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AML MRD by multiparameter flow cytometry using LAIP/Dfn and LSC: Methodological aspects in a multicentric study of the French-Flow MRD AML ALFA Network

AML MRD LSC French Flow Intergroup

ALFA

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INTRODUCTION

MRD follow-up is now mandatory for treatment response evaluation in AML clinical trials (ELN 2017 and 2021 guidelines: *Heuser et al, Blood doi:10.1182/blood.2021013626.*). Beside to molecular biology methods, multiparameter flow cytometry assay represents the most reliable approach. The aim of this study was to implement an harmonized follow up of the patients using a standardized MRD flow approach across 30 French haematology laboratories participating in AML clinical trials

METHOD

To obtain superposable results, the network recommendations were

- Wet labs procedure

Immunostaining was performed after "bulk" lysis technique (NH4Cl).

- Flow cytometers settings

Harmonization of the sensitivity between platforms (18 BD and 12 BC) was performed using 8 Picks Rainbows calibration beads. . A total of minimal 500 000 cells was acquired in each tube.

- Design of a a 2-tubes panel with minimal mandatory 8c markers by tube.

A « backbone » CD34/CD38/CD45/CD117 was used, completed by CD7,CD56, CD13, CD33, CD19 for the 1st tube and CD90(Thy-1), Mix (CD97+CLL1+TIM3), CD123, CD45RA for the 2nd one.

		MIX3Ac=97+TIM3+CLL1		PANEL MRD LAM LSC –CANTO-NAVIOS-LYRIC-DxFLEX									
CANTO 8C/NAVIOS 8c	FITC	PE	ECD	PerCPCy5,5	PECy7	APC	APC-R700/AA700	APCH7	BV421	V500	BV605	BV711	BV786
Tube 1 LAIP 8c	CD7 /CD56	CD13		CD33	CD38	CD34		CD19	CD117	CD45			
Tube 2 LSC 8c	CD90	MIX3		CD123	CD38	CD34		CD45RA	CD117	CD45			
LYRIC 10-12c	FITC	PE	ECD	PerCPCy5,5	PECy7	APC	APC-R700	APCH7	BV421	V500	BV605	BV711	BV786
Tube 1LAIP 12c	CD7	CD13		CD33	CD38	CD34	HLADR	CD19	CD117	CD45	CD56	CD10	CD36
Tube 2 LSC 12c	CD90	MIX3		CD123	CD38	CD34	HLADR	CD45RA	CD117	CD45	CD200	CD19	CD36
NAVIOS 10c	FITC	PE	ECD	PEcy5,5	PECy7	APC	AA700	AA750	BV421/PB	V500/KO	BV605	BV711	BV786
T1 Navios 10c	CD7	CD13	HLADR	CD33	CD38	CD34	CD56	CD19	CD117	CD45			
T2 Navios 10c	CD90 5µL	MIX3	CD19	CD123	CD38	CD34	CD36	CD45RA	CD117	CD45			
DxFLEX 10-12/13c	FITC	PE	ECD	PEcy5,5	PECy7	APC	AA700	AA750	BV421/PB	V500/KO	BV605	BV711	BV786
T1 DxFLEX 12-13c	CD7	CD13	HLADR	CD33	CD38	CD34	CD56	CD19	CD117	CD45	CD36	CD10	/
T2 DxFLEX 12-13c	CD90	MIX3	CD19	CD123	CD38	CD34	CD36	CD45RA	CD117	CD45	CD200	/	HLADR

This panel could be used in 8c,10c,12c (HLADR, CD36, CD10, CD200) implemented on Becton Dickinson (CANTO and LYRIC) or Beckman Coulter (NAVIOS and DxFlex) cytometers.

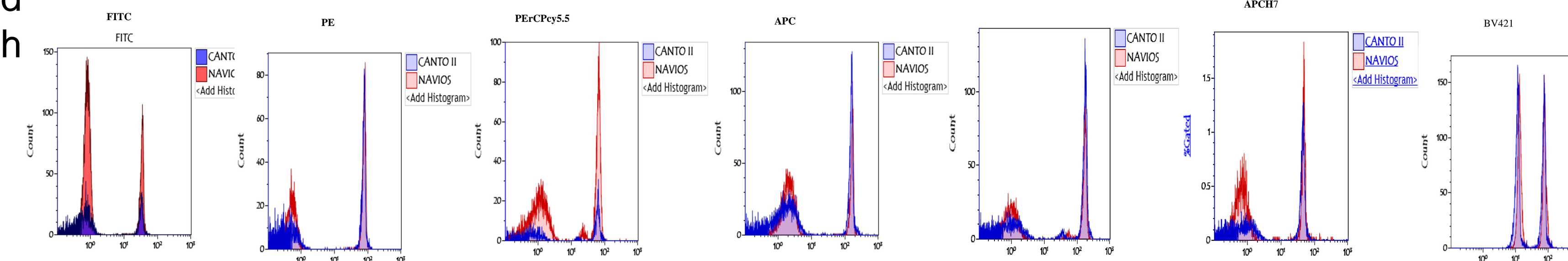
- Common analysis strategy according to ELN recommendations to identify the pattern of LAIP/DfN in bulk cells and the LSC (Leukemia Stem Cells) in CD34+CD38- fraction using DIVA/FACSUITE and KALUZA software.

CONCLUSIONS

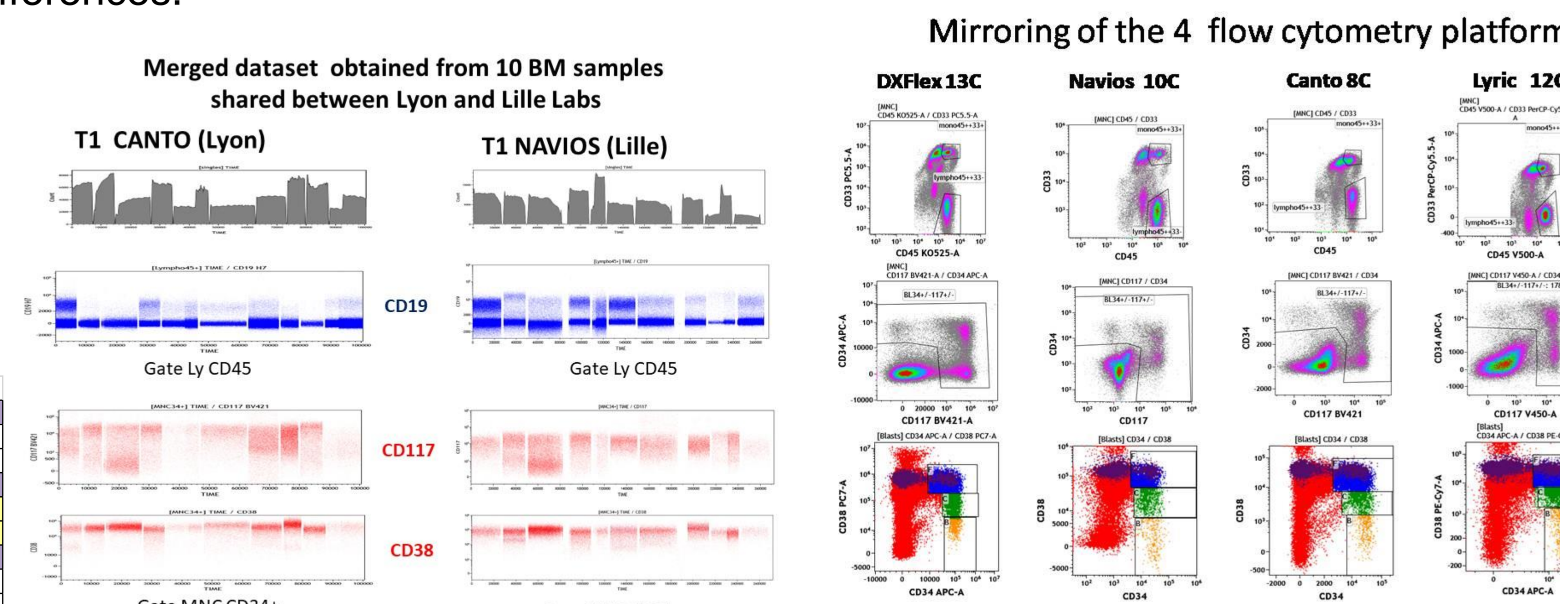
This methodological validation protocol is a mandatory step to consider the use of MRD flow in AML clinical trials. Choosing a multicentric approach could be challenging, but our first results are promising and showed the feasibility of this concept when: (i) a straight harmonisation of the instruments sensitivity and samples preparation are established, and (ii) training and systematic education among the analytical operators are regularly performed. Finally, our pilot study shows a very strong correlation between conventional method and unsupervised analyses using data generated by the multicentric network.

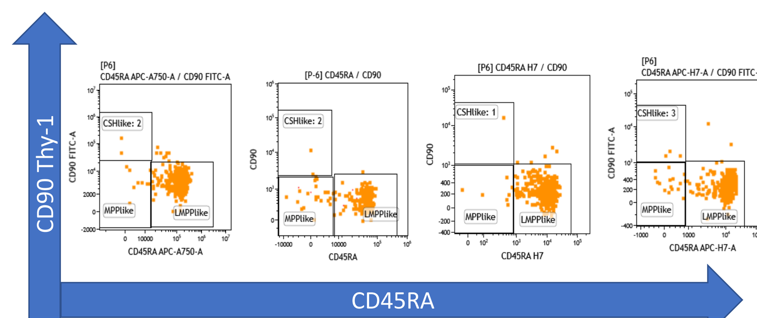
RESULTS

- « 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 Navios MFI Target= CANTO target / 256



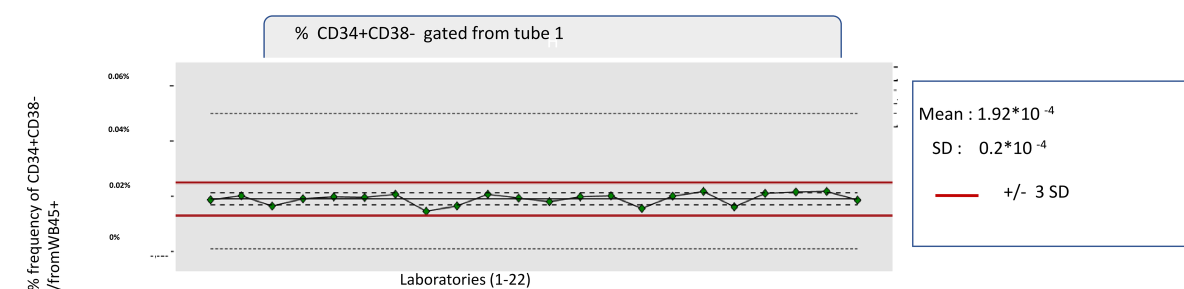
- To detect any bias between the platforms, 10 EDTA fresh regenerative marrow samples were tested in parallel at 2 platforms (CANTO and NAVIOS). Similarity of the staining index between positive and negative population were compared between the samples and the 4 platforms showing no significant differences.



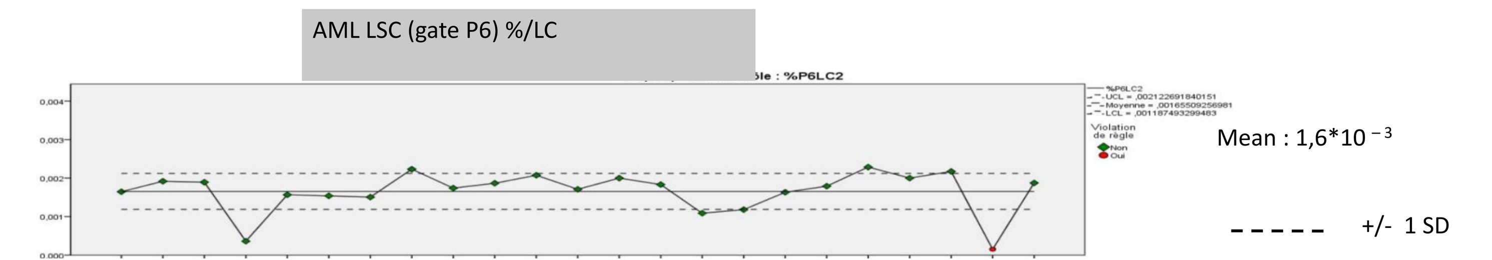


Detection of leukemic stem cells (LSC) across the 4 platform

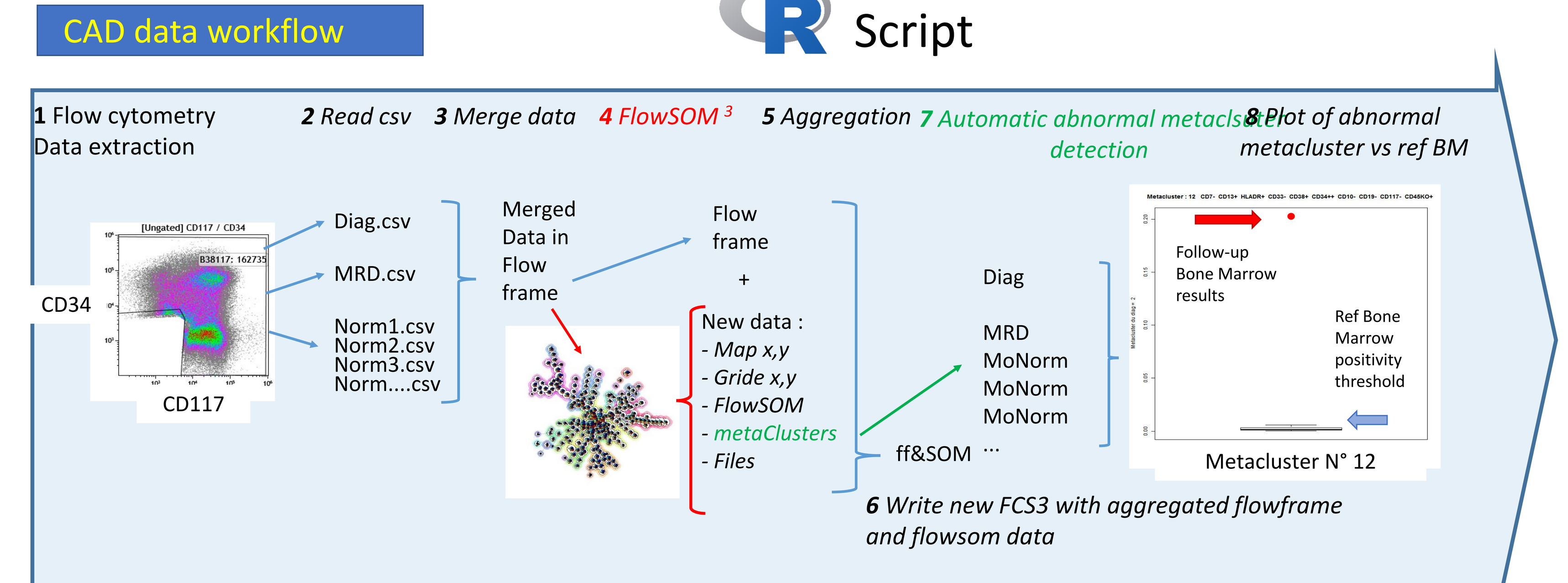
QC (Quality Control Sample), of normal bone marrow (from healthy donor) 1/year (a total of 5 since 2017) was shared among the participants centers The specific events populations (nHSC, MPP, LMPP"like,etc) were evaluated by centers using a common gating strategy.



Standardization of the data analysis strategy obtained in the centers was subsequently evaluated using a virtual quality control(CQA) by sharing MRD FCS data files. About 90% of the locally analyzed data were included in an interval centralized on the average of the series mean +/- 2 SD.

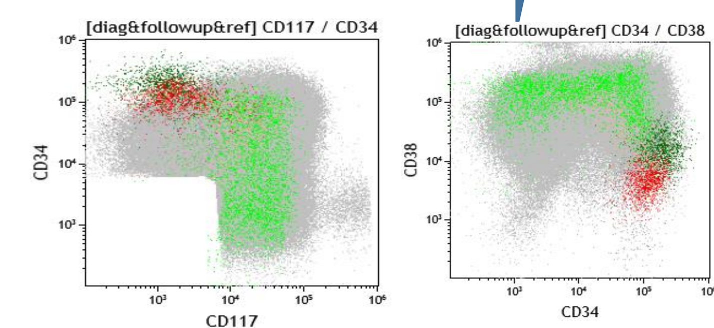


Harmonized data generated allowed to perform a Computer aided design (CAD) in assessment of AML MRD flow using proprietary developed R script based upon FlowSom. It performs an automated definition of metacluster with abnormal population (by comparison to a set of reference bone marrow) which are quantified in the CD34+ CD117+ space for all samples (diagnosis, follow up and references marrow). It then extracts the metacluster significantly different from that observed for the reference samples (> mean+6sd. It calculates the MRD in % of WBC CD45+.

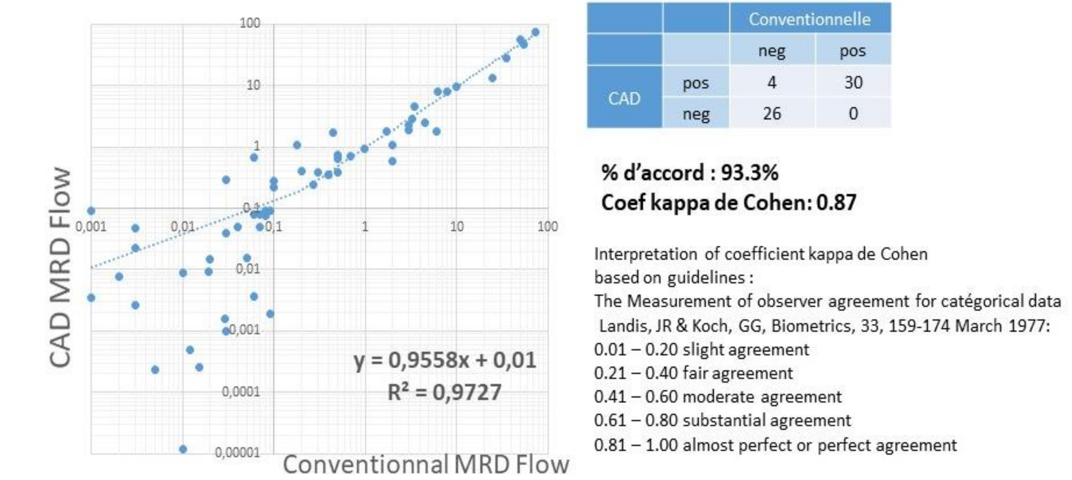


Reading new aggregated data file in conventional cytometry softawre (Kaluza°) MRD visualisation and validation

Colors Legend : Diagnosis, Follow-up, MRD pos:Metacluster 12 in follow-up, Referents BM



The limits of sensitivity of the CAD method shows that a MRD remains evaluable by CAD up to 0.04% (50 events in 1 000 000). We compared the results between the conventional and the CAD method on 60 MRD samples. The slope of the correlation line was 0.95 with an R2 of 0.97. Using the definition threshold of a positive MRD at 0.1%, the agreement of the results between the two methods was evaluated by the Cohen kappa coefficient of 0.87.



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Heuser et al, Blood doi:10.1182/blood.2021013626

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