



Hôpitaux de Lyon



Centre Hospitalier Régional
Universitaire de Lille

Etat des lieux du protocole LAM MRD LSCflow au sein de l'essai clinique de l'ALFA: étude des nouveaux marqueurs LSC et l'intégration de l'approche non supervisée FlowSom et TSNE dans l'analyse des données

Adriana PLESA, CHU Lyon, France

Christophe ROUMIER, CHU Lille, France

Coordinators of Flow Labs for ALFA French MRD centers

ELN Meeting-12th June 2019 Amsterdam

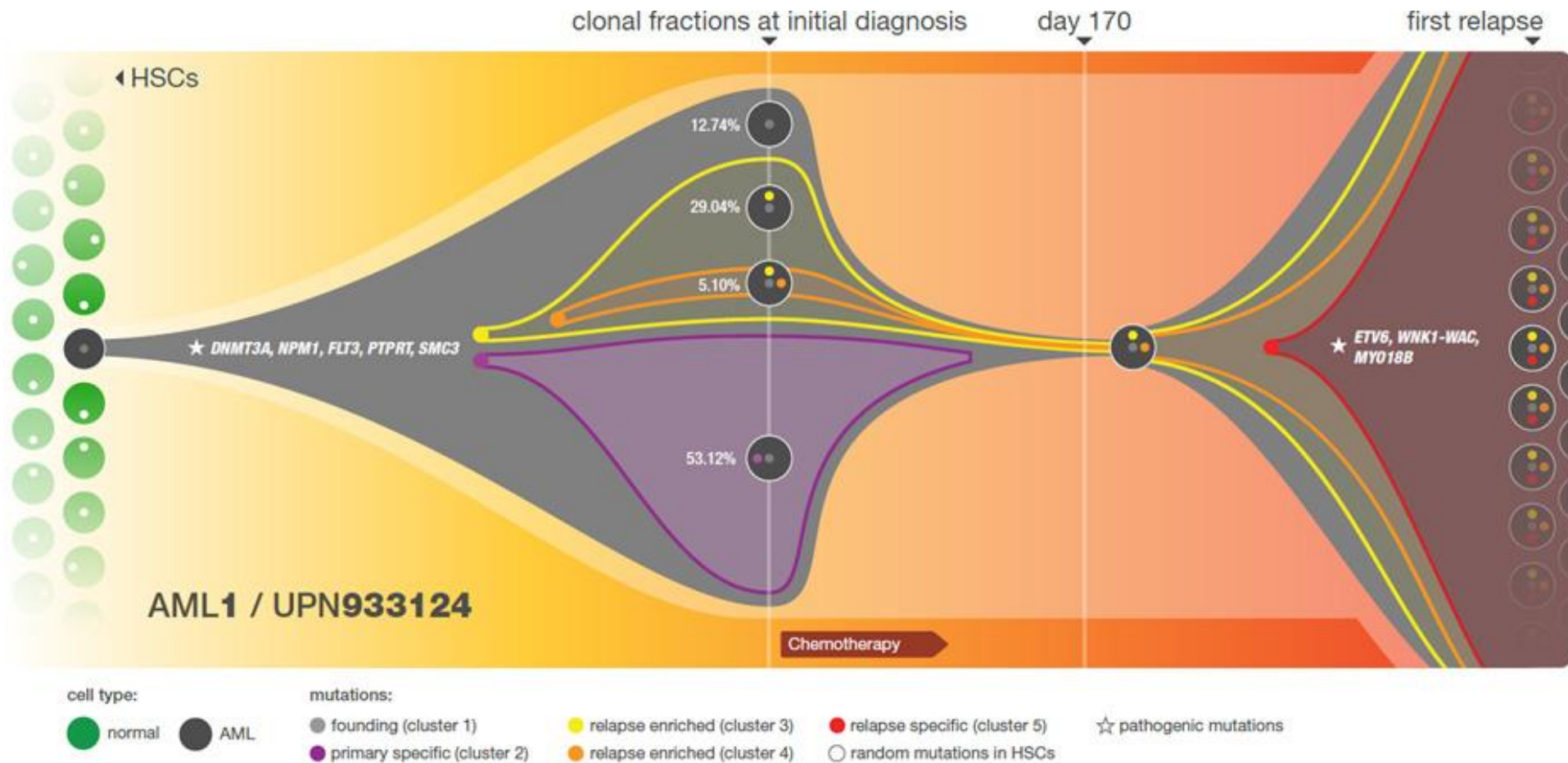


Collaborative InterGroup for Acute Leukemia

AFC 4-6 Novembre 2020

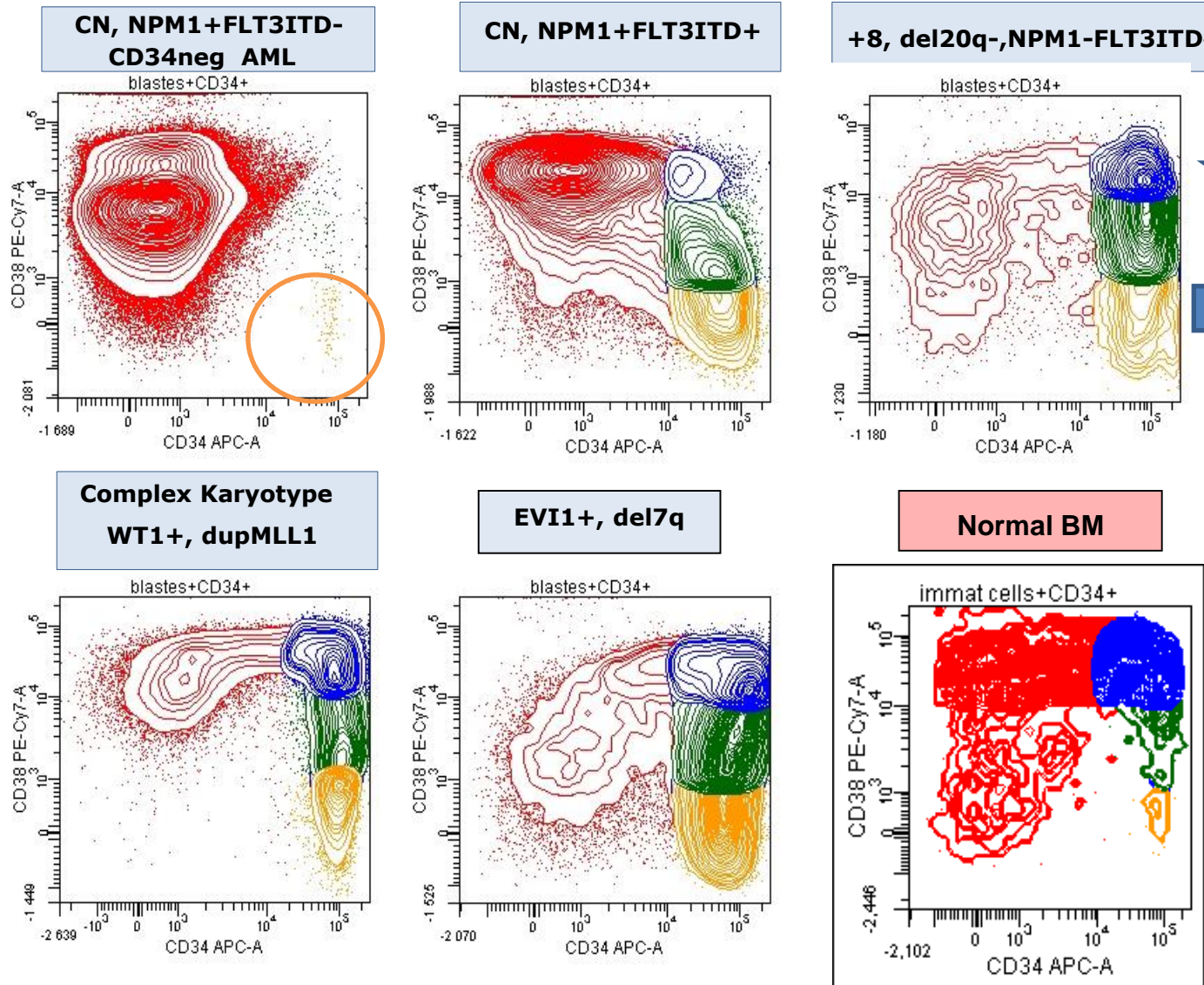


AML clonal heterogeneity

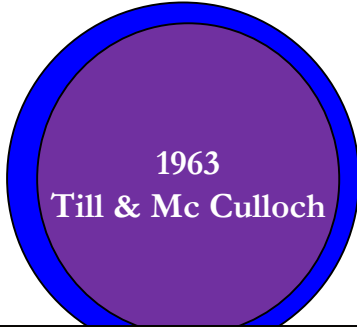


Educational book ASH 2019, Sylvie Freeman

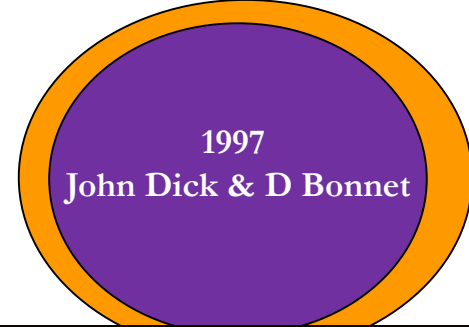
Figure A
PLESA/C
Roumier
ALFA MRD
AML
Group



nHSC



LSC



CD34+		CD34+	
CD38-		CD38-	
HLADR- CD90+CD45RA- CD13+/CD33lo, CD117+	<u>Marqueurs fonctionnels</u> Side Population (SP) Hoechst 33342 ALDH Quiescence G0/G1 5-FU Rhod123 Xenograft, PDX	HLADR- CD90-CD45RA+ CD13+/CD33lo/++CD117-/lo	
CD123-/lo CD44+ VLA4+ CXCR4+ CD47+ CD49f+		CD123++ CD44++ VLA4++ CXCR4++ CD47++ CD49f++	

CLL-1-, TIM3-, CD97-
CD96-, GPR56+, EPCR+, CD93+lo

CLL-1+, TIM3+, CD97+
CD96++, GPR56-/+, EPCR++ CD93++

New paradigm in MRD LAM: LSC approach

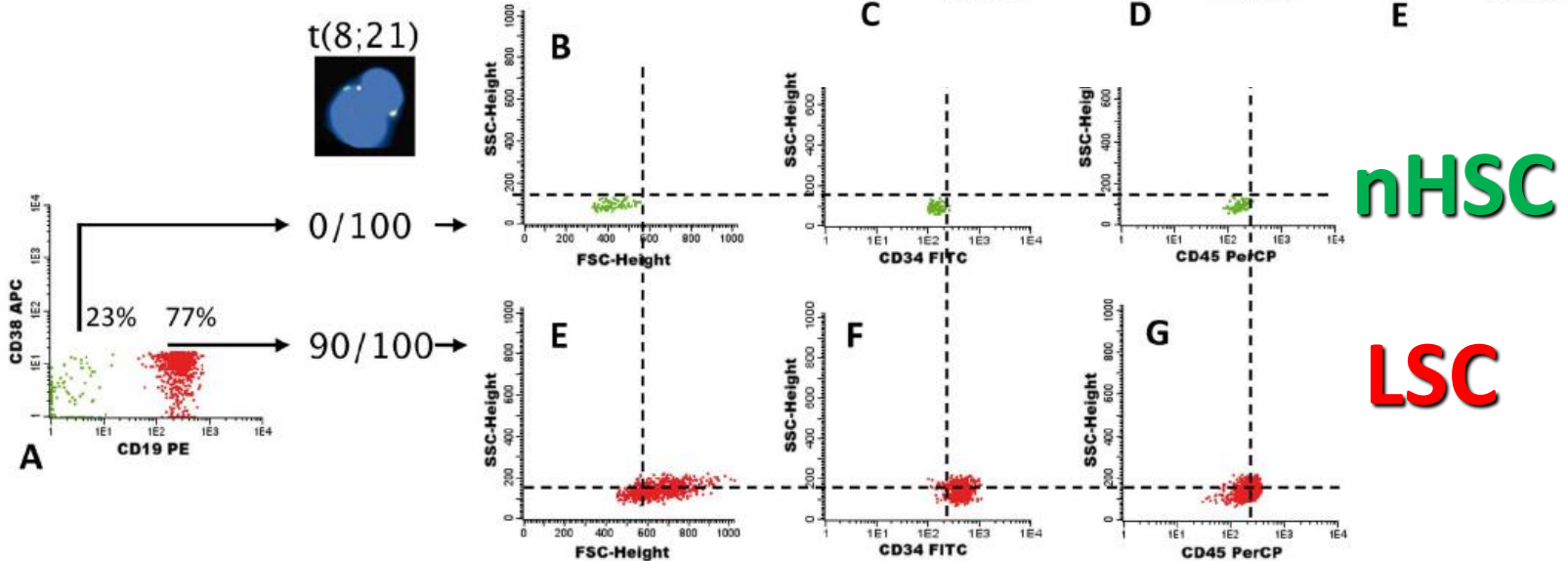
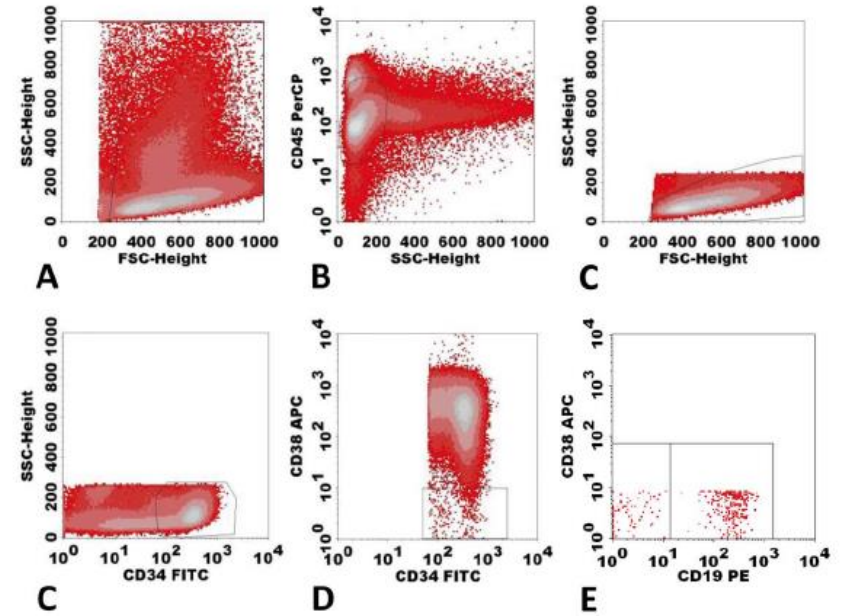
OPEN ACCESS Freely available online

PLOS ONE

Leukemic Stem Cell Frequency: A Strong Biomarker for Clinical Outcome in Acute Myeloid Leukemia

Monique Terwijn¹, Wendelien Zeijlemaker¹, Angèle Kelder¹, Arjo P. Rutten¹, Alexander N. Snel¹, Willemijn J. Scholten¹, Thomas Pabst², Gregor Verhoeft³, Bob Löwenberg⁴, Sonja Zweegman¹, Gert J. Ossenkoppele¹, Gerrit J. Schuurhuis^{1*}

¹Department of Hematology, VU University Medical Center, Amsterdam, The Netherlands, ²Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, ³Department of Hematology, University Hospital Leuven, Leuven, Belgium, ⁴Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

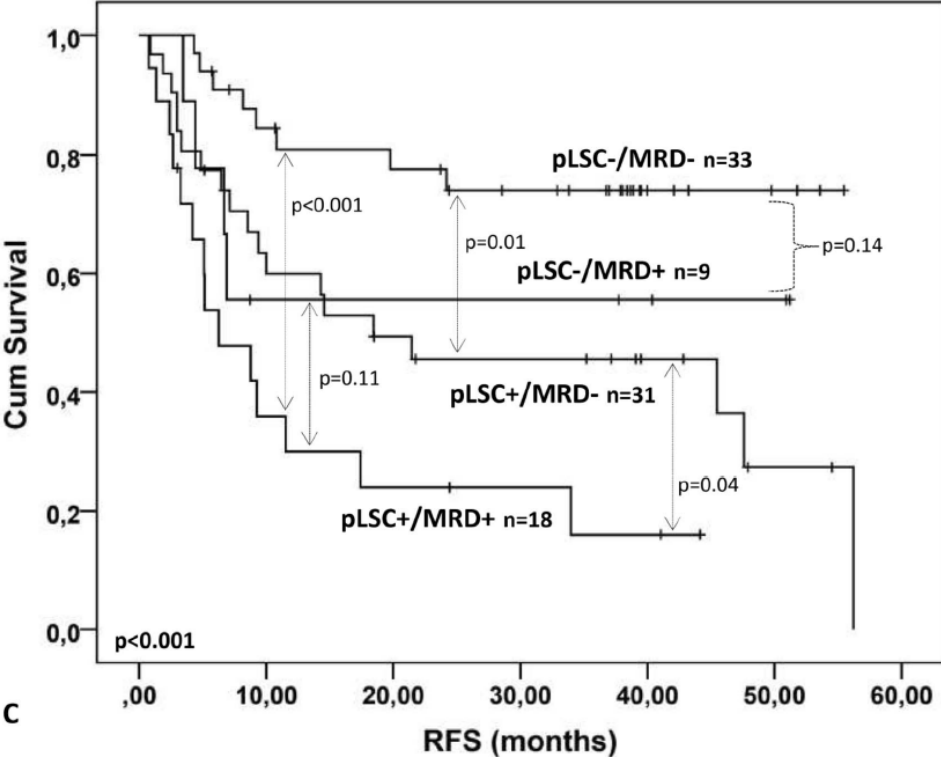
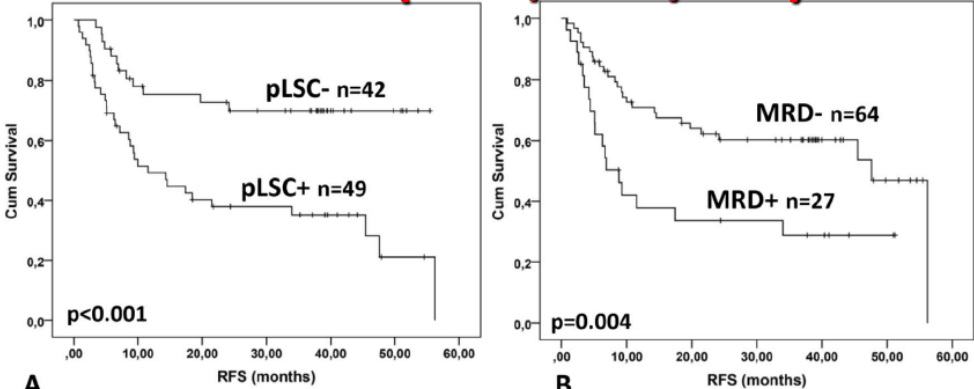


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New concept of AML follow up: Scoring of MRDflow

Leukemic Stem Cell Frequency in AML

(LAIP/DFN/LSC)



AML MRD flow

Strategy of our group (1^{er} présentation internationale du groupe ESCCA 2017)

1) Why?

- Flow Labs and French Clinical Trial organisation
- **ELN 2017 Guidelines requirements- MRD mandatory (molecular and/or flow)**
- Harmonisation of Flow Clinical MRD report

2) How?

Methodology multicentric approach:

- panel design: simple, reproducible, sensitivity, cut
- CANTO vs NAVIOS « miroir »
- gating strategy

3) Ready to start-

- CQA: verify gating strategy
- CQE: global check of panel implementation (n/reg BM)
- Patient follow up

ELN recommendation 2017

Design of MRD studies: **multicenter** versus **single center** approaches

- To facilitate and optimize data from MRD studies, we recommend that for multicenter studies samples may be processed by different centers applying the same MRD panels, according to the recommendations offered in the present paper.
- With insufficient experience in MRD analysis, the final interpretation should be performed at a central institute or in a group workshop.
- For single center studies in institutions with relevant experience, we recommend following the procedures described in this paper.
- Single center studies without relevant experience are strongly discouraged

Clinical Trial ALFA French BIG : MRDFlow LAIP & LSC follow up in AML

Evolution of our group: Coordinators ALFA Lyon/Lille: **Adriana PLESA / Christophe ROUMIER**

ESCCA/AFC 2009

A Plesa

1st LSC study

CD34+CD38- at AML diagnosis

14 Mai 2013

1st

Meeting
AML Group

8 centers

1st version of AML LSC protocole

Lyon, Lille, St Etienne, Marseille IPC,
Grenoble, Nice, Clermont Ferrand,
Trousseau

25 Juin 2014

2nd Training Meeting

10 Février 2015

3rd Training Meeting

2 Nov 2015

4th Training Meeting

18 Mai 2016

5th Training Meeting

7 Juillet 2016

Web Conf Tel

4 Nov 2016 ALFA Clinical Meeting

Implementation of FlowMRD/CMF LAIP+LSC
A Plesa/C Roumier

Requested by Clinical and biological PI of the trial
H Dombret
C Preudhomme
Based on ELN Guidelines
Standardisation /Harmonisation

14 Dec 2016

1st Training Meeting of GroupeMy « large » of BIG
ALFA+FILO

Educational Training Meetings continu

Mai 2017; Dec 2017; Mai 2018; Dec 2018; Mai 2019

9 Mars 2017 CQ1 20 centres

CQA virtual 31 Mai 2017

CQE 20 Juillet 2017

CQE 5 April 2018

CQE 22 Mai 2019

Jun 2019 28 Labs Centers

(14BD: 12Canto+2Lyric and 14BC Navios)

ESCCA 2017 Thessalonique

AFC 2017 Reims

Nov 2017 ESLHO Leiden

EBMT 2018, ASH 2018

Depuis Mars 2018--- Intégration au groupe ELN-AML en tant que
coordinateurs de de la MRDflow du groupe français de ALFA
Meeting Munich 4/4/2018 et 22/2/2019, Amsterdam 12/6/2019
Educational Book ASH 2019-Sylvie Freeman

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MRD evaluation of AML in clinical practice: are we there yet?

Sylvie D. Freeman¹ and Christopher S. Hourigan²

¹Clinical Immunology Service, Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom; and ²Laboratory of Myeloid Malignancies, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

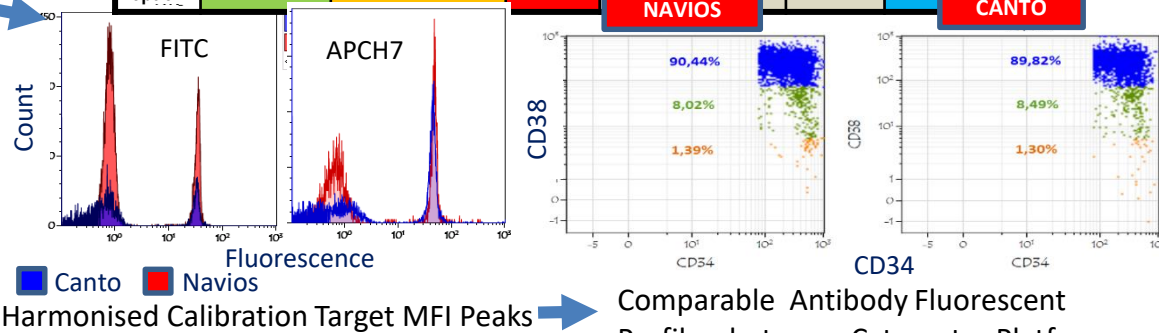
Educational book ASH
2019, Sylvie Freeman

HARMONISATION OF PRE-ANALYTICAL SAMPLE PROCESSING
Bulk Lysis / Staining/Wash/
Acquisition of minimum 500,000 cells

PANEL CONSTRUCTION [1]

	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
T 1	CD7+CD56	CD13	CD33	CD34	CD38	CD117	CD19	CD45
T 2	CD90	MIX LSC: TIM3+CLL1+CD9 7	CD123	CD34	CD38	CD117	CD45RA	CD45
T3 option	CD36	CD11b	CD33	CD34	HLA-	CD117	CD4	CD45

HARMONISATION OF CYTOMETER SETTINGS
to achieve comparable fluorescent profiles [2]
Canto cytometers used by 12 Laboratories
Navios cytometers used by 10 Laboratories

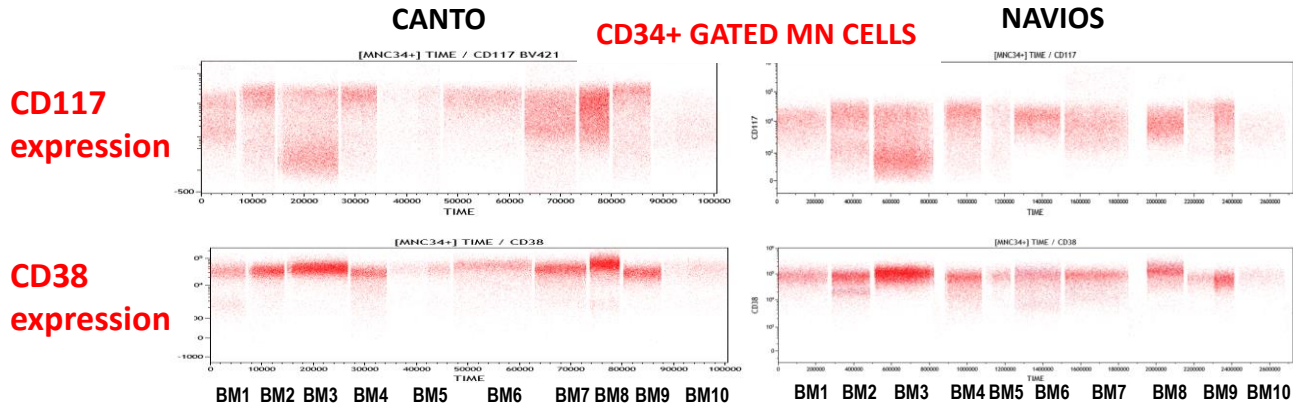


HARMONISATION OF GATING STRATEGY
to achieve reproducibility in detection of
low frequency immunophenotypic aberrant profiles

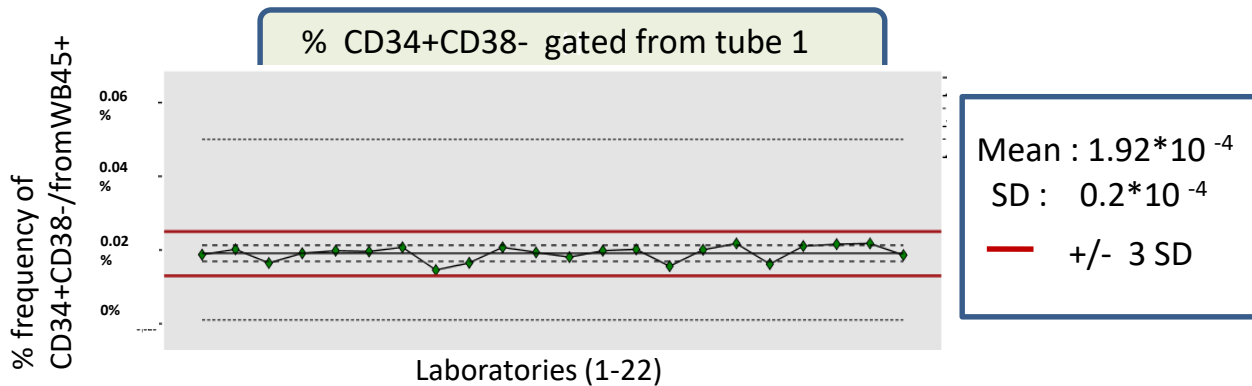
**INTER-LABORATORY COMPARISONS FOR
QUALITY ASSESSMENT OF FLUORESCENT
PROFILES AND GATING**

B Examples of Inter-Laboratory Quality Assessments

QA of marker expression profiles for 10 shared normal bone marrow samples [3]



QA from normal BM sample shared among 22 centers [4]



Minimal information should be given in **Clinical FlowReport**:

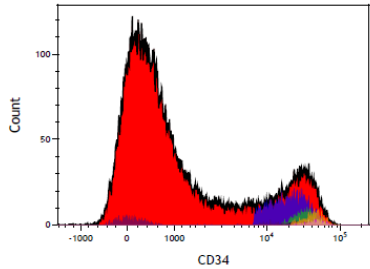
- Quality of BM (dilution)
- Strategy of identification of MRD: LAIP/DFN/LSC
- Description of the LAIP used
- LOD based on nBM
- Thresholds $< 10^{-3}$ for LAIP or $< 10^{-4}$ for LSC
- Interpretation of the result:
 - MRD+
 - MRD- with LOD value
 - MRD detectable but nonquantifiable

CQE Mai 2019: Collaboration au sein de l'ELN avec Sylvie Freeman, Birmingham UK

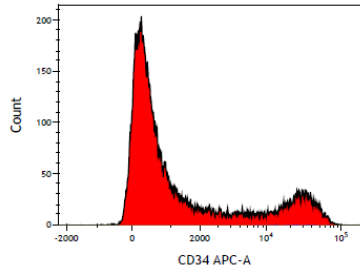
ALFA Tube T1 EEQ nBm healthy donor shared between French Labs and Birm/UK

	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
T 1	CD7/56	CD13	CD33	CD34	CD38	CD117	CD19	CD45

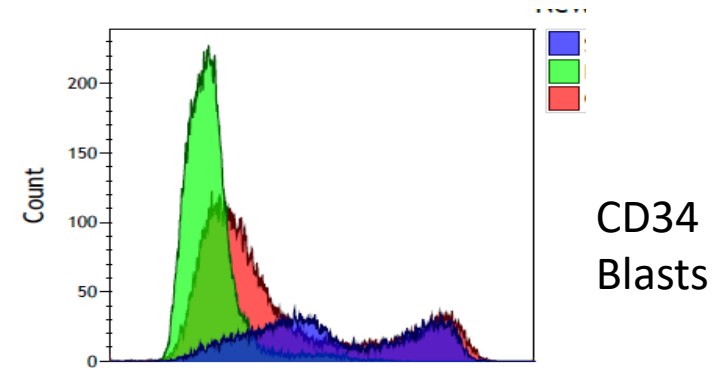
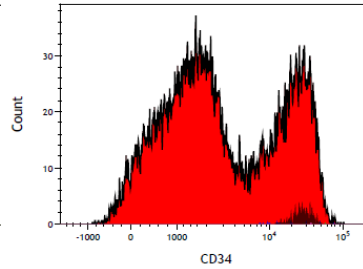
Lyon Canto



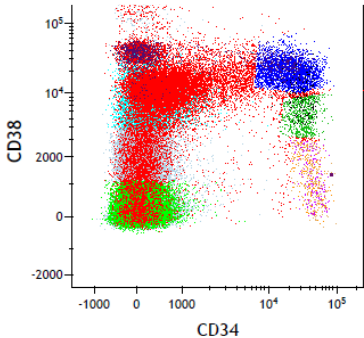
Lyon Lyric



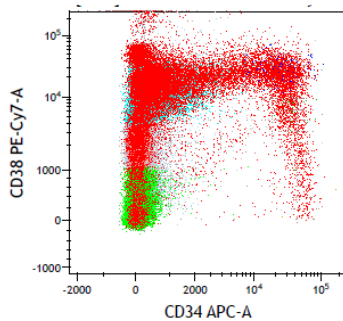
Birm Canto



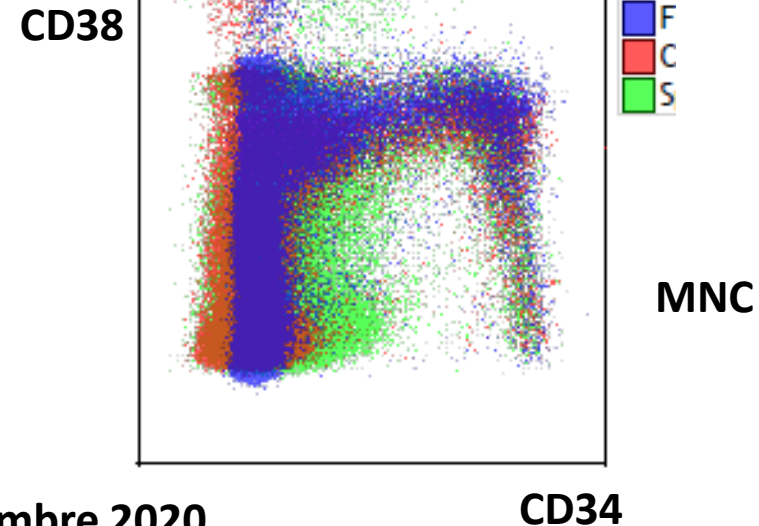
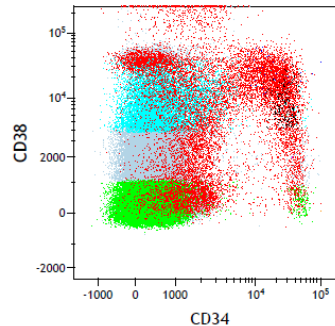
Lyon Canto



Lyon Lyric



Birm Canto



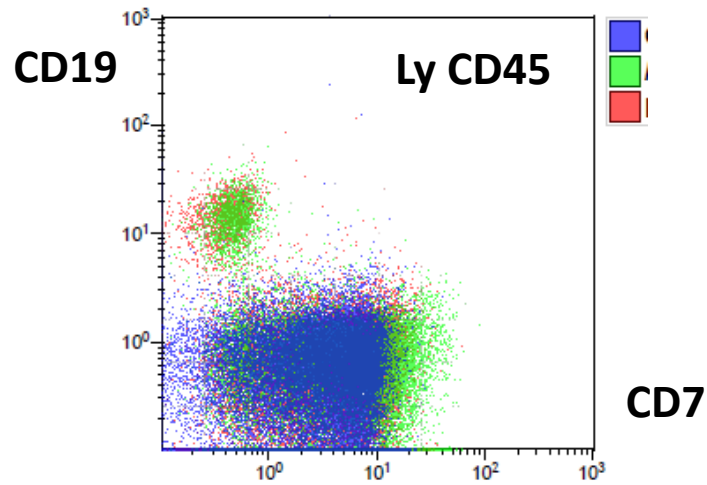
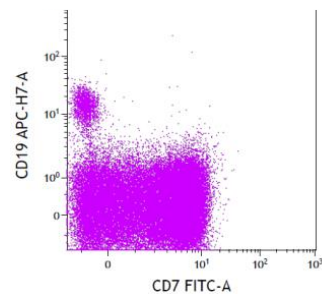
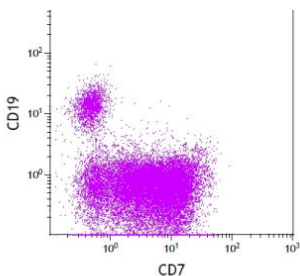
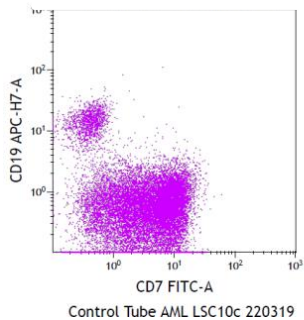
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CD34

Lyric Birm

Lyric Lyon

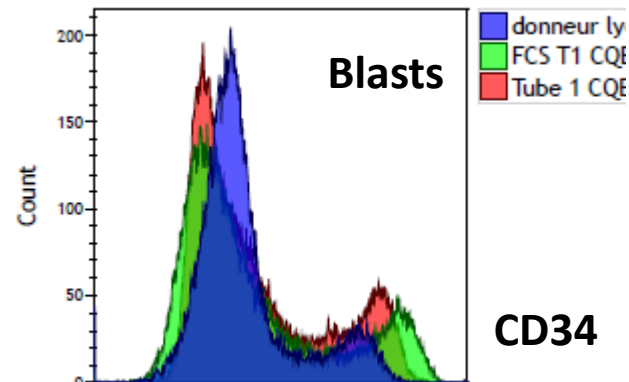
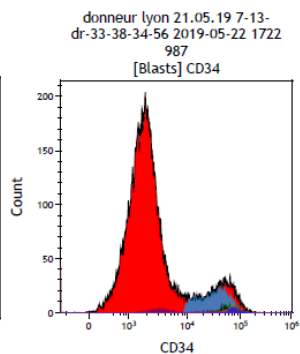
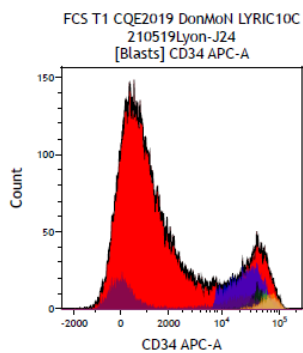
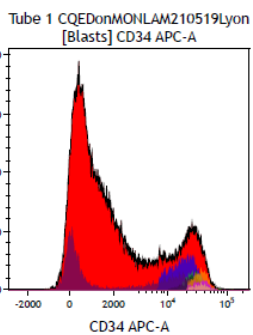
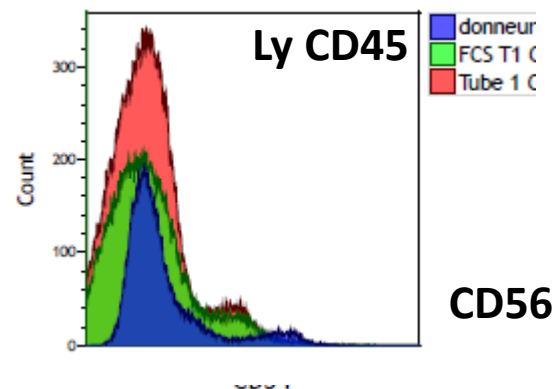
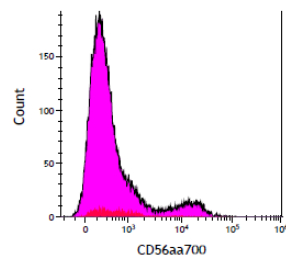
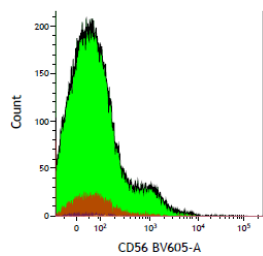
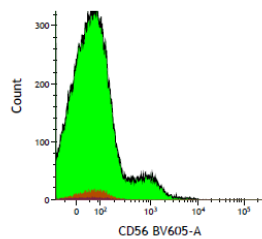
Navios Lille



Lyric Birm

Lyric Lyon

Navios Lille



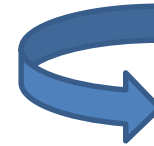
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MRD AML ALFA Panel evolution 8c to 10c-Lyric12C

Standardisation Lyon-Lille-Birmingham

CANTO-NAVIOS-LYRIC; Analyse masque harmonisé DIVA-KALUZA-FLOWJO

	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
T 1	CD7+CD56	CD13	CD33	CD34	CD38	CD117	CD19	CD45
T 2	CD90	MIX LSC: TIM3+CLL1+CD97	CD123	CD34	CD38	CD117	CD45RA	CD45
T3 option	CD36	CD11b	CD33	CD34	HLA-DR	CD117	CD4	CD45



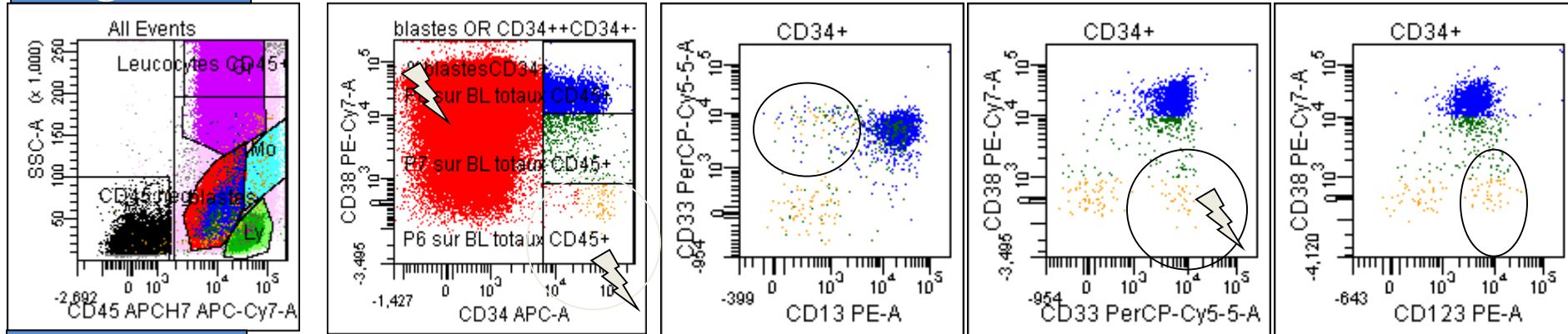
MAJ Juin 2020 Panel 12C	
Protocole MRD LAM LSC-ALFA	
A Plesa(Lyon-Lyric) C Roumier(Lille-Navios)	

NewALFA Tube1 10C LYRIC/ Navios	Lyric8C								LYRIC 10C		LYRIC 12C	
	Lyric8C	Lyric8C	Lyric8C	Lyric8C	Lyric8C	Lyric8C	Lyric8C	Lyric8C	LYRIC 10C	LYRIC 10C	LYRIC 12C	LYRIC 12C
	FITC	PE	PerCPCy5,5	APC	PECy7	APCH7	BV421	V500	APC-R700	BV605	BV711	BV786
Tube 1 LAIP	CD7	CD13	CD33	CD34	CD38	CD19	CD117	CD45	HLADR	CD56	CD10	CD36
Tube 2 LSC	CD90	MIX3	CD123	CD34	CD38	CD45RA	CD117	CD45	HLADR	CD36	CD19	CD133
Tube 3 Mono	CD13	IL1 RAP	CD11b	CD34	CD64	CD4	CD117	CD45	HLADR	CD14	CD33	CD16
T4 LSC new marq GPR56	MIX3	GPR56	CD90	CD34	CD38	CD45RA	CD117	CD45	HLADR	CD9	CD19	CD81

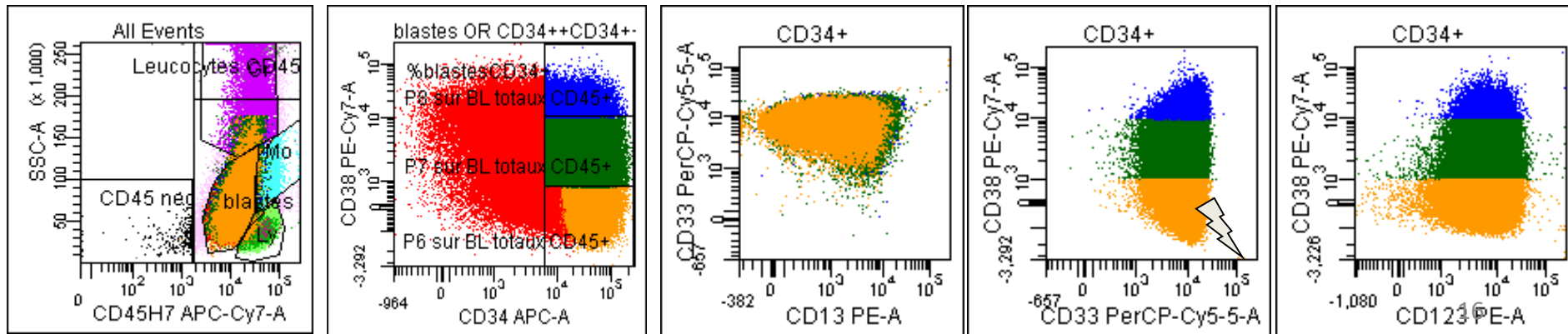
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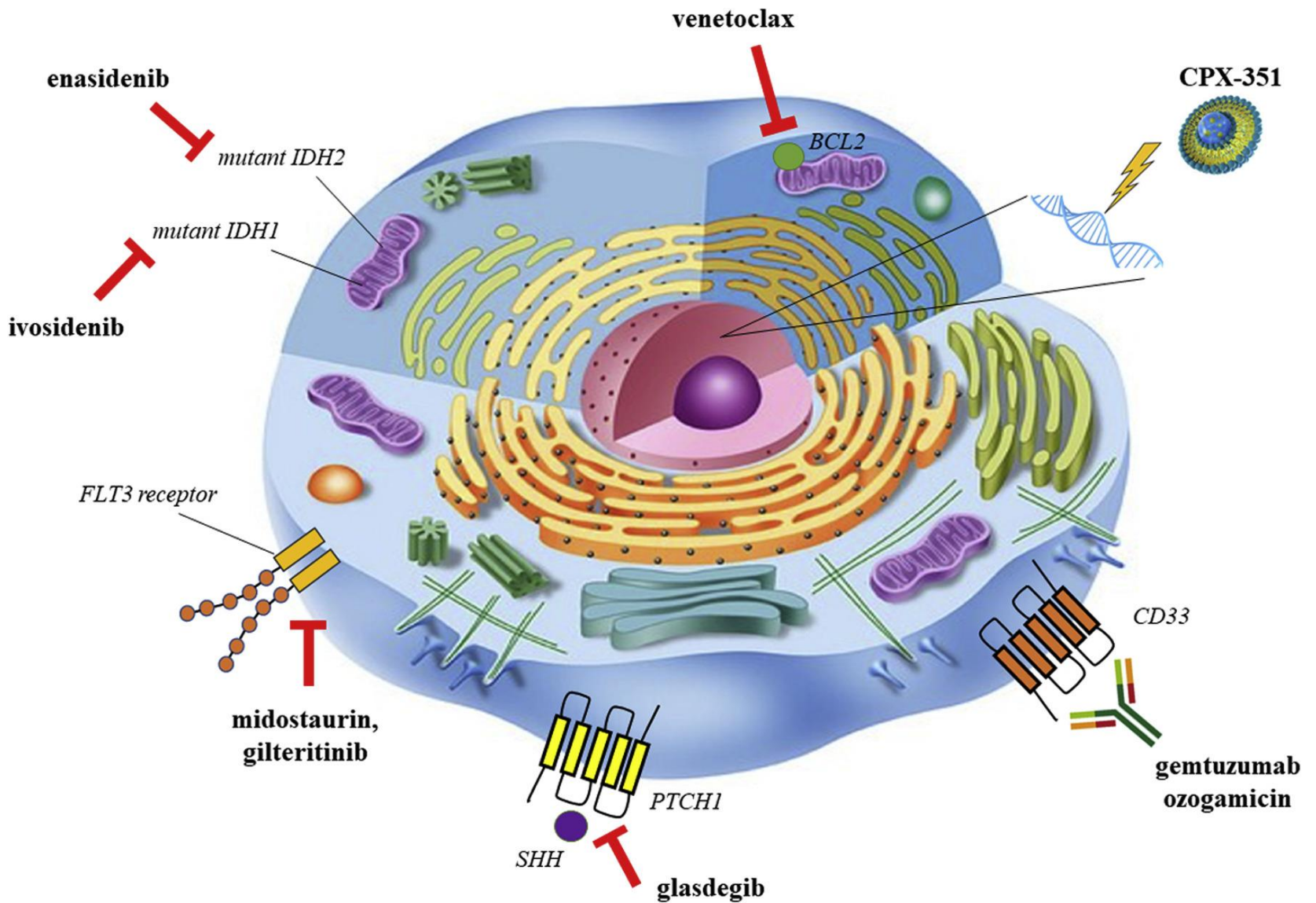
Patient 64y, AML4 dg15/06/2012 relapse 07/01/2013 (ALFA 1200)
 minority subclone 34+38- du dg (0,4% from blasts)
 at dg: WT1+, NPM1+, **FLT3ITD-**, EVI1-, CEBPa-, DNMT3A+, N caryotype
 relapse: WT1+, NPM1+, **FLT3ITD+!!!** N caryotype
 Architecture clonale « moléculaire » & « Phénotypique »

Diagnostic



Relapse



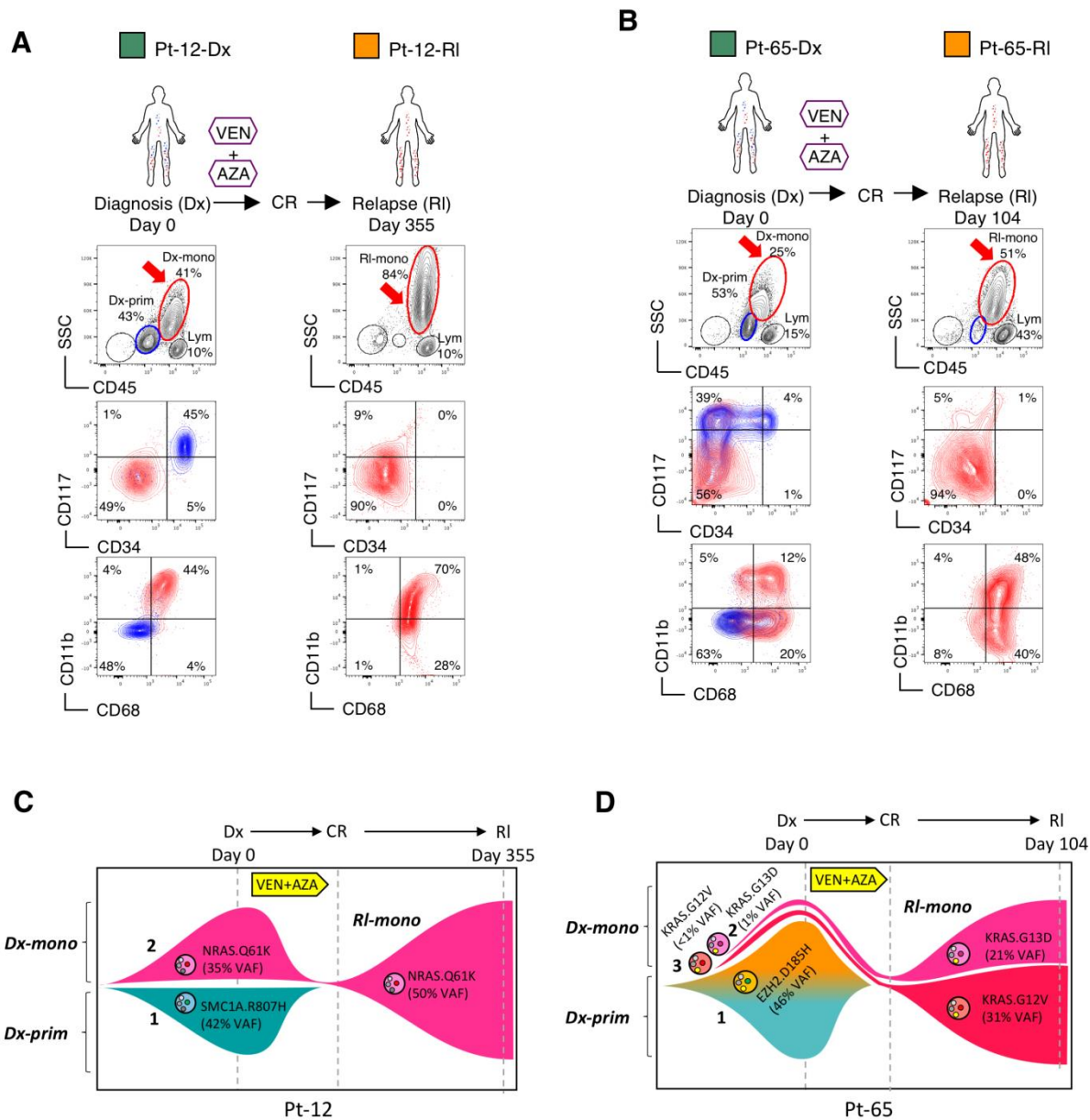


Shyam A. Patel, Jonathan M. Gerber Clinical Lymphoma, Myeloma & Leukemia May 2020-279

<https://doi.org/10.1016/j.clml.2020.01.011>

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Figure 4



Universal monitoring of minimal residual disease in acute myeloid leukemia

Elaine Coustan-Smith,¹ Guangchun Song,² Sheila Shurtleff,² Allen Eng-Juh Yeoh,^{1,3} Wee Joo Chng,³ Siew Peng Chen,¹ Jeffrey E. Rubnitz,^{4,5} Ching-Hon Pui,^{2,4,5} James R. Downing,^{2,5} and Dario Campana^{1,3}

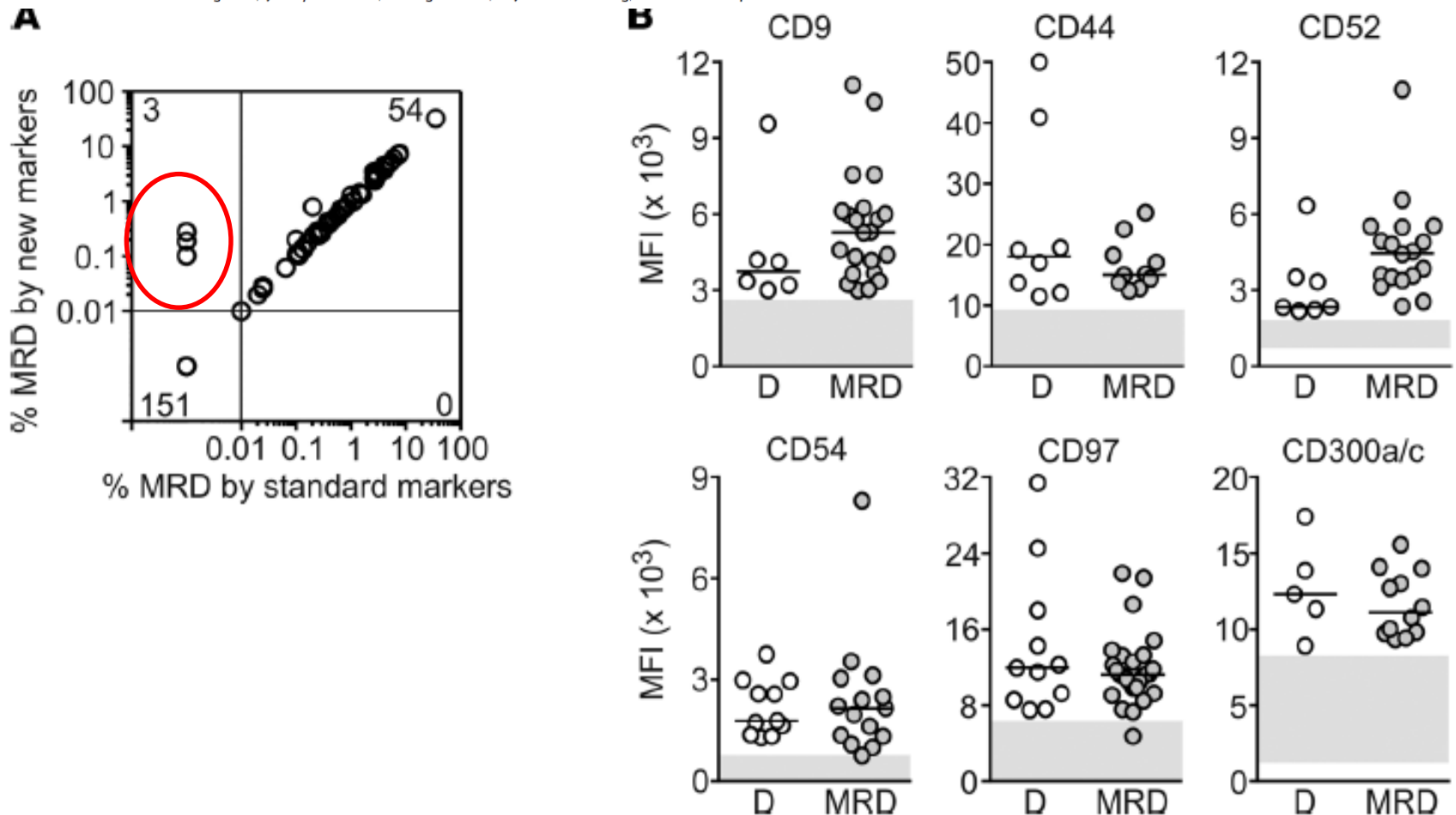


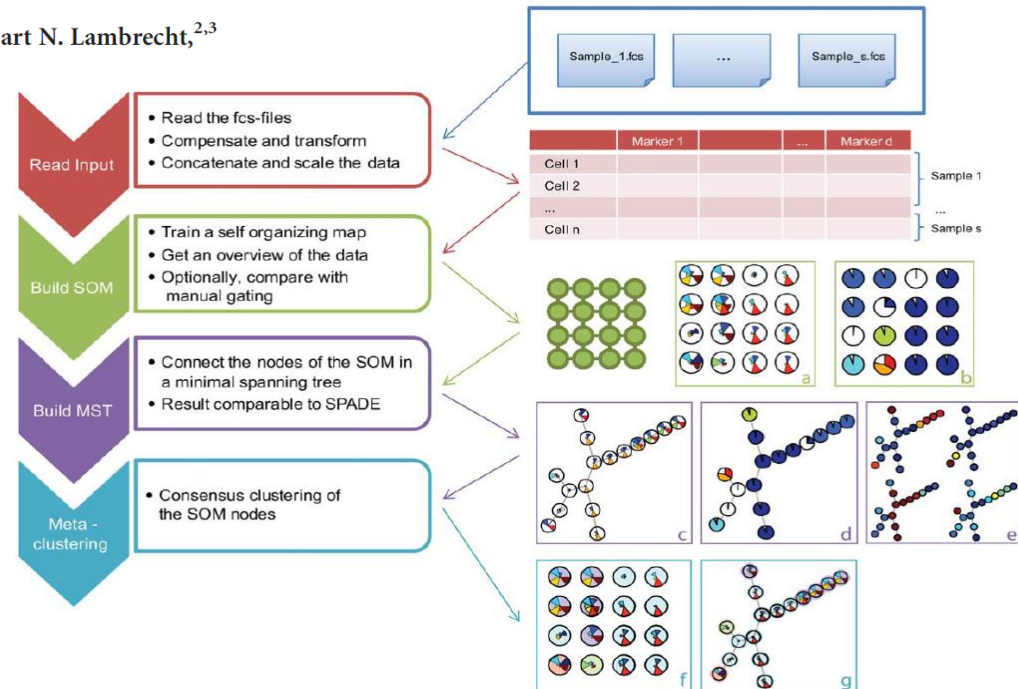
Figure 5. The selected markers allow detection of MRD and remain aberrantly expressed during chemotherapy. (A) Relation between MRD levels measured using the markers identified in this study versus those measured with standard markers (see Supplemental Table 5). Spearman correlation of positive MRD results by both methods: $r = 0.9816$, $P < 0.0001$. (B) Median fluorescence intensity (MFI) of the indicated markers measured at diagnosis (D) and during chemotherapy (MRD). Horizontal bars correspond to the median value in each group. Gray areas include the MFI of normal CD34⁺ myeloid progenitors.

Analyse non supervisée et MRD dans les LAM

FlowSOM: Using Self-Organizing Maps for Visualization and Interpretation of Cytometry Data

Cytometry Part A • 87A: 636–645, 2015

Sofie Van Gassen,^{1,2,3*} Britt Callebaut,¹ Mary J. Van Helden,^{2,3} Bart N. Lambrecht,^{2,3}
Piet Demeester,¹ Tom Dhaene,¹ Yvan Saeys^{2,3}



Application de Flowsom en cytométrie clinique



Modalités de traitement des données : scrip R : F Dumezy, A Plesa, C Roumier

- ✓ LMD or FCS files (Navios, Lyric, Canto)
- ✓ Export des données des « populations sélectionnées» via KALUZA ou FLOWJO: obtention d'un fichier CSV
- ✓ Console R ou R studio
- ✓ script Running :
 - Parameters selection (F11, FL2, ...). Eviter FS or SS
 - Définition du Nombre de clusters
 - FlowSOM (SOM and Map)
 - TSNE graph
 - Obtention d'un **nouveau fichier FCS** ajoutant de nouveaux paramètres aux paramètres initiaux : **Flowsom metacluster, FlowSOM cluster, size, x and y of the SOM grid, xNodes and yNodes of the map, TSNE1 and TSNE 2.**
- ✓ Ouverture et analyse de ce nouveau fichier via **Kaluza ou FlowJo** , autorisant fenêtrage sur les anciens et nouveaux paramètres et le calcul des statistiques

FCS/LMD files Parameters

FS PEAK	▶
FS INT	▶
FS TOF	▶
SS INT	▶
<input checked="" type="radio"/> FL1 INT: 90	▶
FL2 INT: mix	▶
FL3 INT: 19	▶
FL4 INT: 123	▶
FL5 INT: 38	▶
FL6 INT: 34	▶
FL7 INT: 36	▶
FL8 INT: 45RA	▶
FL9 INT: 117	▶
FL10 INT: 45	▶
TIME	

Script
FlowSOM/TSNE



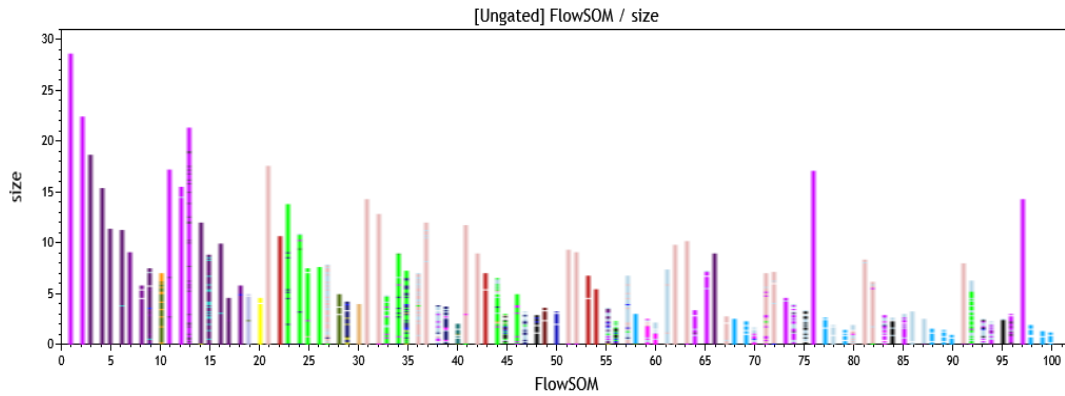
FlowSOM/TSNE script files Parametres

FS.PEAK	▶
FS.INT	▶
FS.TOF	▶
SS.INT	▶
X.90	▶
mix	▶
X.19	▶
X.123	▶
X.38	▶
X.34	▶
X.36	▶
TIME	
tsne.1	▶
tsne.2	▶
FlowSOM	▶
<input checked="" type="radio"/> xNodes	▶
yNodes	▶
size	▶
xGrid	▶
yGrid	▶
metaClustFSOM	▶

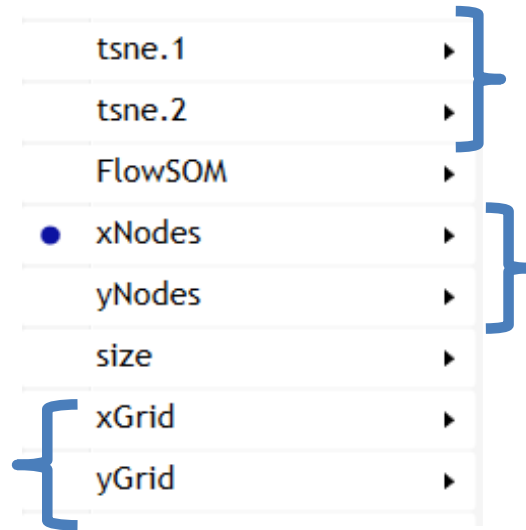
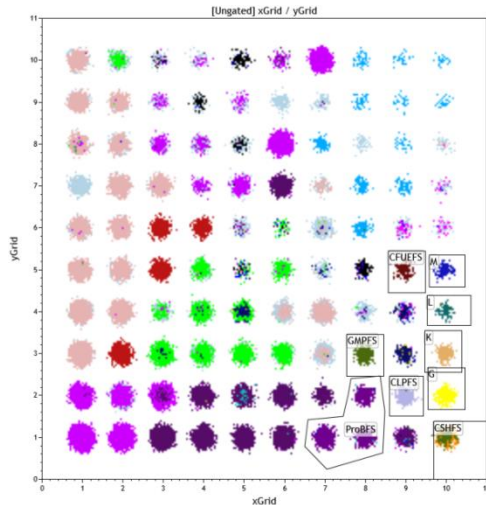
Nouveaux
parametres

Exemple d'histogrammes réalisés avec les nouveaux paramètres

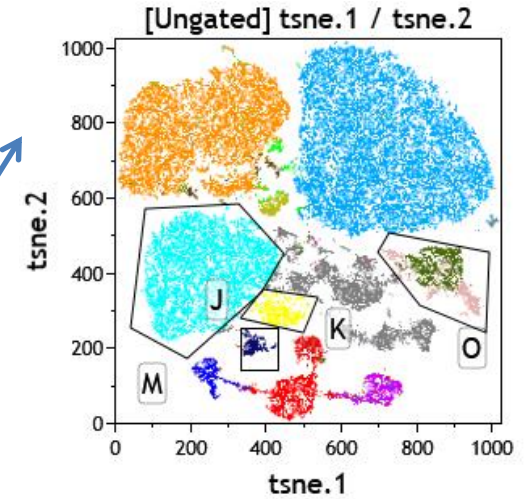
1: Taille et numéro du cluster



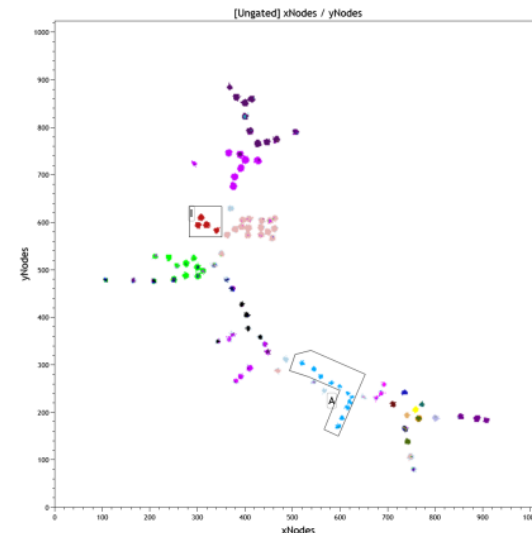
2: Coordonnées grille



3: Coordonnées TSNE



4: Coordonnées carte SOM



Application dans un diagnostic de LAM

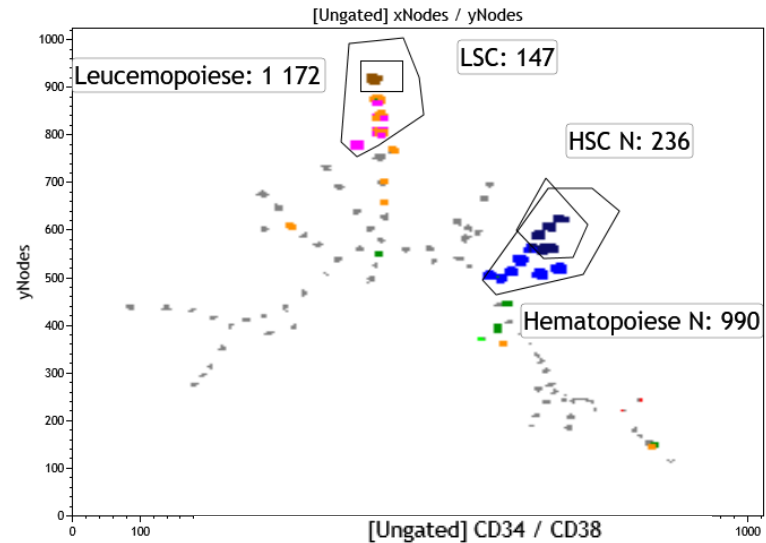
Fichier LMD

Script R
FlowSom

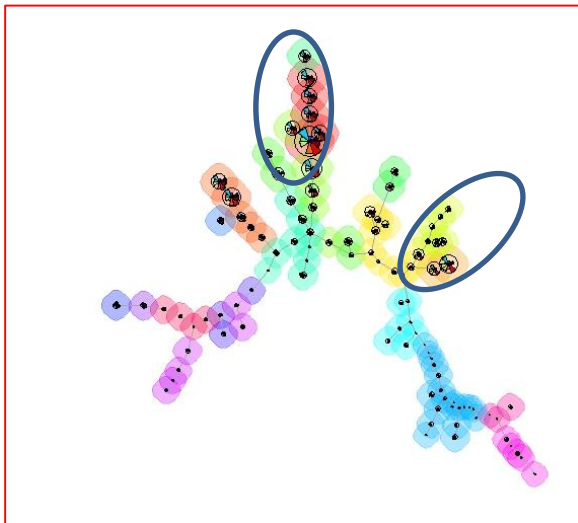
Production du Fichier FCS

Ouverture du fichier FCS (Kaluza, Flowjo, Diva ...

•Fonctionnalités habituelles: statistiques, gating etc

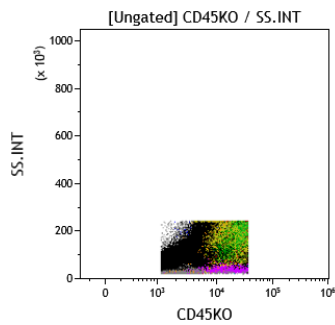


Graphe Flowsom sous R



Cartographie SOM et différentiation Myéloïde

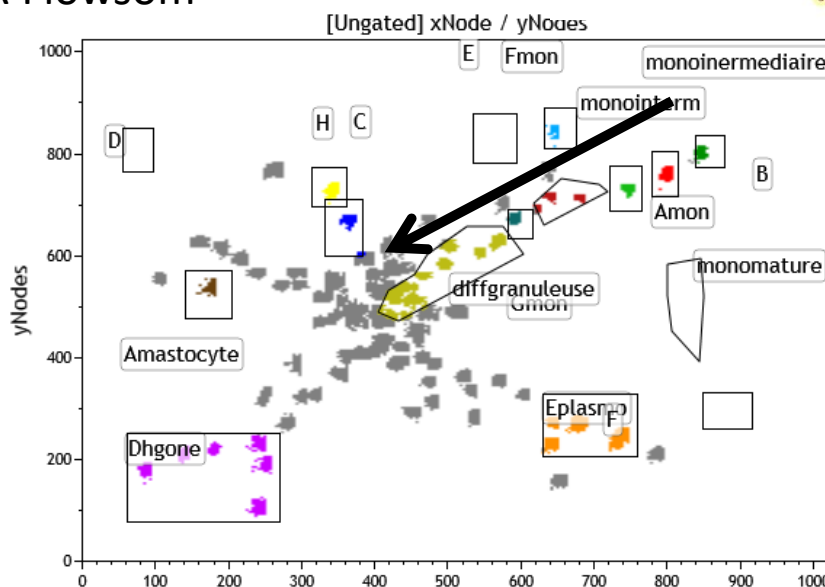
Tubes	FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KO
T1	7	13	HLADR	33	38	34	10	19	117	45



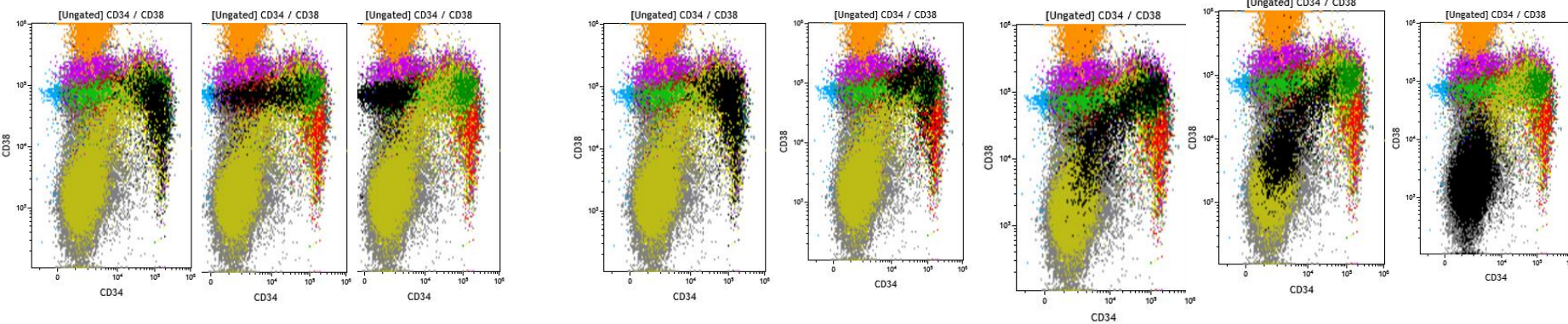
Export fichier FCS
depuis kaluza

R Flowsom

Importation
dans Kaluza



Retro
gating



Flowsom et analyse de MRD dans les LAM

Logiciels de clusterisation automatique :

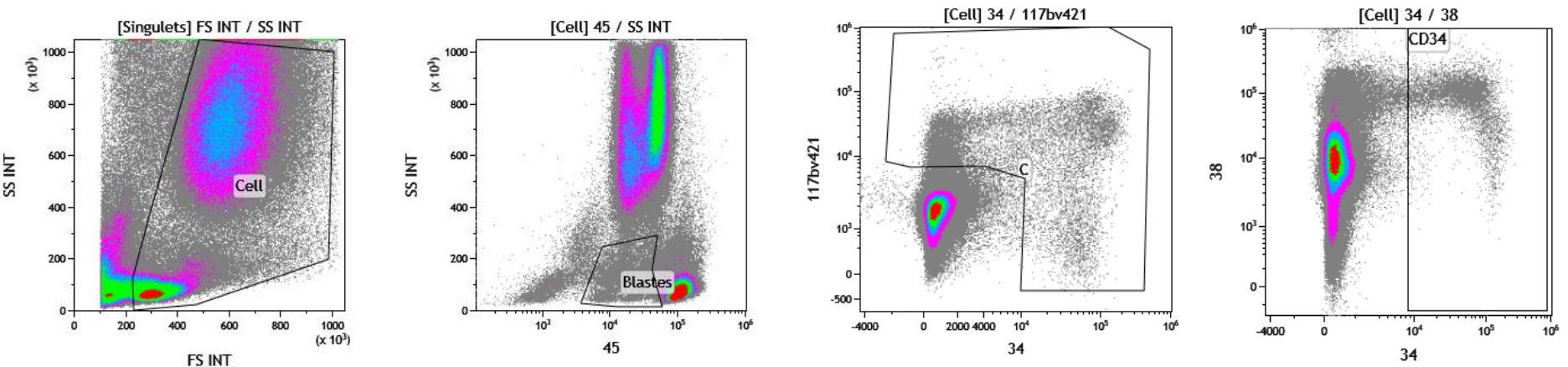
- Définir le nombre de clusters pour séparer les blastes leucémiques, les progéniteurs physiologiques, les autres cellules de la MO.
- Comparer la MO du diagnostic avec le ou les points de suivi, et des MO de Référence
- Vérifier le contenu de chacun de ces clusters au diagnostics et en suivi
- Pour les clusters leucémiques vérifier si on peut les voir dans les MO de références
- Calculer la valeur de la MRD en fonction du dénominateurs choisi.

Définir le nombre de clusters pour rassembler les blastes leucémiques, les progéniteurs physiologiques, les autres cellules de la MO.

Adéquation entre les Ag testés dans le tube, le nombre de populations cellulaires identifiables, Les caractéristiques des cellules leucémiques vs les cellules physiologiques

Tubes	FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KO
T1	7	13	HLADR	33	38	34	10	19	117	45

Définir la population étudiée



- Exhaustifs mais bcp de cellules analysées sans intérêt pour la MRD

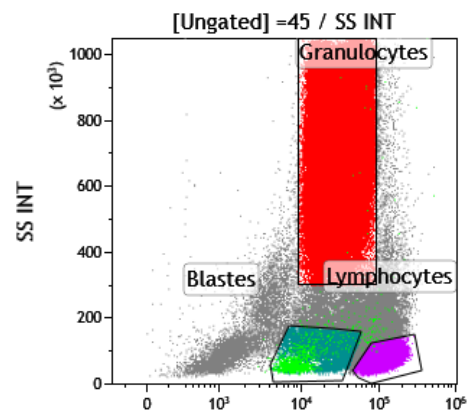
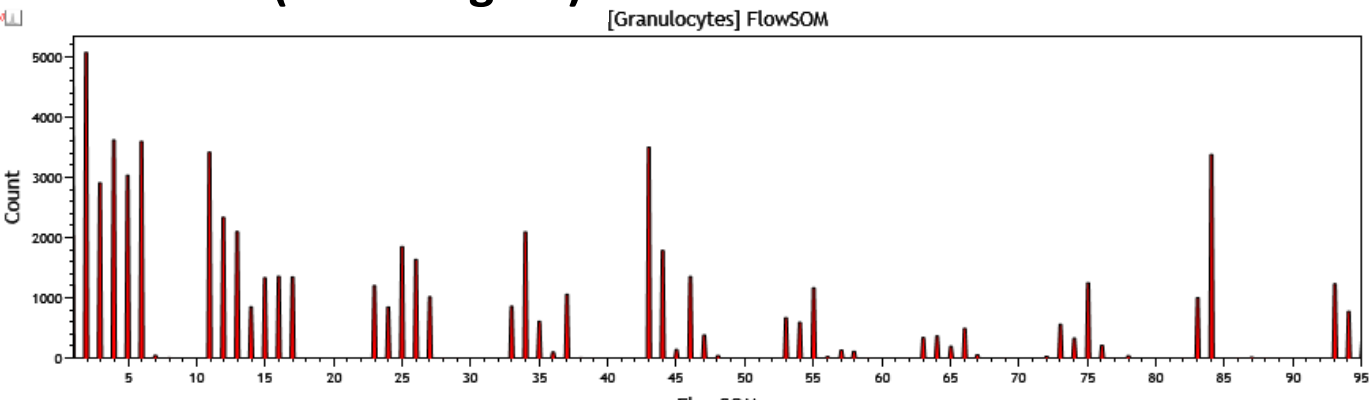
- Sélection difficile à standardiser: M4, M5...

- Reproductible mais quid des LAM CD117- CD34-

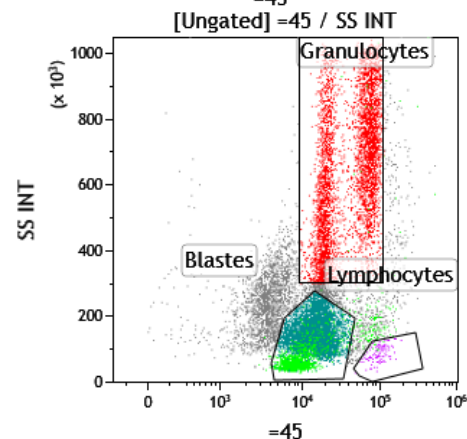
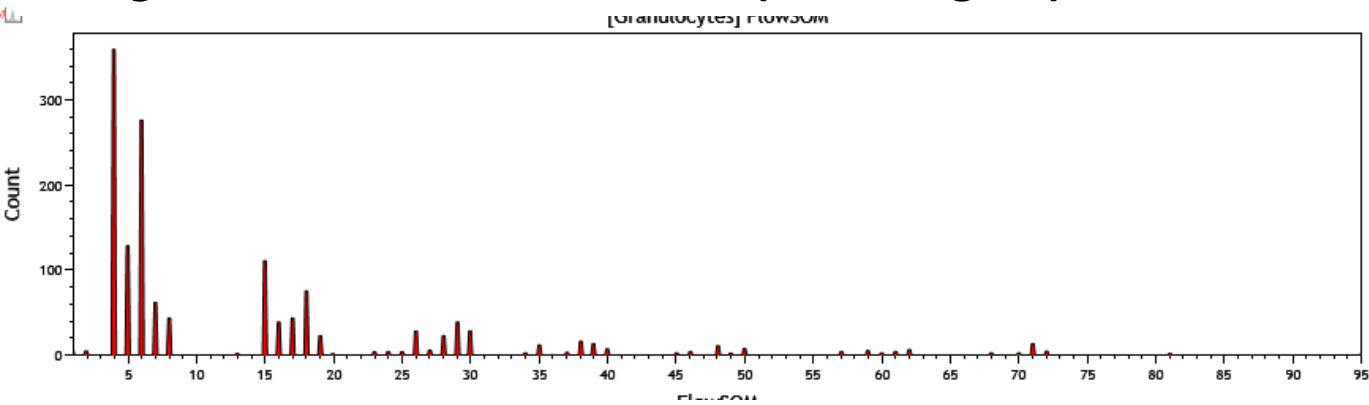
- Intérêt majeur par ex dans l'étude des LSC

FSOM 100 clusters

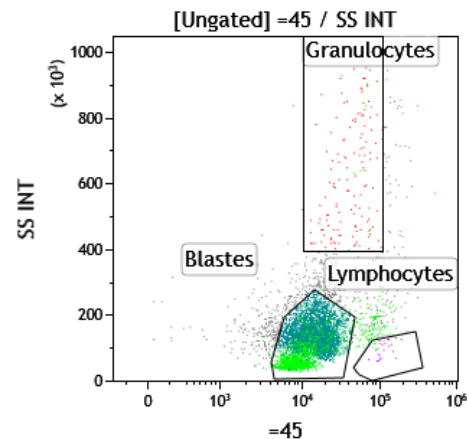
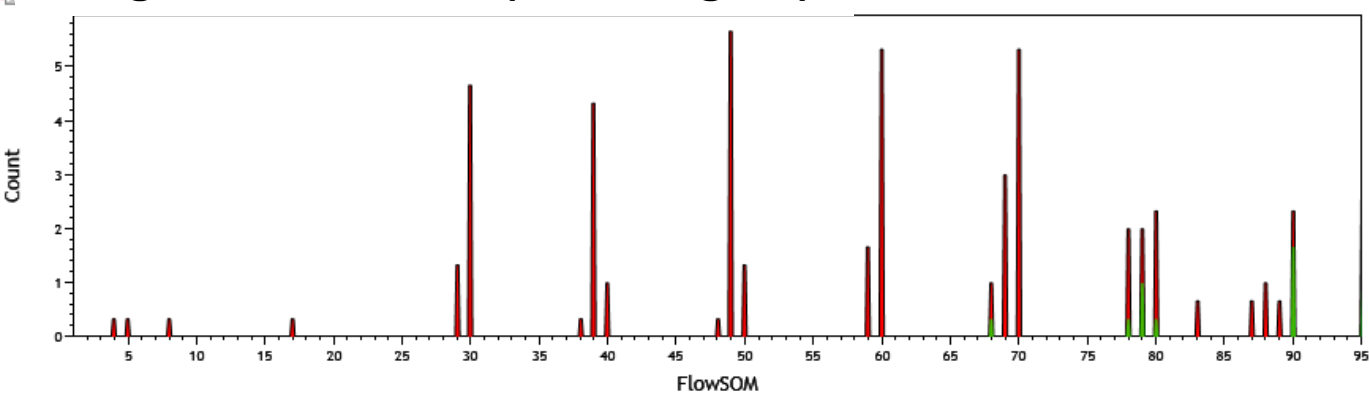
•Live cells (clusters gran)



•Progéniteurs CD34+ ou CD117+ (clusters gran)

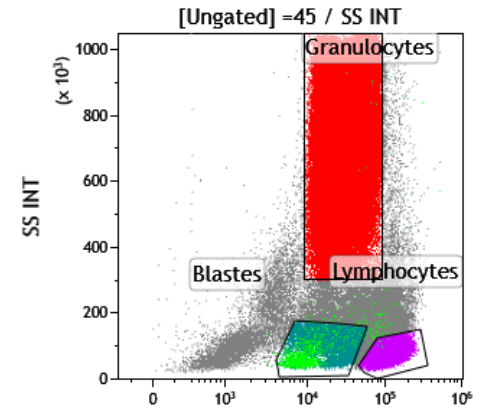
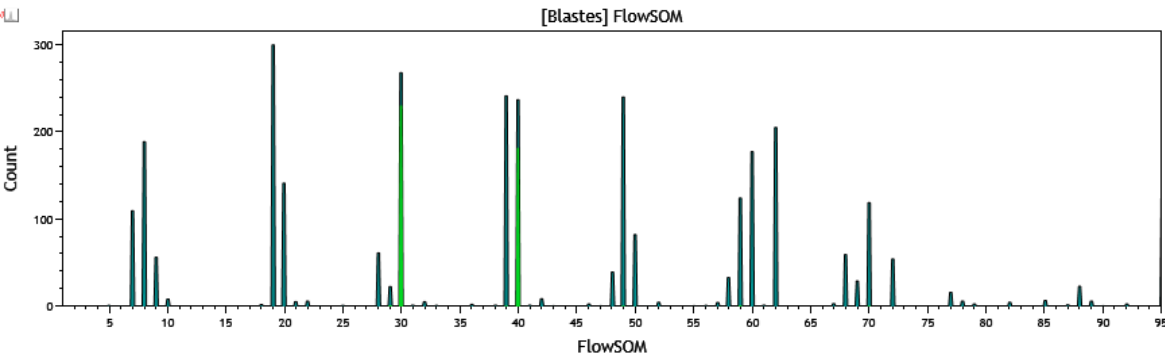


•Progéniteurs CD34+ (clusters gran)

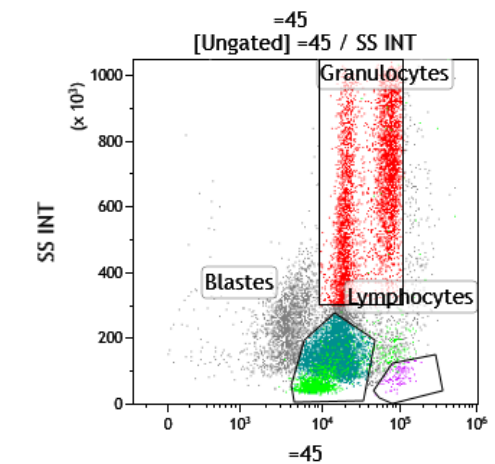
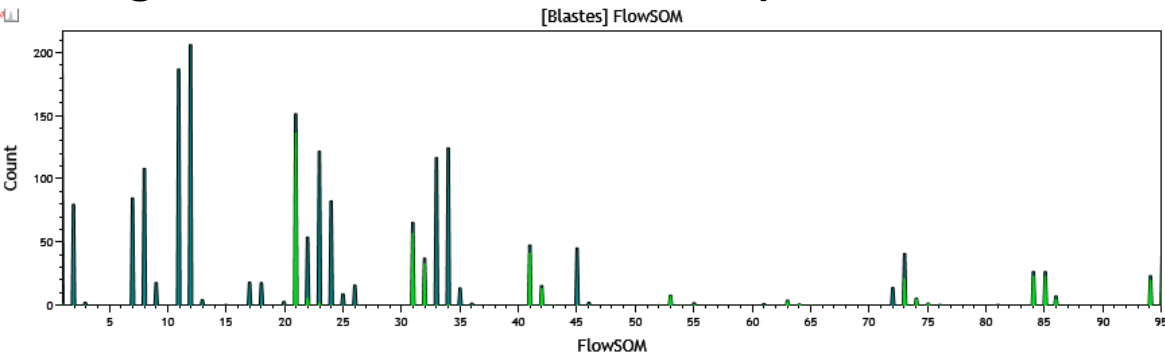


FSOM 100 clusters

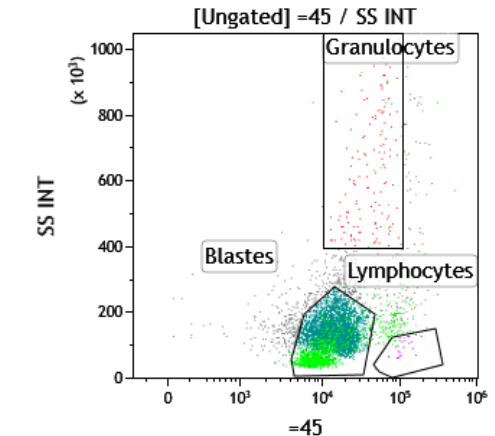
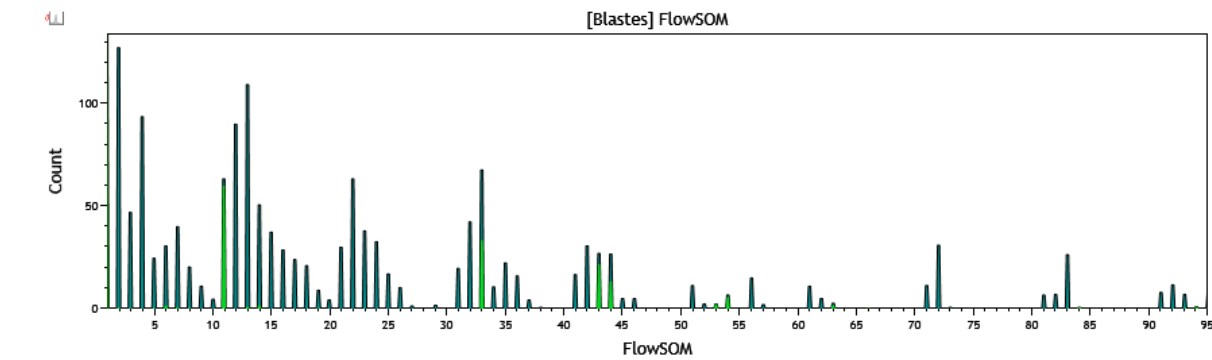
• Live cells (clusters « blastes »)



• Progéniteurs CD34+ ou CD117+ (clusters « blastes »)



• Progéniteurs CD34+ (clusters « blastes »)



➤ Comparer la MO du diagnostic avec le ou les points de suivi, et des MO de Références

Fichiers composés (merge)

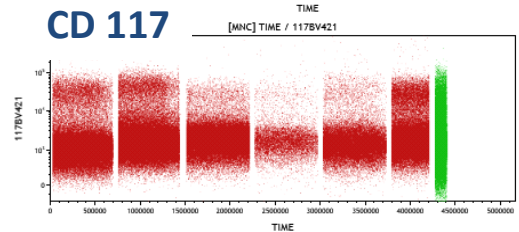
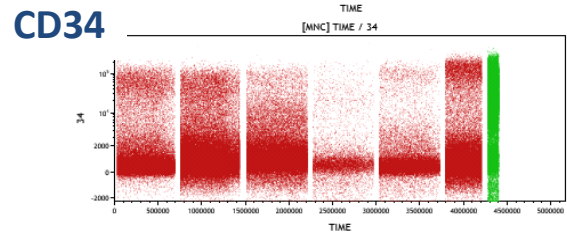
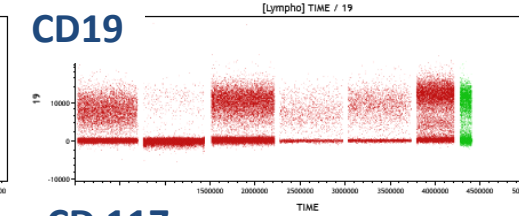
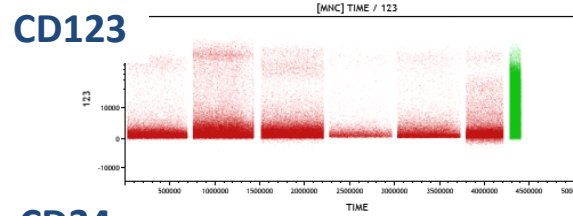
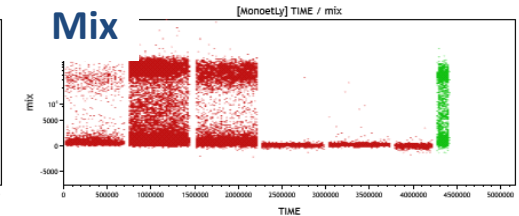
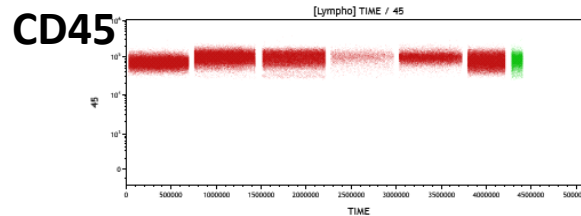
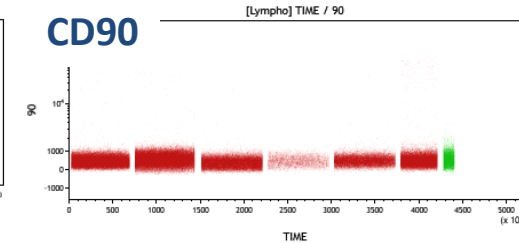
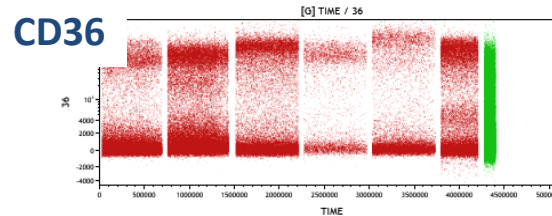
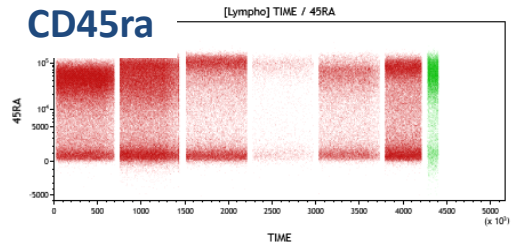
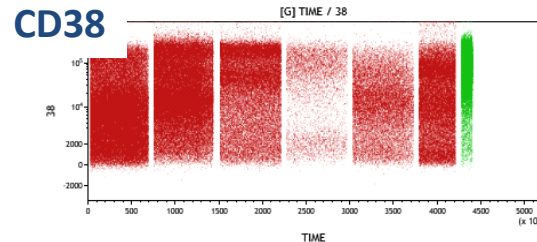
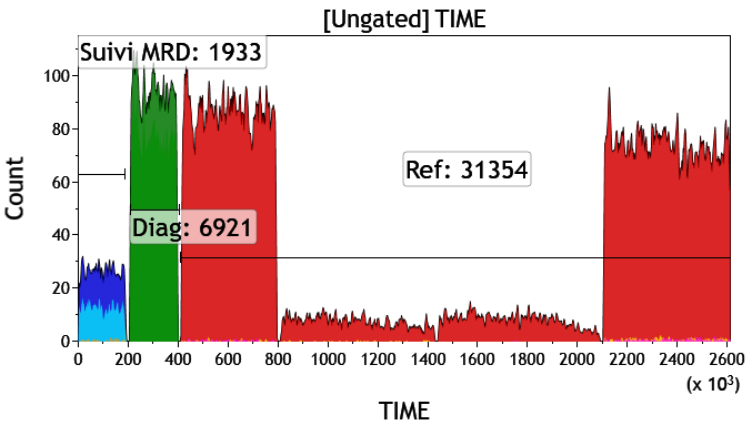
- Normalisation mathématique

Ou

- Harmonisation des sensibilités de chaque canal sur tous les fichiers dès l'acquisition

1 AML dg

Référence BM



Gating assisté par ordinateur

Point principaux conditionnant le résultats

Fichier simple

- Sélection de la population cellulaire à analyser (Intérêt de garder des cellules de fin de différenciation de lignée pour étendre la carte) en fonction du panel et de la question biologique
- Elimination de toutes contaminations (artéfact, amas plaquettes, doublons....)
- Vérification primordiale de l'ensemble des compensations (éviter surcompensations) problèmes des valeurs négatives
- Nombre de clusters demandés (49, 64, 81, 100...) : éviter suréchantillonnage ou sous échantillonnage

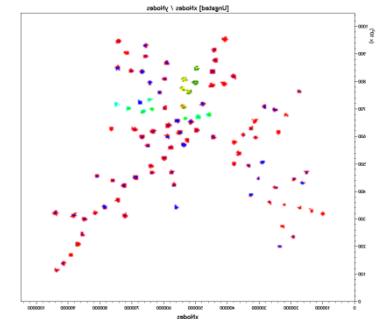
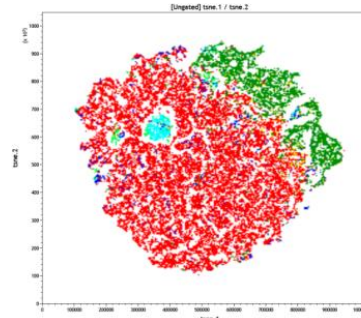
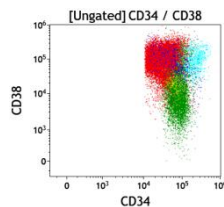
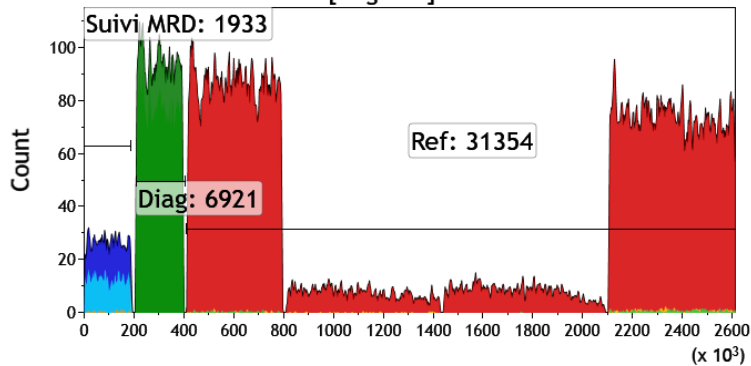
Fichiers composés (merge)

- ✓ Normalisation mathématique

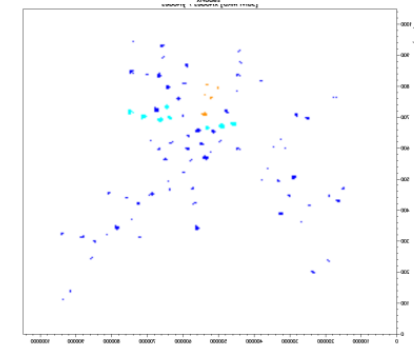
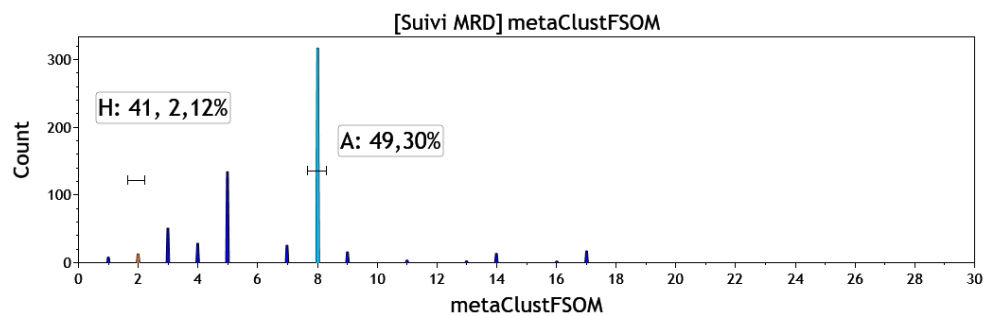
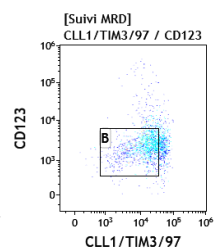
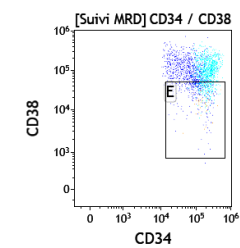
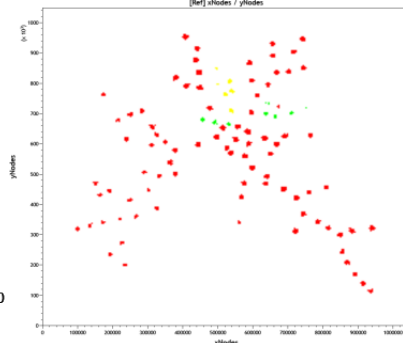
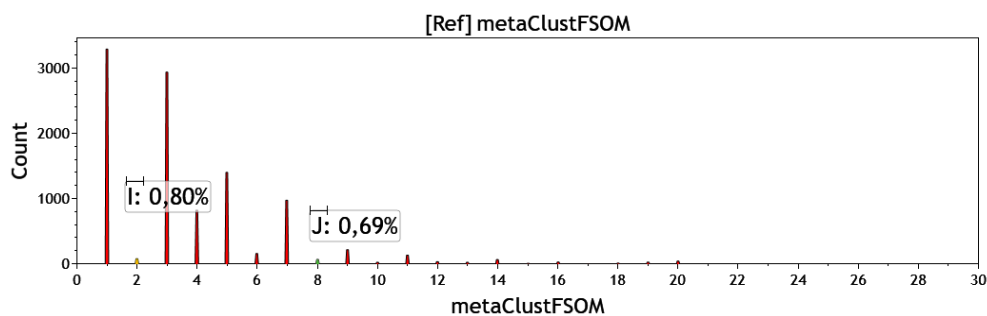
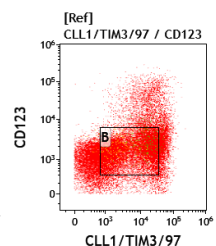
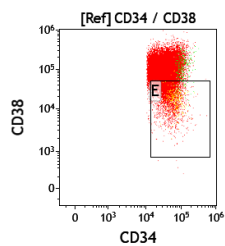
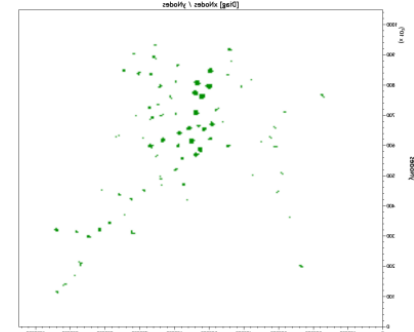
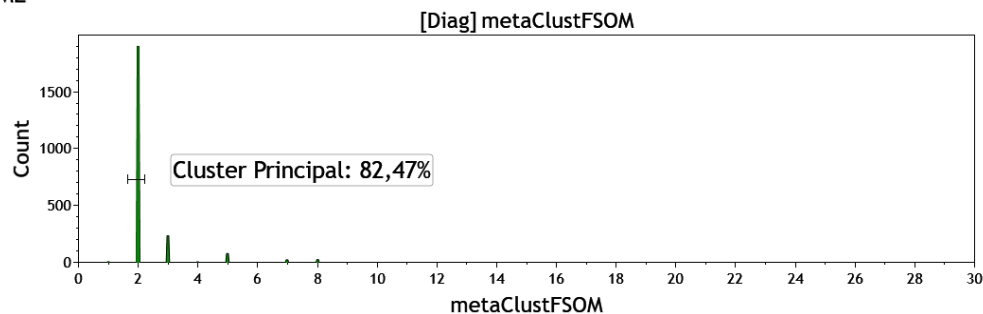
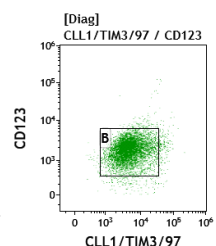
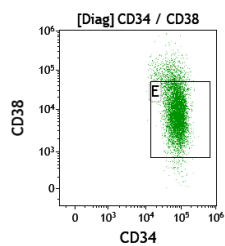
Ou

- ✓ Harmonisation des sensibilités de chaque canal sur tous les fichiers dès l'acquisition

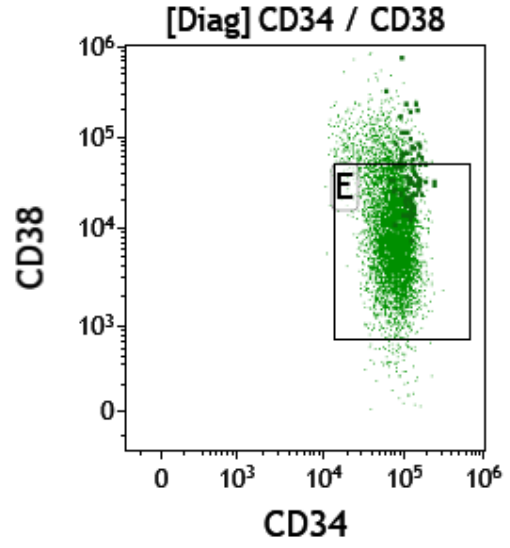
[Ungated] TIME



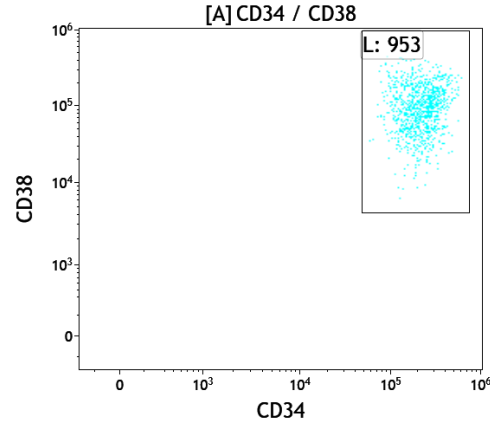
TIME



**LAM diag mutations:
IDH1 VAF 31%, DNMT3a VAF 35%**



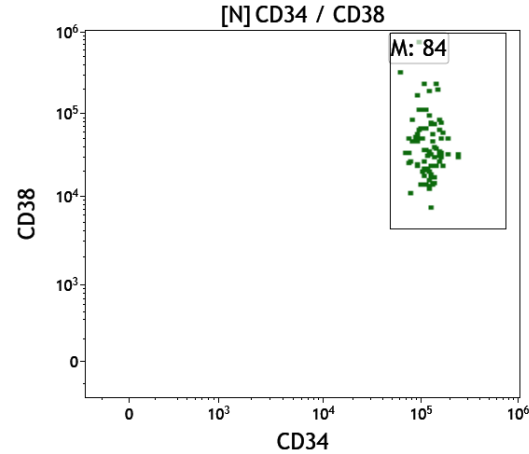
LAM suivi



**Suivi
IDH1 mut VAF 5%
DNMT3a VAF 4%
NIBL VAF 1%**

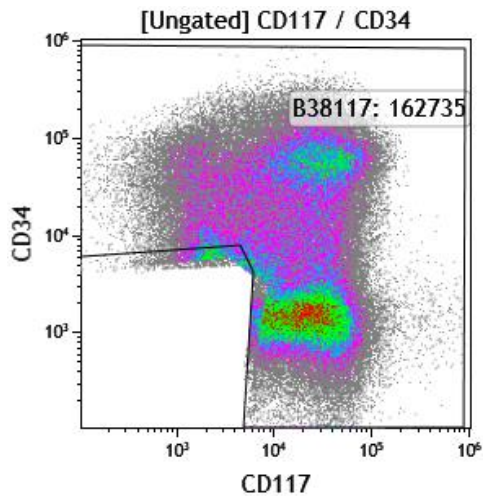
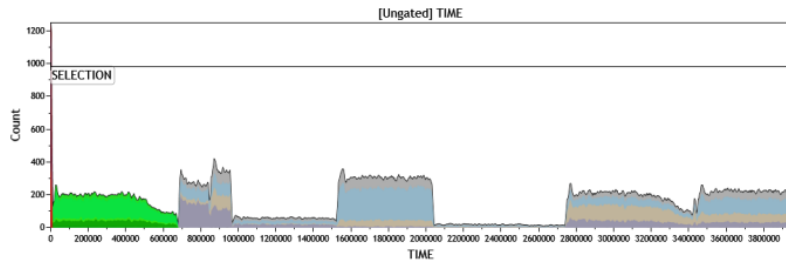
**49% des MNC CD34+, MNC34+ : 5,5% des leucocytes
MRD FS : 2,7%
MRD CD34+ CD38+ CD117+ Mix+ CD45ra+: 3,2%**

Shift ou sous clone?

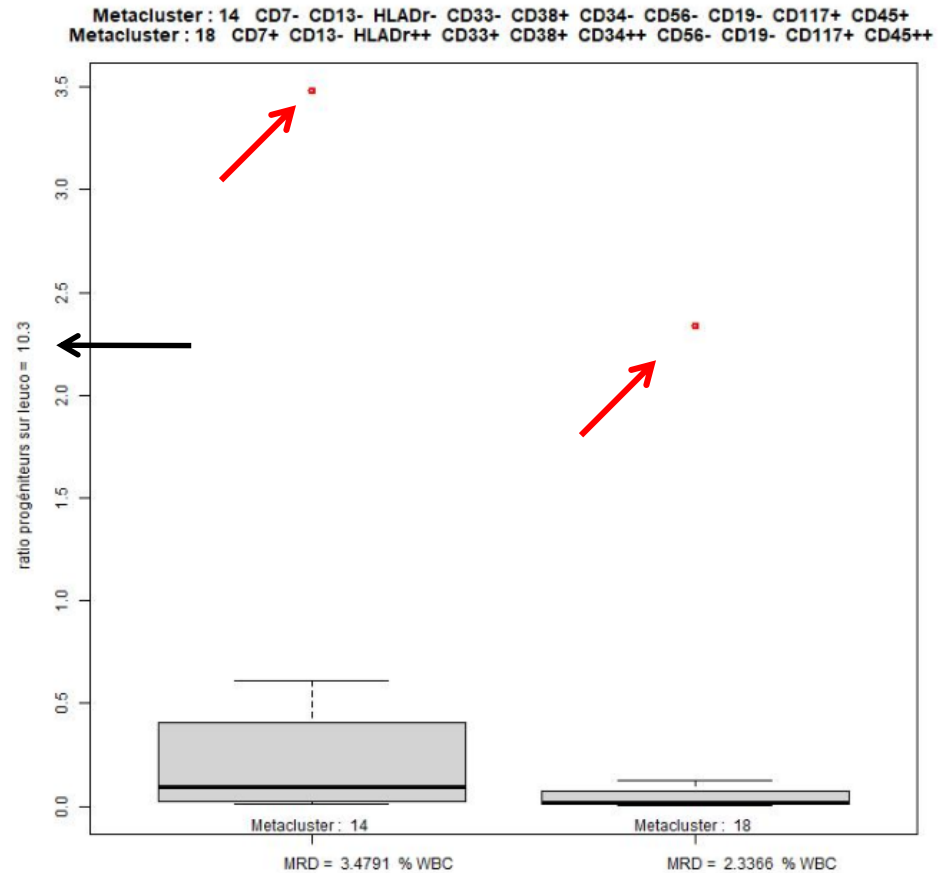


**Au diag: 1,2% des MNC CD34+
Soit 0,06% des WBC CD45+**

Analyse MRD, T1 fenêtre CD34+ et/ou CD117+

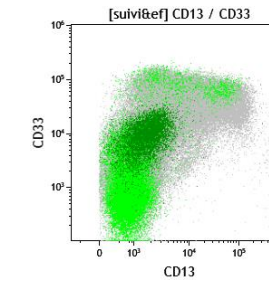
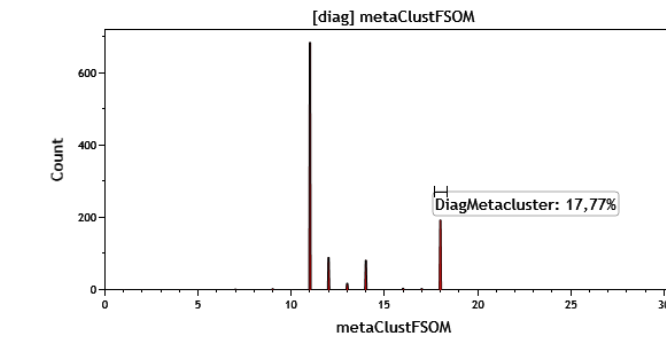
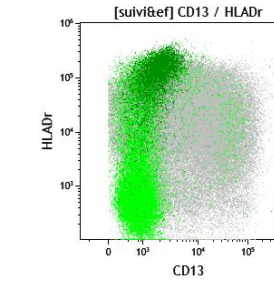
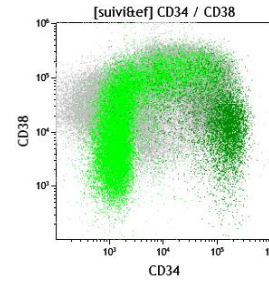
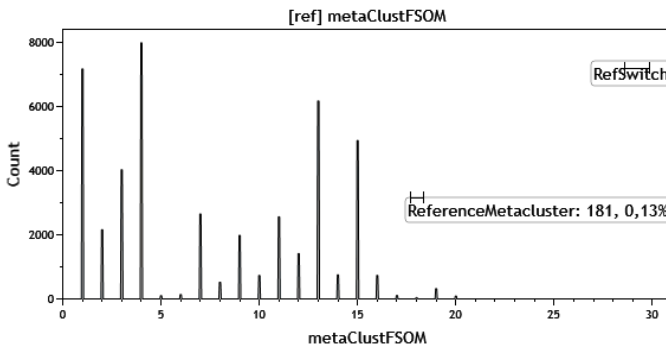
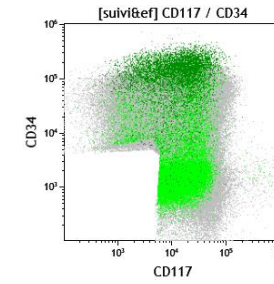
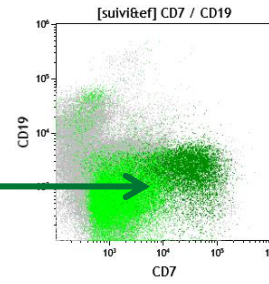
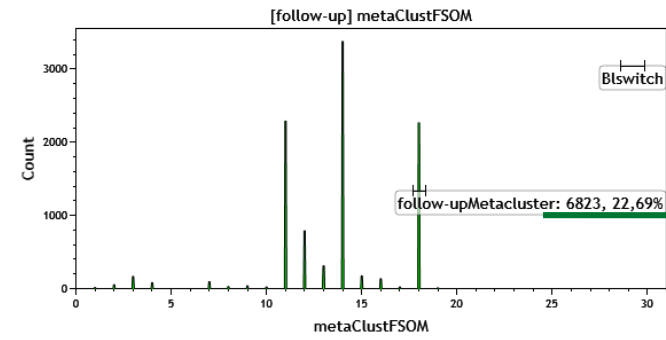


Script R



- 2 metaclusters observés avec une fréquence plus élevée dans MO de MRD que dans Moelle de référence : Métacluster 14 et Métacluster 18

- La fenêtre CD34+ ou CD117+ représente 10,3% des WBC45+ de la MO de suivi



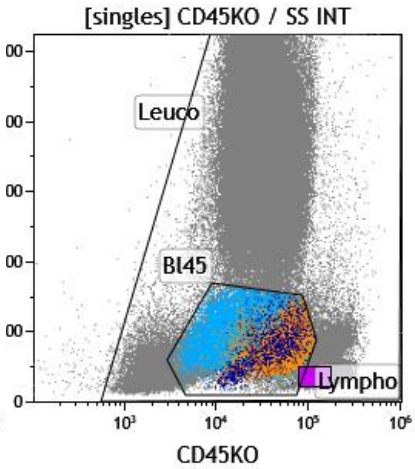
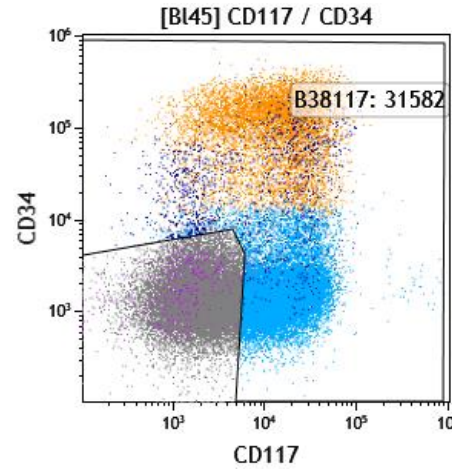
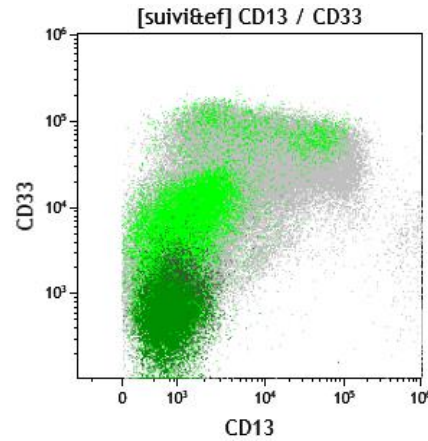
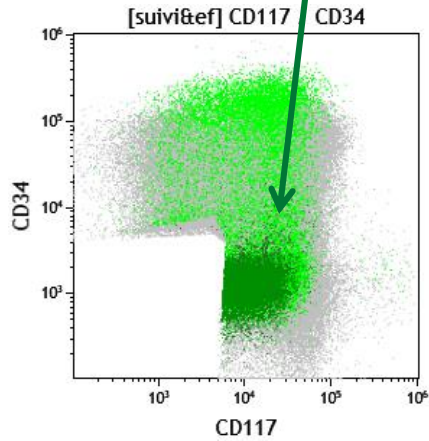
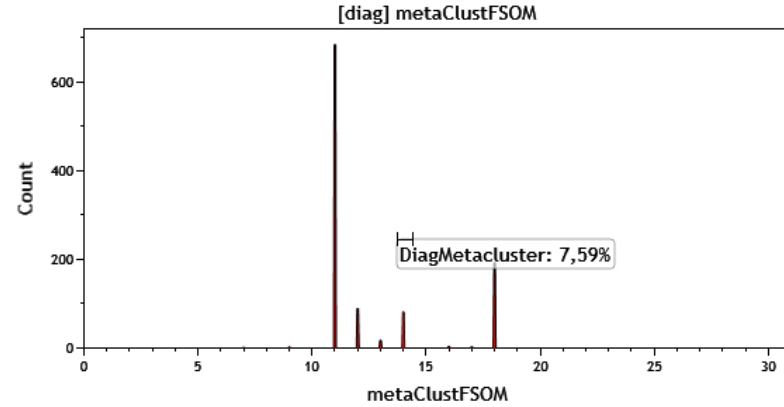
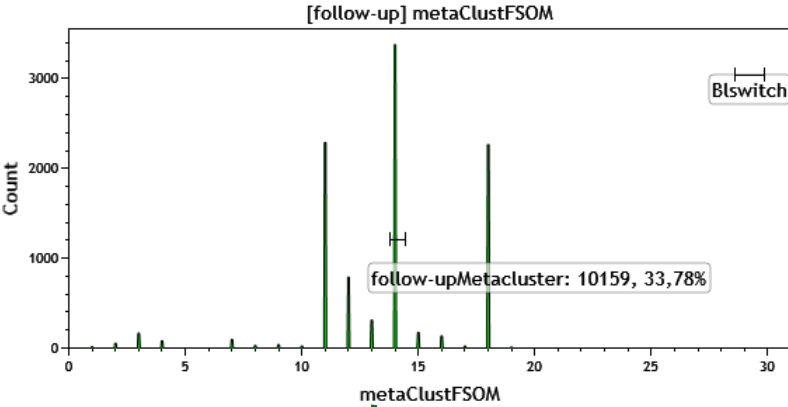
MRD flowsom: $0,22 \times 0,103 = 0,02$ soit 2%

MRD conventionnelle : CD34++ CD38- CD117+ CD13- CD33+ CD7+ HLADR+: 1,2%

Cluster 14 ???



Peu fréquent au diag!!!!

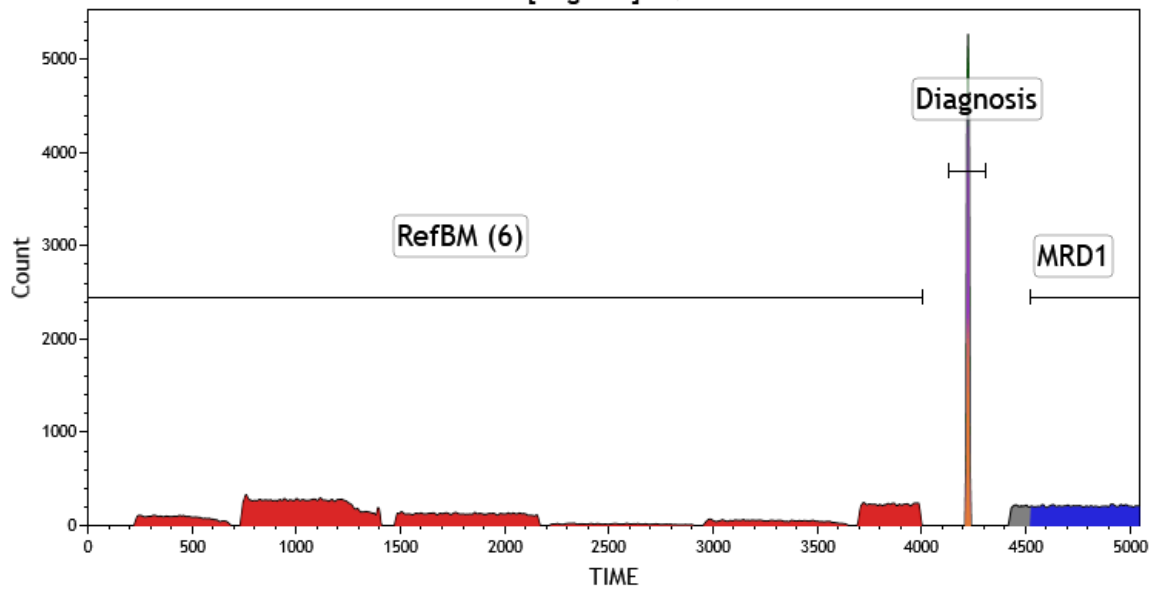


CD45 faible CD34- CD33- CD13-CD36+

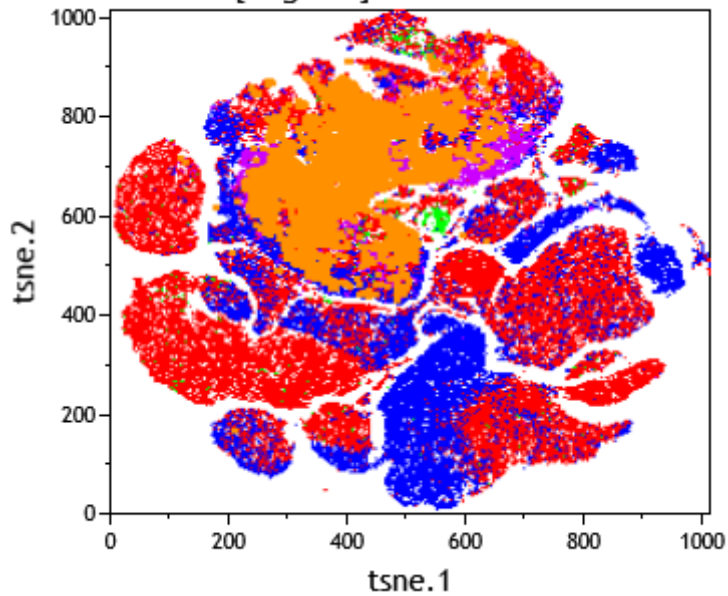


Proerythroblastes

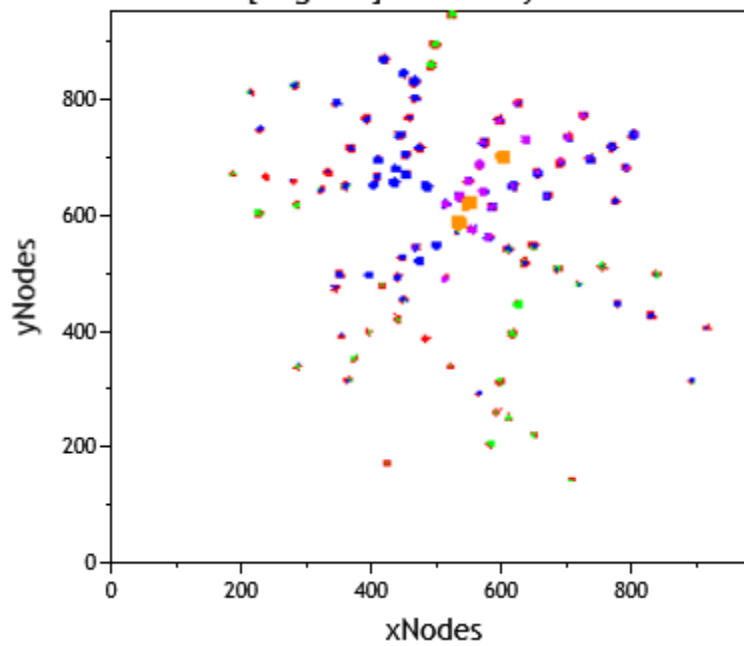
[Ungated] TIME

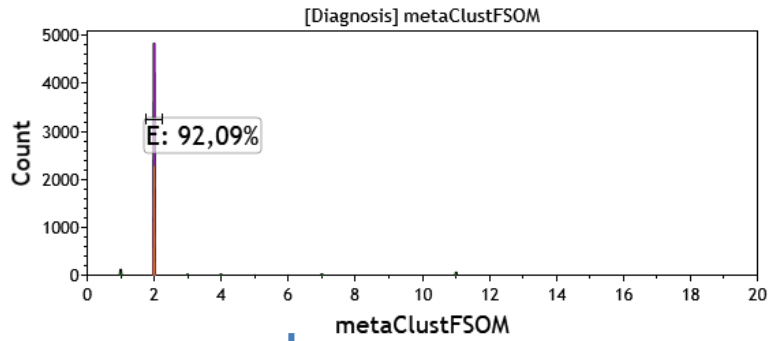
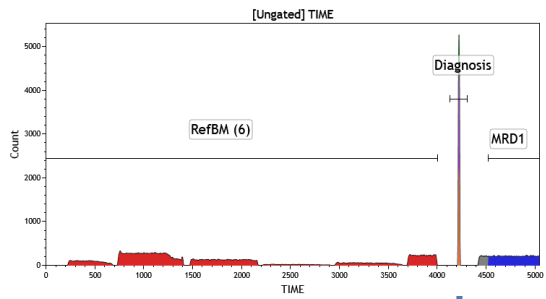


[Ungated] tsne.1 / tsne.2

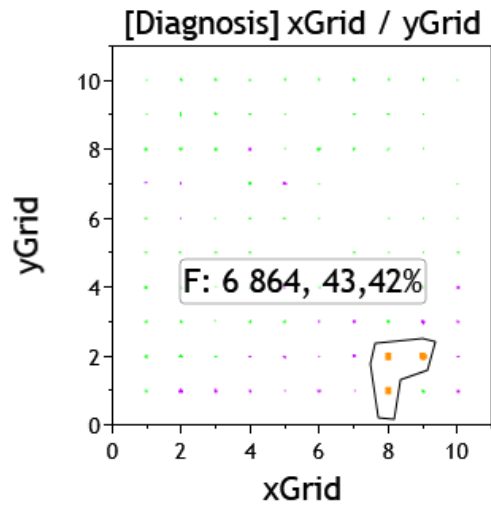
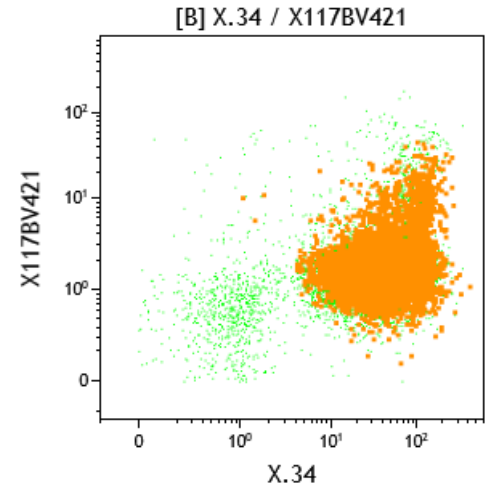
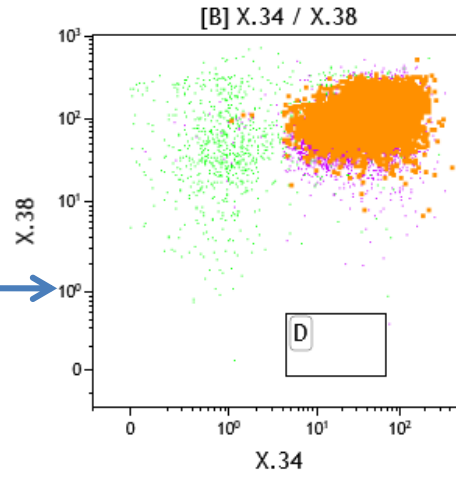


[Ungated] xNodes / yNodes



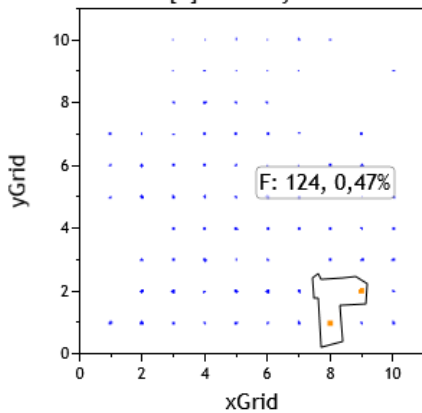


Diag

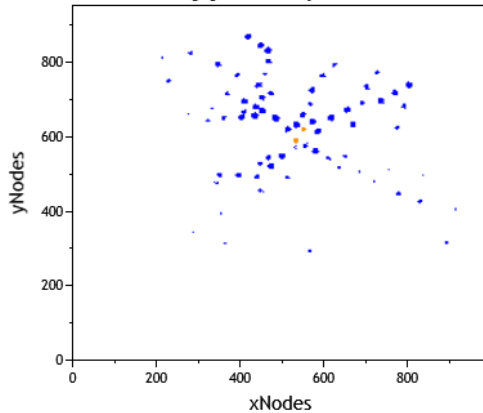


Patient- MRD1

[C] xGrid / yGrid

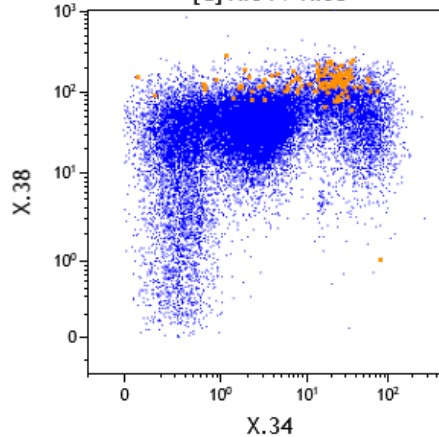


[C] xNodes / yNodes

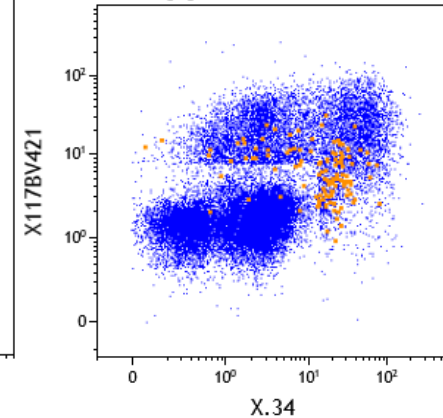


MRD1

[C] X.34 / X.38

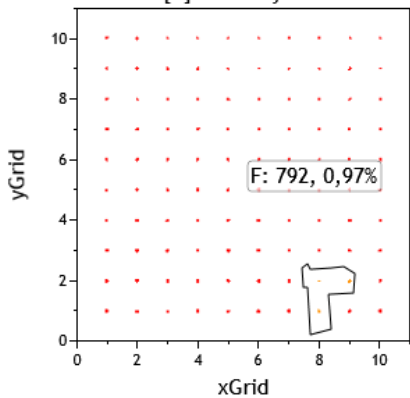


[C] X.34 / X117BV421

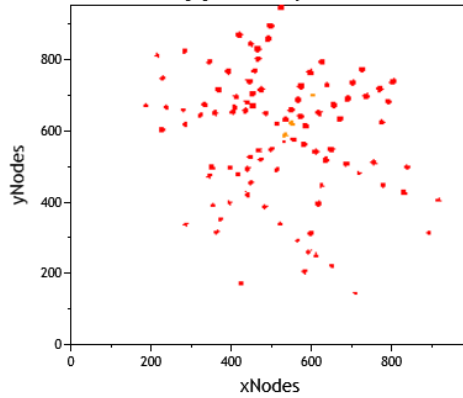


Reference BM

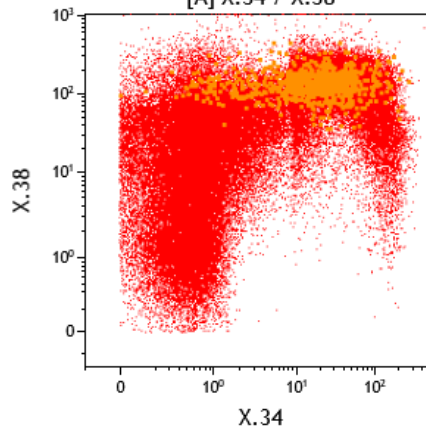
[A] xGrid / yGrid



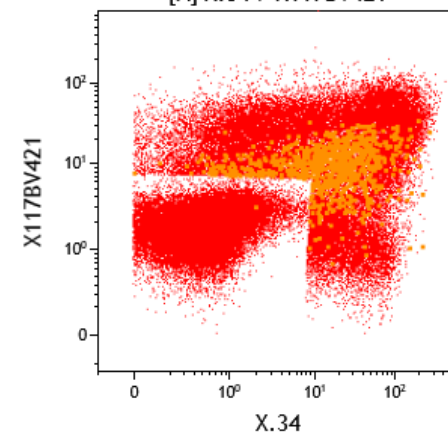
[A] xNodes / yNodes



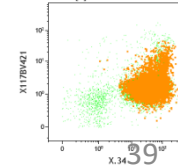
[A] X.34 / X.38



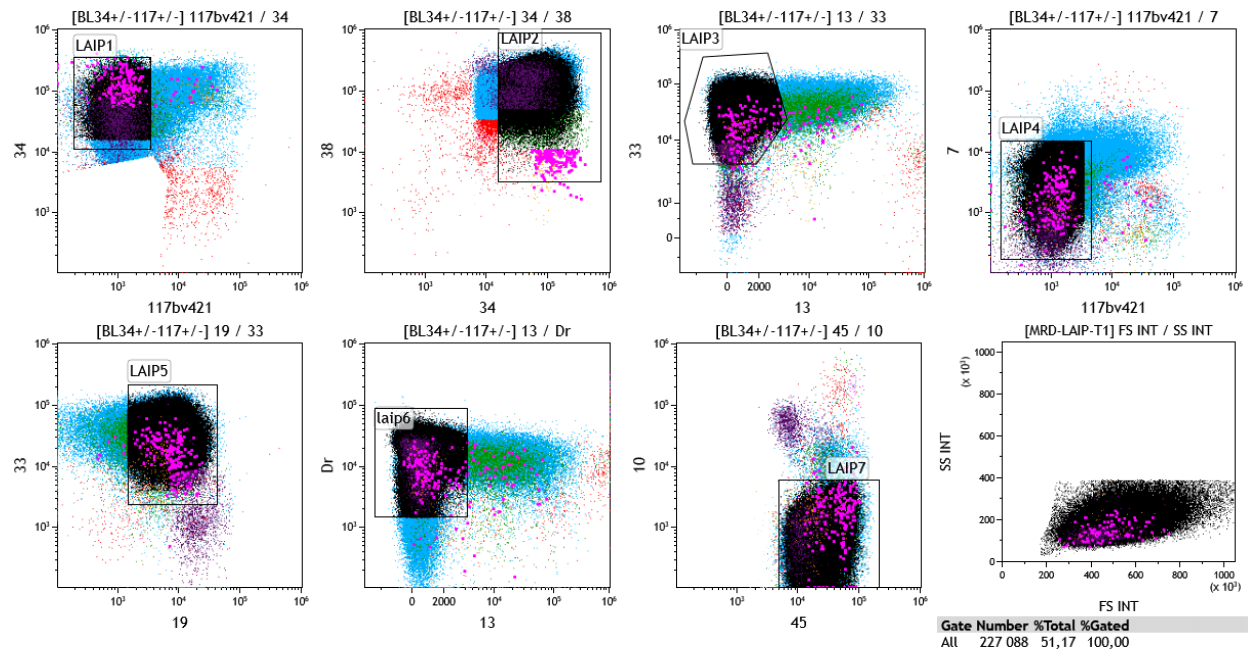
[A] X.34 / X117BV421



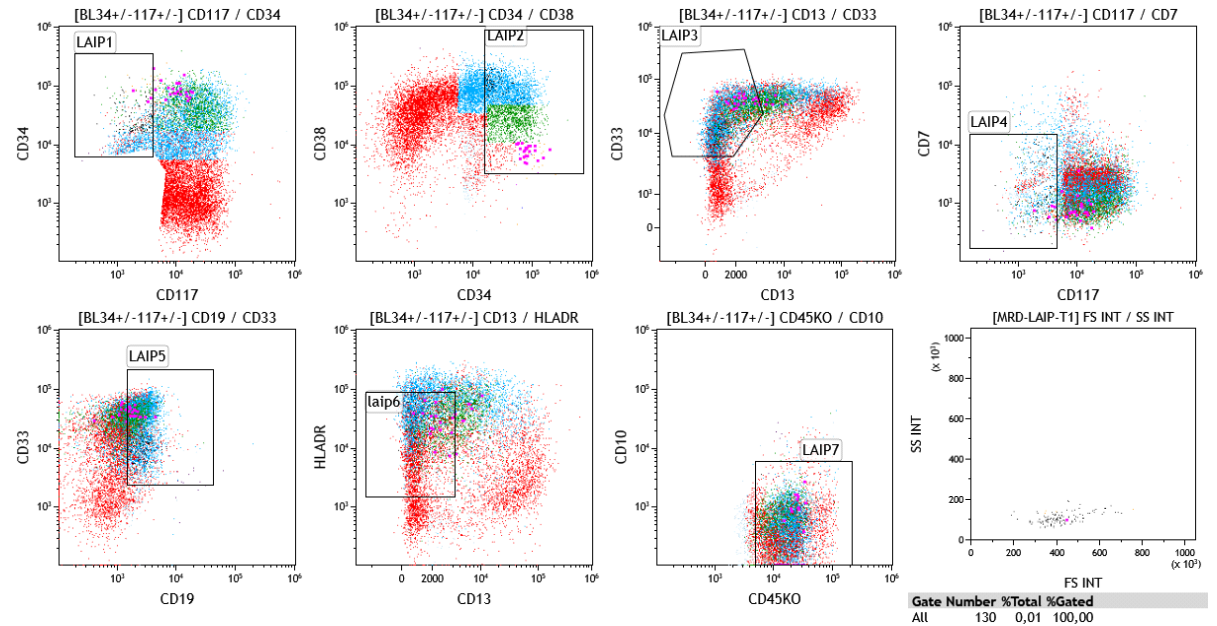
[B] X.34 / X117BV421



diag LAIP



MRD1 LAIP



Classical MRD Analyse:
MRD LAIP dif Norm: 2×10^{-4}

MRD LOD: 2×10^{-4}

RESULTATS LAIP

The unique cytometer ID specified by the user AN52248

Name of the data file containing the data set

Date of data set acquisition

purete medullaire CD34 % 22,92

[WBC45+] Number 848 011

[MNC34+] Number 7 130

[hematogones19+33-] Number 113

"Description LAIP" CD7 CD13 HLADR CD33 CD38 CD34 CD10 CD19 CD117 CD45KO

[MRD-LAIP-T1] Number 130

MRD-LAIP-T1 en % des WBC45+ 0,02

LOD en % pour 50ev (sans correction de dilution) 0,01

FlowSOM MRD Analyse :

Number WBC CD45 MRD1 : 781292

Number of 34+ ou CD117+ cells MRD1 : 12193

MRD in CD34+orCD117+: 0,47%

MRD among WBC CD45+: 7,3 exp-5

FlowSOM Analyse Reference BM:

Number WBC CD45 Ref BM: 2571130

Number of 34+ ou CD117+ cells MRD1 : 56553

LAIP in Ref BM CD34+orCD117+ cells : 0,97%

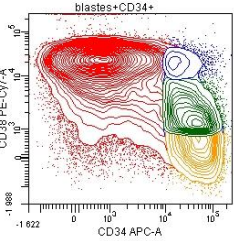
Sensitivity/LOD in WBC CD45+: 2,1 exp -4

Conclusion/Perspectives

- Clonal heterogeneity in AML (phenotype, genotype, fonction) improve LSCflow profile in CD34+CD38-
- New markers nHSC vs LSC definition: dg, pronostic, MRD
- GPR56: very good LSC marker at dg---testing stability for MRD LSC
- Using unsupervised analyse for standard vs New MRD LSC markers (FlowSom, TSNE)
- Using PDX, LSC17 scoring Nanostring in combination with new LSCflow

Jun 2020: 28 Flow Labs: 14 BD (Canto / Lyric) - 14 BC (Navios)

BIG Trial: ALFA & FILO



Flow Cytometric Labs ALFA Clinical Trial (18 Labs)

Lyon: Adriana Plesa, Delphine Manzoni

Lille: Christophe Roumier, Florent Dumezy

Paris St Louis: Stephanie Mathis, Anna Raimbault

Paris St Cloud: Valerie Bardet

Paris Creteil: Orianne Wagner Ballon

Paris Versailles: Victoria Raggiueneau

Paris IGR: Veronique Saada

Paris Pitie Salpetriere: Magali Le Garf Tavernier

Paris Bobigny: Remi Letestu

Paris St Antoine: Frederic Feger

Lille St Vincent: Agnes Charpentier

Amiens: Veronique Harrivel

Rouen: Elsa Bera

Caen: Veronique Salaun, Edouard Cornet

Dijon: Julien Guy

Marseille CHU: Isabelle Arnoux

Limoges: Jean Feuillard, Estelle Guerin

Pontoise: Stéphanie Barriere

Valenciennes: Claire Hemar

REMERCIEMENTS

Clinical coordinators: **Hervé Dombret**, Cristian Recher

Biological coordinator: **Claude Preudhomme**

ALFA coordinator: **Karine Celli-Lebras**, Renaud Bufet

Yves Bertrand, IHOP, Lyon

Xavier Thomas, CHLS, Lyon

Flow Cytometric Labs

Part of FILO Clinical Trial (10 Labs)

Toulouse: Francois Vergez

Marseille IPC: Anne Catherine Lhoumeau

Angers: Franck Geneviève

Paris Cochin: Nicolas Chapuis

St Etienne: Lydia Campos, Jeremie Stagnara

Grenoble: Marie Christine Jacob, Tatiana

Raskovalova

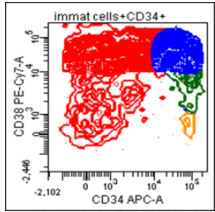
Clermont Ferrand: Richard Veyrat Masson

Rennes: Mikael Roussel

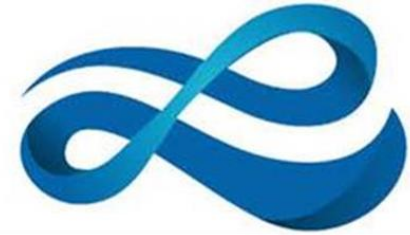
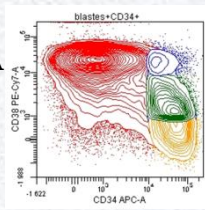
Moulhouse: Agathe Debliquis

Strasbourg: Caroline Mayeur-Rousse

AFC 4-6 Novembre 2020



Flow AML MRD Intergroup ALFA



AML MRD LSC
French Flow Intergroup

MERCI à TOUS!!!

AFC 4-6 Novembre 2020