AML MRD by multiparameter flow cytometry using LAIP and LSC: Methodological aspects in a multicentric study of the French AML Intergroup

Adriana PLESA , Lyon , France

ESCCA, Thessaloniki, Greece, 27 Septembre 2017

Hôpitaux de Lyon

CIGAL
Collaborative InterGroup for Acute Leukemia

Centre Hospitalier Régional Universitaire de Lille

Map of the centers involve on French AML Clinical Trial

AML Clinical Centers: 54

Patients inclusion		Nb of center
	45%	9
	14%	5
48%		
	34%	25
7%	7%	15

AML MRD flow

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?

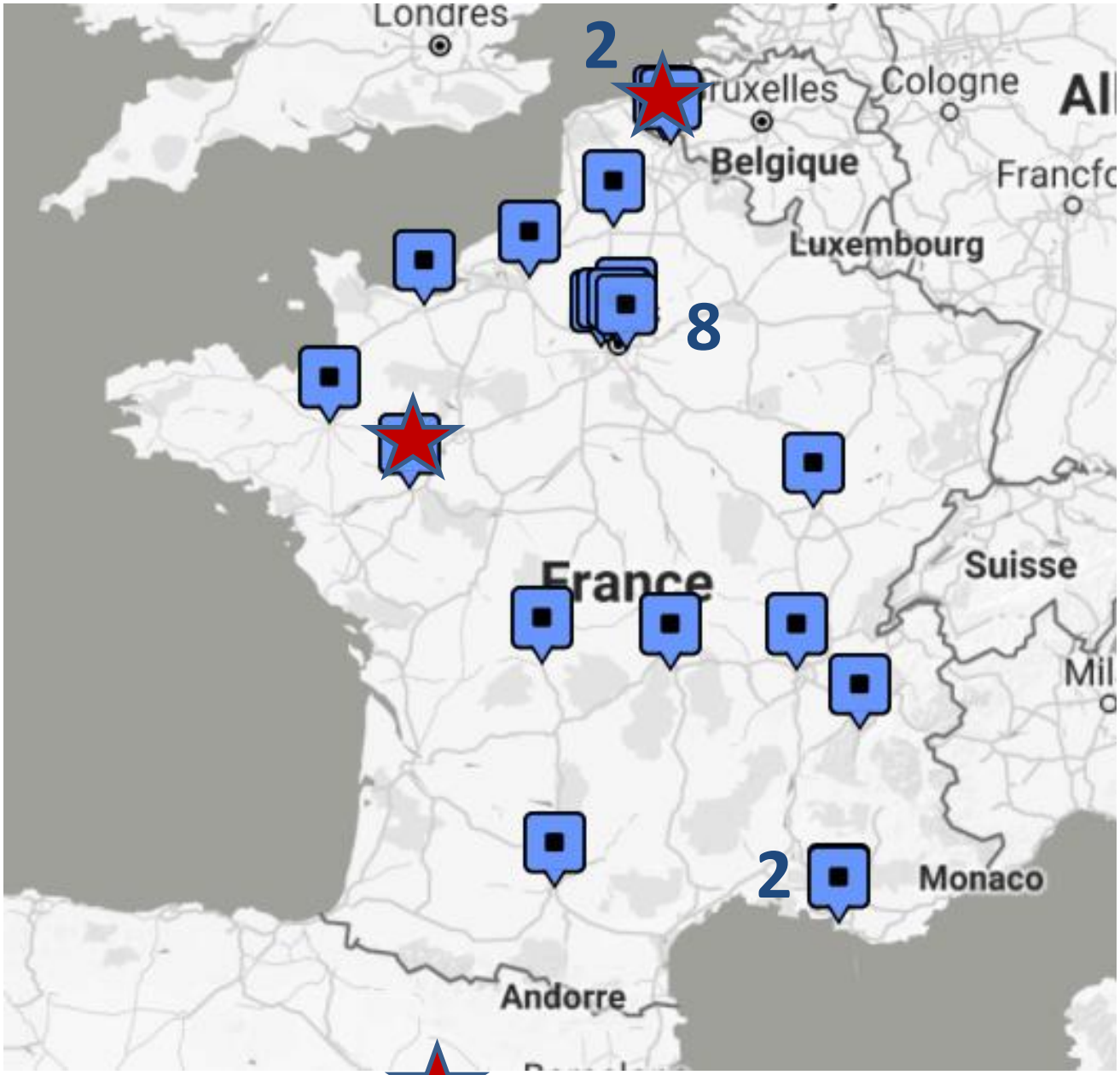
Methodology multicentric approach:

- panel design: simple, reproductible, sensitivity, cut
- CANTO vs NAVIOS « mirroir »
- gating strategy

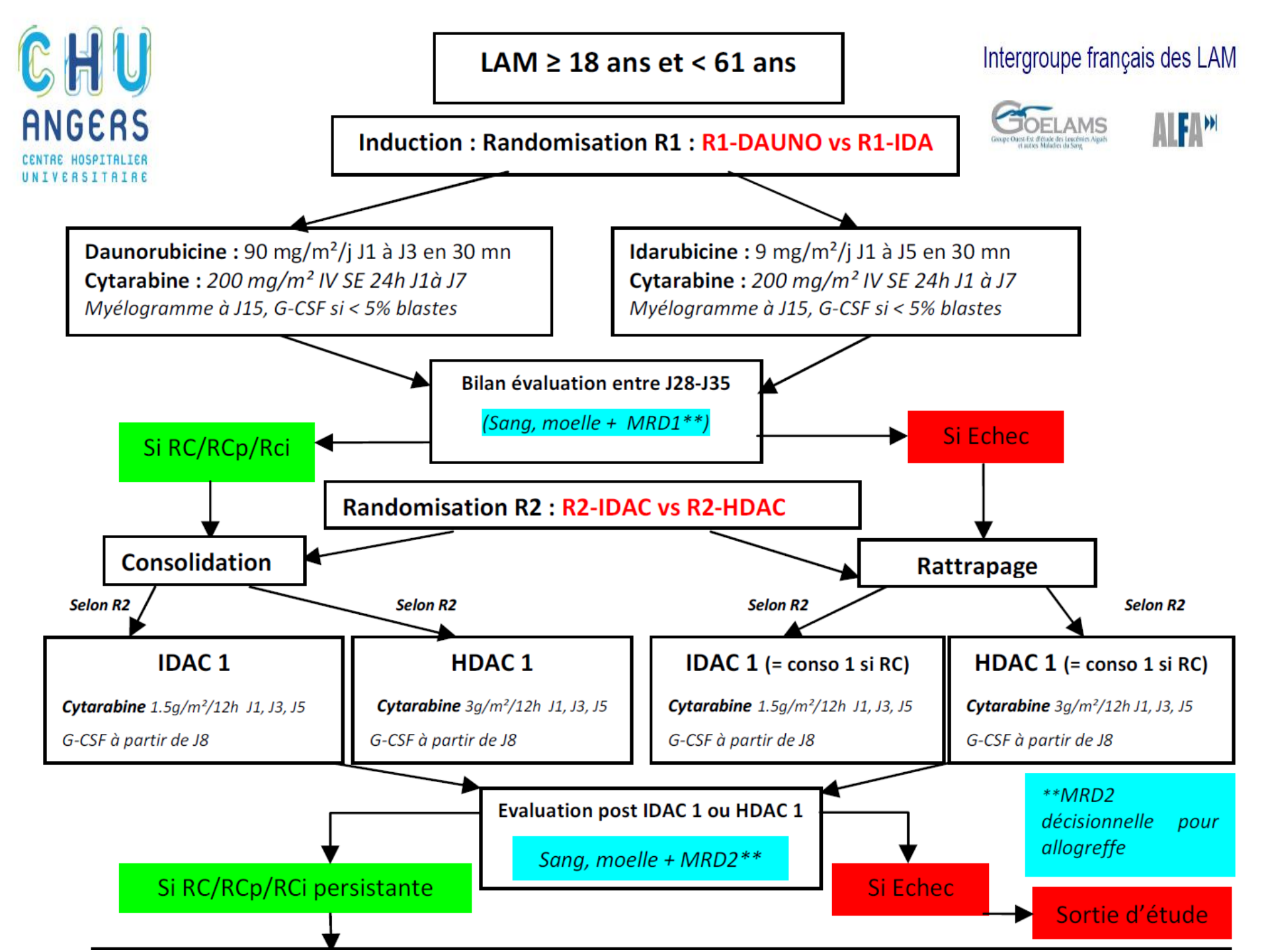
3) Ready to start

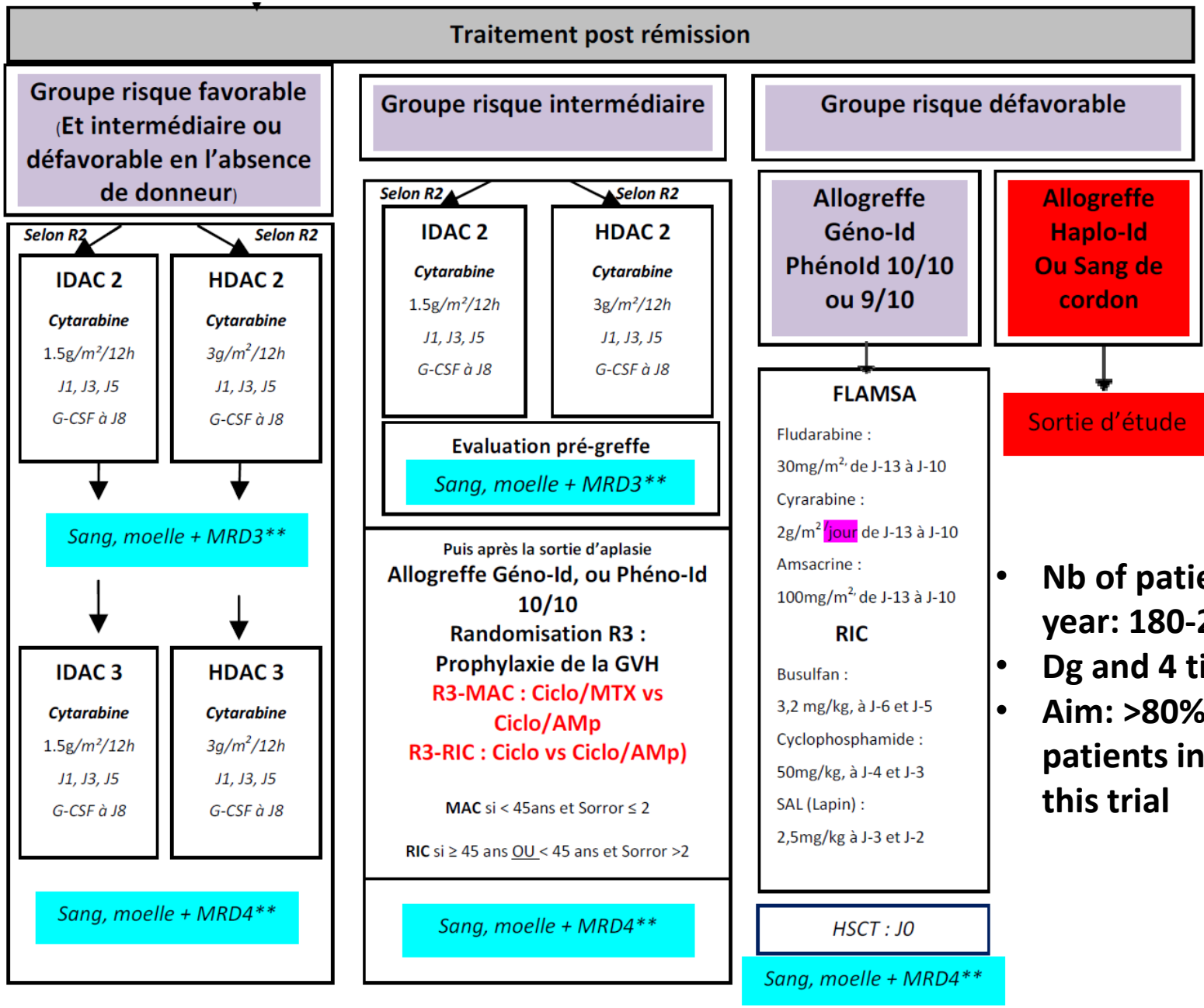
- CQA: verify gating strategy
- CQE: global check of panel implementation (n/reg BM)
- Patient follow up

25 Flow Labs: 13 BD (Canto / Lyric) - 12 BC (Navios)



2 Molecular Labs


LAM ≥ 18 ans et < 61 ans
Intergroupe français des LAM
Induction : Randomisation R1 : R1-DAUNO vs R1-IDA
Daunorubicine : 90 mg/m²/j J1 à J3 en 30 mn
Cytarabine : 200 mg/m² IV SE 24h J1à J7
Myélogramme à J15, G-CSF si < 5% blastes
Idarubicine : 9 mg/m²/j J1 à J5 en 30 mn
Cytarabine : 200 mg/m² IV SE 24h J1 à J7
Myélogramme à J15, G-CSF si < 5% blastes
Bilan évaluation entre J28-J35
(Sang, moelle + MRD1**)
Si RC/RCp/Rci
Si Echec
Randomisation R2 : R2-IDAC vs R2-HDAC
Consolidation
Rattrapage
Selon R2
Selon R2
Selon R2
Selon R2
IDAC 1
Cytarabine 1.5g/m²/12h J1, J3, J5
G-CSF à partir de J8
HDAC 1
Cytarabine 3g/m²/12h J1, J3, J5
G-CSF à partir de J8
IDAC 1 (= conso 1 si RC)
Cytarabine 1.5g/m²/12h J1, J3, J5
G-CSF à partir de J8
HDAC 1 (= conso 1 si RC)
Cytarabine 3g/m²/12h J1, J3, J5
G-CSF à partir de J8
Evaluation post IDAC 1 ou HDAC 1
Sang, moelle + MRD2**
**MRD2 décisionnelle pour allogreffe
Si RC/RCp/RCi persistante
Si Echec
Sortie d'étude



- **Nb of patients / year: 180-200**
- **Dg and 4 time MRD**
- **Aim: >80% of patients included in this trial**

*** : MRD centralisée (ALFA ou GOELAMS) pour NPM1mut (en local pour les autres marqueurs)*

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

Hartmut Döhner,[1] Elihu Estey,[2] David Grimwade,[3] Sergio Amadori,[4] Frederick R. Appelbaum,[2] Thomas Büchner,[5] Hervé Dombret,[6] Benjamin L. Ebert,[7] Pierre Fenaux,[8] Richard A. Larson,[9] Ross L. Levine,[10] Francesco Lo-Coco,[4] Tomoki Naoe,[11] Dietger Niederwieser,[12] Gert J. Ossenkoppele,[13] Miguel Sanz,[14] Jorge Sierra,[15] Martin S. Tallman,[10] Hwei-Fang Tien,[16] Andrew H. Wei,[17,18] Bob Löwenberg,[19] and Clara D. Bloomfield[20]

Monitoring of MRD. Two approaches can be used to detect MRD, that is, multiparameter flow cytometry (MFC) and molecular techniques, including real-time quantitative PCR (RT-qPCR), digital PCR, and next-generation sequencing–based technologies. Standardized RT-qPCR assays are now available to detect AML-associated genetic lesions (Table 4). Each methodology

Applicability **Sensitivity** **Applicability** **Sensitivity**

Table 6. Response criteria in AML

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)

New paradigm in MRD LAM: LSC approach

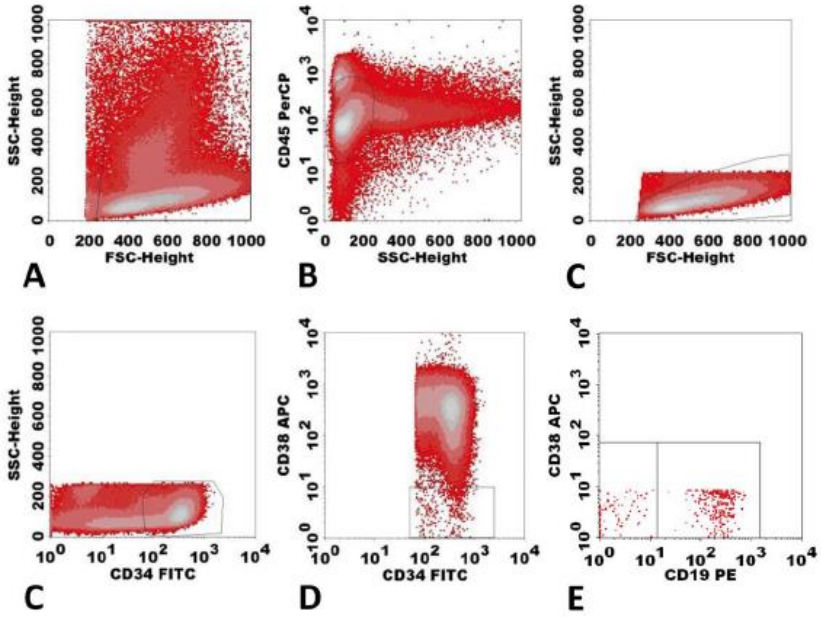
OPEN ACCESS Freely available online

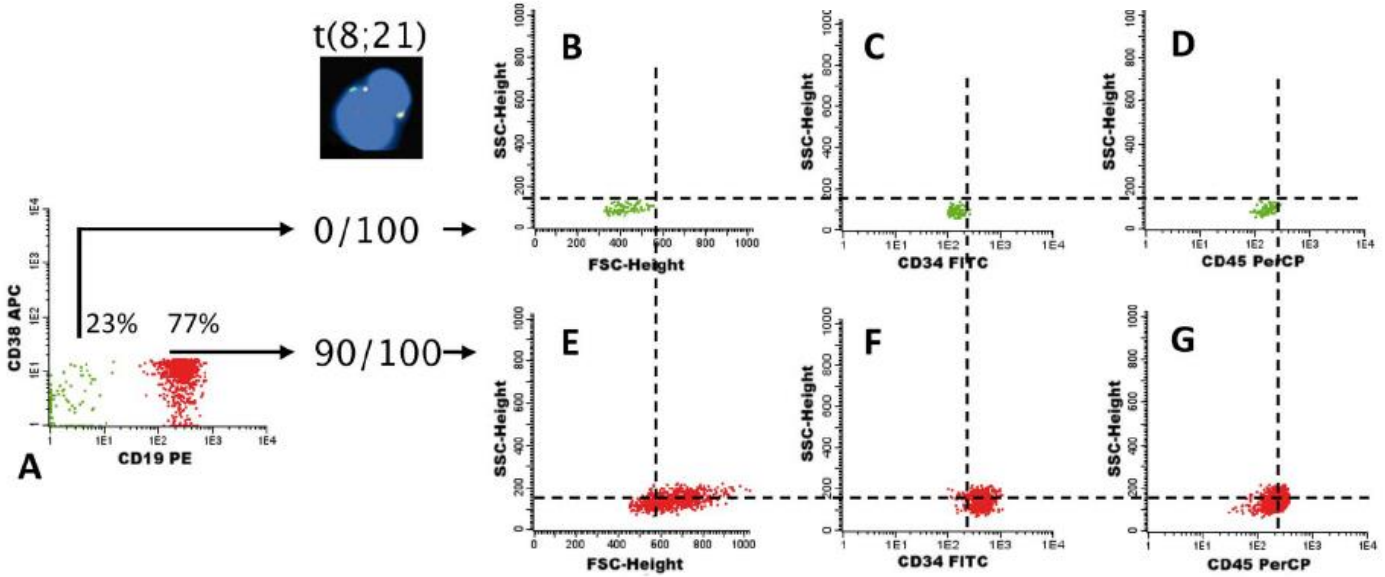
PLOS ONE

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

Monique Terwijn[1], Wendelien Zeijlemaker[1], Angèle Kelder[1], Arjo P. Rutten[1], Alexander N. Snel[1], Willemijn J. Scholten[1], Thomas Pabst[2], Gregor Verhoef[3], Bob Löwenberg[4], Sonja Zweegman[1], Gert J. Ossenkoppele[1], Gerrit J. Schuurhuis[1]*

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

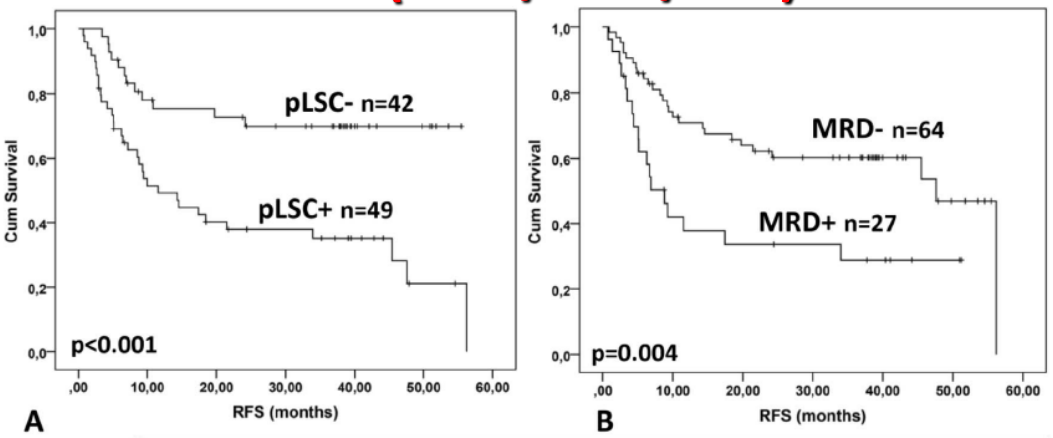


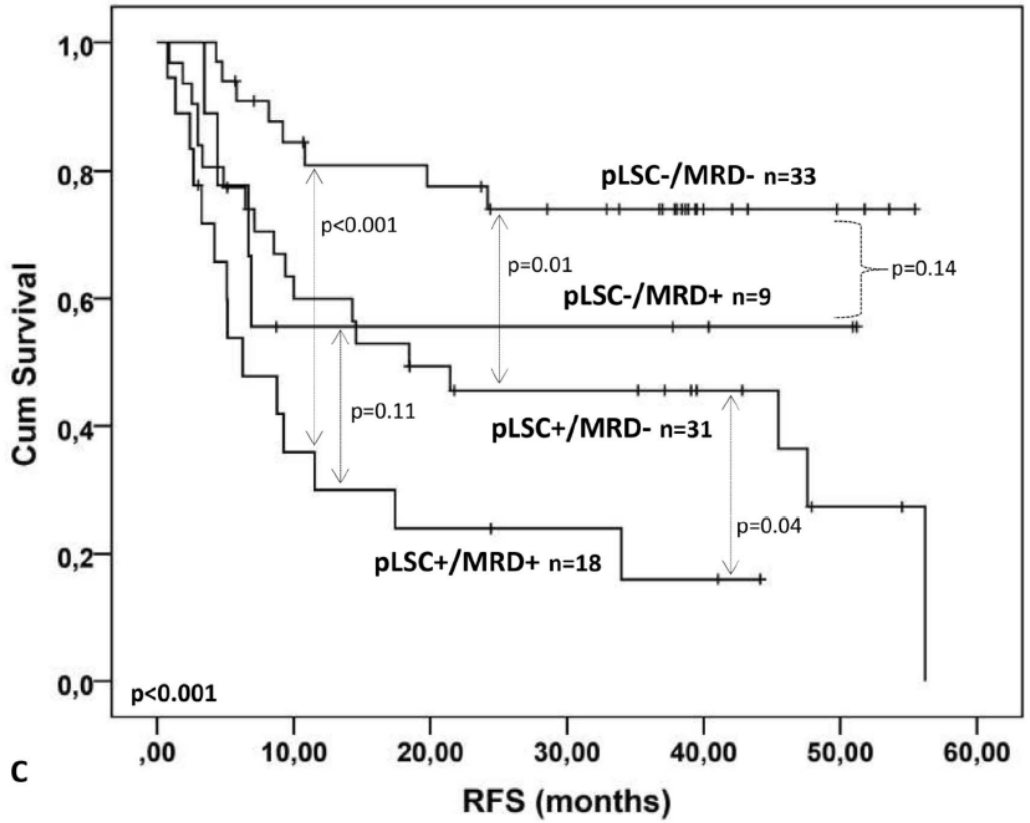


nHSC

LSC

New concept of AML follow up: Scoring of MRDflow (LAIP/DFN/LSC)





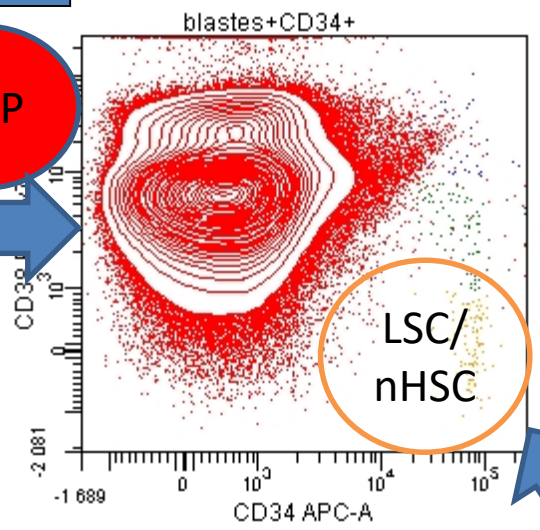
M Terwijn, PLOSOne 2014

AML dg CN, NPM1+FLT3ITD-

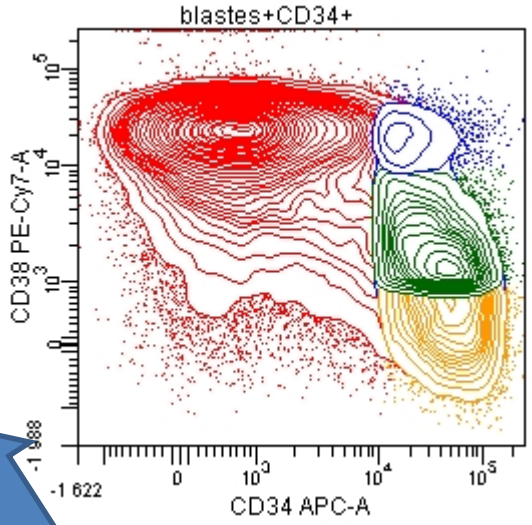
CN, NPM1+FLT3ITD+

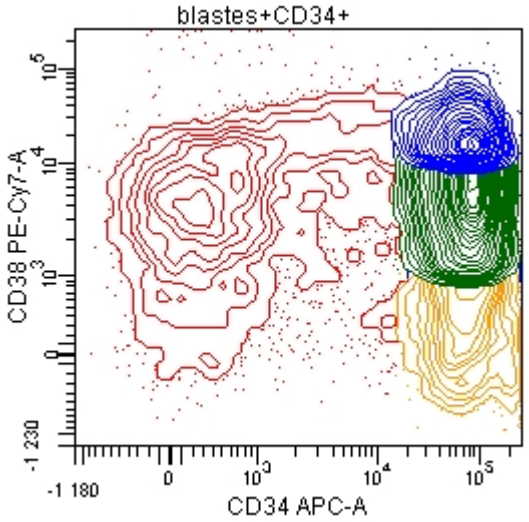
+8, del20q-,NPM1-FLT3ITD+

LAIP



LSC/
nHSC

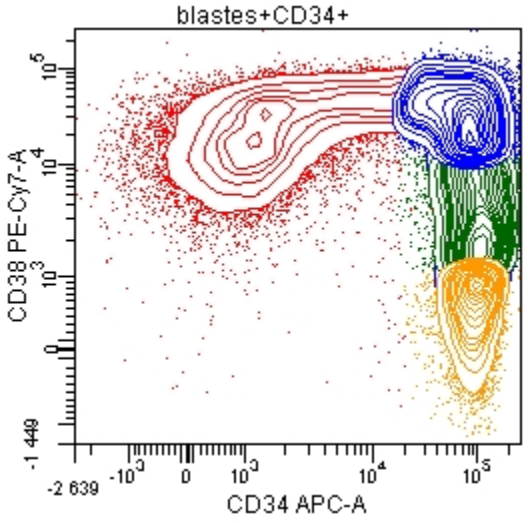


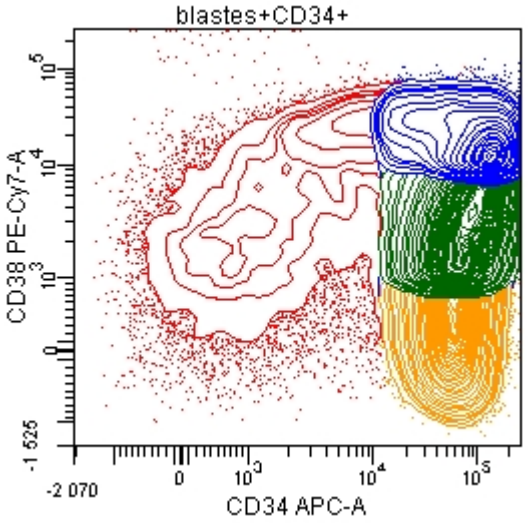


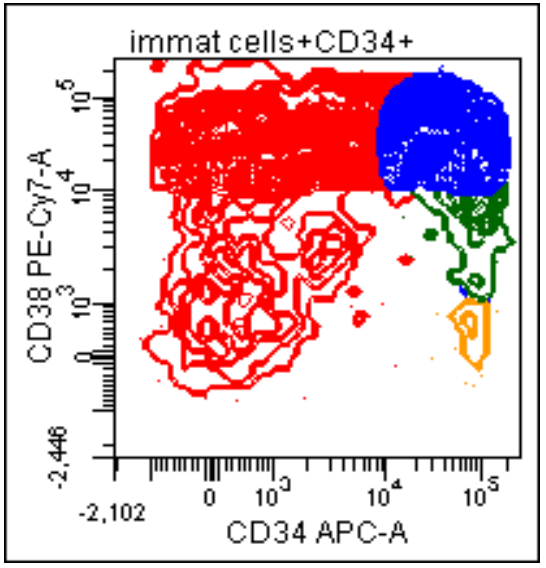
Caryotype CX>5, WT1+, dupMLL

EVI1+, del7q

nBM







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- ***CANTO vs NAVIOS « mirroir »***
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MRD Flow panel design rational

Aim: **simple, reproductible, sensitivity, cost**

1) **LAIP approach** *(Leukemia Associated ImmunoPhenotype)- at diagnosis*

2) **DFN approach-** *Different of Normal- pattern of normal myeloid differenciation (reactionel vs regeneration BM)*

3) **LSC approach** : 34+38-

Rational

- LAIP (Kern, Venditti, Wood, Schuurhuis, Freeman …)
- Backbone 34/38 CSH/LSC approach (Dick&Bonnet, Nature 2009)
- LMPP-like/GMP-like LSC approach (Goardon, Cancer Cell 2011)
- LSC Flow Signature (Schuurhuis, PlosOne2014): quantification at dg and in MRD follow up

	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
T 1	CD7/56	CD13	CD33	CD34	CD38	CD117	CD19	CD45
T 2	CD90	MIX* TIM3+CLL1	CD123	CD34	CD38	CD117	CD45RA	CD45

*New markers of LSC prospectively evaluated in addition to TIM3 and CLL1: CD97, GPR56…

Supp Tube	CD36	CD11b	CD33	CD34	HLADR	CD117	CD4	CD45

Int J Hematol (2017) 105:549–557
DOI 10.1007/s12185-017-2221-5

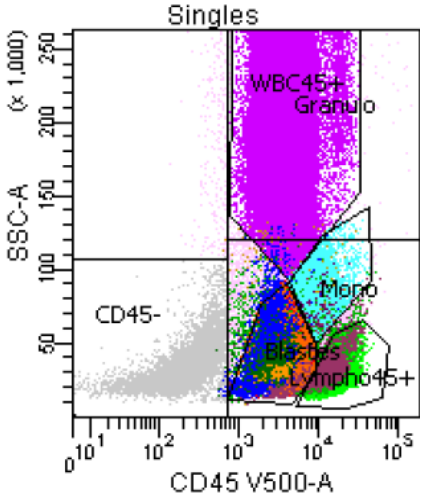
Leukemic stem cells: identification and clinical application

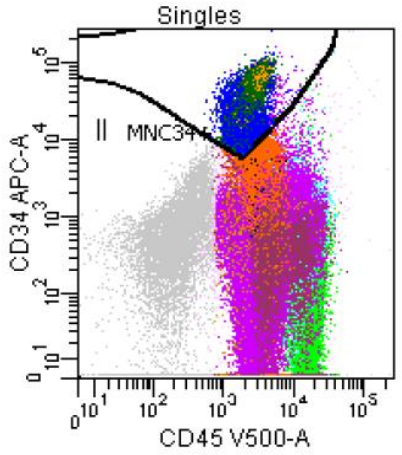
Diana Hanekamp[1] · Jacqueline Cloos[1,2] · Gerrit Jan Schuurhuis[1]

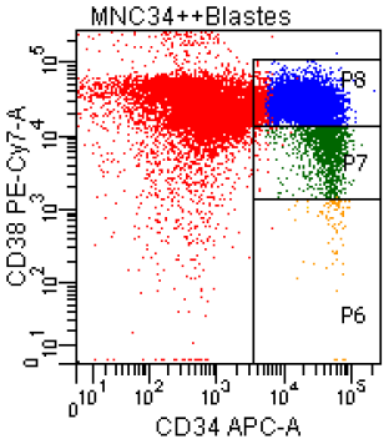
Table 1 Distinct leukemic stem cell markers

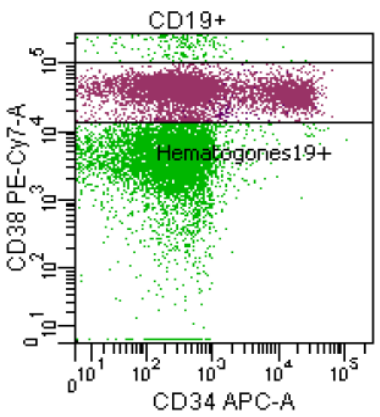
Marker	Identified as	Expression				References
		Normal	In AML (%)	HSC	CD34+ CD38− LSC	
IL1RAP	IL1R3	T cells	79	−	+	[70–72]
CLL-1	CLEC12A, MICL, DCAL-2	Myeloid cells	70	−	+	[6]
TIM-3	T-cell Ig Mucin 3	Activated T cells, NK cells	91	−	+	[73]
CD2	SRBC, LFA2, T11	T cells, NK cells	87	−	+	[14]
CD7	GP40, TP41, LEU-9	T cells	43	−	+	[6]
CD11b	Integrin alpha M, Mac-1	Myeloid cells	55	−	+	[6]
CD22	BL-CAM, Siglec-2	B cells	51	−	+	[6]
CD25	IL2RA, TAC	Activated B and T cells	25	−	+	[74]
CD33	P67, Siglec-3	Myeloid cells, NK cells	82	+	++	[6] [75]
CD44	Adhesion molecule	Ubiquitously	100	+	++	[6]
CD45RA	Tyrosine phosphatase receptor type C	T cells, myeloid cells	65	−	+	[76]
CD47	Integrin-associated protein (IAP)	Ubiquitously	100	+	++	[77]
CD56	N-CAM, MSK39	NK cells, activated T cells	32	−	+	[6]
CD96	TACTILE	Activated T cells	33	−	+	[6]
CD99	MIC2, single-chain type-1 glycoprotein	Myeloid cells	83	−	+	[78]
CD123	IL3R	Myeloid cells	82	+	++	[6] [48] [79]

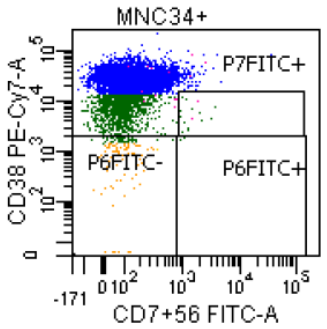
LSC Gating Strategy (nBM)

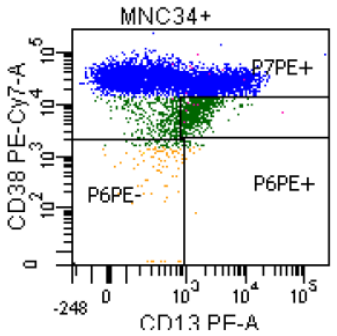


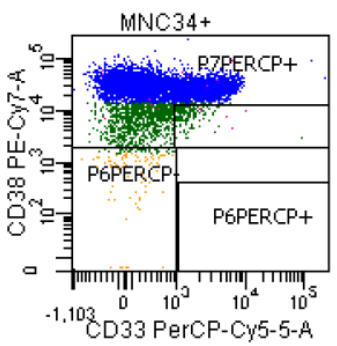


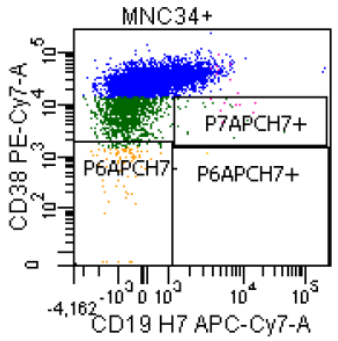


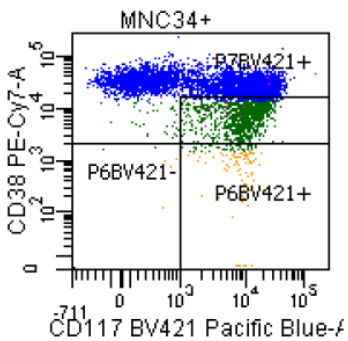


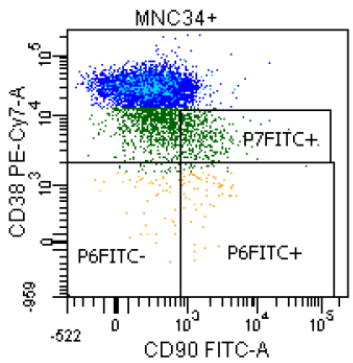


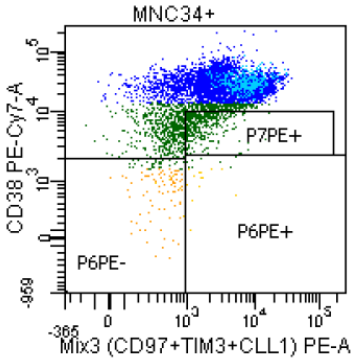


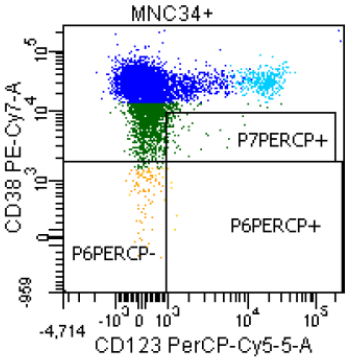


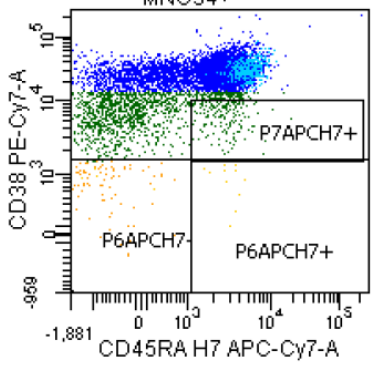


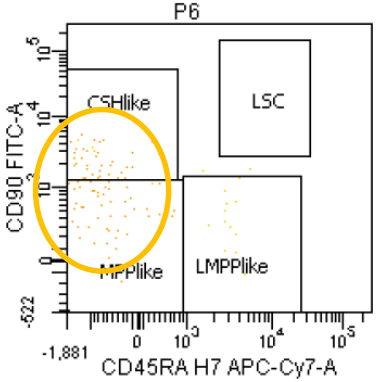




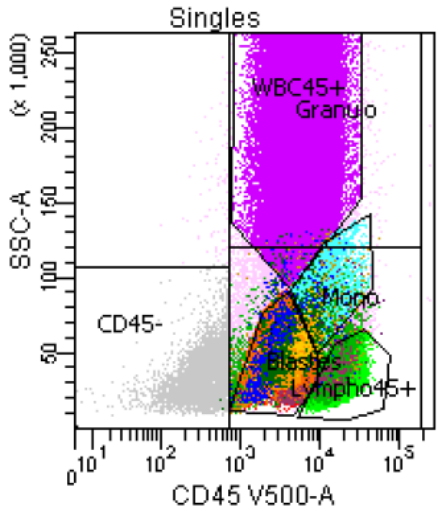


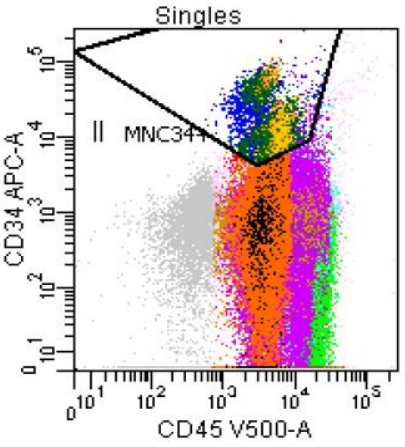


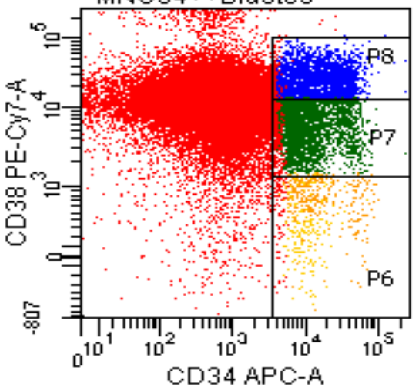


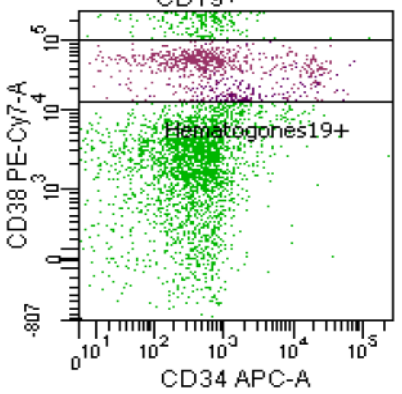


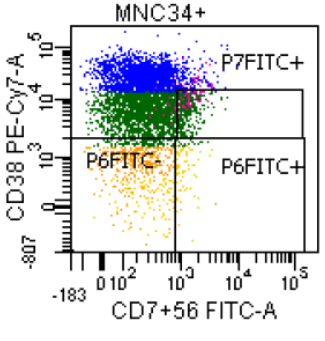
LSC Gating Strategy (AML patient)

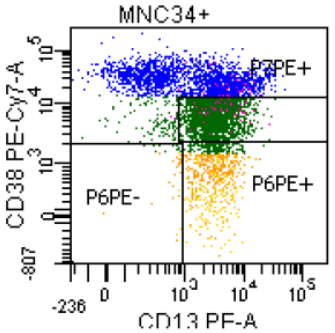


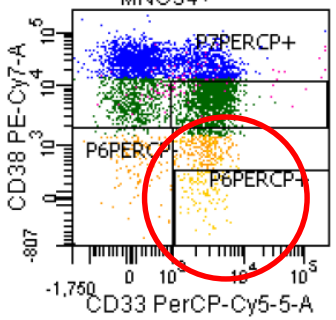


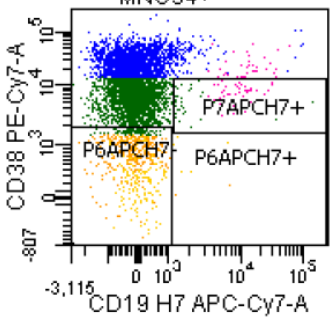


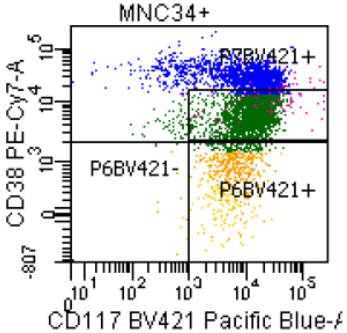


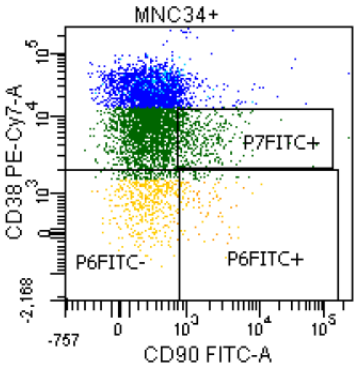


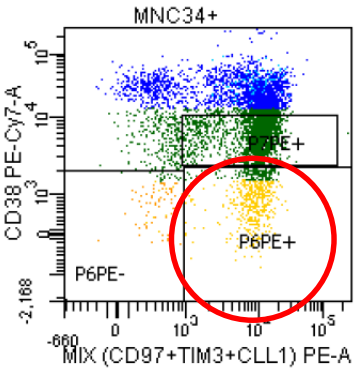


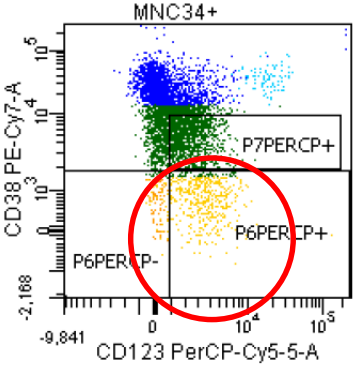


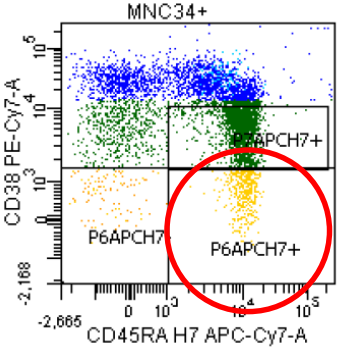


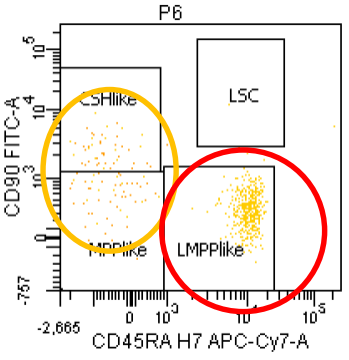








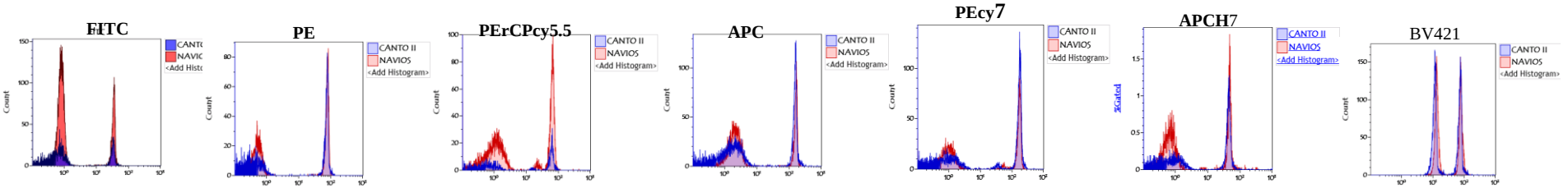




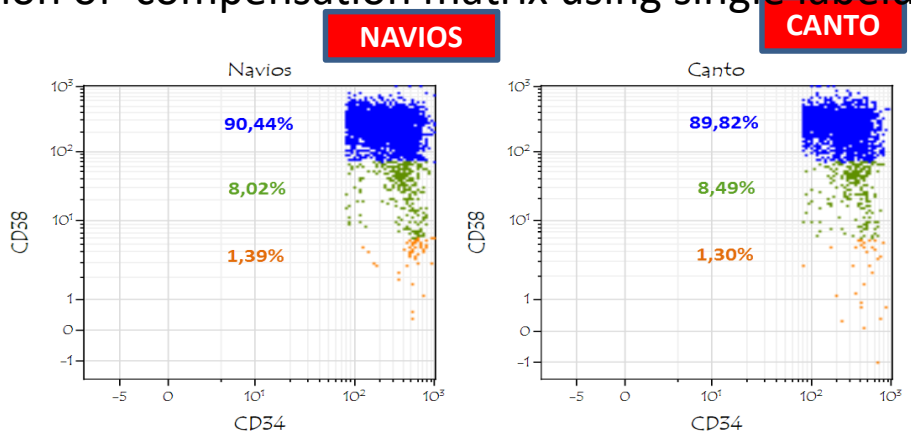
Challenge : to achieve harmonisation of MRD results between 25 FlowLabs CANTO vs NAVIOS « mirroir »

1) Harmonisation of preanalytical treatment of the sampels: BM dilution ctr, Bulk Lysis, nb of events aquisition (**mandatory >500 000/tube**)
2) « Mirroring » CANTO vs NAVIOS platforms

- Setting of voltage for the chosen chanel trough acquisition of rainbow beads without compensation to reach target of MFI values Multicentrique on the CANTO platform;
- transposition to NAVIOS platform by: New MFI Target= CANTO target / 256



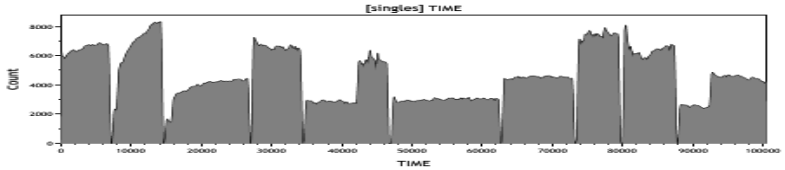
3) Evaluation of the homogeneity of the resultas using normal BM cells labeld with the panel after elaboration of compensation matrix using single labelds Ab

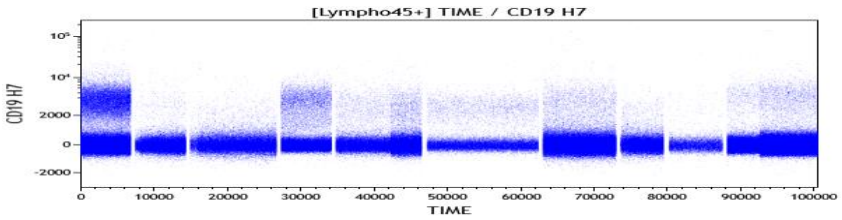


Comparable flow cytometry data can be obtained with two types of instruments, Canto II, and Navios. A GEIL study.
Solly F, Rigollet L…Lacombe F, Béné MC. **Cytometry A. 2013** Dec;83(12):1066-72.

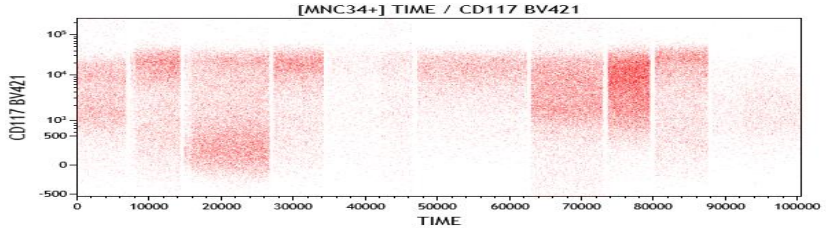
Merged dataset obtained from 10 BM samples shared between Lyon and Lille Labs

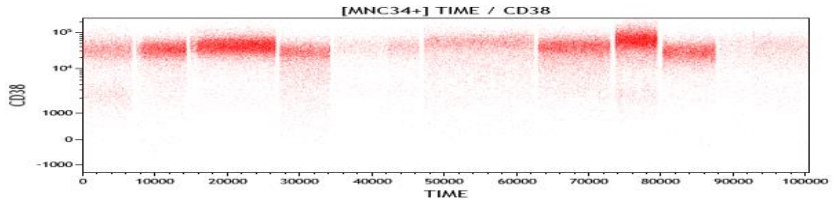
T1 CANTO (Lyon)





Gate Ly CD45





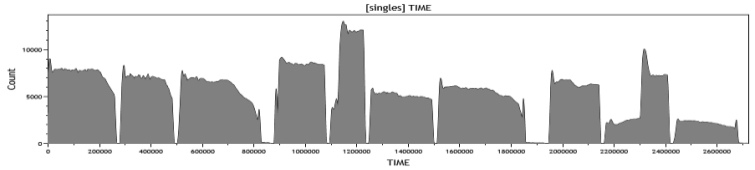
Gate MNC CD34+

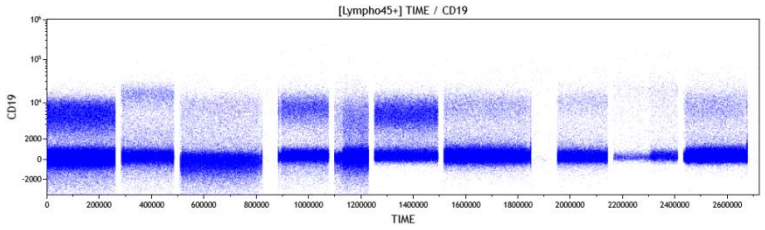
CD19

CD117

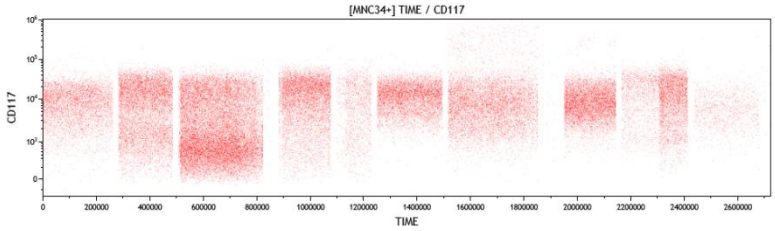
CD38

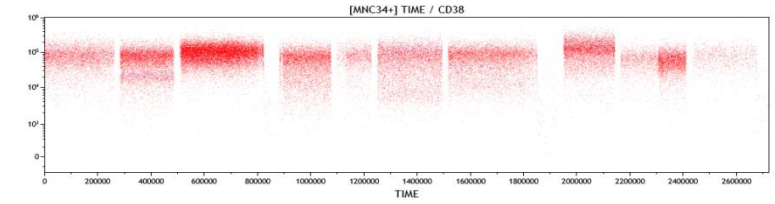
T1 NAVIOS (Lille)





Gate Ly CD45

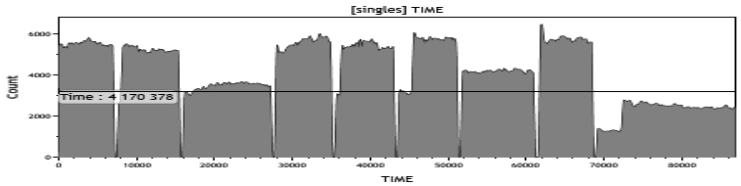


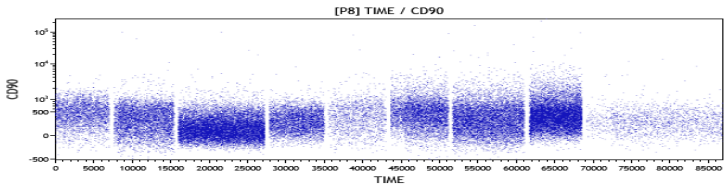


Gate MNC CD34+

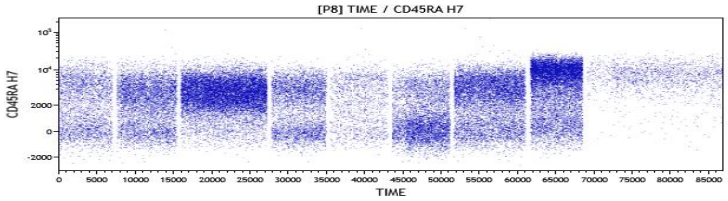
Merged dataset obtained from 9 BM samples shared between Lyon and Lille Labs

T2 Canto (LSC markers)

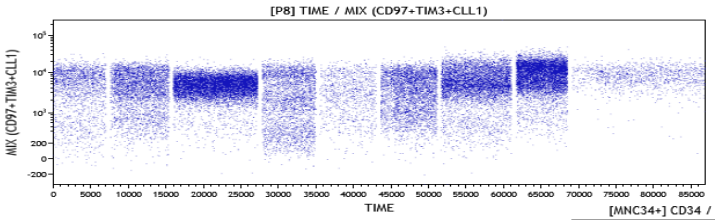




CD90

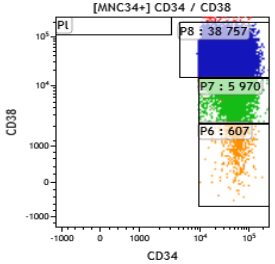


CD45RA

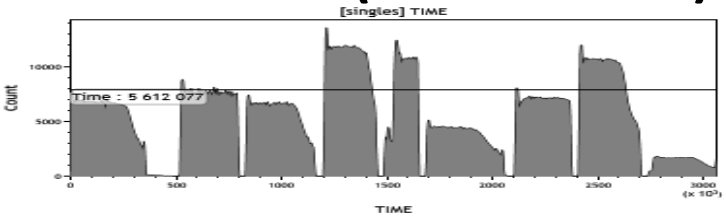


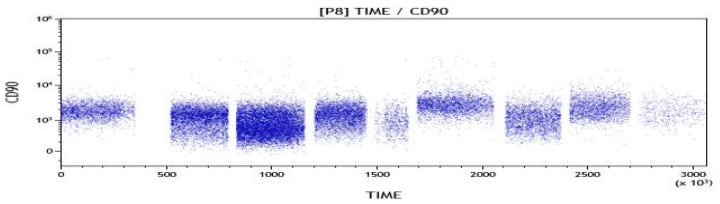
CLL1/Tim3

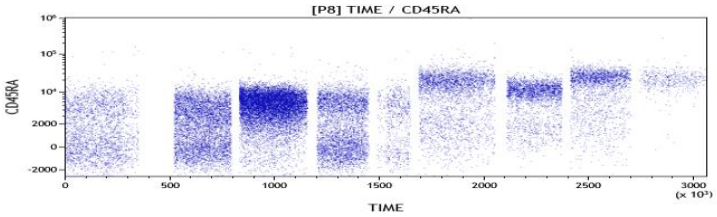
Gate CD34+CD38+hi

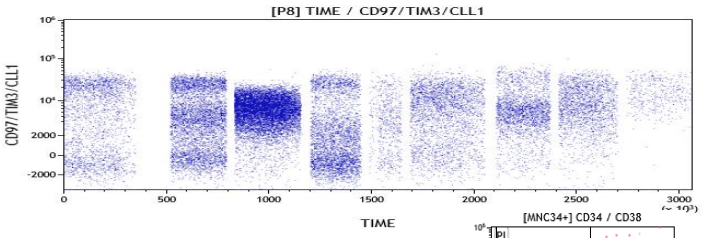


T2 Navios (LSC markers)

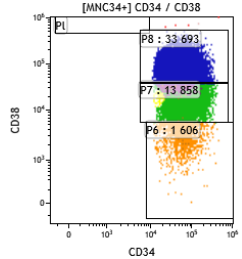


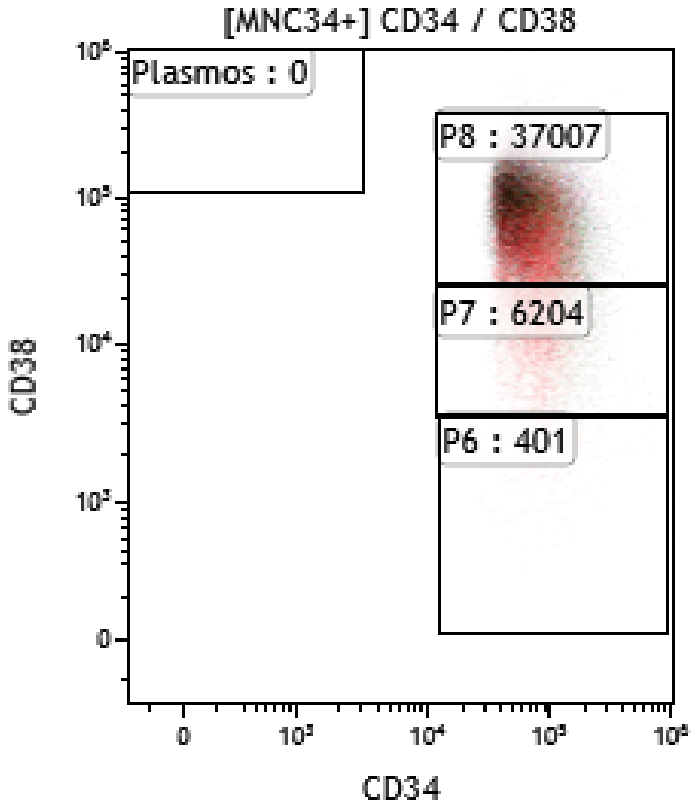




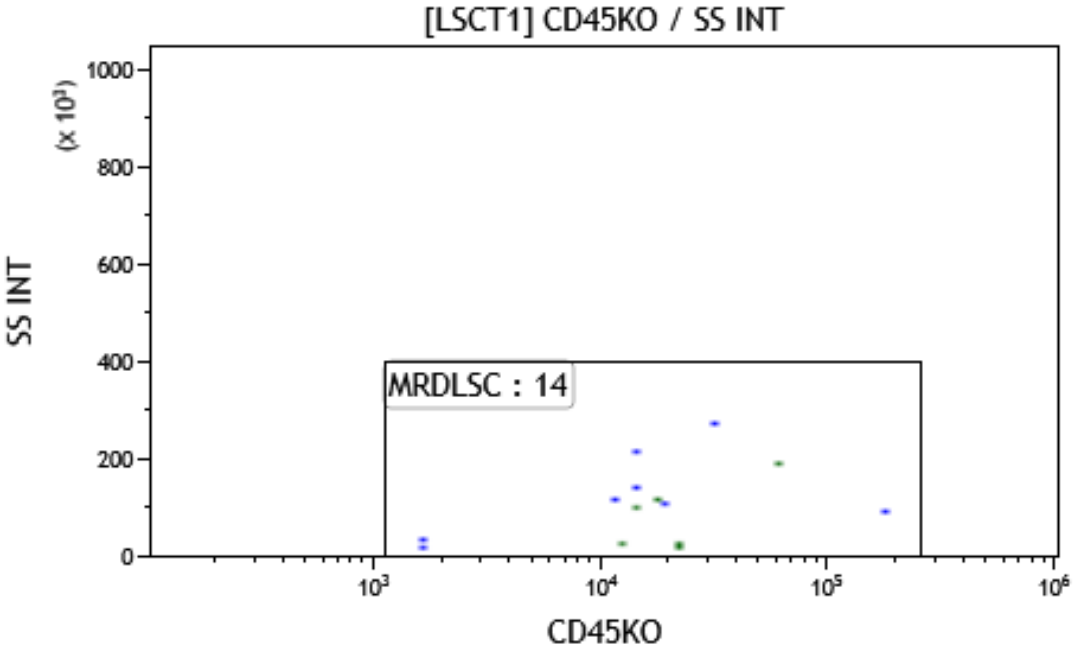


Gate CD34+CD38+hi





LAIP-P6(34+38-) Boleene MRD
LOD<0,0003%(3x10-6)
5X10 6 ev



MRD LSC LOD

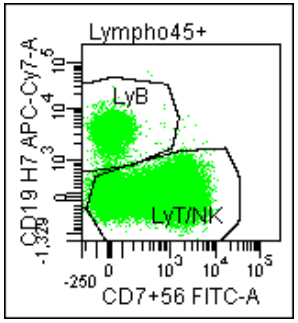
14 ev
0,0003%

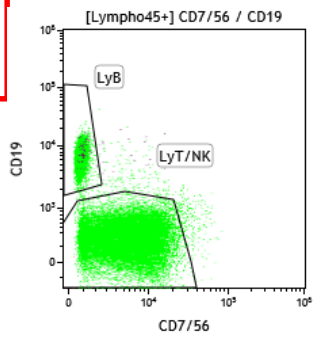
Gate	Number	%Total
All	14	0,0002
MRDLSC	14	0,0002

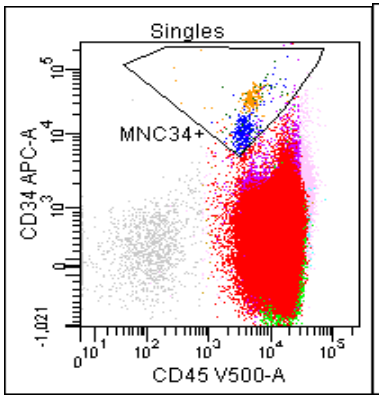
CANTO/
DIVA

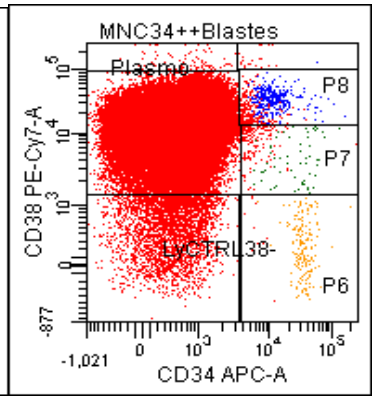
Harmonisation of detection of LSC using gating strategy adaptated to CANTO and NAVIOS softwares in an AML CD34- patient (BM sample shared between Lyon and Lille Labs)

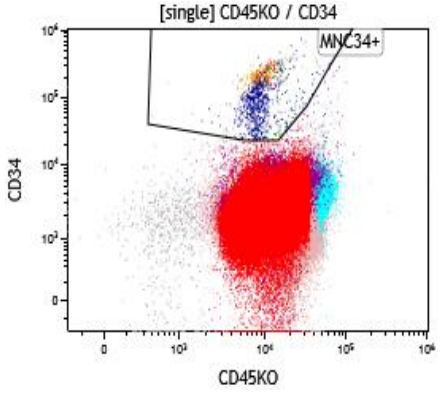
NAVIOS/
KALUZA

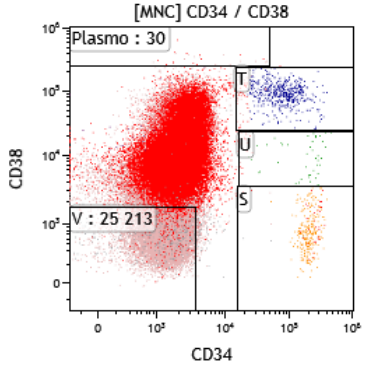


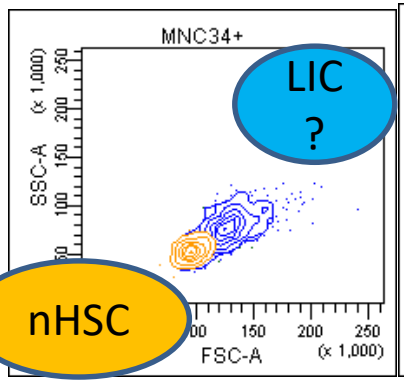


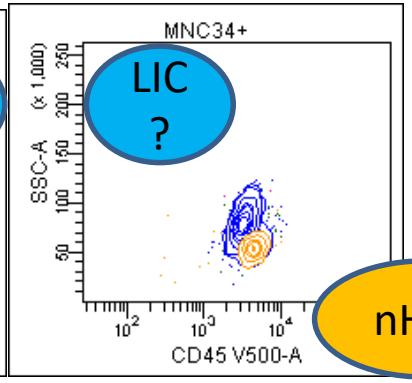


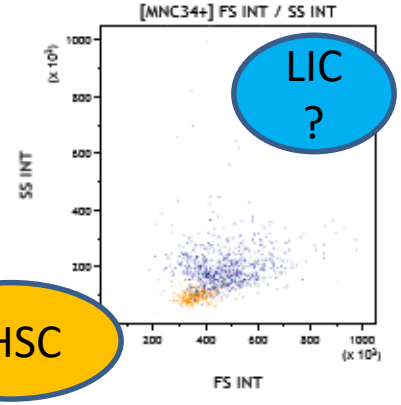


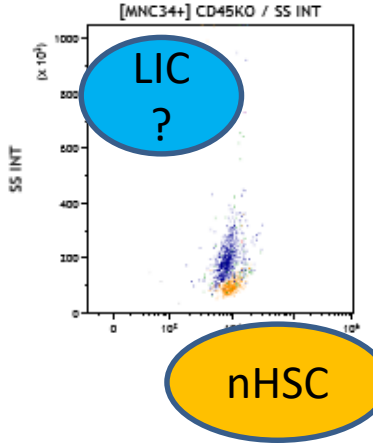




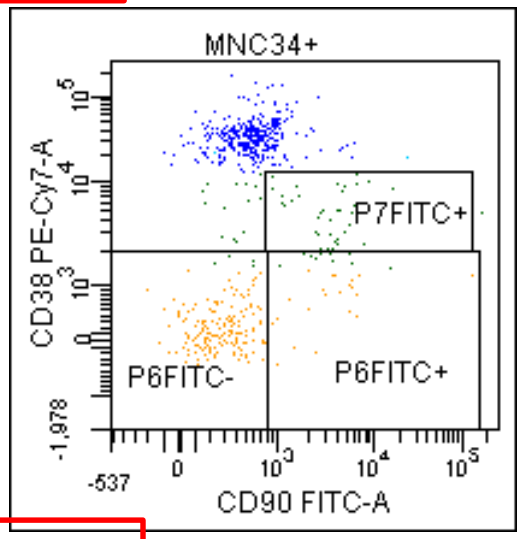


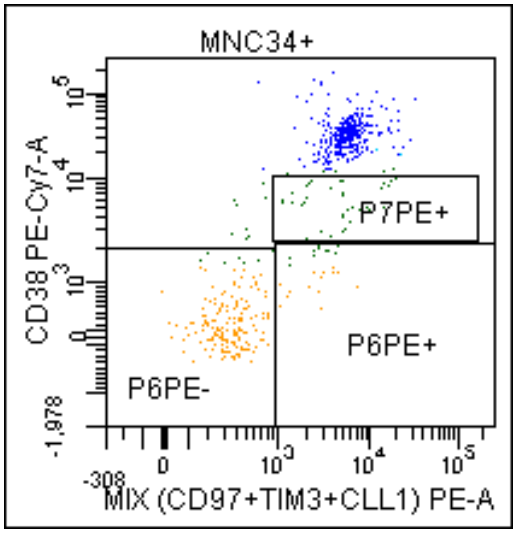


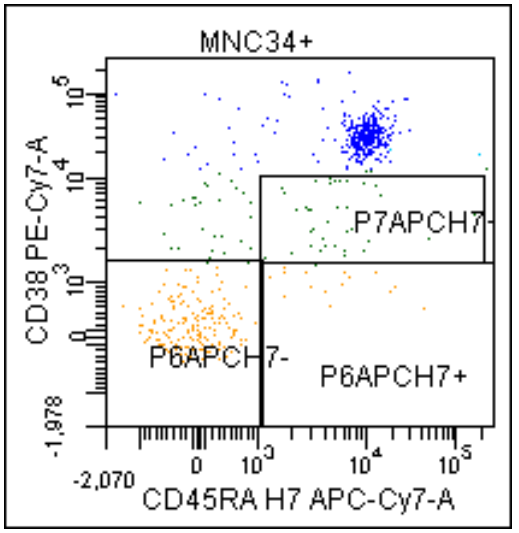




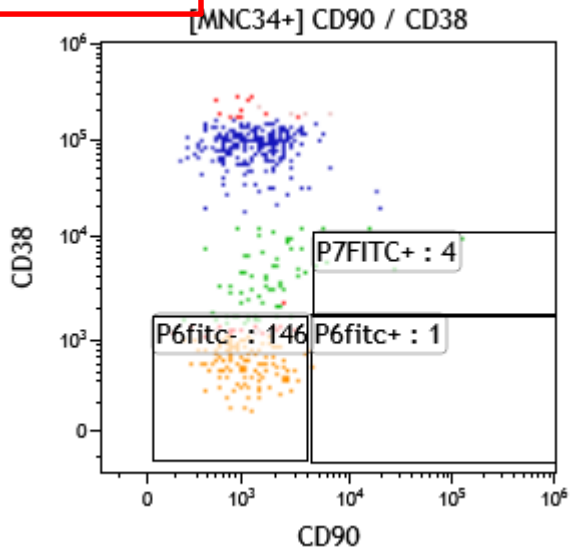
CANTO/
DIVA

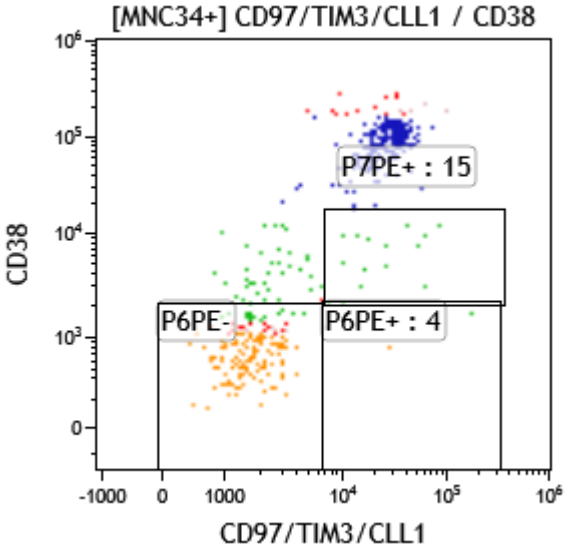


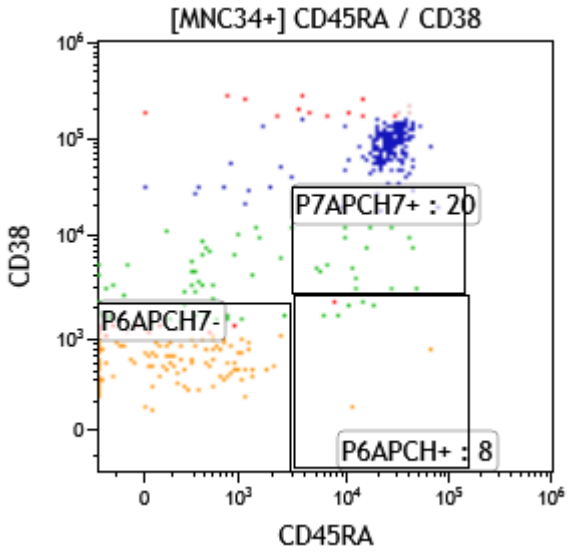




NAVIOS/
KALUZA







AML MRD flow

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, reproductible, sensitivity, cut
- CANTO vs NAVIOS « mirroir »
- gating strategy

3) ***Ready to start***

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

Original Article

A QA Program for MRD Testing Demonstrates That Systematic Education Can Reduce Discordance Among Experienced Interpreters

Michael Keeney,[1*] Brent L. Wood,[2,3] Benjamin D. Hedley,[1] Joseph A. DiGiuseppe,[4] Maryalice Stetler-Stevenson,[5] Elisabeth Paietta,[6] Gerard Lozanski,[7] Adam C. Seegmiller,[8] Bruce W. Greig,[8] Aaron C. Shaver,[8] Lata Mukundan,[9] Howard R. Higley,[9] Caroline C. Sigman,[9] Gary Kelloff,[10] J. Milburn Jessup,[11] and Michael J. Borowitz[12]

[1]Pathology and Laboratory Medicine, London Health Sciences Centre, London, Ontario, Canada
[2]Seattle Cancer Care Alliance, Seattle, Washington
[3]University of Washington, Seattle, Washington
[4]Department of Pathology, Hartford Hospital, Hartford, Connecticut
[5]National Cancer Institute, National Institutes of Health, Bethesda, Maryland
[6]Oncology, Montefiore Medical Center, Bronx, New York
[7]Department of Pathology, Ohio State University, Columbus, Ohio
[8]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee

Concordance before training

Table 2
Dry Challenges 1–3

# Attempted % Attempted	15 100%	15 100%	15 100%	15[a] 100%	15 100%	15 100%	15[a] 100%
# Positive	7	9	7	11	12	9	8
# Negative	8	6	8	4	3	6	7
False positive	0	1	2	2	4	0	0
False negative	2	1	4	1	2	2	1
Overall concordance (%)	87%	87%	60%	80%	60%	87%	93%
Outside ½ log	2	4	6	4	7	3	1
½ log Concordance (%)	87%	73%	60%	73%	53%	80%	93%

[a]The 3rd round of results for two laboratories were submitted after the deadline results were due.

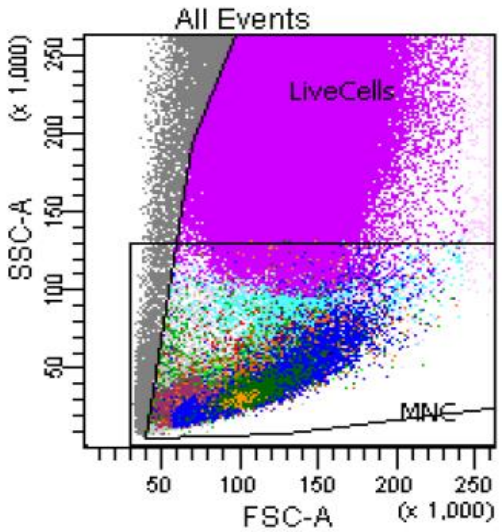
Concordance after training

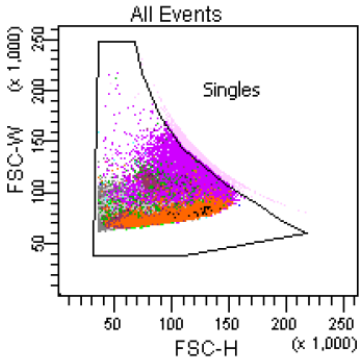
Table 3
Dry Challenges 4–5

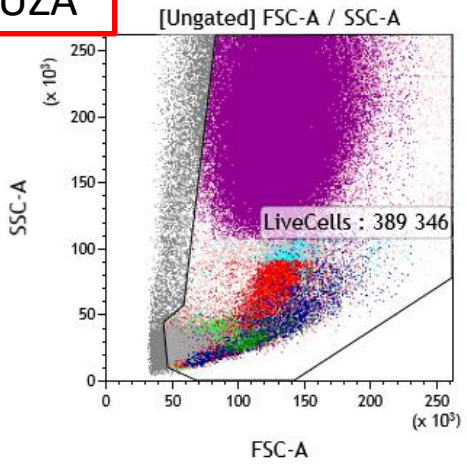
	Statistics for 7 participating laboratories						
# Attempted % Attempted	10 100%	10 100%	10 100%	10 100%	10 100%	10 100%	10 100%
# Positive	5	5	7	6	5	6	5
# Negative	5	5	3	4	5	4	5
False POS	0	0	3	1	0	1	0
False NEG	0	0	1	0	0	0	0
Overall Concordance (%)	100%	100%	60%	90%	100%	90%	100%
Outside ½ log	0	0	4	1	0	1	0
½ log Concordance (%)	100%	100%	60%	90%	100%	90%	100%

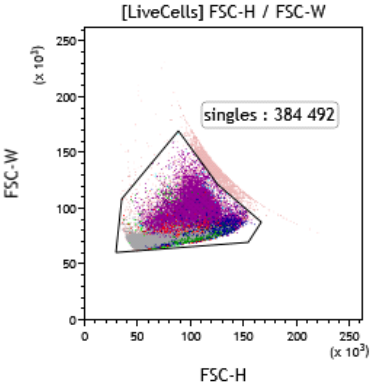
DIVA

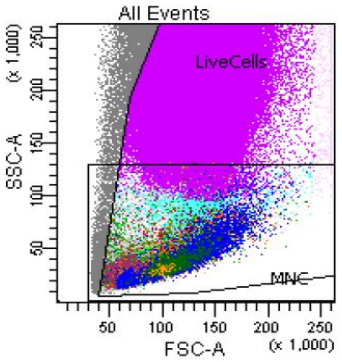
KALUZA

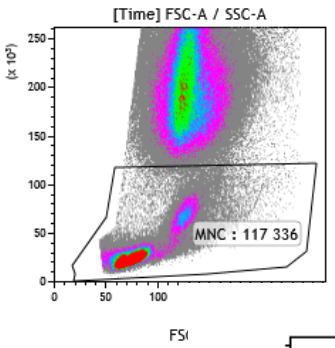


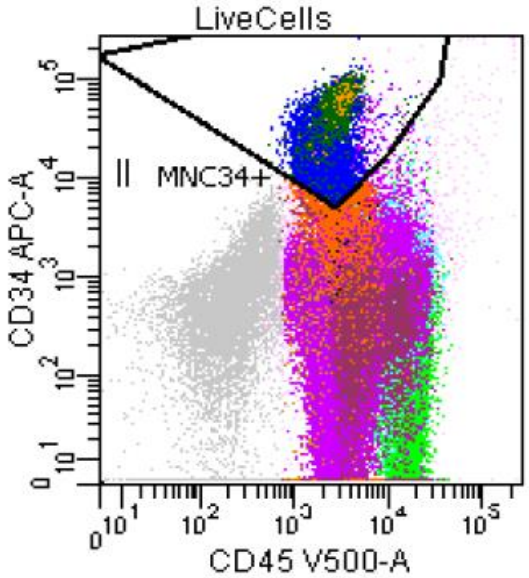


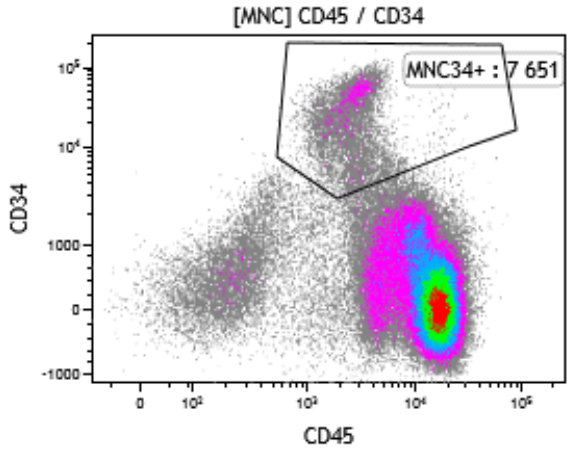








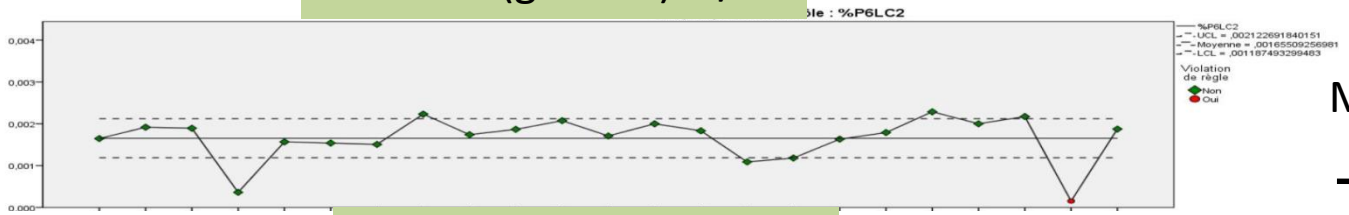




CQA 23 centers (2 sets of data not integrated)
DIVA vs KALUZA
LMD nBM

CQA LMD Files shared among 23 centers

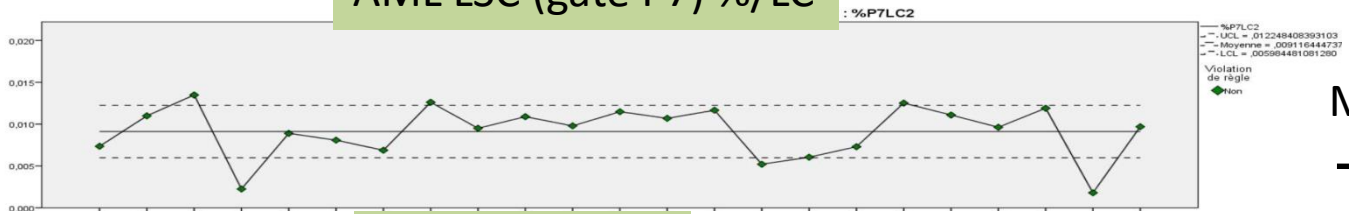
AML LSC (gate P6) %/LC



Mean : $1,6*10^{-3}$

– – – +/- 1 SD

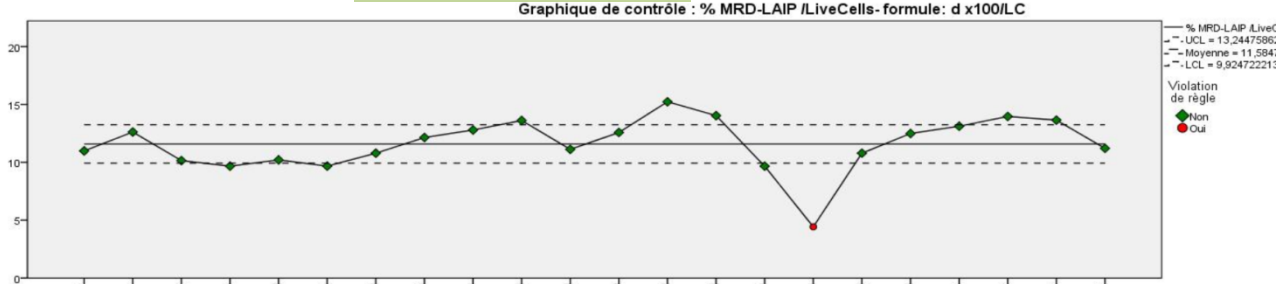
AML LSC (gate P7) %/LC



Mean : $9,1*10^{-3}$

– – – +/- 1 SD

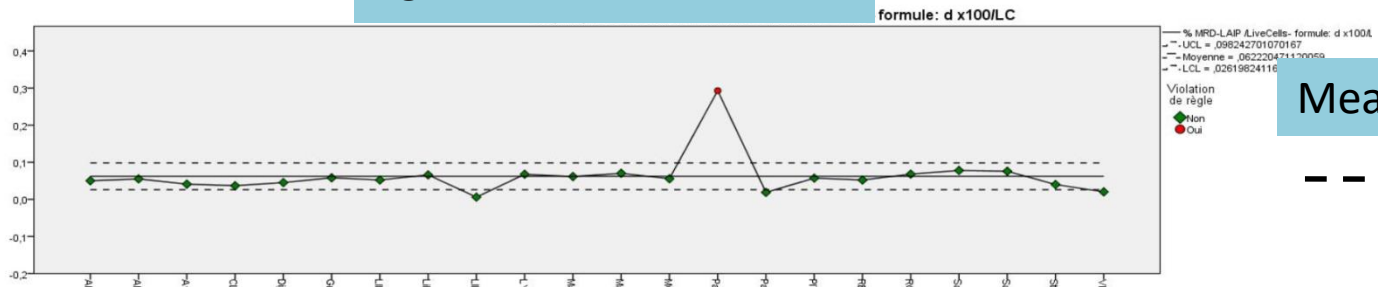
AML LAIP %/LC



Mean 11,9%

– – – +/- 1 SD

LOD AML LAIP in NBM

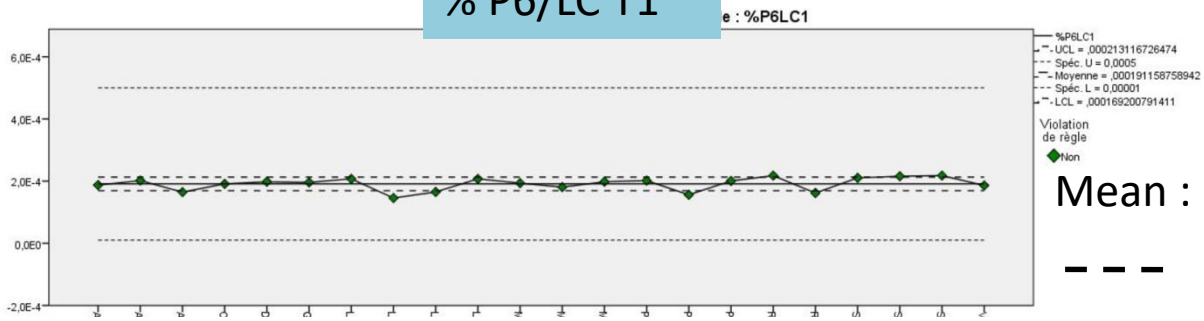


Mean : $6,2*10^{-4}$

– – – +/- 1 SD

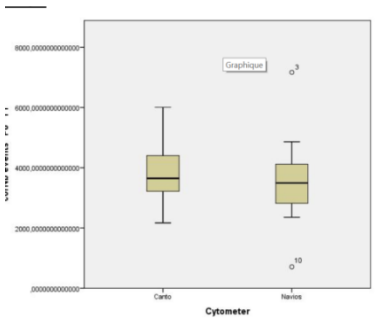
CQE nBM sample shared among 22 centers

% P6/LC T1



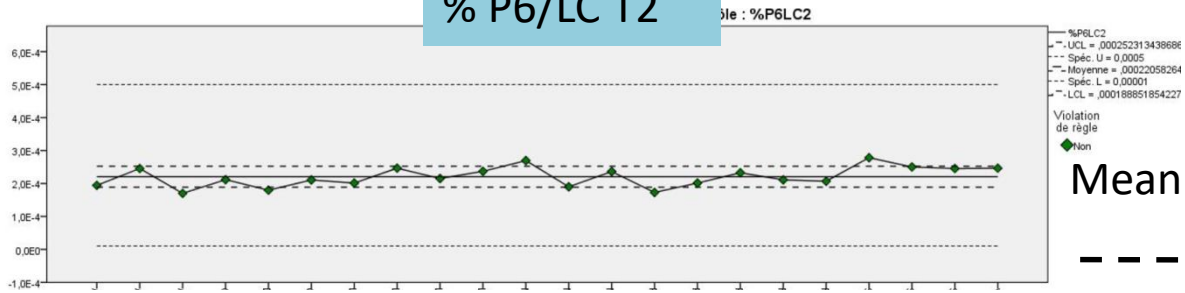
Mean : 1,92*10 $^{-4}$

– – – +/- 1 SD



Canto Navios

% P6/LC T2

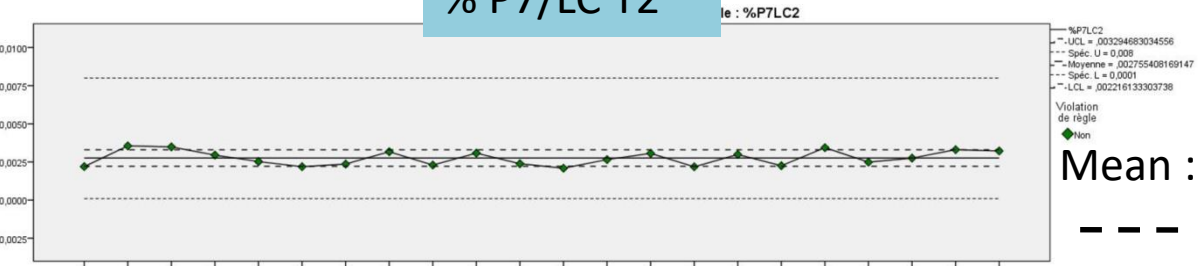


Mean : 2,2*10 $^{-4}$

– – – +/- 1 SD

- staining validation
- stability at 24h
- homogeneity of the results

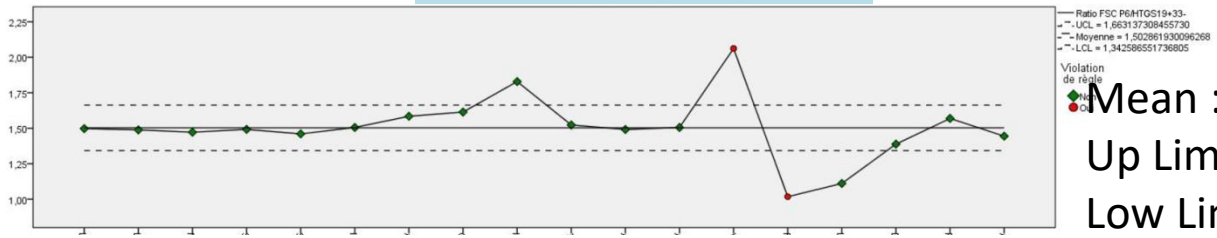
% P7/LC T2



Mean : 2,75*10 $^{-3}$

– – – +/- 1 SD

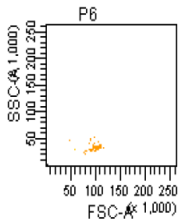
Ratio FSC of P6/HTG

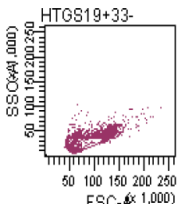


Mean : 1,50

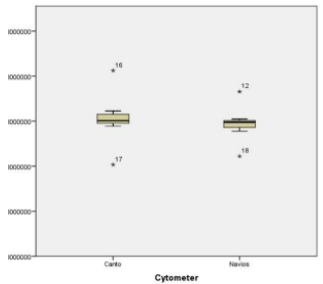
Up Limit: 1,66

Low Limit: 1,34





Canto Navios



Ready to start

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

Take home message

- This methodological validation protocol is a mandatory step to consider the use of MRD flow in AML clinical trials;
- 3 MRD approach are complementary: LAIP/DFN/LSC
- Prospectively Lyofilised Ab approach
- Choosing a multicentric approach could be challenging, but our first results are promising and showed the feasibility of this concept when:

(i) a straight harmonisation of the instruments sensitivity and samples preparation are established, and
(ii) training and systematic education among the analytical operators are regularly performed.

Thanks!

Flow Cytometric Labs

AFC (French Association of Cytometry)

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