphologie
«Standard» mais…

Quel panel ?
EuroFlow

FC Scoring Systems
Oui, car manque de marqueurs discriminants dans les SMD

Quelle est la place de la CMF dans les SMD ?
Certainement d'importance grandissante

Conclusions

Multicentre validation of a reproducible flow cytometric score for the diagnosis of low-risk myelodysplastic syndromes: results of a European LeukemiaNET study

by Matteo G. Della Porta, Cristina Picone, Cristiana Pascutto, Luca Malcovati, Hideto Tamura, Hiroshi Handa, Magdalena Czader, Sylvie Freeman, Paresh Vyas, Anna Porwit, Leonie Saft, Theresia M. Westers, Canan Alhan, Caludia Cali, Arjan A. Van de Loosdrecht, and Kiyoyuki Ogata

Haematologica 2012 [Epub ahead of print]

- Il a été montré que la CMF est capable d'apporter une aide précieuse dans le diagnostic différentiel des syndromes myélodysplasiques.

- **<u>Avantages du score</u>** :
  - Peut être facilement implanté dans beaucoup de laboratoires et ne demande pas un panel d'anticorps extensif !
  - Les quatre paramètres possèdent une variabilité inter-opérateurs très faible, et leur expression est corrélée à la sévérité de la maladie.
  - Résultats facilement compris par les cliniciens.
  - Si le score est positif (≥2), probabilité post-test d'avoir une pathologie est > 90%.
  - Si le score est négatif, on ne peut exclure un diagnostic de SMD —> observation clinique pendant 6 mois suivie par une nouvelle évaluation médullaire ?

Peut-on utiliser le scoring system pour diagnostiquer un SMD ?

- Notre étude montre que le score donne une bonne performance pour le diagnostic des syndromes myélodysplasiques —> utilisé dans notre laboratoire...
- Avec un seul tube (AML-1) ! Rapide, « pas cher » et standardisé…
- Améliorations diagnostiques :
  - Etudier plus de populations (monocytes, ,érythrocytes, …)
  - Intérêt pour des marqueurs additionnels : CD7, CD15, CD56, ...

En seconde intention : HLA-DR/CD45/**CD15**/**CD56**/CD117/CD34/**CD7**/CD19

- Besoin de logiciels informatiques permettant une analyse multidimensionnelle —> peut aider à repérer les irrégularités dans les patterns de maturation, et aider lorsque l'on se retrouve devant des population se superposant.

L'immunophénotype peut aider à établir le diagnostic d'un SMD, en particulier quand la morphologie et la cytogénétique sont non discriminantes

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