



# Immunophénotypage des LALB au diagnostic et en suivi MRD à l'ère de l'immunothérapie

*Adriana PLESA , CHU Lyon , France*

*Saint-Malo , 29 Juin 2026*





## Activités du groupe CytHem LAL

Un update



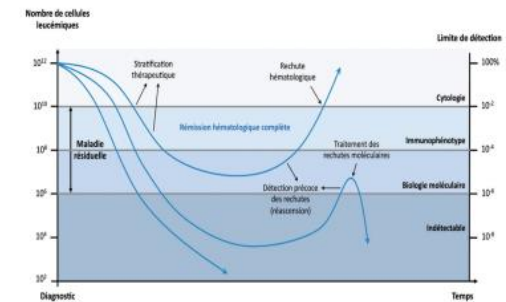
Isabelle Arnoux / Adriana Plesa  
Marie Loosveld / Ludovic Lhermitte

Table 2  
B-ALL Leukemia Associated Immunophenotypes (LAIP)

LAIP expression type	Markers	Frequency of expression (%)
Overexpressed	CD58++	63
	CD10++	49
	CD34++ homogeneous	49
	CD123++	37
Underexpressed	CD45-/+ dim	49
	CD38+dim	57
	CD24+dim	13
	CD20-/CD10+	49
Asynchronous	CD21+/CD10+	10
	CD20-/CD10-	10
Crosslineage	CD13+/CD15+/CD33+	22
	CD2+/CD4+/CD5+	6

Table 3  
T-ALL Leukemia Associated Immunophenotypes (LAIP)

LAIP expression type	Markers	Frequency of expression (%)
Immature	TdT	88
	CD5+/cCD3+/CD3-	70
	CD45	51
	CD34	29
Overexpressed	CD1a	50
	CD99	58
Underexpressed	CD7	42
	CD4+/cCD8+	26
Asynchronous	CD2 neg	23
	CD10	37
Crosslineage	My (13, 15, 33, 117)	16



Duployer N et al, Revue Francophone des Laboratoires, avr 2015;2015(471):51-64

Fossat C et al, Cytometry B 2015

## CytHem LAL : Le Projet

## Expérience du STIC 06

## MRD des LAL

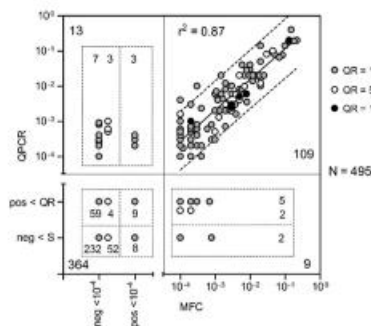
5 juin 2024

Lancement du groupe LAL

Présentation du projet

Axe Maladie Résiduelle

Axe diagnostique



Garand R et al, Leuken

### Lower Limit Of Quantification

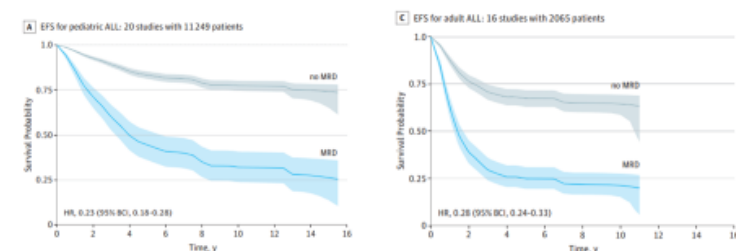
LLOQ= 40/N\*100

N=nombre d'évènements totaux vivants, en excluant l'érythropoïèse

### Limit Of Detection

LOD=10/N\*100

MRD: Facteur pronostique **le plus puissant**: association très significative entre la réponse au traitement (MRD) et la survie



Berry DA et al, JAMA Oncol. 13 juill 2017;3(7):e170580.

Ludovic Lhermitte, APHP-Hôpital Necker

# Diagnostic des LAL-T

## Diagnostic de LAL-T : affiliation au lignage T

Table 13. Lineage assignment criteria for mixed-phenotype acute leukaemia.

	Criterion
<b>B lineage</b>	
CD19 strong <sup>a</sup>	1 or more also strongly expressed: CD10, CD22, or CD79a <sup>c</sup>
or,	
CD19 weak <sup>b</sup>	2 or more also strongly expressed: CD10, CD22, or CD79a <sup>c</sup>
<b>T lineage</b>	
CD3 (cytoplasmic or surface) <sup>d</sup>	Intensity in part exceeds 50% of mature T-cells level by flow cytometry or, Immunocytochemistry positive with non-zeta chain reagent
<b>Myeloid lineage</b>	
Myeloperoxidase	Intensity in part exceeds 50% of mature neutrophil level
or,	
Monocytic differentiation	2 or more expressed: Non-specific esterase, CD11c, CD14, CD64 or lysozyme

<sup>a</sup>CD19 intensity in part exceeds 50% of normal B cell progenitor by flow cytometry.

<sup>b</sup>CD19 intensity does not exceed 50% of normal B cell progenitor by flow cytometry.

<sup>c</sup>Provided T lineage not under consideration, otherwise cannot use CD79a.

<sup>d</sup>Using anti-CD3 epsilon chain antibody.

Alaggio, *Leukemia* 2022  
WHO-HAEMR5, part B, p.203

## CytHem-QUESTIONS

Comment classer une LAL-T en 2024 : « tips & tricks »

	WHO-HAEMS	EGIL
<b>Classification</b>	<p><b>ETP (Early T-cell progenitor)</b> CD1a- et CD8- et CD5dim/neg ⊛ &lt;75% des blastes</p> <p><b>ET</b> Expression ≥1 marqueur myéloïde ou souche - My : CD13, CD33, CD11b et/ou CD65 - Stem : CD117, CD34, HLA-DR ⊛ ≥25% des blastes</p> <p><b>NOS (Not Otherwise Specified)</b> Ne remplit pas les critères ETP (ou near-ETP [voir page suivante])</p>	<p>Pro-T-ALL (TI) CD7+</p> <p>Pre-T-ALL (TII) CD2+ et/ou CD5+ et/ou CD8+</p> <p>Cortical-T-ALL (TIII) CD1a+</p> <p>Mature-T-ALL (TIV) TCRαβ+ ou TCRγδ+ (surface)</p>
<b>Pertinence</b>	<p>Qualification diagnostique requise par WHO2023 (WHO-HAEMS)</p> <p>En 2024, le phénotypage est central pour - le diagnostic des LAL-T (cyCD3+) et - la classification (ETP/NOS)</p>	<p>Aucun intérêt diagnostique en 2023</p> <p><i>"In the past, immunophenotypic features that define maturational stages of T cells in the thymus were applied to T-ALL in efforts to identify prognostically important subtypes, but such classifications (eg, pro-T, pre-T, cortical T, and mature T-ALL) proved to be clinically unhelpful."</i> E. Coustan-Smith, <i>Blood</i> 2023</p>
<b>Le point de vue de Necker</b>	<p>Intérêt au diagnostic et à la rechute indispensable à rapporter: ETP vs NOS</p>	<p>Aucun intérêt diagnostique mais certainement un intérêt à la rechute. La connaissance du stade d'arrêt de maturation est importante pour l'orientation vers des thérapies ciblées</p> <p>NB : l'EGIL est une très mauvaise classification du stade d'arrêt de maturation mais a l'avantage d'être connue de tous (cliniciens &amp; biologistes) et de comporter les marqueurs les plus importants.</p>
	<p>Il est essentiel de rendre selon WHO-HAEMR5, et il reste utile de classer selon l'EGIL.</p> <p>Exemples de Rendu préconisé: Exemple 1: Phénotype de LAL-T ETP selon WHO et TI selon EGIL. Exemple 2: Phénotype de LAL-T NOS selon WHO et TIII selon EGIL</p>	
	<p>ATTENTION : contrairement à l'idée reçue, le phénotype WHO-HAEMS et EGIL ne se superposent pas nécessairement. Il est possible de retrouver occasionnellement un phénotype ETP selon WHO et non-TI selon EGIL. Exemple: LAL-T ETP selon WHO-HAEMS et LAL-T TIV γδ selon EGIL.</p>	

Ludovic Lhermitte, APHP-Hôpital Necker

# A2G French Flow ALL Team-2026

Flow Measurable Residual Disease (MRD) in era of immunotherapy  
Experience of French Flow MRD ALL Group

**A2G BlinaFlowBCP Group**

**Madrid, 28th September 2025**

*Adriana PLESA , CHU Lyon , France*

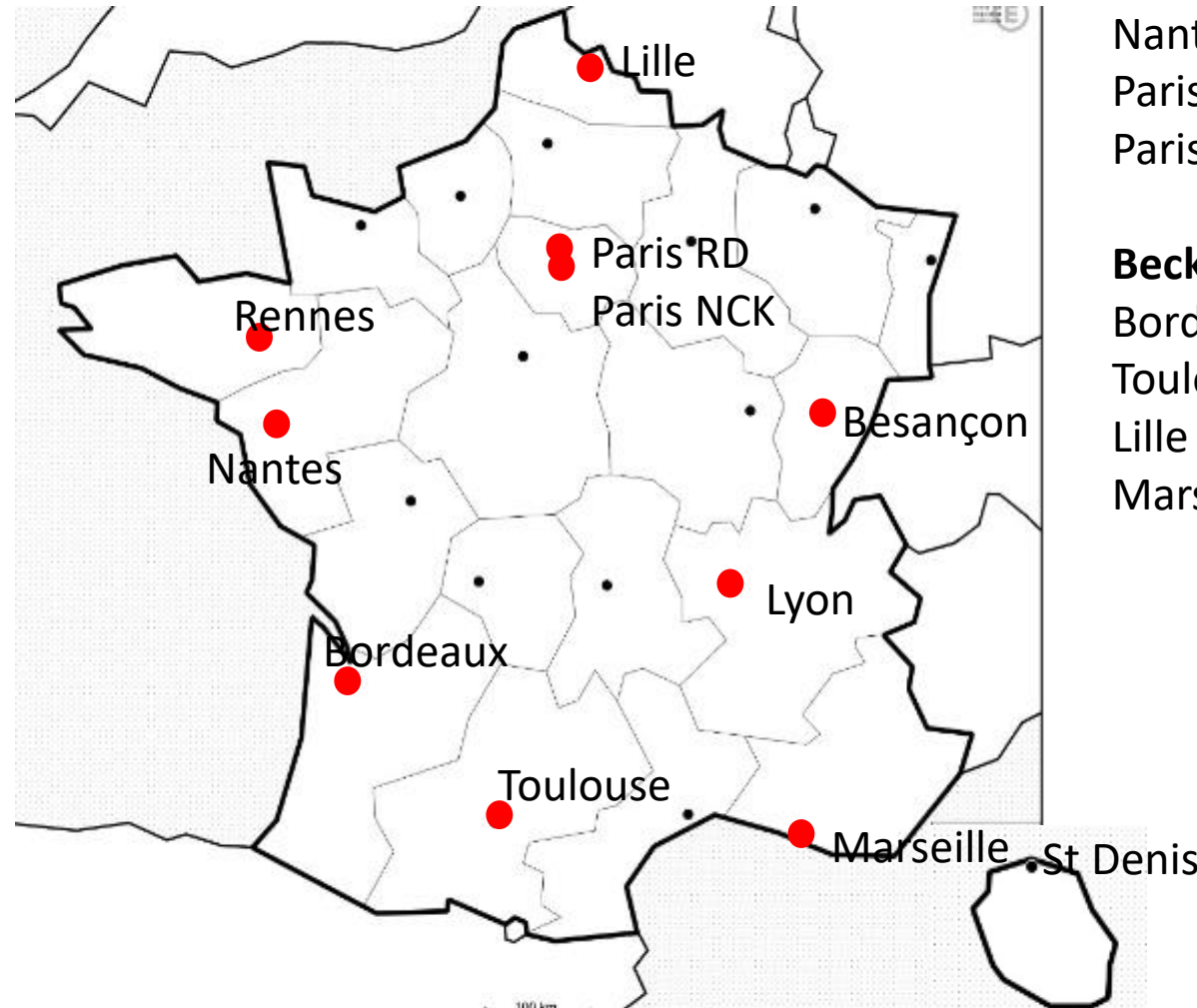
**French A2G BCPblina group**

*Adriana PLESA , CHU Lyon , France*

*Isabelle Arnoux, CHU Marseille, France*

*Elodie Lainey, APHP Paris Robert Debré, France*

*Christophe Roumier, CHU Lille, France*



**BD Platform :**

Besançon LYRIC

Lyon LYRIC/Spectral A8 Discover

Rennes NL Spectral Cytek

Nantes LYRIC

Paris RD CANTO---DxFlex

Paris NCK CANTO---DxFlex

**Beckman Platform :**

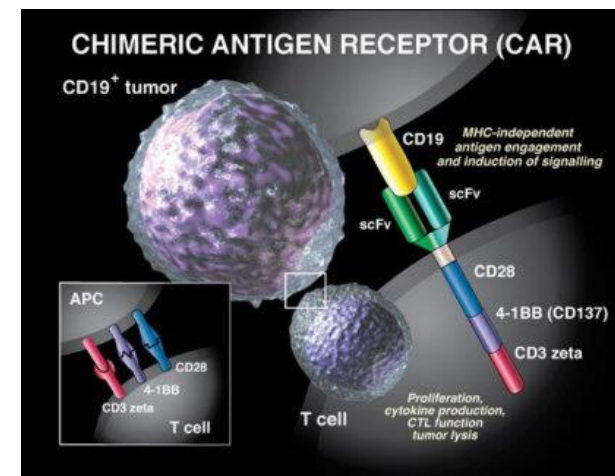
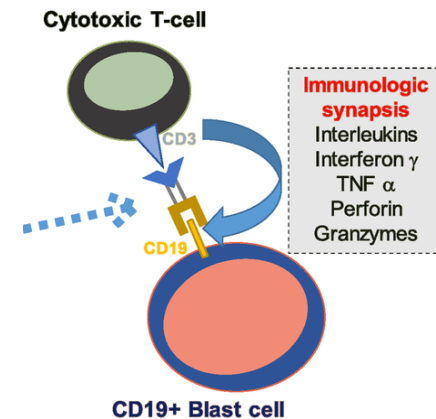
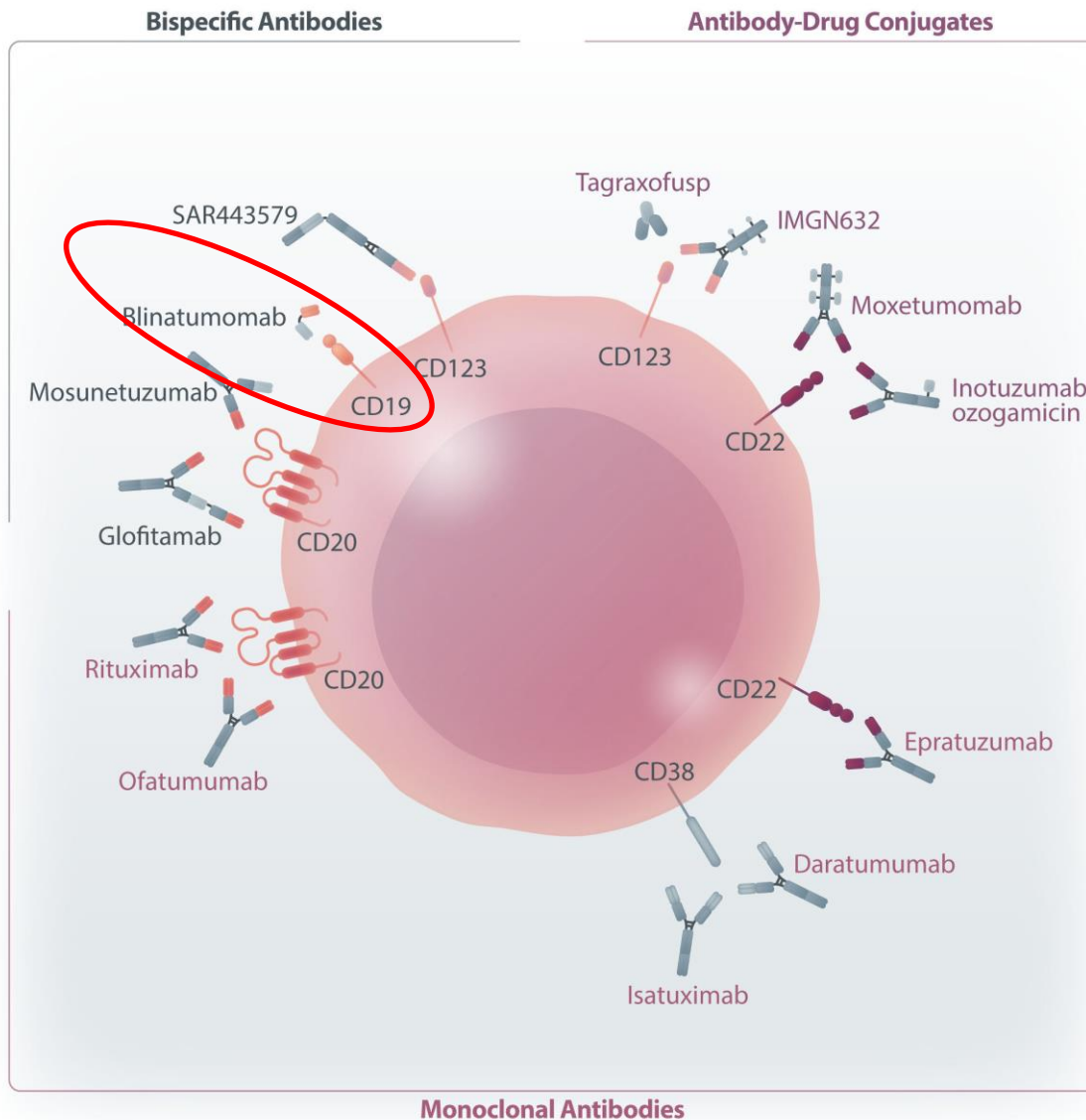
Bordeaux DxFlex

Toulouse DxFlex

Lille DxFlex

Marseille Navios---NL Spectral Cytek

# Naked antibodies and antibody-drug conjugates: targeted therapy for childhood acute lymphoblastic leukemia



New in A2G2 :MRDflow  
J15  
Blina in 1st line induction

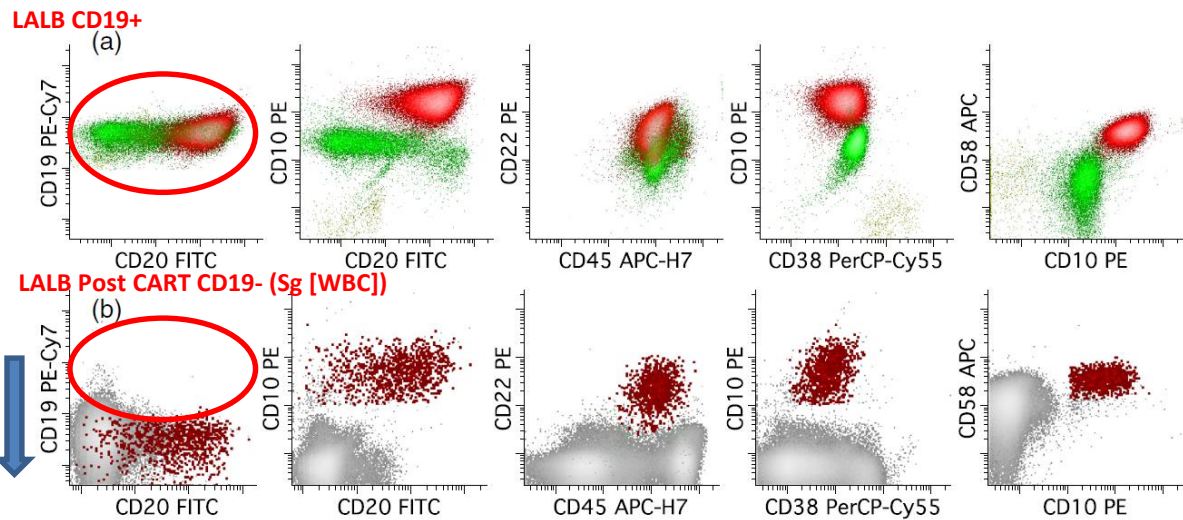
Figure 1. Targets and mechanism of actions of antibodies for pediatric acute lymphoblastic leukemia.

Lost of « input » gating MRD target CD19  
1)Adapted panel design & Gating strategy  
2)New Markers for 1st « on » gating analyse



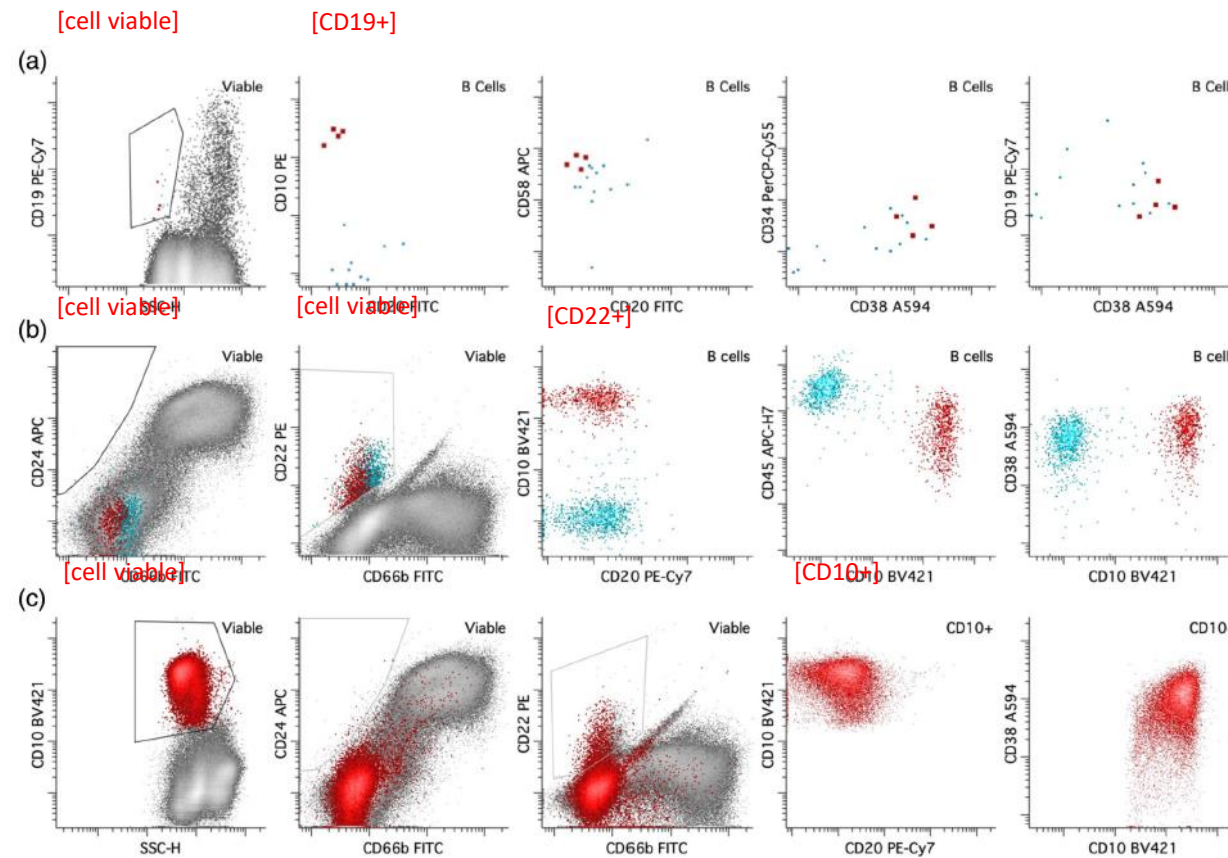
# Flow cytometric assessment for minimal/measurable residual disease in B lymphoblastic leukemia/lymphoma in the era of immunotherapy

Xueyan Chen<sup>1</sup> | Qi Gao<sup>2</sup> | Mikhail Roshal<sup>2</sup> | Sindhu Cherian<sup>1</sup>



**FIGURE 3** CD19 negative relapse post CD19-directed CAR-T cells. (a) Row 1: All plots show CD19-positive cells. Plots illustrate leukemic blasts (red) present in a patient with relapsed/refractory B-lymphoblastic leukemia, present in a background of left shifted hematogones (green). The abnormal blasts comprise ~7% of the white blood cells and demonstrate aberrant expression of CD10 (bright), CD20, CD38 (mildly decreased), and CD58 (increased). (b) Row 2: All plots show mononuclear cells. Although no CD19-positive cells are evident, an abnormal blast population is evident (0.25% of the white blood cells) that lacks CD19 but expresses B cell antigens CD20 and CD22 and shares the immunophenotypic abnormalities seen in this patient prior to therapy. The findings represent a CD19 negative relapse. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

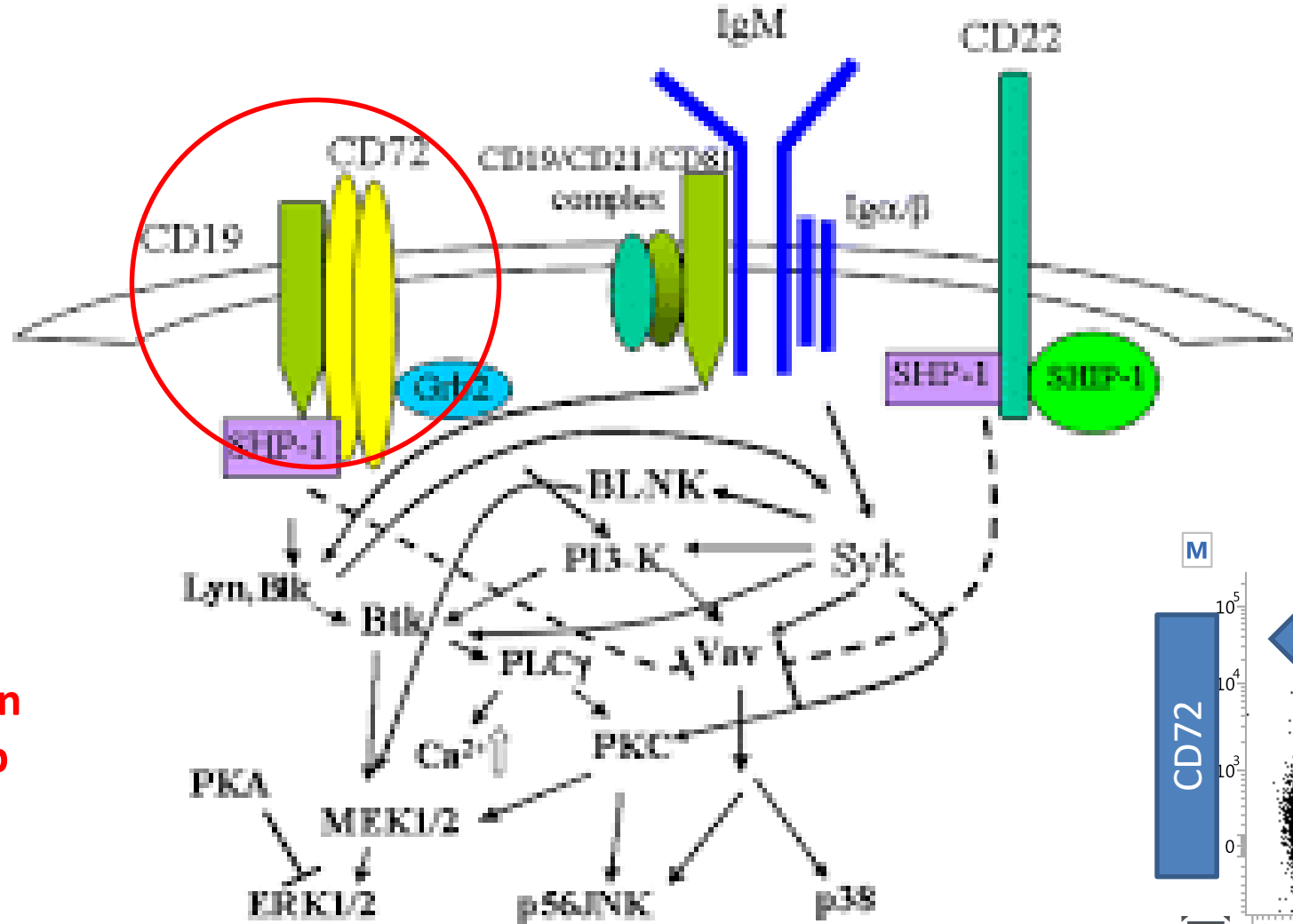
## 1st Adapted Gating Strategy



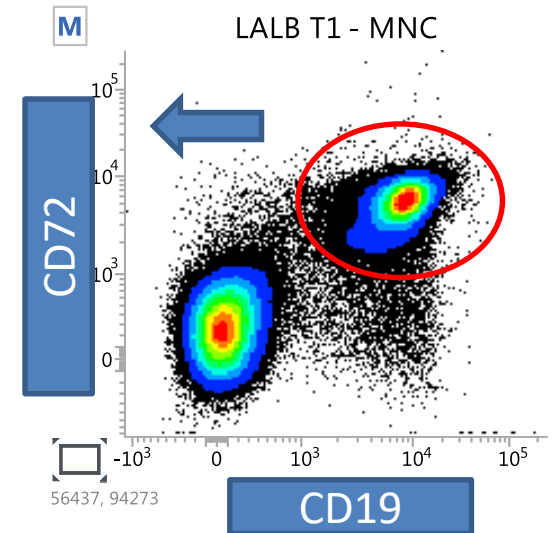
**FIGURE 7** Alternative gating strategy to identify B cells after immunotherapies targeting different B cell markers. (a) Only few B cells are identified using CD19 gating. Red dots represent immature B cells with an aberrant immunophenotype suspicious for MRD. (b) There are more B cells identified by CD22 and CD24 gating than by CD19 gating. The subset colored red represents abnormal immature B cells with an immunophenotype similar to that seen in A. (c) Gating with CD10 generates a much larger population of abnormal immature B cells (colored red) with an immunophenotype similar to that seen in A and B. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Adapted gating strategy based on most robust LAIP : CD34, CD38, CD10, CD22, CD24

2<sup>nd</sup> New markers as initial gating when CD19 is lost  
 CD72 CoReceptor in B cell Lymphopoiesis



CD72 vs CD19  
 BCP ALL  
 Level of co-expression  
 Stability in follow-up





**CD72 is a pan-tumor antigen associated to pediatric acute leukemia**

Barbara Buldini<sup>1,2</sup> | Giovanni Faggin<sup>1</sup> | Elena Porcù<sup>1</sup> | Pamela Scarparo<sup>1</sup> |  
 Katia Polato<sup>1</sup> | Claudia Tregnago<sup>2</sup> | Elena Varotto<sup>1</sup> | Paolo Rizzardi<sup>3</sup> |  
 Carmelo Rizzari<sup>4</sup> | Franco Locatelli<sup>5</sup> | Alessandra Biffi<sup>1,2</sup> | Martina Pigazzi<sup>1,2</sup>

**TABLE 1** CD72 expression by flow cytometry in BCP-ALL samples collected at diagnosis and relapse, as defined according to the AIEOP-BFM and WHO classifications [6, 11].

CD72 expression according to WHO	CD72 expression according to AIEOP-BFM	Diagnosis		Relapse		Total	
		n	%	n	%	n	%
<b>Total</b>		<b>139</b>		<b>76</b>		<b>215</b>	
Strong	Bright	105	76	53	70	158	73
	Medium	8	6	7	9	15	7
	Heterogeneous	21	15	11	15	32	15
	PP2	0	0	0	0	0	0
Weak	DIM	2	1	4	5	6	3
	PP1	0	0	0	0	0	0
Negative	Negative	3	2	1	1	4	2

Note: PP2, partial positive 2; PP1, partial positive 1.

**Total 495 pathological pediatric BM aspirates, including: 215 (BCP-ALL), 156 (AMLs), 88 T-lineage ALLs or LBL, 13 B-lineage lymphoblastic lymphomas, 9 MDS (5%–9% blasts on BM: MDS-IB1) and 14 nonhematopoietic solid tumors infiltrating BM.**



- **CD72 is highly expressed in almost all BCP-ALL** and the majority of AML at diagnosis, including BCP-ALL cases characterized by CD19 loss.
- support a potential role for advanced diagnostics and novel immunotherapy approaches, providing a pan-ALL and AML target.

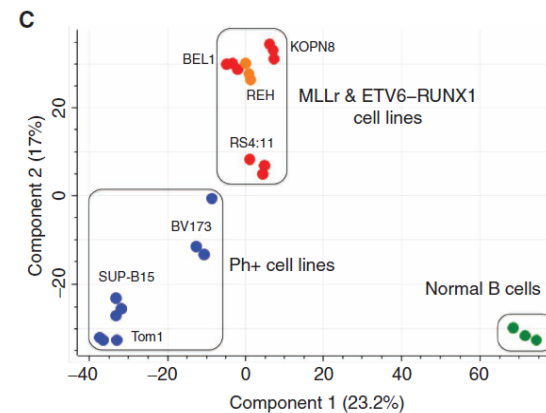
**2<sup>nd</sup> New markers as initial gating when CD19 is lost**

RESEARCH ARTICLE

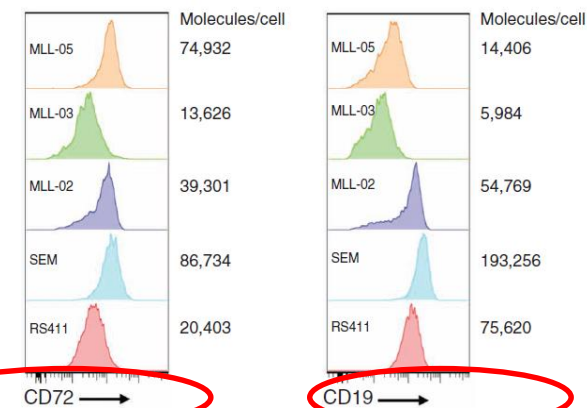
**Surface Proteomics Reveals CD72 as a Target for *In Vitro*-Evolved Nanobody-Based CAR-T Cells in *KMT2A/MLL1*-Rearranged B-ALL**

Matthew A. Nix<sup>1</sup>, Kamal Mandal<sup>1</sup>, Huimin Geng<sup>1</sup>, Neha Paranjape<sup>1</sup>, Yu-Hsiu T. Lin<sup>1</sup>, Jose M. Rivera<sup>2</sup>, Makeba Marcoulis<sup>1</sup>, Kristie L. White<sup>1</sup>, Jeffrey D. Whitman<sup>1</sup>, Sagar P. Bapat<sup>1</sup>, Kevin R. Parker<sup>3</sup>, Jonathan Ramirez<sup>4</sup>, Anne Deucher<sup>1</sup>, Paul Phojanokong<sup>5</sup>, Veronica Steri<sup>6</sup>, Faranak Fattahi<sup>1</sup>, Byron C. Hann<sup>5</sup>, Ansuman T. Satpathy<sup>3</sup>, Aashish Manglik<sup>6,7</sup>, Elliot Stieglitz<sup>2</sup>, and Arun P. Wiita<sup>1</sup>

**CD72 when CD19 is lost good to integrate into MRD BCP-ALL panel design (up-regulated in *KMT2A/MLL1*)**



B



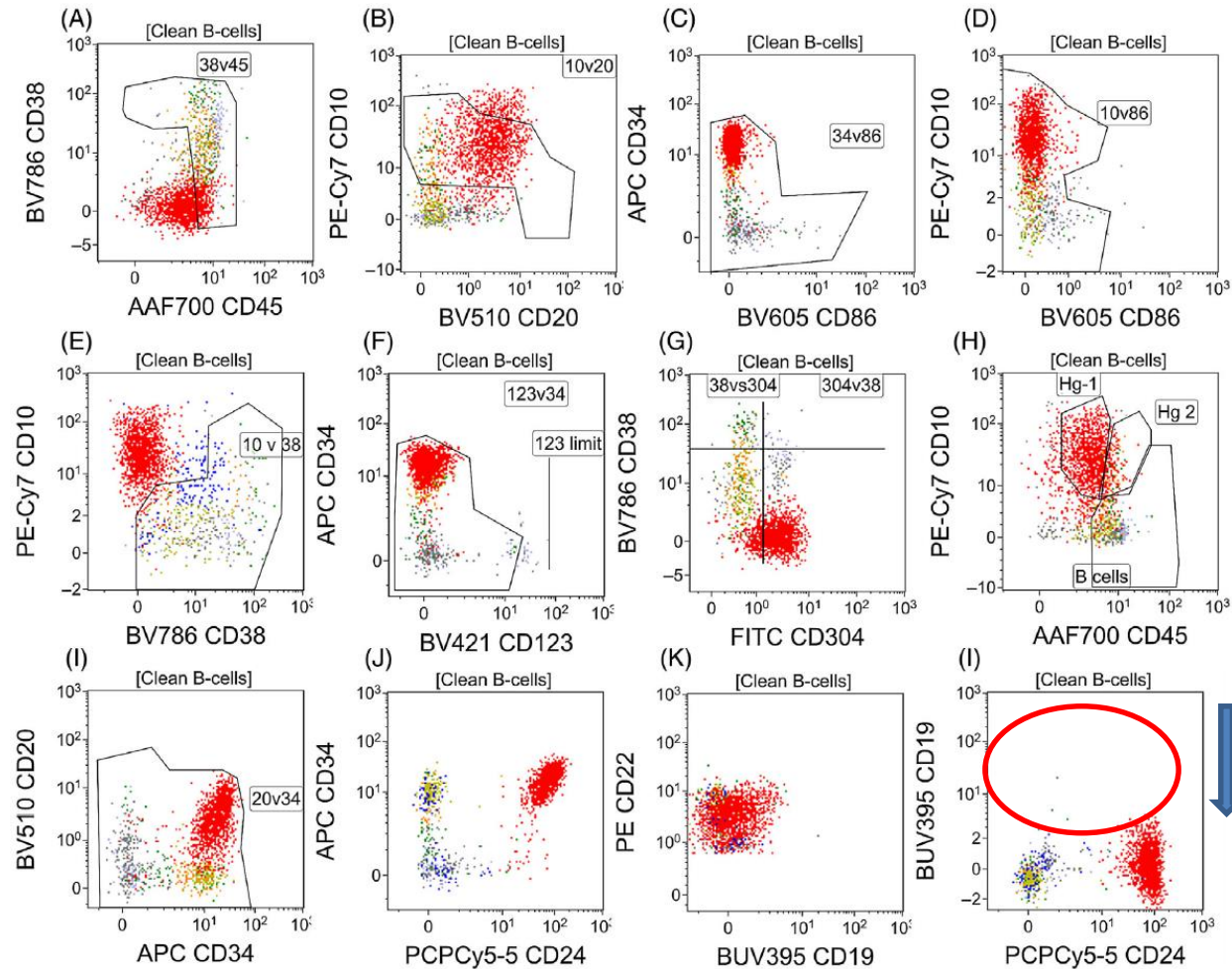


# 15-color highly sensitive flow cytometry assay for post anti-CD19 targeted therapy (anti-CD19-CAR-T and blinatumomab) measurable residual disease assessment in B-lymphoblastic leukemia/lymphoma: Real-world applicability and challenges

Gaurav Chatterjee<sup>1</sup> | Priyanka Dhende<sup>1</sup> | Simpy Raj<sup>1</sup> | Vruksha Shetty<sup>1</sup> | Sitaram Ghogale<sup>1</sup> | Nilesh Deshpande<sup>1</sup> | Karishma Girase<sup>1</sup> | Jagruti Patil<sup>1</sup> | Aastha Kalra<sup>1</sup> | Gaurav Narula<sup>2</sup> | Kajal Dalvi<sup>1</sup> | Chetan Dhamne<sup>2</sup> | Nirmalya Roy Moulik<sup>2</sup> | Sweta Rajpal<sup>1</sup> | Nikhil V. Patkar<sup>1</sup> | Shripad Banavali<sup>2</sup> | Sumeet Gujral<sup>3</sup> | Papagudi G. Subramanian<sup>1</sup> | Prashant R. Tembhare<sup>1</sup>



Multicolor panel design 15c seems improve specificity and sensitivity for LAIP capture in CD19 target lost (including MyCD33), but also « LL » low level normal cells missinterpreted as MRD (pDC, MSC/Endoth, ELP-1, ELP-2)

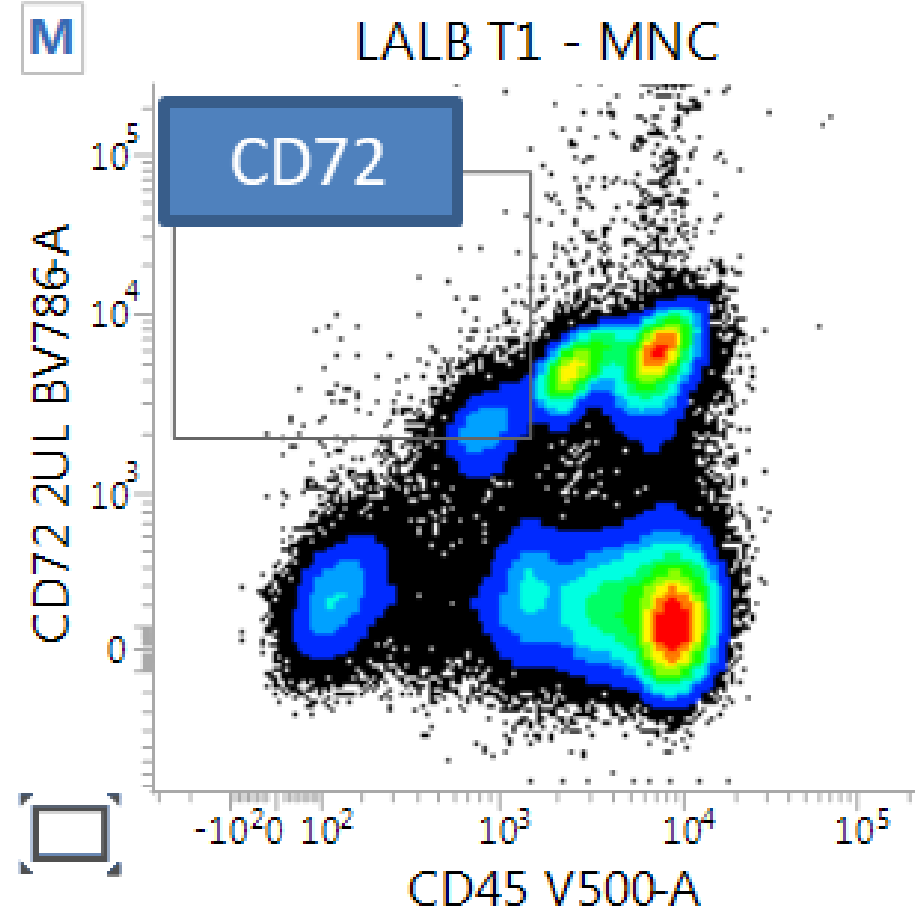
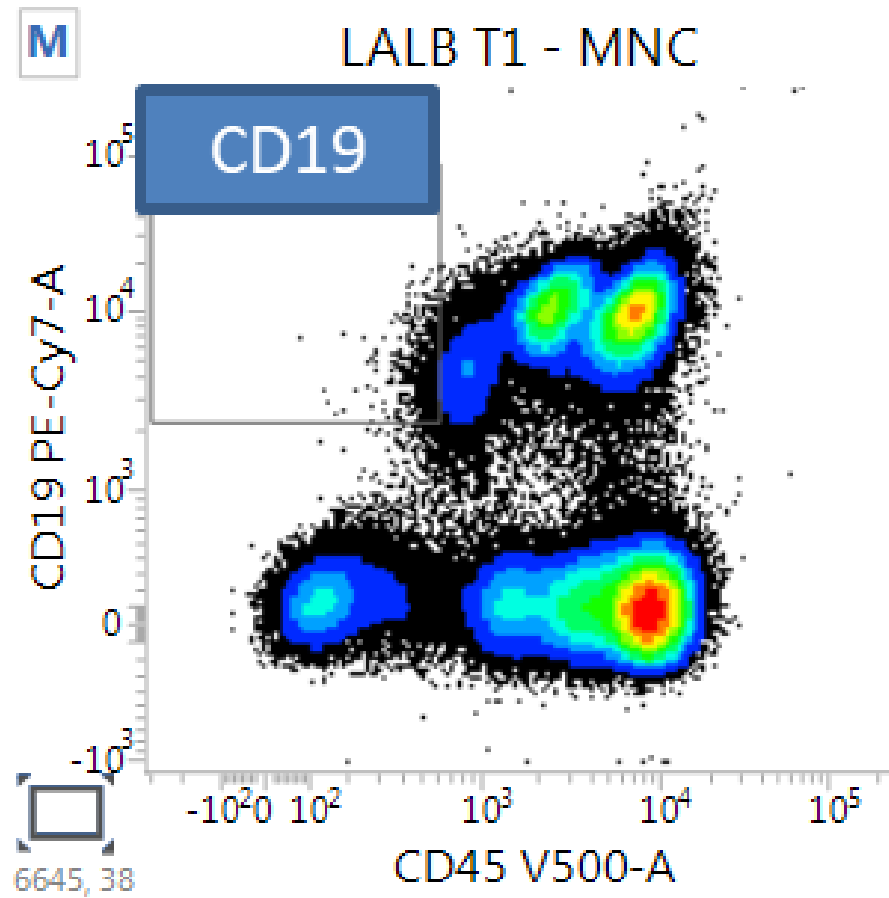


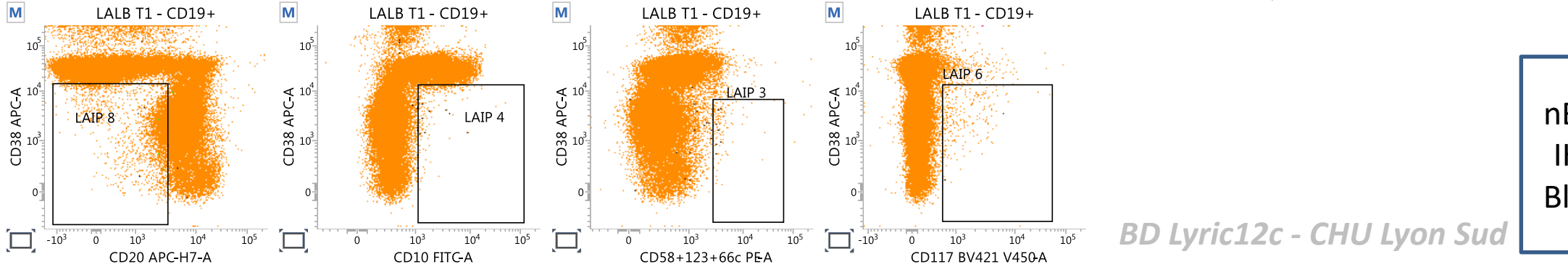
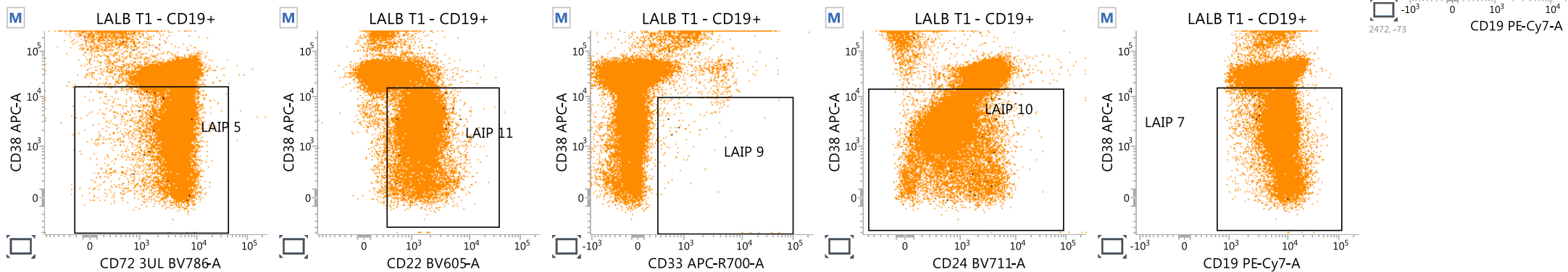
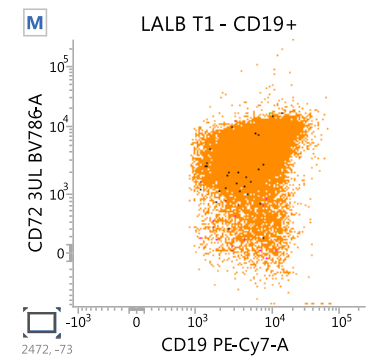
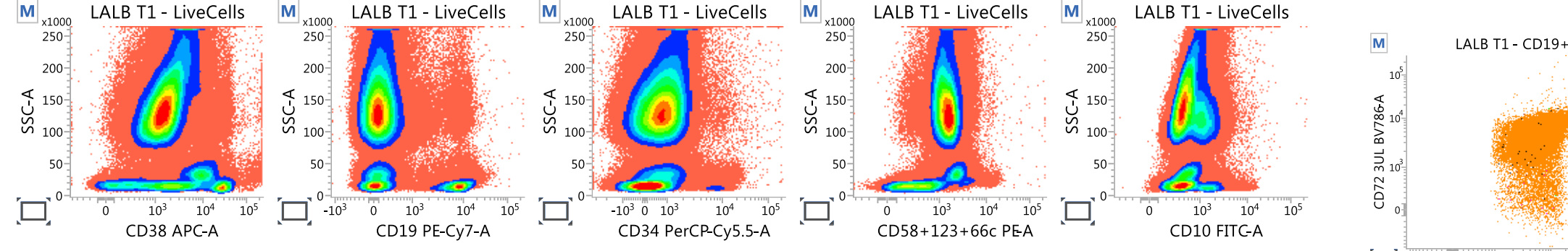
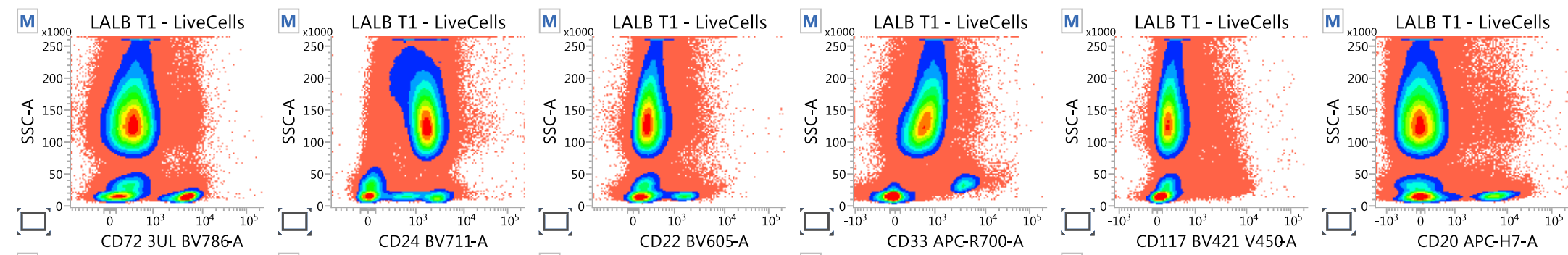
**FIGURE 3** Another example of clear MRD positive (0.044%) in patient treated with anti-CD19-CAR-T therapy. MRD is highlighted with “red colored” dots show bright CD10, dim CD20, moderate CD22, bright CD24, moderate CD34, dim CD45 and dim CD304 expression but are negative for CD19, CD28, CD73, CD84, and CD123. It can be noted that all B-cell precursors are negative for CD19.

**TABLE 1** 15-color BMRD panel for post anti-CD19 immunotherapy samples.

Fluorochrome	BUV395	BV786	BV711	BV650	BV605	BV510	BV421	FITC	PE	PE-CF594	PC5.5	PC7	APC	APC-AF700	APC V 770
Markers	CD19	CD 38	CD33	HLADR	CD86	CD20	CD123	CD304	CD22	CD73	CD24	CD10	CD34	CD45	CD81
Clone	HIB19	HIT2	WM53	G46-6	2331 (FUN-1)	2H7	9F5	BDCA2	REA340	AD2	ALB9	ALB1	581	J33	REA513

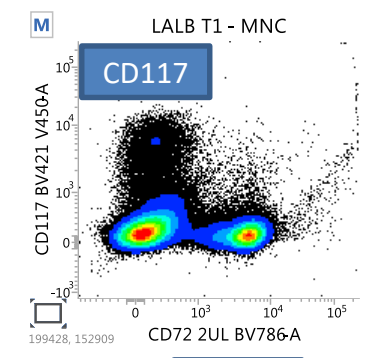
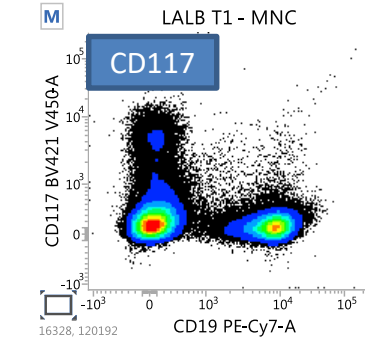
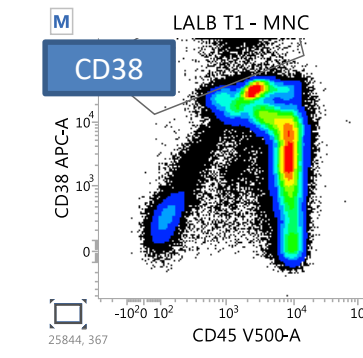
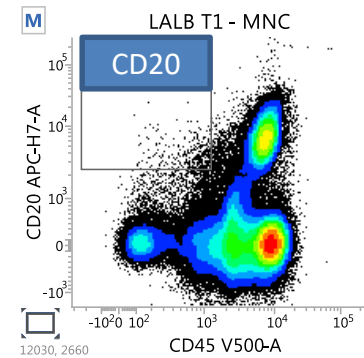
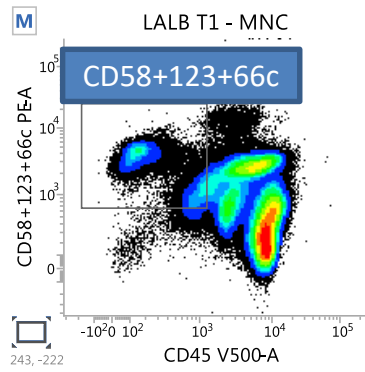
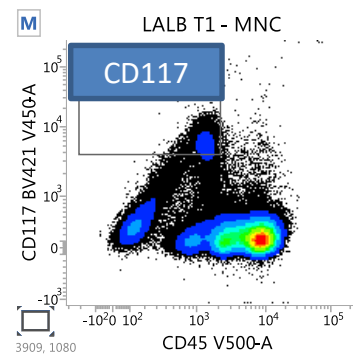
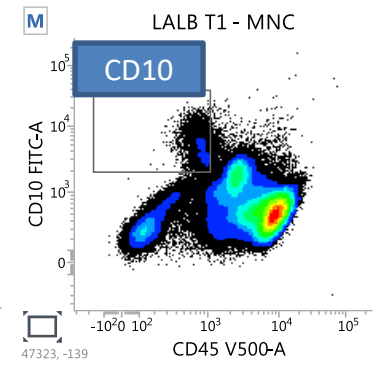
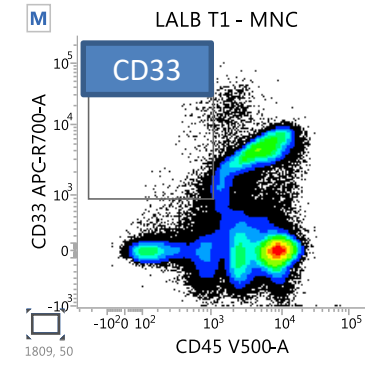
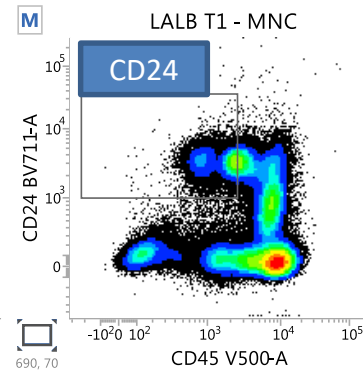
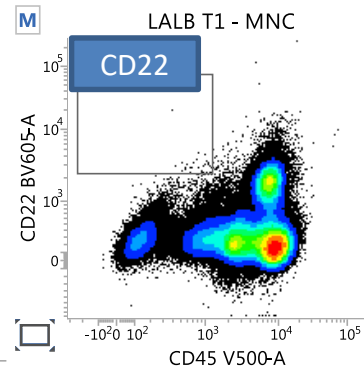
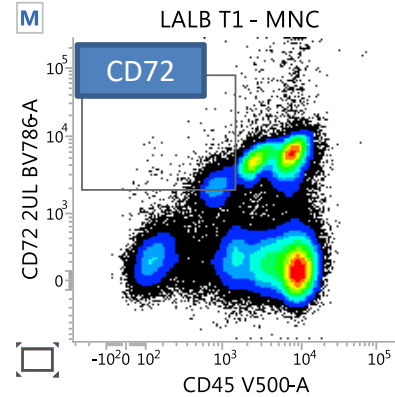
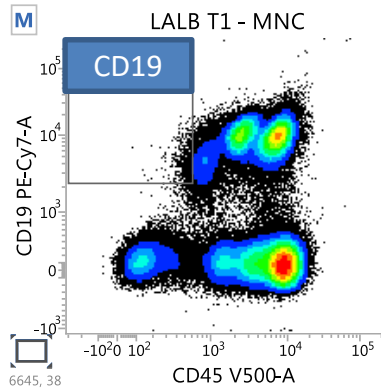
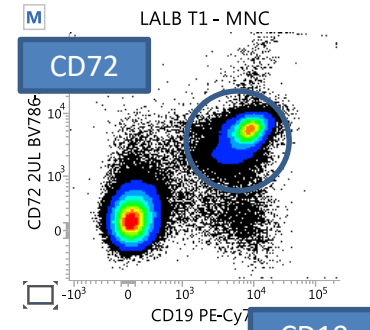
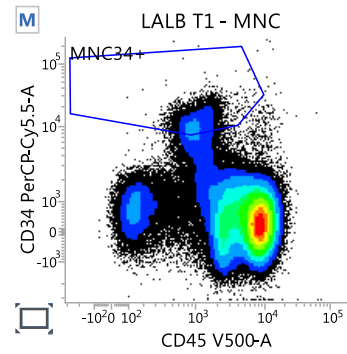






nBM, 10 Y, Anemia  
IHOP/Lyon (Panel  
Blina BCP Lyric12c)

nBM, 10 Y, Anemia  
IHOP/Lyon (Panel  
Blina BCP Lyric12c)



CD19

CD72

# Panels standardisation in 4 Platforms : CANTO/NAVIOS/LYRIC/DxFlex (8c/10c/12c/13c)



## Ongoing Test -1st Test Lyon Spectral-BD Discover/Cytek

Panels BCP-ALL: according to STIC and A2G ( **Backbone** commun STIC06 & add Marqueurs A2G)

WebTeam FlowA2G 8-1-2025

Panel BCP-ALL Blina MRD

HARMONISATION OF PRE-ANALYTICAL SAMPLE PROCESSING  
Bulk Lysis NH4Cl/ Staining/Wash/  
Acquisition of minimum 800,000 -1 000 000cells



HARMONISATION OF CYTOMETER SETTINGS  
to achieve comparable fluorescent profiles



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

A2G France BCP blina ALL 2025		Backbone "core markers"								dropping Ab					
A2G BCPblina 2025 Lyon/Lille Standardization/Harmonization															
LYRIC 12c	Tubes		FITC	PE		PerCpCy5.5	PC7	APC	APC R700	APCH7	BV421	V500	BV605	BV711	BV786
		10µl	10µL	5µL+5µL+5µL		10µL	5µL	5µL	5µL	5µL	5µL	5µL	5µL	5µL	2µL
	Tube BlinaMRD	BSB+	CD10	CD58+CD123+CD66c		CD34	CD19	CD38	CD33	CD20	CD117	CD45	CD22	CD24	CD72
	Tube 2	BSB+	CD10	CD304+73 (2,5+2,5 µL)		CD34	CD19	CD38	CD200	CD20	CD49f	CD45	CD9	CD371	CD81
DxFlex 13c	Tubes		FITC	PE	ECD	PC5,5	PC7	APC	APC R700	A750	BV421	KO	BV605	BV660	BV786
		10µl	10µL	5µL+5µL+5µL			5µL	5µL		5µL	5µL	5µL	5µL	2µL	2µL
	Tube BlinaMRD	BSB+	CD10	CD58+CD123+CD66c	CD19	CD22	CD34	CD38		CD33	CD117	CD45	CD24	CD20	CD72
	Tube 2	BSB+	CD10	CD304+73 (2,5+2,5 µL)	CD19	CD20	CD34	CD38		CD200	CD49f	CD45	CD9	CD371	CD81
BD Pharm Lyse™ Lysing Buffer #555899															
BD Horizon™ Brilliant Stain Buffer Plus #566385															

A2G France Panel LALB MRD Blina (teste/titration ok, prêt mise en routine)  
Lyric12c Lyon-DxFlex13c Lille/RBD-Navios10c Marseille  
Suite Utrecht 30 Sept 2024: 2 WebTeam (Oct/Nov 2024) Adriana/Isabelle (France) avec Hanne (Nopho) et Lucy (UK)  
Proposition collaboration/harmonisation Panel NextA2G



HARMONISATION OF CLINICAL FLOW REPORT  
MRD /integrated Cytomorphology

LOQ 0,01% 1x10<sup>-4</sup> (min 10 ev cluster MRD)-LOD 0,001%

### Proposal Schema of a Flow Cytometric ALL MRD Harmonisation Strategy

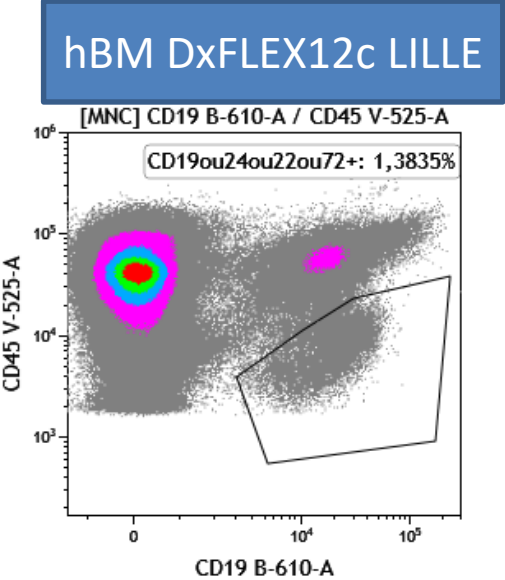
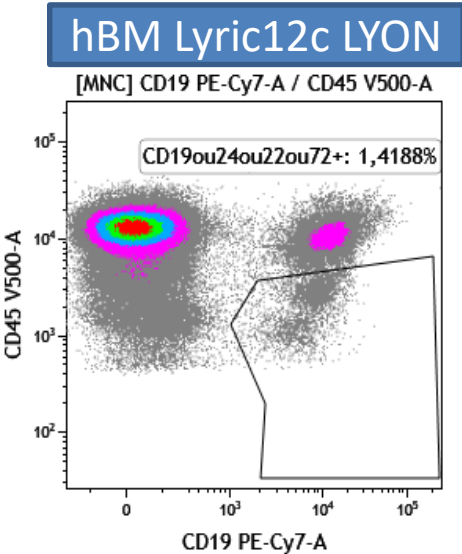


Comments:

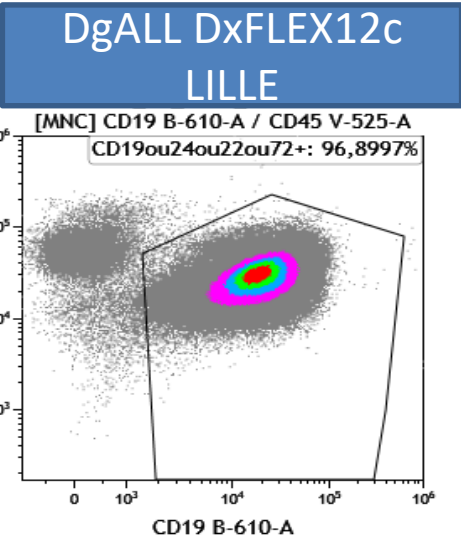
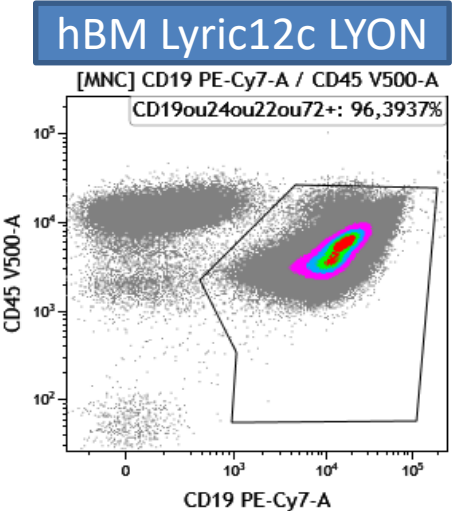
-Standardization : using same panel and setting for same platforms/machines (ex Lyric1 , Lyric2, Lyirc3, etc)

-Harmonization: same panel for a clinical Trial but 2 platforms (ex BD Lyric12c vs BC DxFlex 12c)

-Optimization: a panel adapted according to the platform (ex DxFlex CD19 ECD is better than PEcy7, but well mirroring with Lyric CD19 PEcy7)



hBM



Dg BCP ALL





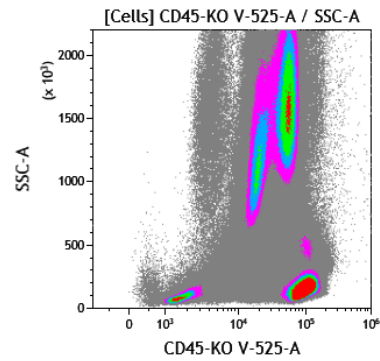
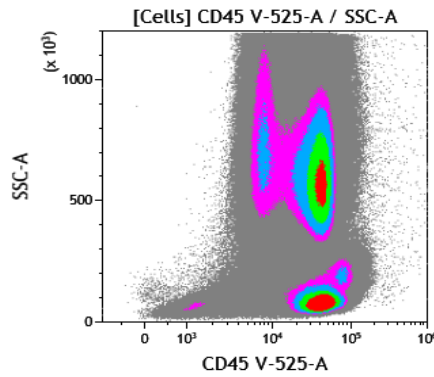
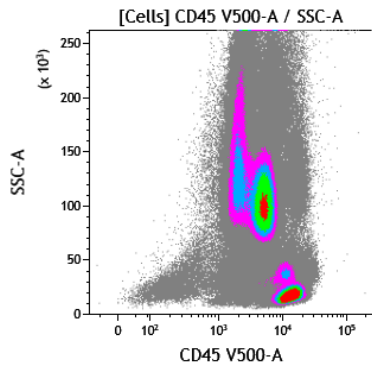
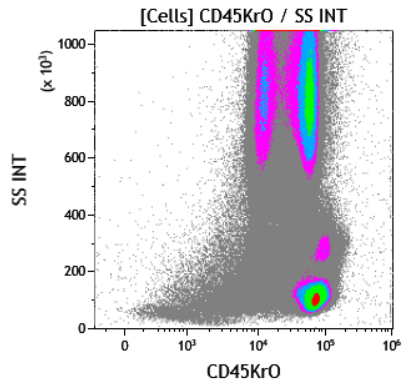
**hBM (ECQ 11/9/2025)  
From Lyon-to-  
Marseille/Lille/ParisRBD**

**Navios 10c  
Marseille**

**Lyric12c  
Lyon**

**DxFlex  
Lille 12c**

**DxFlex 12C  
APHP Paris**

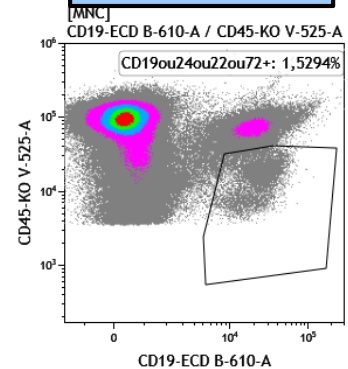
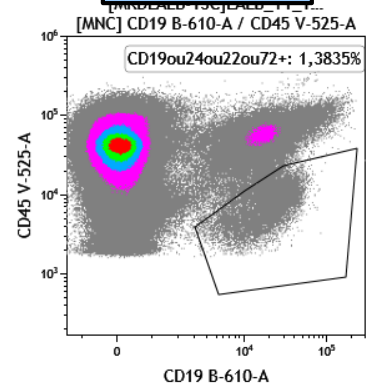
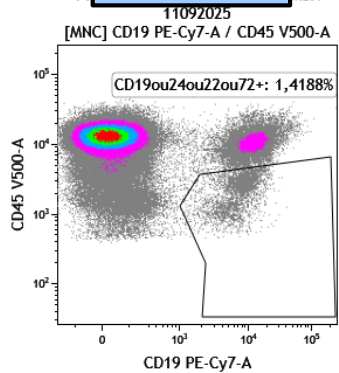
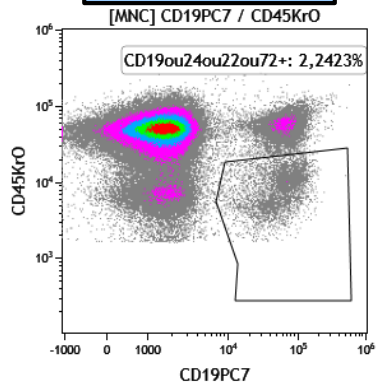


**Navios 10c  
Marseille**

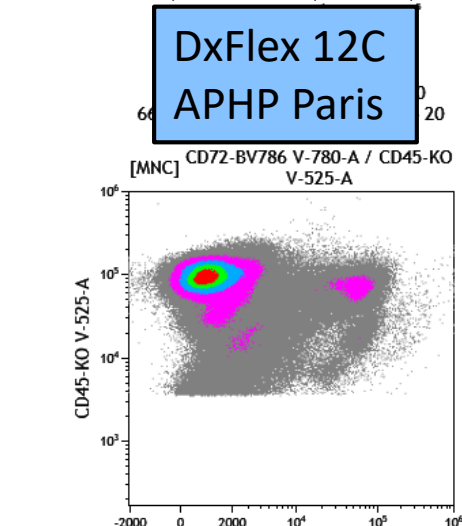
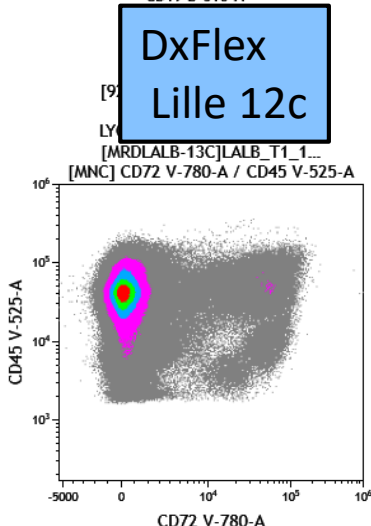
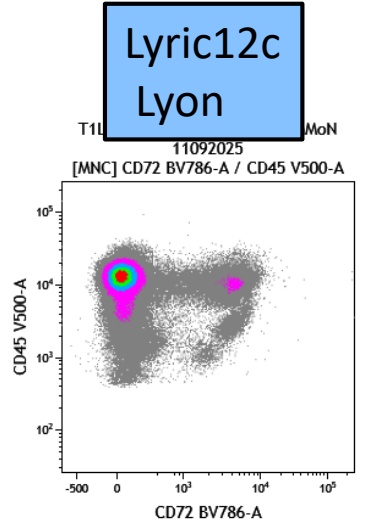
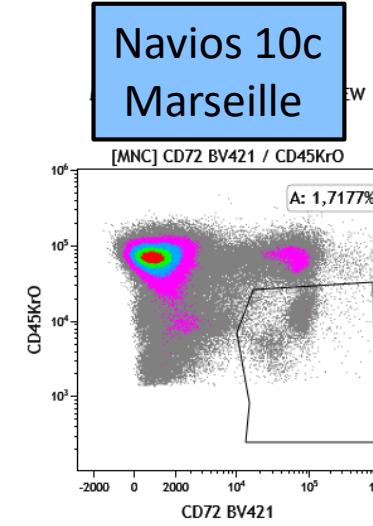
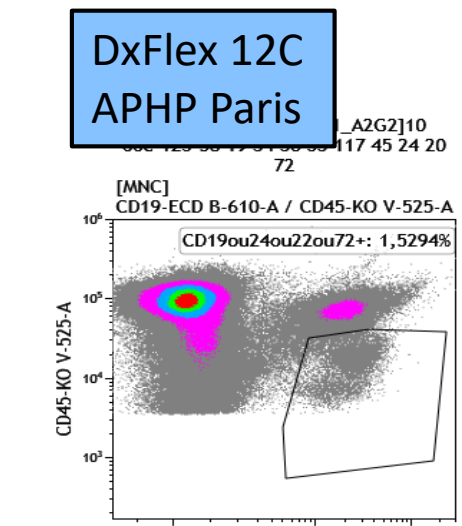
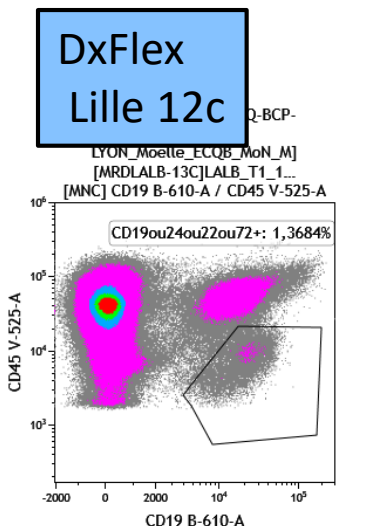
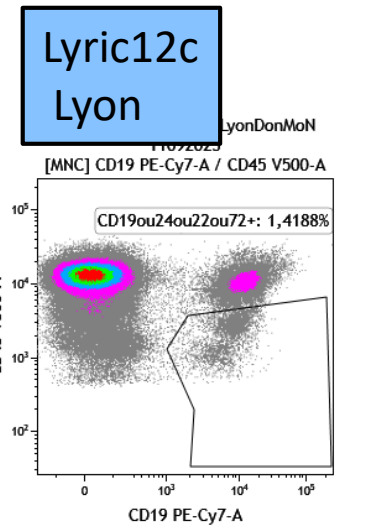
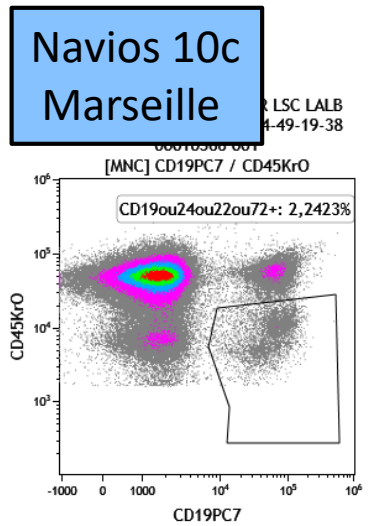
**Lyric12c  
Lyon**

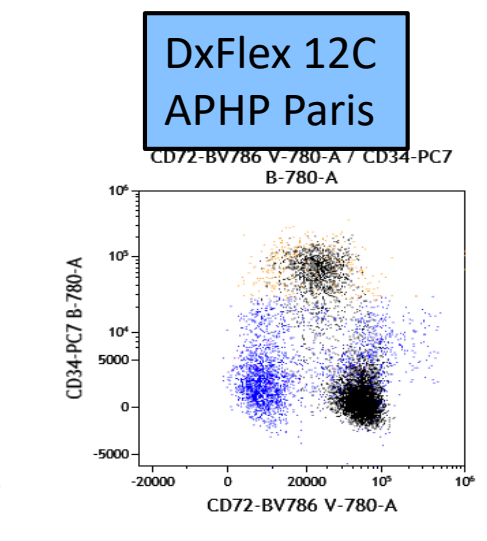
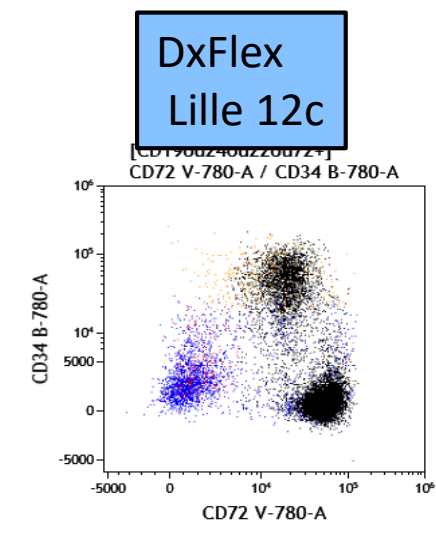
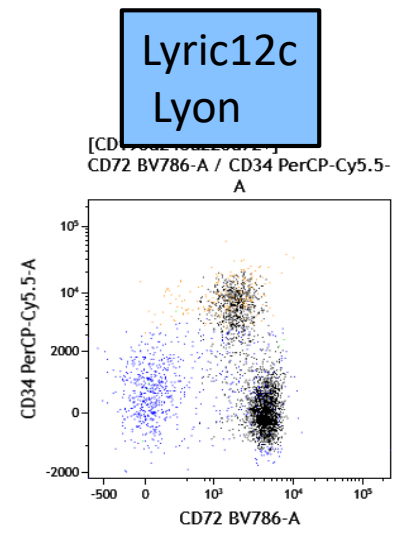
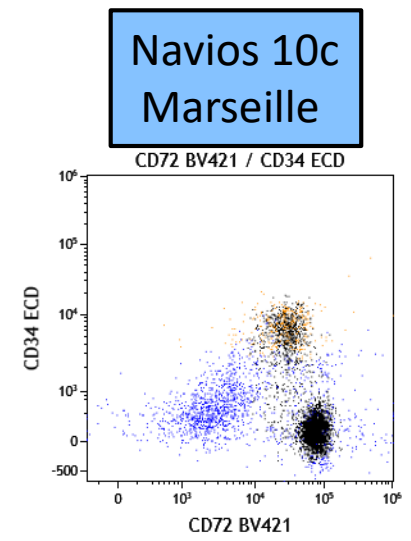
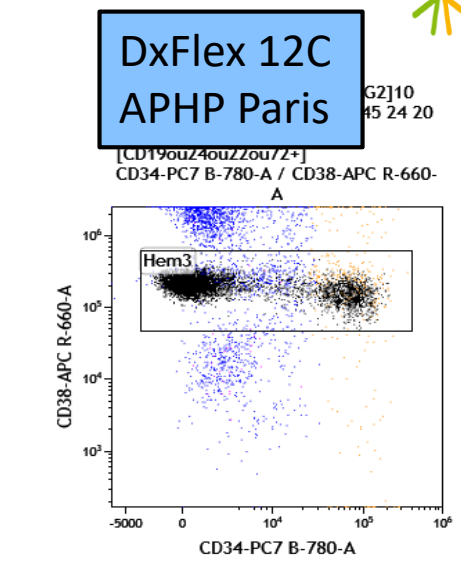
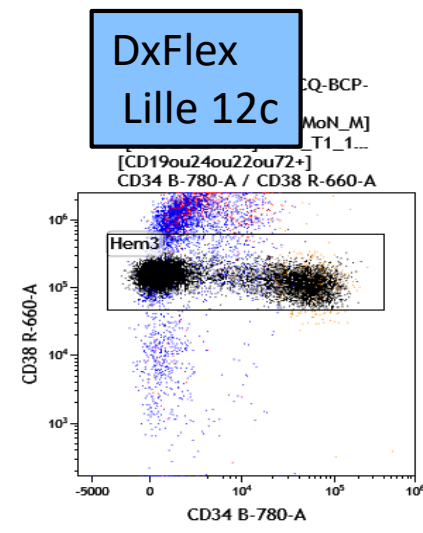
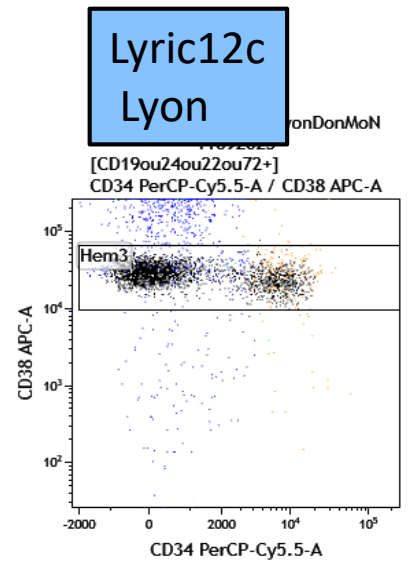
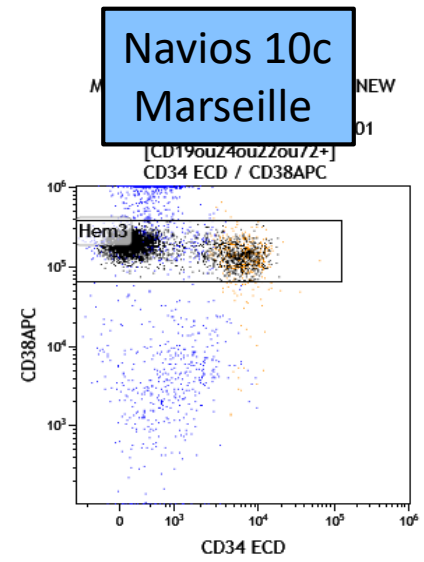
**DxFlex  
Lille 12c**

**DxFlex 12C  
APHP Paris**

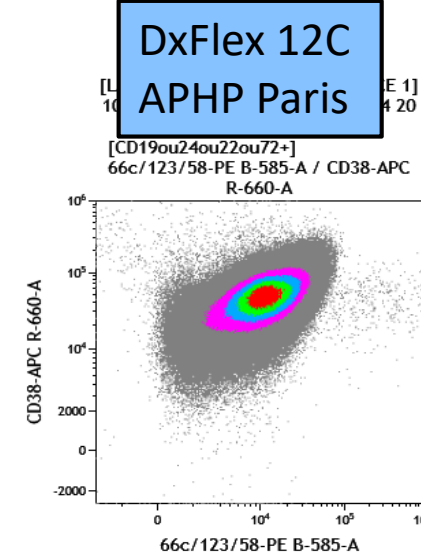
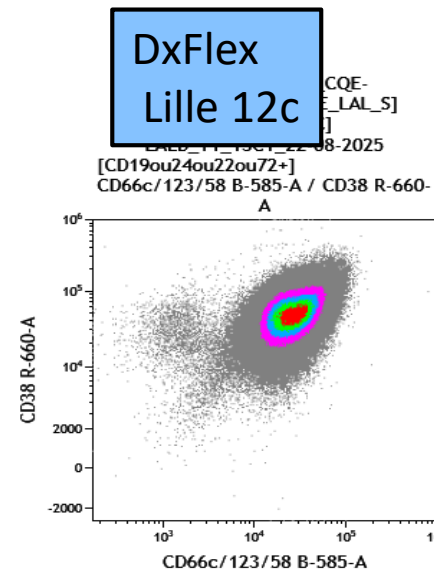
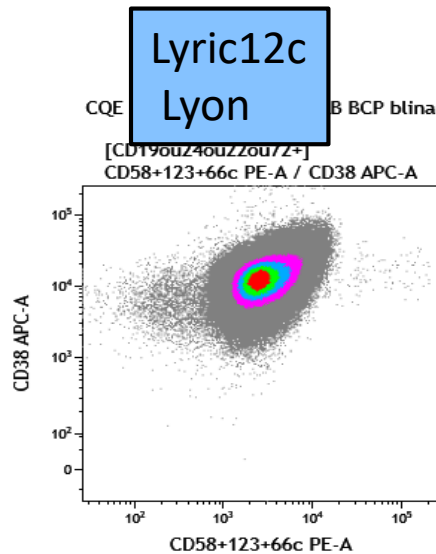
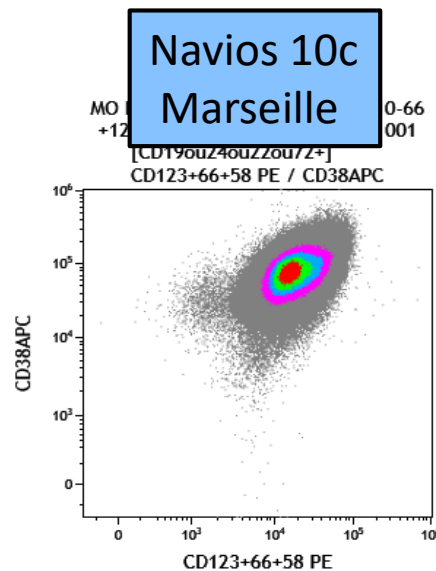
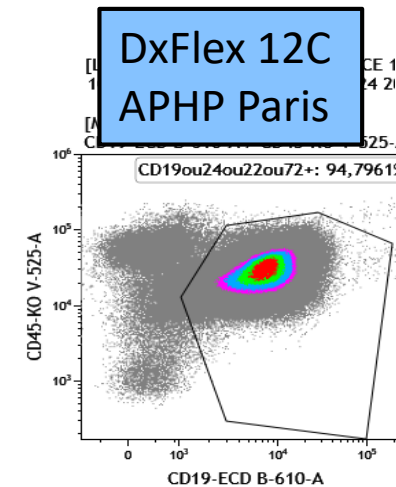
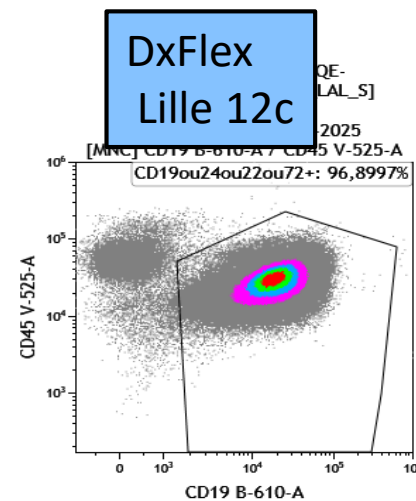
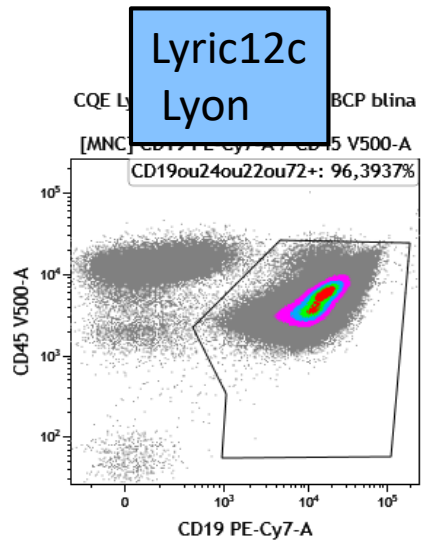
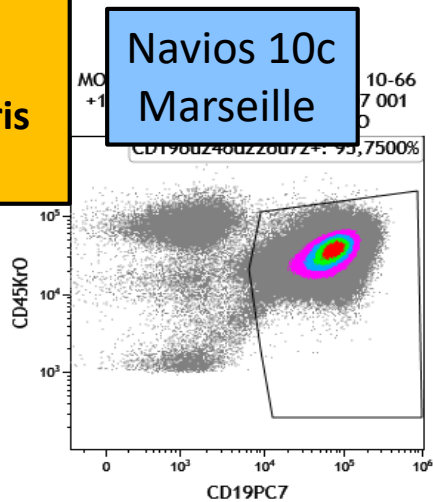


**ECQ hBM - BCP Blina panel Lyon/Marseille/Lille/ParisRBD  
Adriana Plesa (Lyon)/Isabelle Arnoux (Marseille)/Christophe Roumier (Lille)/Elodie Lainey (Paris)**





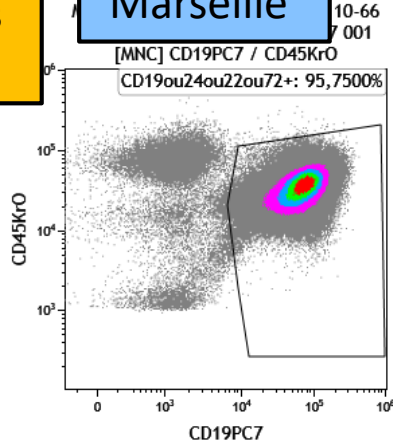
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(ECQ 22/8/2025)  
From Lyon-to-  
Marseille/Lille/Paris  
RBD**



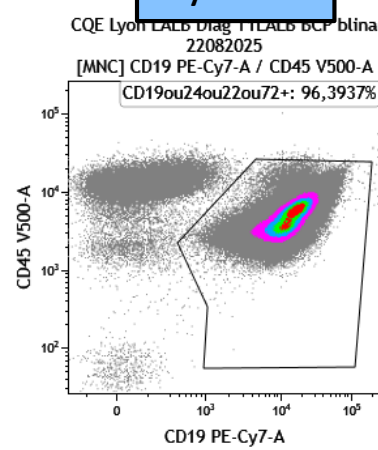
ECQ BCPALL Dg (ProBI, KMT2A, t(9;11) - BCP Blina panel Lyon/Marseille/Lille/ParisRBD  
Adriana Plesa (Lyon)/Isabelle Arnoux (Marseille)/Christophe Roumier (Lille)/Elodie Lainey (Paris)

**BCP ALL diagnosis  
(ECQ 22/8/2025)  
From Lyon-to-  
Marseille/Lille/Paris  
RBD**

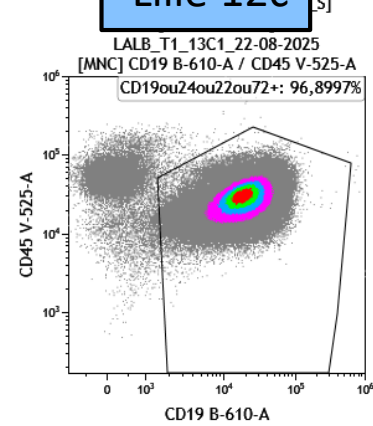
**Navios 10c  
Marseille**



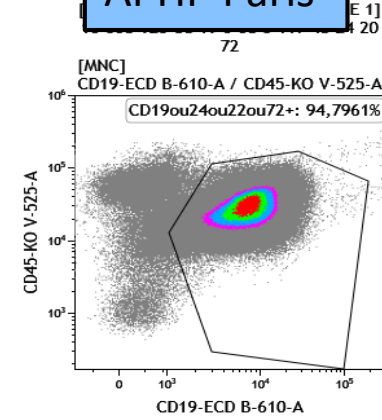
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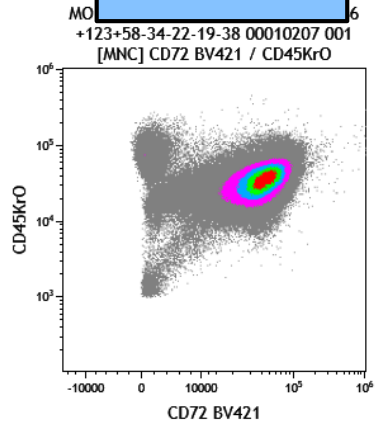
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Lille 12c**



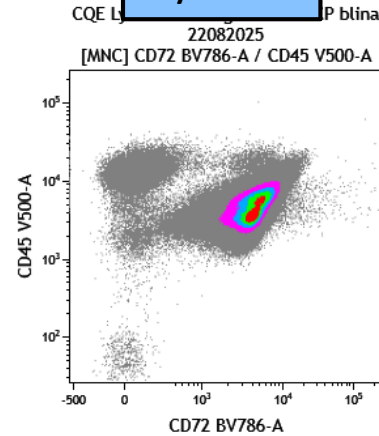
**DxFlex 12C  
APHP Paris**



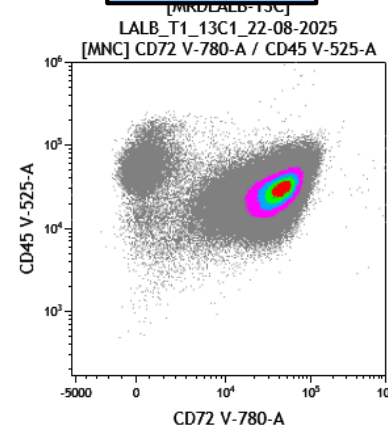
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Marseille**



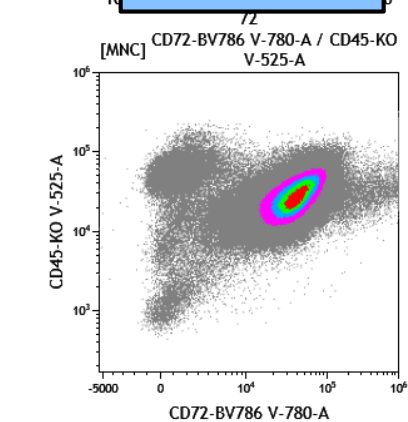
**Lyric12c  
Lyon**



**DxFlex  
Lille 12c**

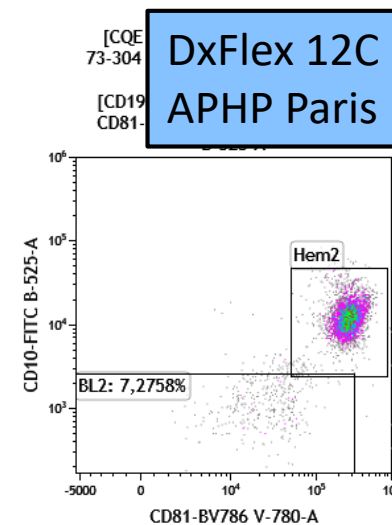
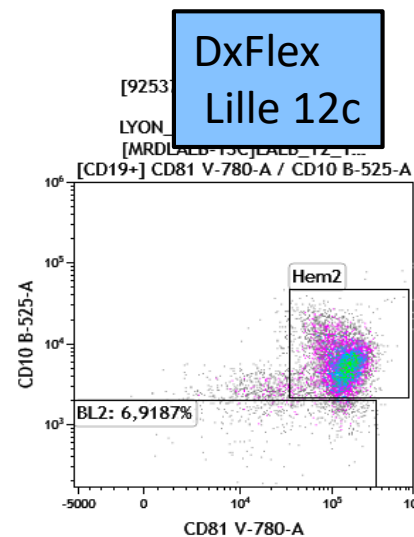
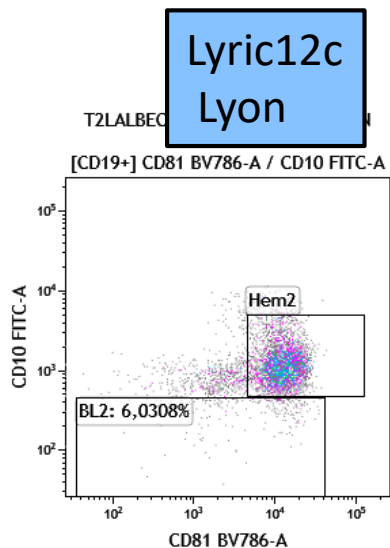
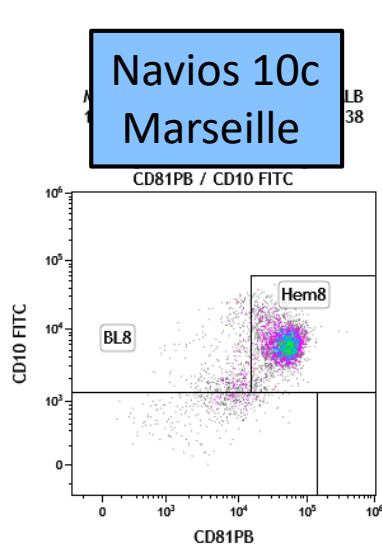


**DxFlex 12C  
APHP Paris**

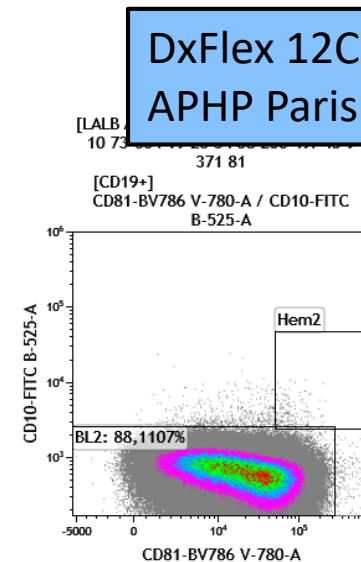
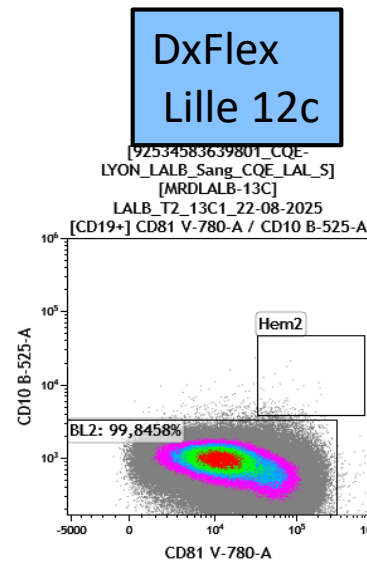
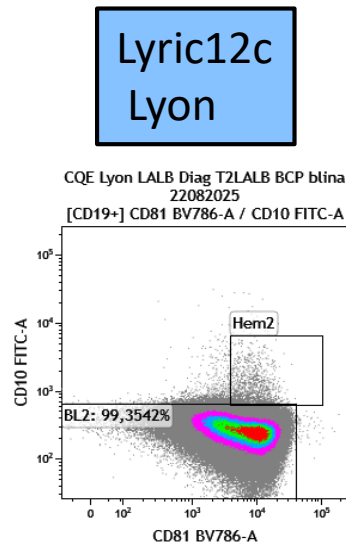
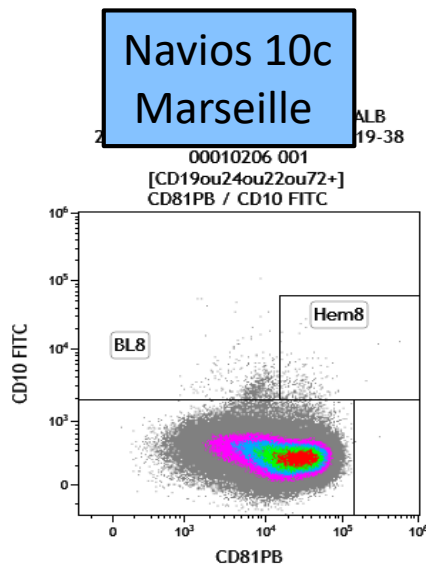


ECQ BCPALL Dg (ProBI, KMT2A, t(9;11) - BCP Blina panel Lyon/Marseille/Lille/ParisRBD  
Adriana Plesa (Lyon)/Isabelle Arnoux (Marseille)/Christophe Roumier (Lille)/Elodie Lainey (Paris)

**hBM**

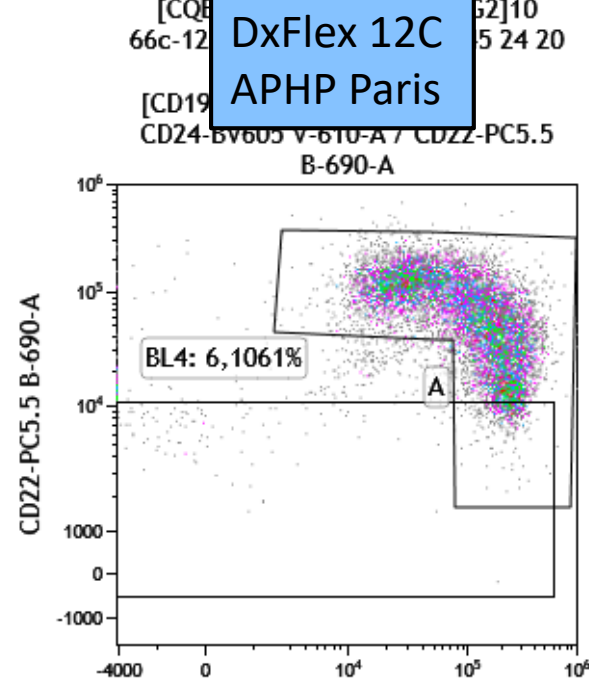
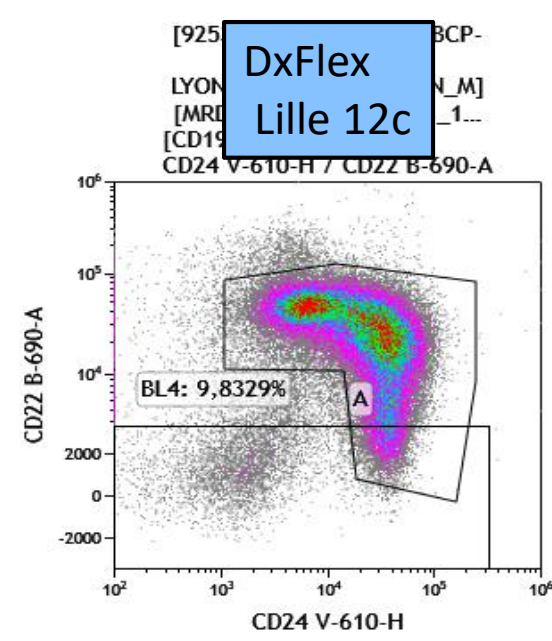
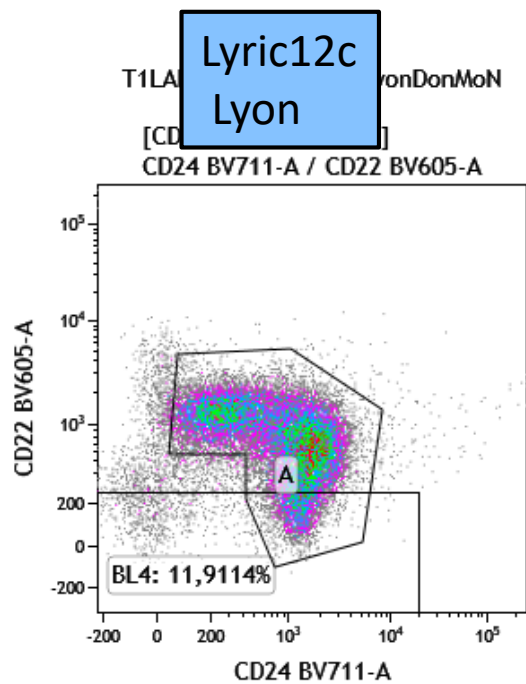


**BCP ALL Dg**

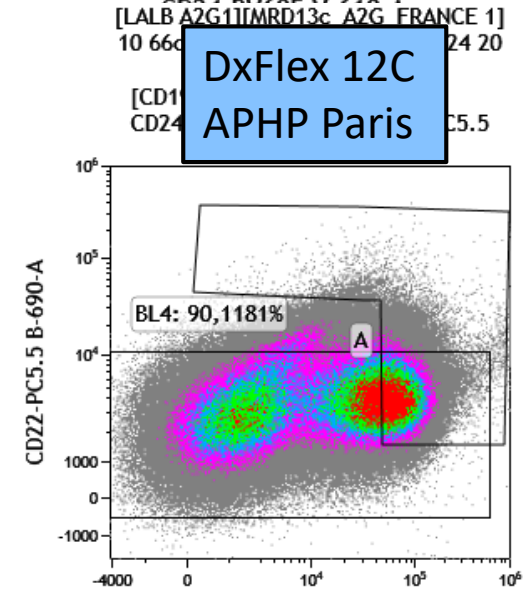
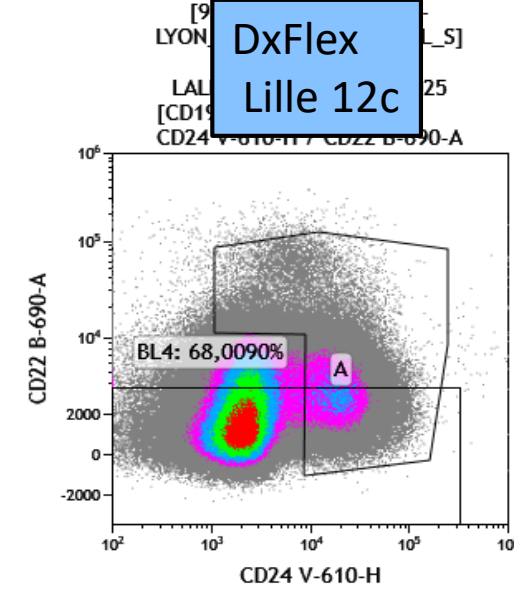
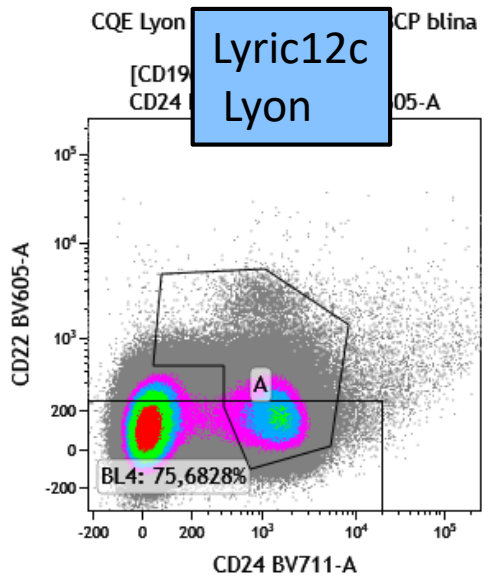


ECQ hBM/BCPALL Dg (ProBI, KMT2A, t(9;11) - BCP Blina panel Lyon/Marseille/Lille/ParisRBD  
Adriana Plesa (Lyon)/Isabelle Arnoux (Marseille)/Christophe Roumier (Lille)/Elodie Lainey (Paris)

**hBM**



**BCP ALL Dg**



ECQ hBM/BCP ALL Dg (ProBI, KMT2A, t(9;11) - BCP Blina panel Lyon/Lille/ParisRBD  
 Adriana Plesa (Lyon)/Christophe Roumier (Lille)/Elodie Lainey (Paris)

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

- Quality of BM (dilution)
- Strategy of identification of MRD: LAIP/DFN
- Description of the LAIP used
- LOD based on nBM
- Thresholds < 10<sup>-4</sup> for LAIP
- Interpretation of the result (Castor)
  - MRD+
  - MRD- with LOD value
  - MRD detectable but nonquantifiable

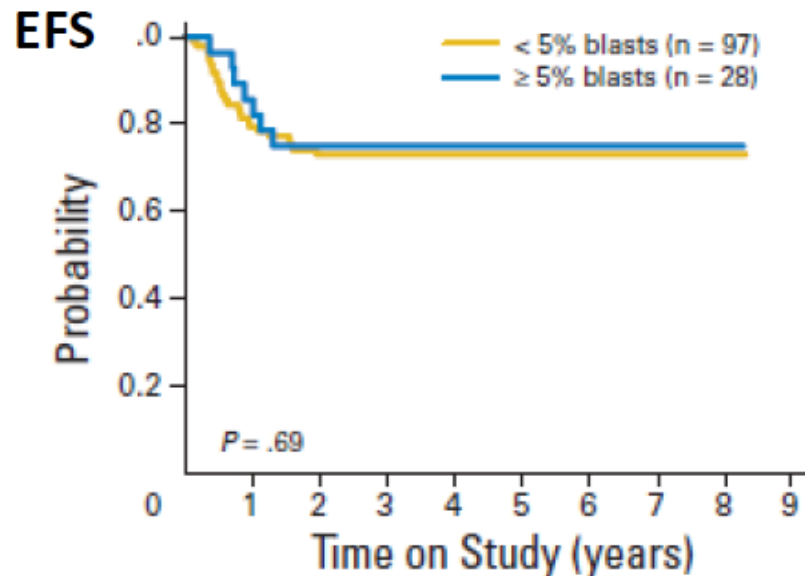
**BM Control DILUTION - mandatory for MRD AML evaluation**  
 One of this methods should be use for each MRD flowClinical Report

Methods	validation
Holdrinet RS et al. Exp Hematol 1980, 8: 103-107 Brooimans RA et al. Cytometry part B 2009, 76B: 18-26 Pureté de la moelle = $(1 - (GR_{MO} / GR_{SG}) \times (GB_{SG} / GB_{MO})) \times 100$	> 80%
Björklund E et al. Leukemia 2003, 17: 138-148	GPA > 15% CD3 < 20%
Loken MR et al. Cytometry part B 2009, 76B: 27-36	CD16high < 30%
STIC 2006 MRD LAL-French Group (Leukemia 2012; 27: 370-76, Cytometry part B 2015; 88B: 21-29) <u>Flow or Ctr DIL cytologique: PNN&lt;40%, Ebl&gt;5%, Ly&lt;20%</u>	CD16++ CD11b++ < 40% GPA > 5% CD3 < 20%
<b>BM Purity % : MNC 34+/WBC45+</b> French ALFA AML MRD LSC Group-APlesa/CRoumier; 2017	<b>BM Purity 100-10%</b>

## Blasts $\geq 5\%$ but MRD negative test in AML

Flow MRD negativity confers same good outcomes whether  $>5\%$  or  $<5\%$  by morphology in AML

- Paediatric and Adult real world and trial cohorts.



	Refractory post 1 <sup>st</sup> Course	Younger Adults	
<b>Trial</b>		UK AML 17 <sup>2</sup>	HOVON AML 42A2 <sup>3</sup>
<b>No. with Flow MRD results</b>		259	81
<b>% with Flow MRD- test</b>		10%	31%
		<b>Equivalent to CR MRD-</b>	
<b>CIR at 3yrs</b>	Flow MRD-	26%	32%
	Flow MRD+	52%	54%
<b>OS at 3yrs</b>	Flow MRD-	61%	60%
	Flow MRD+	33%	27%

## Reporting Blast % with Integrated Diagnostics

FDA                      Blasts: \_\_\_\_\_ % ????????

How are blasts reported at your institution - morphology ? flow? IHC?

For case report forms, which number do you tell the research nurse to use for answer the question  
"% blasts \_\_\_\_"

FDA mini symposium May 2024

*"the Methodology of Quantitating Blasts in Acute Leukemias for Response Assessments"*

Impacts: trial endpoints and inclusion (R/R studies)  
also comparison of real world data and training

➤ [Set-up of Working Group \(International / ELN / EHA\) for consensus recommendations](#)



*Reporting blast percentage for response assessment in acute leukemias: recommendations from an EHA/ELN expert panel.*

Wang SA, Arenillas L, Buccisano F, Bruggemann M, Kern W, Menes M, Plesa A, Stone L, Wellnitz D, Westerman DA, Wood BL, Freeman SD. *Haematologica*; <https://doi.org/10.3324/haematol.2025.288228>

### Response Assessment in ACUTE LYMPHOBLASTIC LEUKEMIA

Response Assessments	Report
MRD <0.01%	Report Remission, blasts <0.01% + Cytomorphology Quality
Cytomorphology <5% blasts + MRD positive ≥ 0.01%	Report % blasts by Flow Cytometry or IG/TR MRD + Cytomorphology Quality
Cytomorphology ≥5% blasts + MRD positive ≥ 0.01%	Report % blasts by Cytomorphology if major discrepant excess blasts by Cytomorphology with potential MRD target loss  Otherwise, report % blasts by Flow cytometry or IG/TR MRD + Cytomorphology Quality

### Response Assessment in ACUTE MYELOID LEUKEMIA (INCLUDING MDS/AML)

Response Assessments	Report
MRD negative	Report Remission, blasts <0.1% or <LOD + Cytomorphology Quality
Cytomorphology <5% blasts + MRD positive	Report % blasts by Flow Cytometry (MRD) assay <sup>5</sup> + Cytomorphology Quality
Cytomorphology ≥5% blasts + MRD positive	Report % blasts by Cytomorphology for Erythroid, Monocytic or Megakaryocytic AML  Otherwise report % blasts by Flow Cytometry (MRD) assay + Cytomorphology Quality

# A2G Spectral Flow Meeting French Experience

- WebTeam 6-3-2026
- Adriana Plesa for French A2G Team

**Clinical Add-On : BCP ALL >20c Panel**  
*The challenge of standardization in clinical  
cytometry*

# What is the challenge in the clinic? an example from AML will illustrate this

Three goals in the clinic:

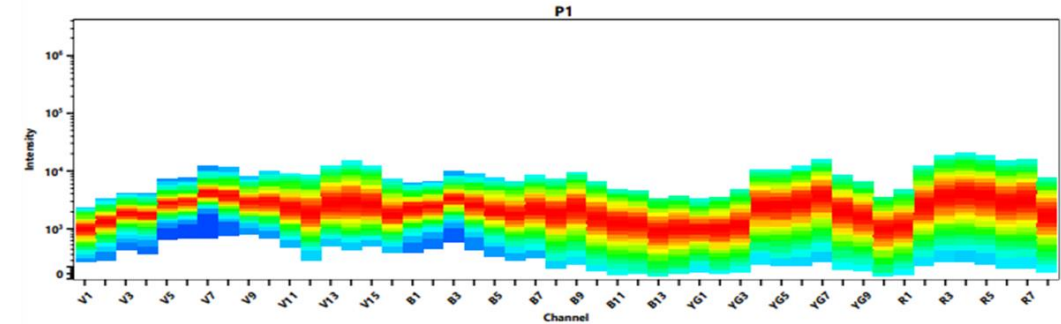
① Preserve the diagnostic resolution **12C → 26C** in MRD or immune profiling over time

② Increase analytical depth : add analytical depth and rare marker exploration

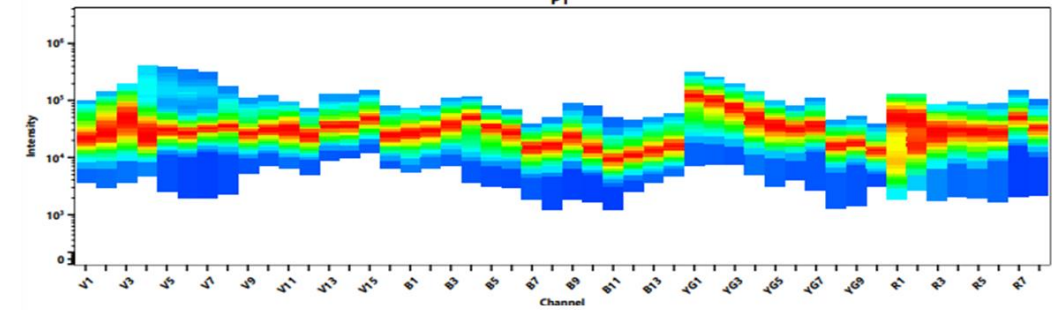
③ Maintain cross-site/instruments reproducibility

***The triad of "Resolution – Depth – Reproducibility » defines clinical robustness***

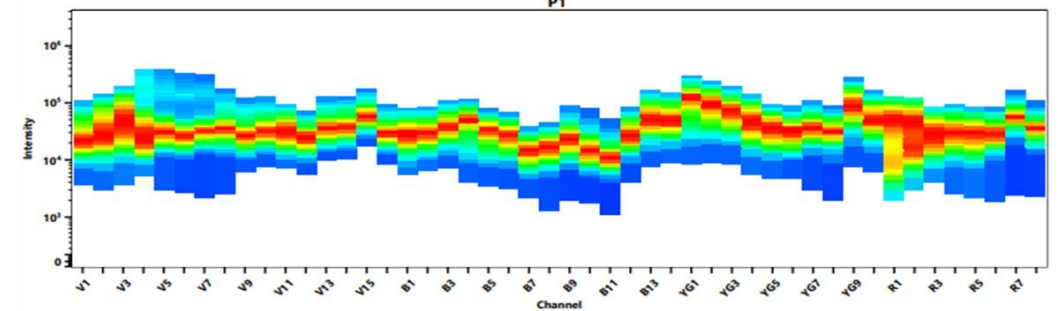
UNSTAIN



FMO38



LSC 26c



# Flow MRD new : Spectral Flow and Unsupervised Analysis

Spectral Flow AML Panel: The challenge of standardization in clinical cytometry (“All-In-One” Tube)

4xTubes 12c

1xTube 21c ou 26c

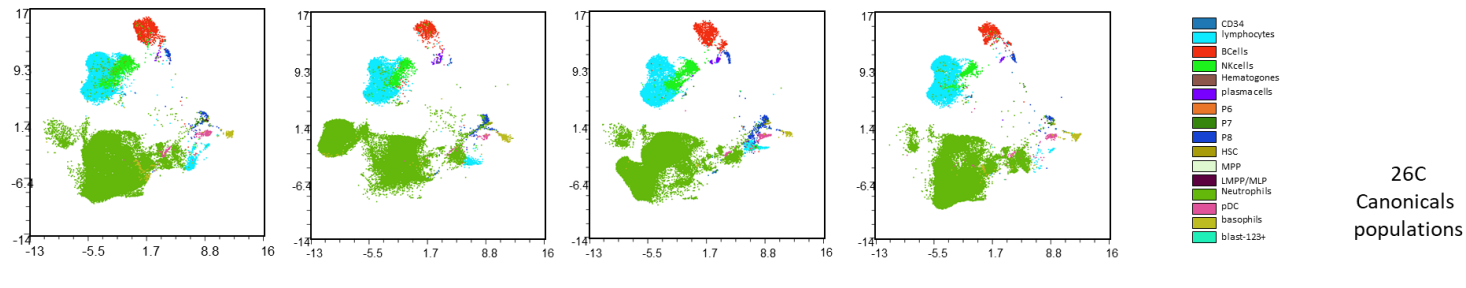
Routine 12-color myeloid assay (BD LYRIC12c)				New 26-color myeloid assay in twosteps	
12-color Panel				21-color	26-color
T1 UAIP	T2 LSC	T1 AML Mono/Gran	T2 AML LSC GPR56/CD81		
CD45	CD45	CD45	CD45	CD45	CD45
CD34	CD34	CD34	CD34	CD34	CD34
CD38	CD38	CD38	CD38	CD38	CD38
CD117	CD117	CD117	CD117	CD117	CD117
	MIX (CLL1+TIM3+CD97)		TIM3	MIX (CLL1+TIM3+CD97)	TIM3
			CD97 RY586		CD97 RY586
			CLL1(CD371)		CLL1(CD371)
HLA-DR	HLA-DR	HLA-DR	HLA-DR	HLA-DR	HLA-DR
		CD15		CD15	CD15
		CD14		CD14	CD14
CD33		CD33		CD33	CD33
		CD11b		CD11b	CD11b
CD36	CD36	CD36		CD36	CD36
CD13				CD13	CD13
CD7				CD7	CD7
CD56 PE-Fire810				CD56 PE-Fire810	CD56 PE-Fire810
CD19	CD19			CD19	CD19
	CD45RA		CD45RA	CD45RA	CD45RA
	CD90		CD90	CD90	CD90
			GPR56	GPR56	GPR56
			CD81	CD81	CD81
		CD64		CD64	CD64
				Zombie ViaDyeRed	Zombie ViaDyeRed
	CD123			CD123	CD123
CD10	CD200	CD4		CD10	CD10
				CD200	CD200
				CD4	CD4

- 12-color panels (n=3) : established for routine clinical immunophenotyping
- 26-color panels: add analytical depth and rare marker exploration

**Capturing LSC « All-in-One » Tube combine**

**Immunotherapy Target and VEN+AZA Primary Resistance at diagnosis or Monocytic clonal selection and « Switch » inducing resistance**

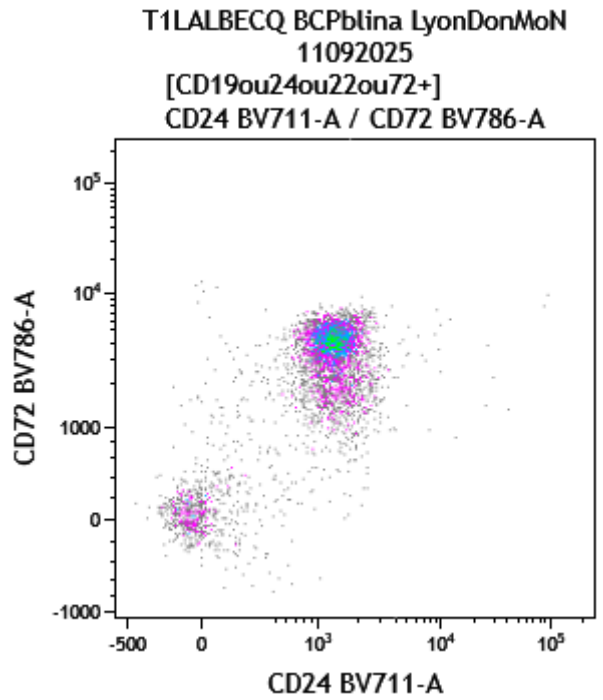
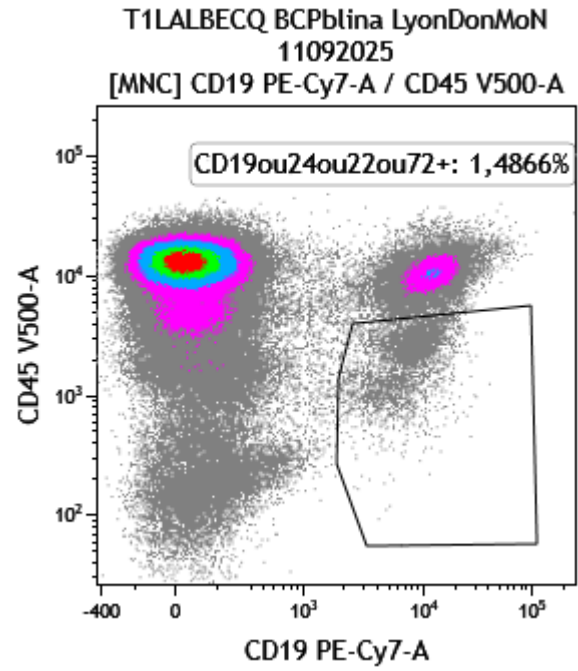
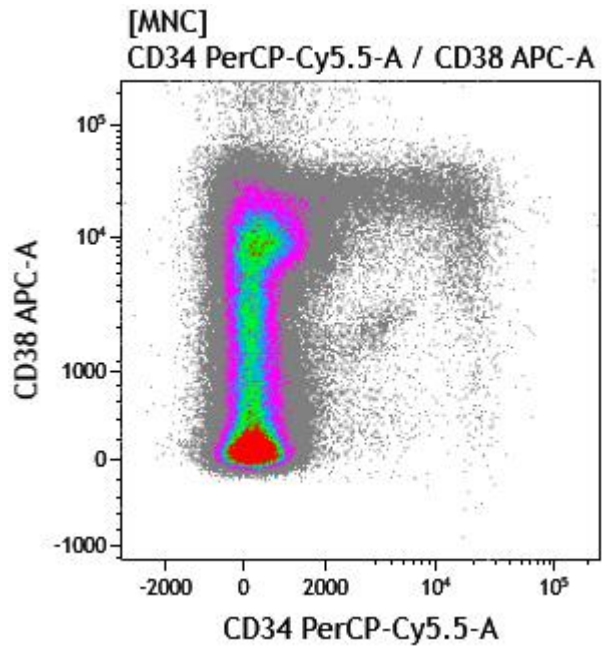
Homogeneity of the TOPOGRAPHY of the data over time – 4 samples, 4 acquisitions



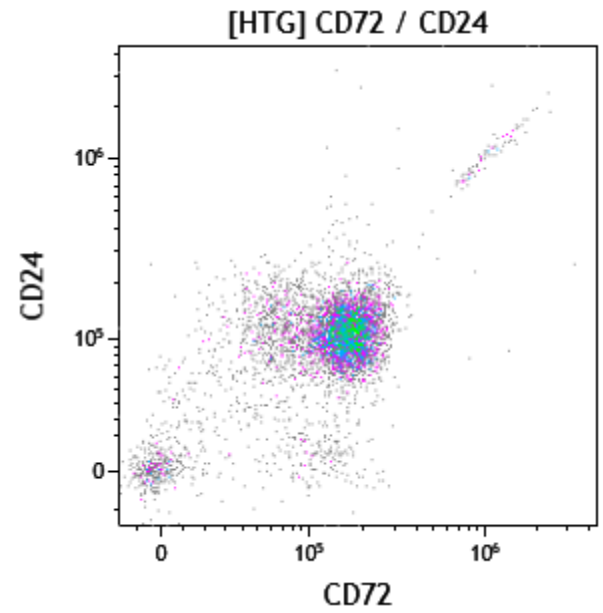
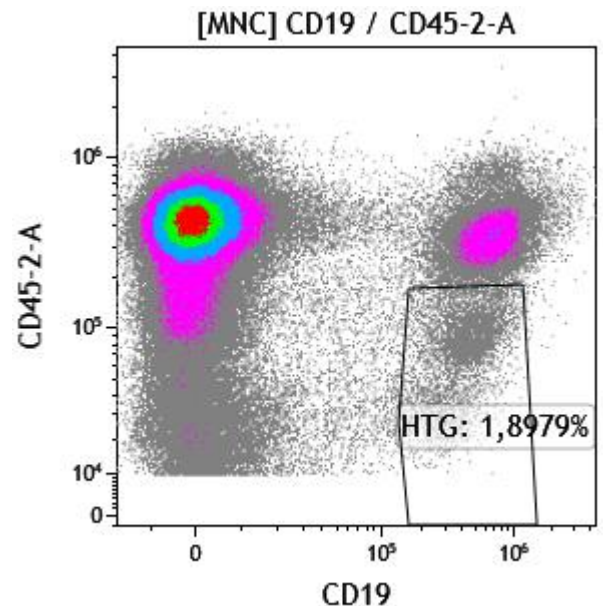
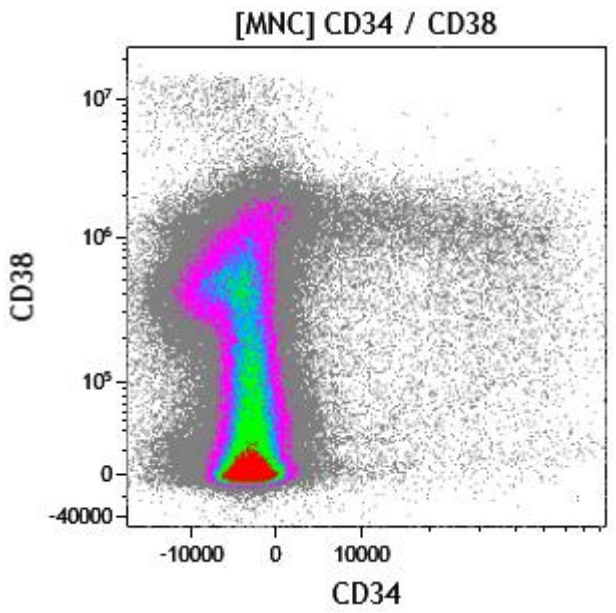
Sous-group Spectral Flow 2026: A Plesa, C Roumier, I Oster, T Andrieu (CRCL Lyon)  
7 Labo CMF : Lyon, Lille, Rennes, Marseilles (CHU), Nancy, Clermont F, Nice,...

Lyric 12C T1 NBM 11/09/2025

LYRIC 12c  
VS  
SPECTRAL12c

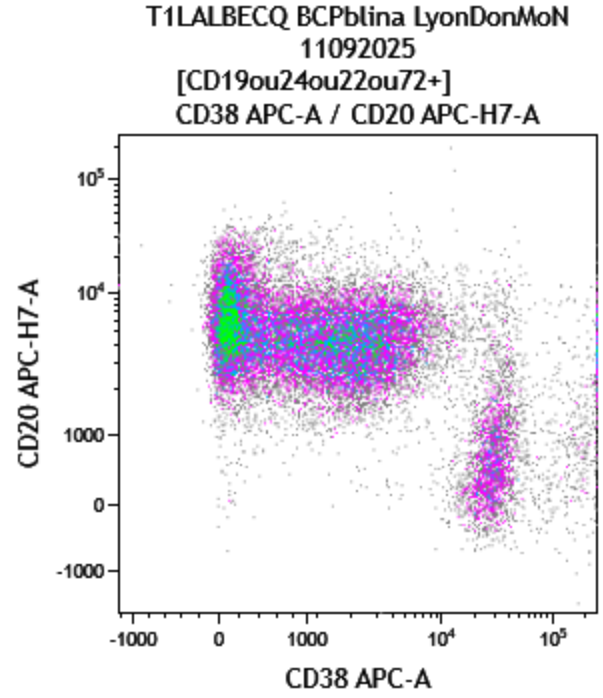
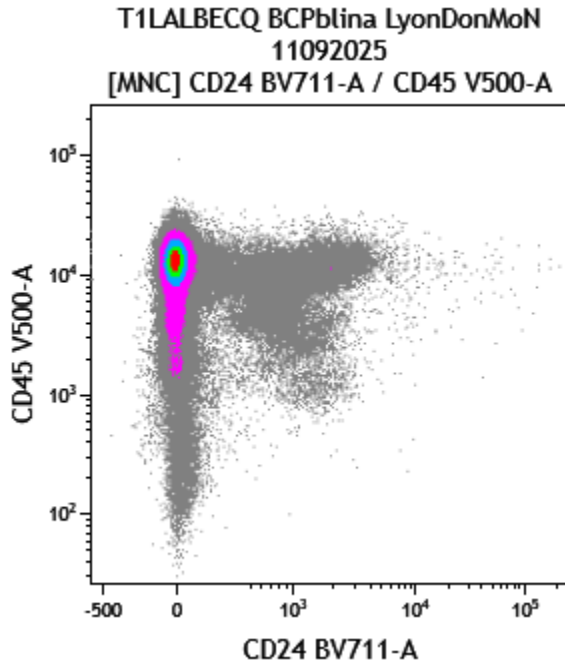
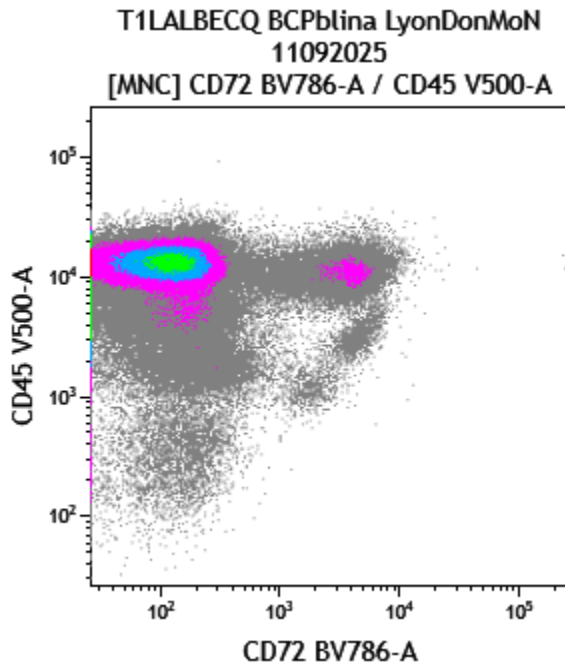


SpectralBD Discover S8 12C T1 NBM 11/09/2025

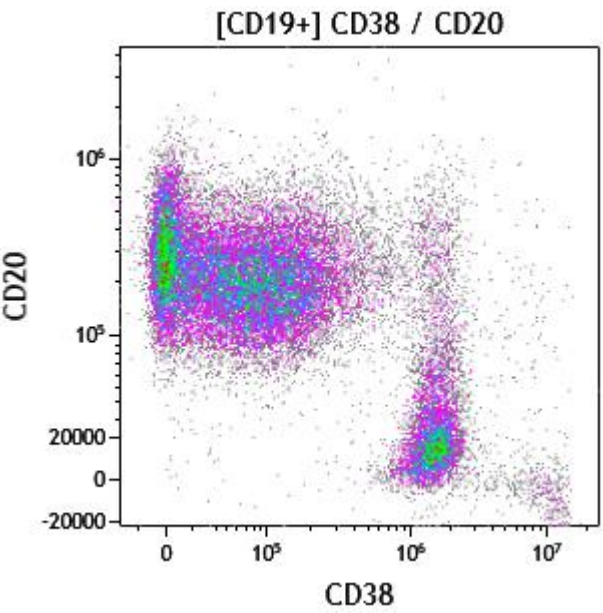
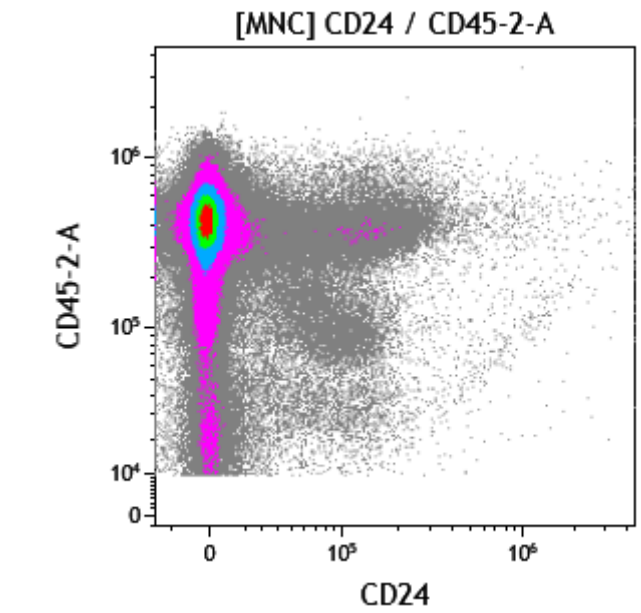
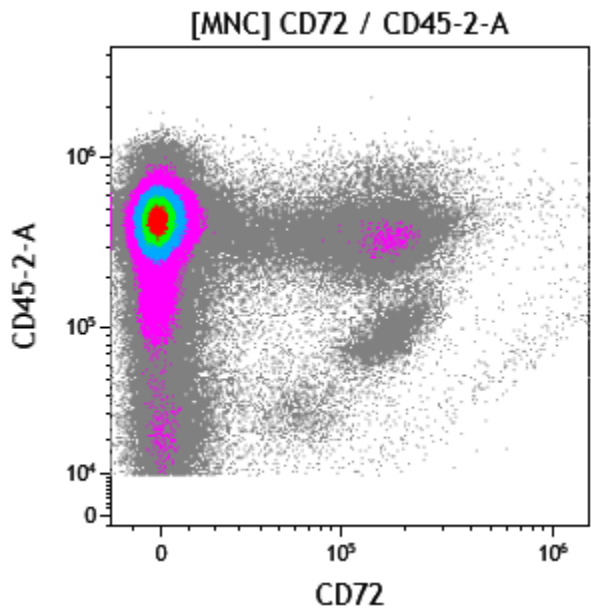


Lyric 12C T1 NBM 11/09/2025

LYRIC 12c  
vs  
SPECTRAL12c



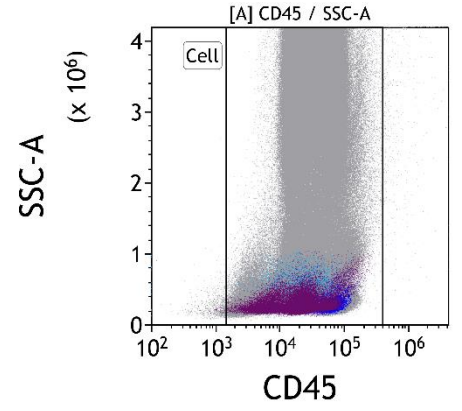
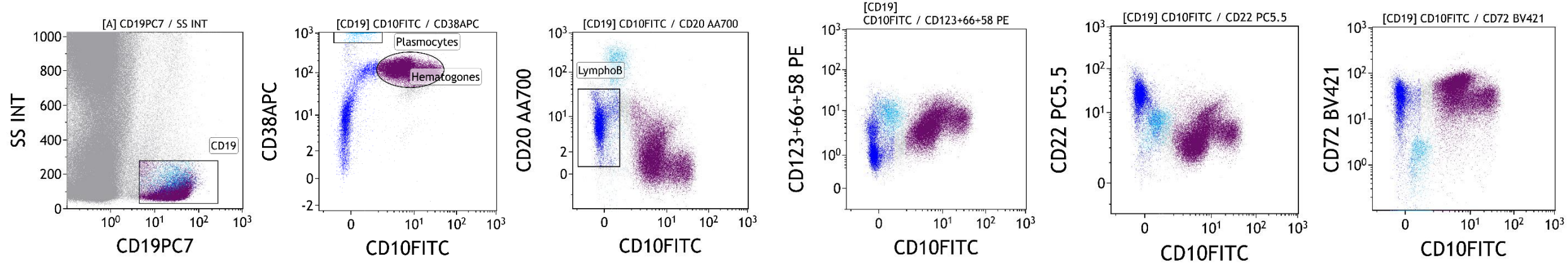
SpectralBD Discover S8 12C T1 NBM 11/09/2025



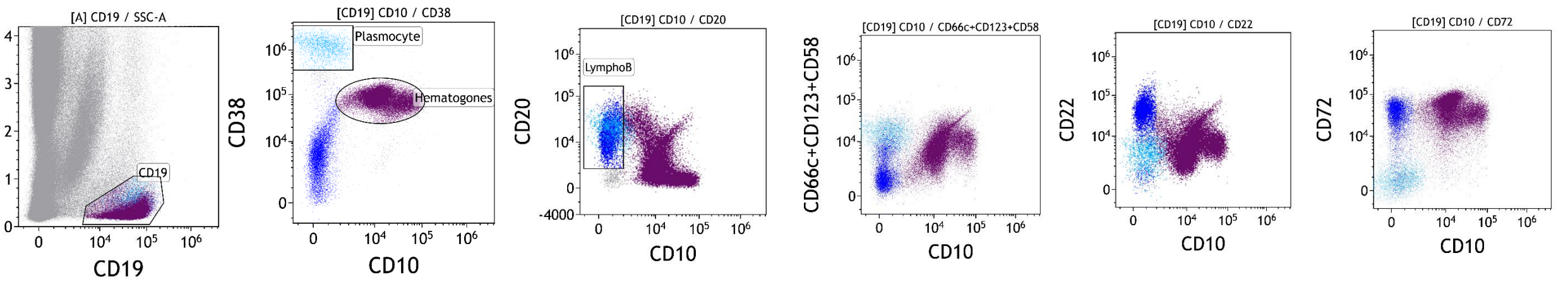
NAVIOS 10c  
vs  
SPECTRAL10c

# Moelle normale

Navios



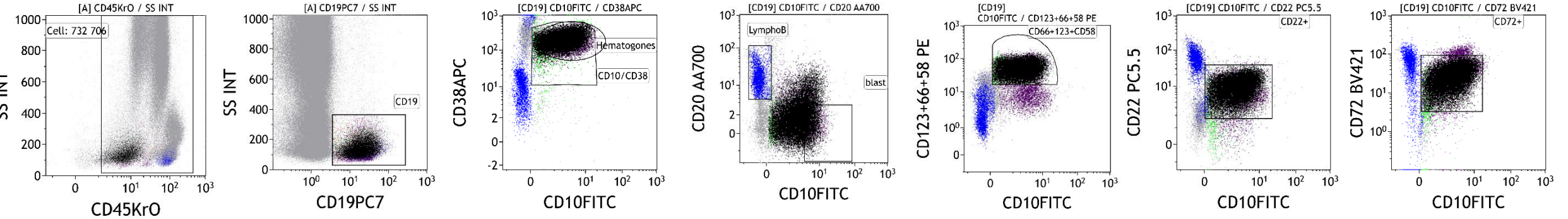
Cytek NL



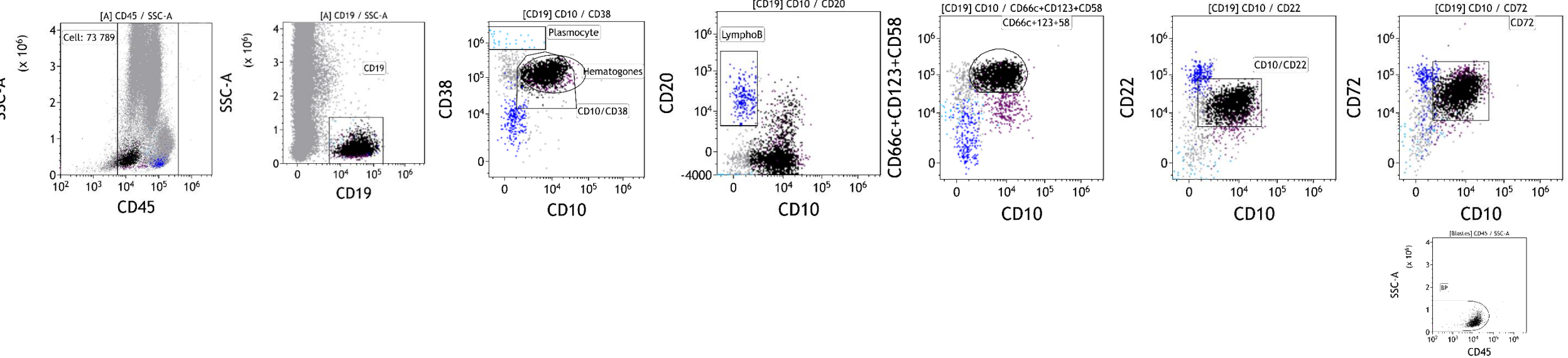
NAVIOS 10c  
vs  
SPECTRAL10c

# MRD moelle – rechute LAL B (cas I...)

Navios



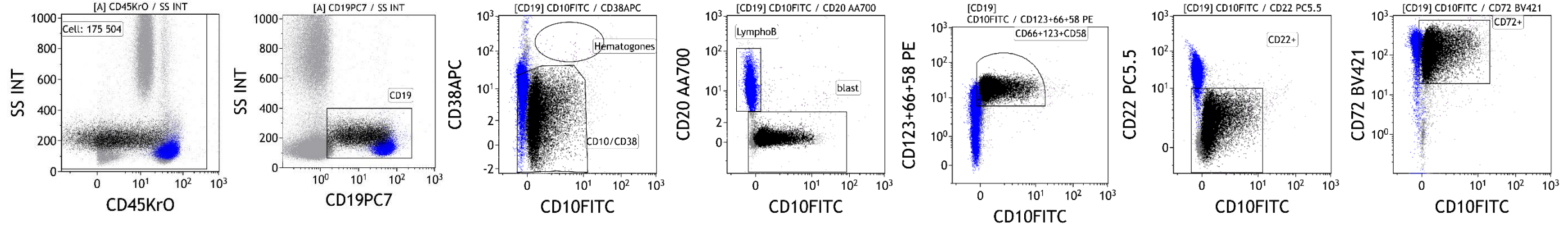
Cytek NL



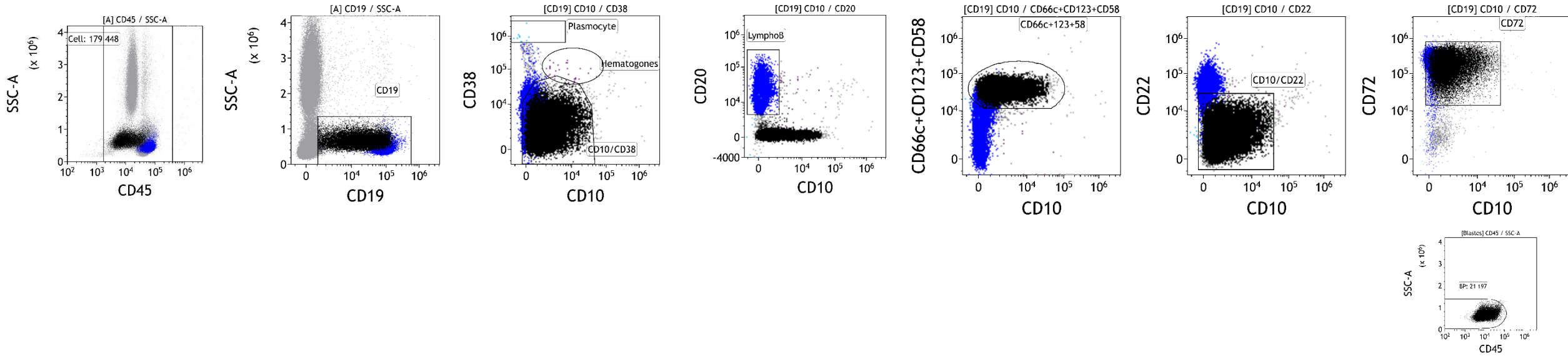
NAVIOS 10c  
VS  
SPECTRAL10c

# MRD sang – rechute LAL B (cas M...)

Navios



Cytek NL



## Why use CAD in ALL



- Flow data are more and more complex: 8c 10C 12/13C spectral >20c
- Save time
- Increase inter-operator reproducibility
- Overcome the difficulties of conventional analysis
- Have the computer handle the complexity of windowing
- Increase the completeness of windowing of malignant events



- Replace the pre-analytical homogenization work
- Perform analysis with any panel of antibodies
- Free yourself from automated adjustment issues
- Free yourself from knowledge of normal and malignant phenotypes
- Think that ANS is capable of doing better than the expert

## How to use CAD in MRD ALL

1: choice of methods : supervised or unsupervised?

2: choice of algorithm

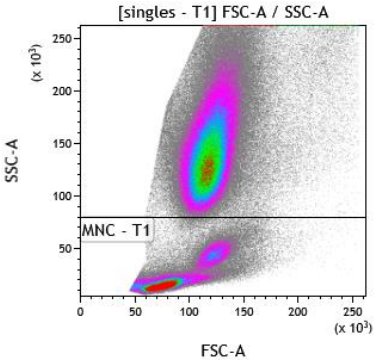
FCS : diag file, MRD file, set of  $n$  files of Ref Bone marrow ( $n > 10$ )

Flow cytometry  
Data extraction

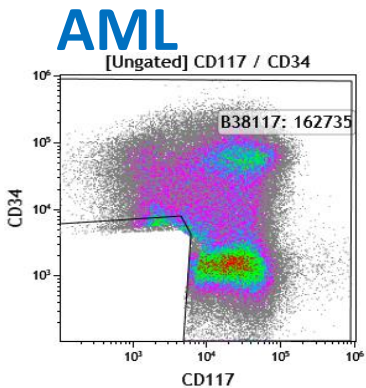
1 Read  $n+2$  csv

2 Merge data

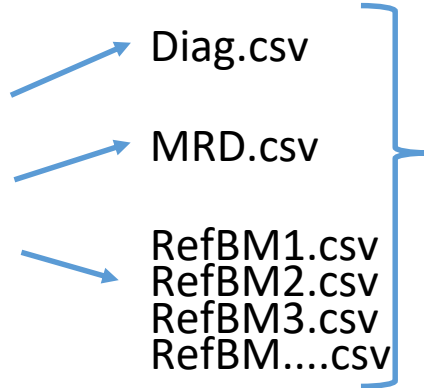
3 FlowSOM \*



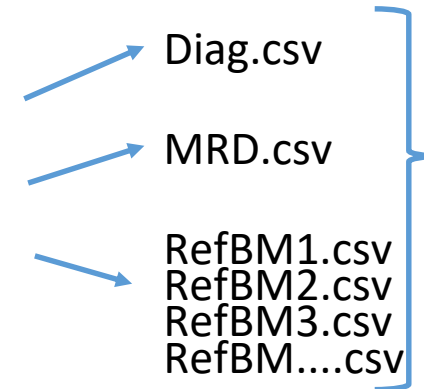
Input gate MNC



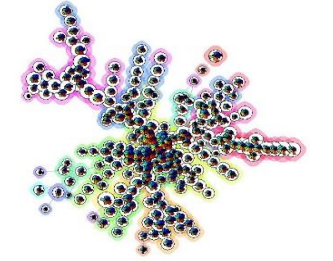
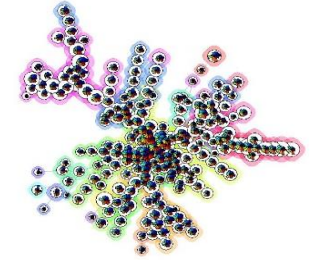
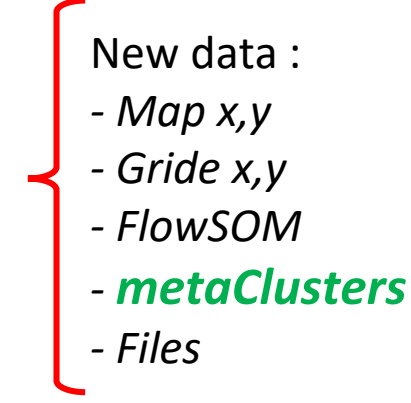
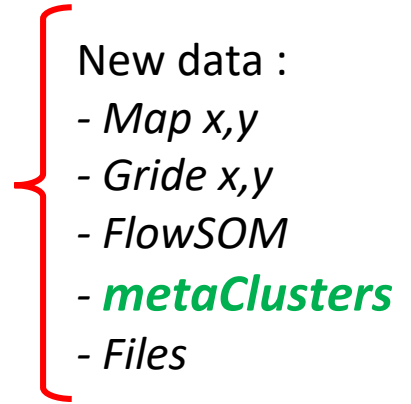
Input gate CD34/CD117



Merge  
Data in  
Flow  
frame

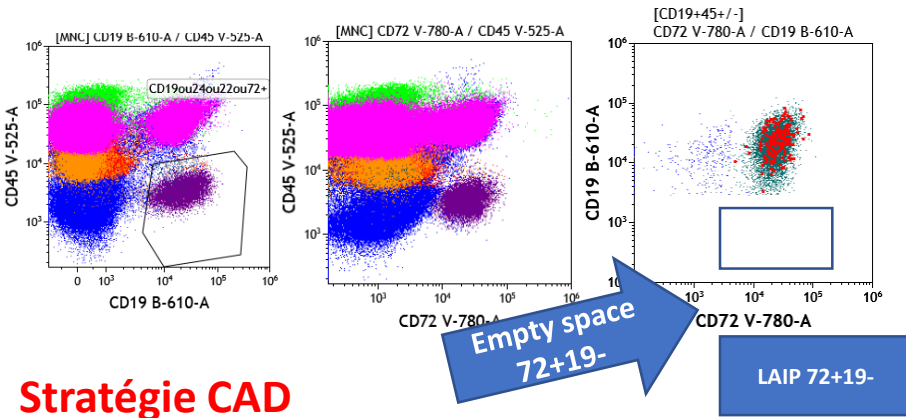


Merge  
Data in  
Flow  
frame

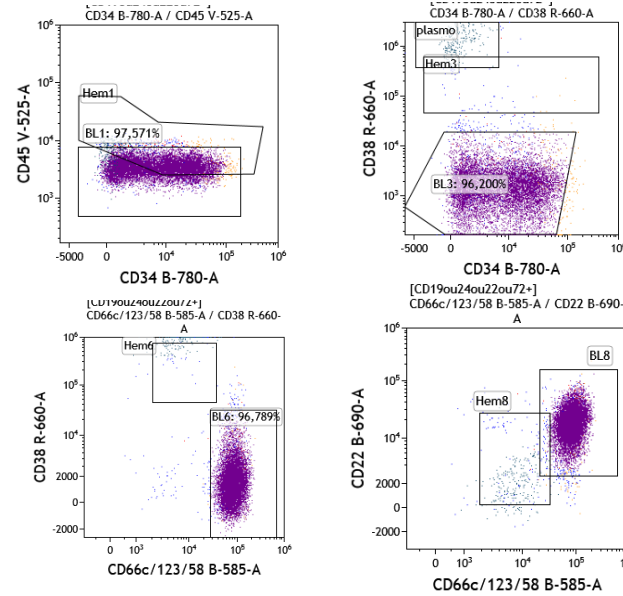
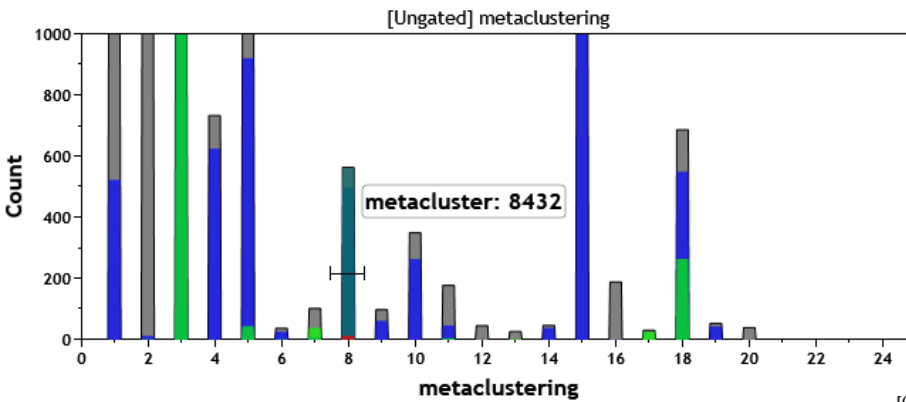


# Ex cas DxFlex12c CHU Lille BCP ALL MRD1 pos –Script CAD Lille

## Stratégie conventionnelle

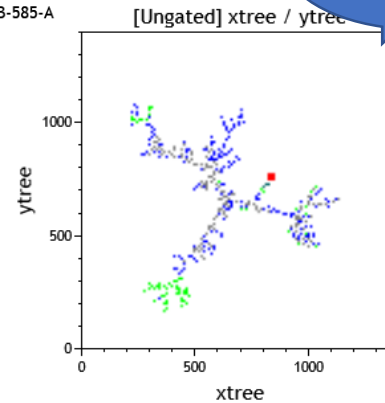
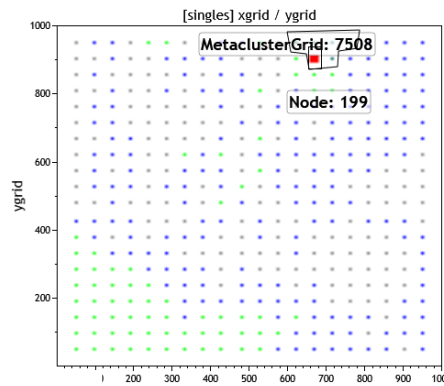


## Stratégie CAD

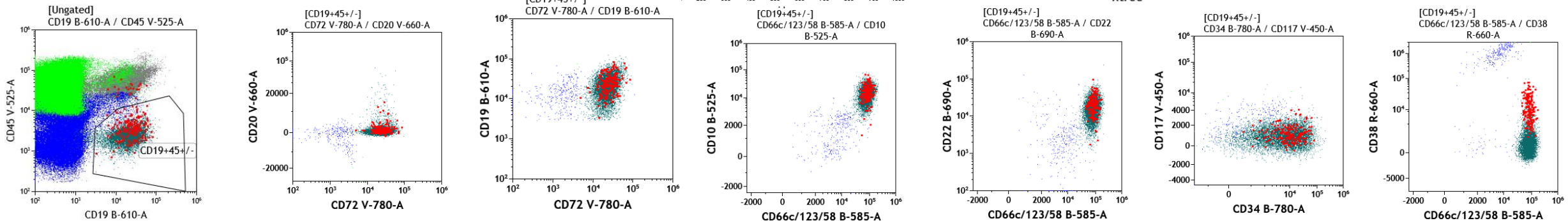


MRDR1	MRDR1	0,449
MRDS1	MRDS1 (seuil 30ev)	0,001
MRDRES	MRDRES	0,449
MRDSD	MRDSD	0.01
	test Seuil si mo diluée	0.01

72+19- LAIP peu informatif chez les patients sans Blina  
10++20-34+38+/-45-58/66c/123++

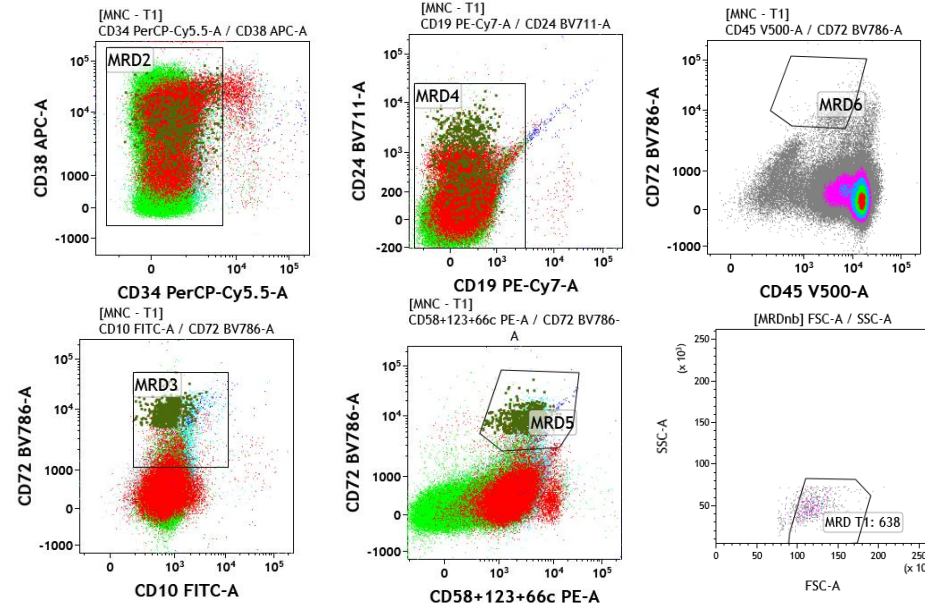
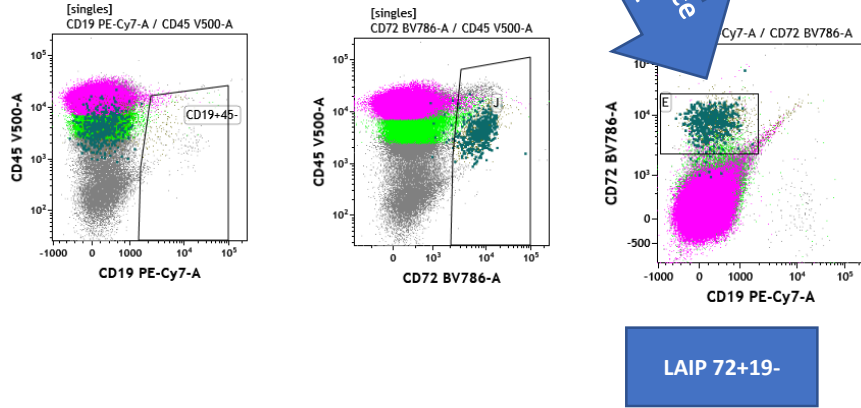


Name	Value
metacluster/leuco tot	0,357



# Ex cas LYRIC12c (CHU Lyon) BCP ALL MRD pos et Rechute postBlina CD19 neg -Script CAD Lille

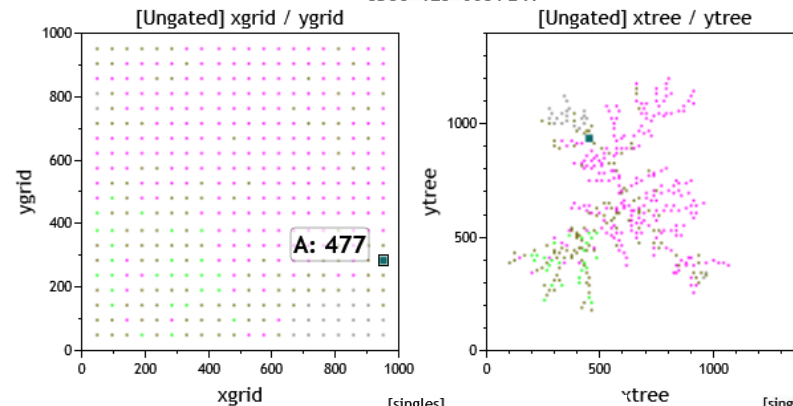
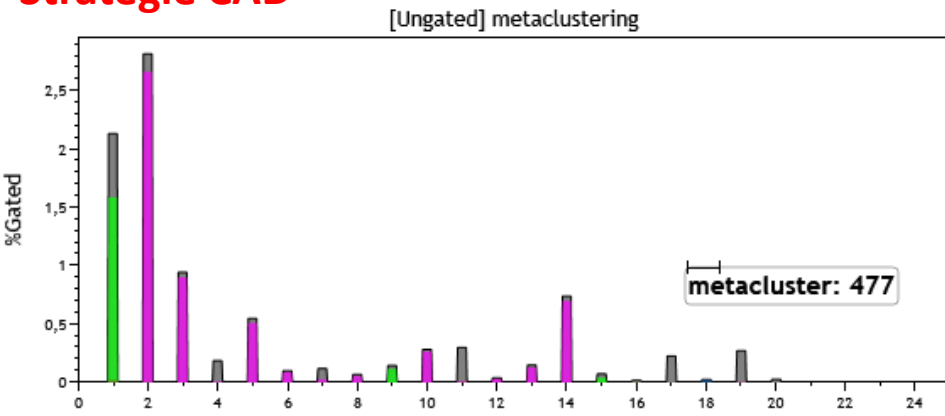
## Stratégie conventionnelle



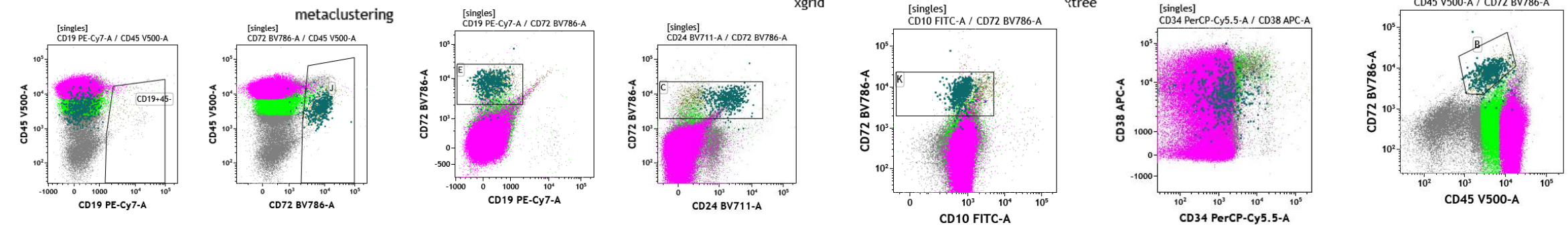
Date of data set acquisition	12-MAY-2026
MRD T1 %	0,094
[MRD T1] Number	638
[WBC45+ - T1] Number	676465

72+19- LAIP très puissant chez les patients sous Blina  
10-20+/-34-38+f45+f24++  
58/66c/123++

## Stratégie CAD

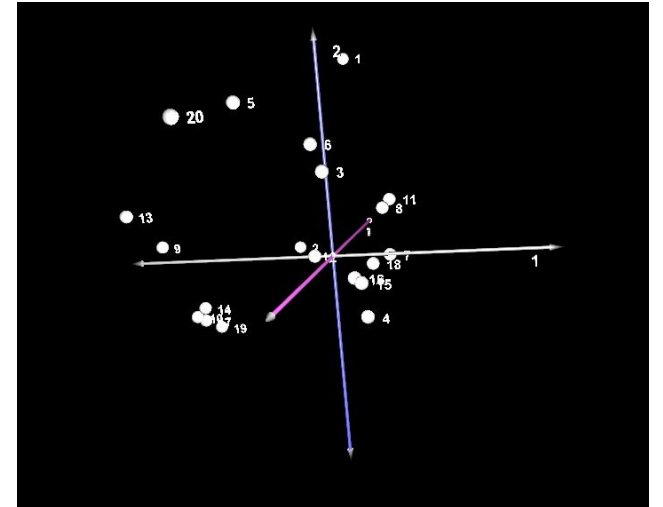


Name	Value
WBC recopié du FCS initial	710615
MNC	230850
metacluster	477
metacluster/ WBC %	0,067



# CONCLUSIONS

- Prospectif project ongoing with first results(Lyon/Lille/Marseille/ParisRBD) analysis in progress on LAIP pattern at diagnosis for Lyric/DxFlex/Navios Platforms from 2 tubes BCPblina Panel
  - Pattern of modulation on Day 15 evaluation
  - Day 33 MRD1 and Day 78 MRD2
  - Combining Flow with Oncogenetic and mutational architecture
  - Unsupervised Strategy in progress in Lille/Lyon for both platforms
  - Prospectif Spectral flow BCP ALL
- Project in progress to evaluate the switch impact with Blina/CART « on therapy »
- **CQE BCP ALL –hBM (Juin 2026 –à venir A2G French group)**



# Remerciements

- Christophe Roumier, Florent Dumezy, Wissal Chkili (CHU Lille)
- Iole Oster, Delphine Manzoni (CHU Lyon SUD)
- Isabelle Arnoux, Vanessa Nivaggioni, Marie Loosveld (APHM, Marseille)
- Elodie Lainey (APHP, Hôpital Robert Debré)
  
- A2G France LAL flow Group: Ludovic Lhermitte, Francine Garnache, Anne Roggy, Aguirre Mimoun, Jean-Philippe Vial, François Vergez, Mikael Roussel, Marion Eveillard
  
- Florent Navarro (BD Biosciences)
- Antoine Pacheco (Beckman Coulter)
- Olivier Jean (Cytek bio)
  
- Spectral (Thibault Andrieu , Priscillia Battiston (CRCL/CLB-Lyon))

