

-
28 & 29
SEPTIEMBRE
2023

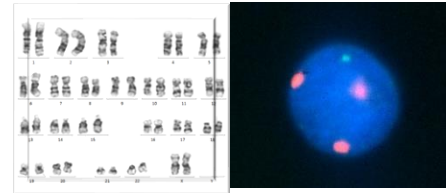
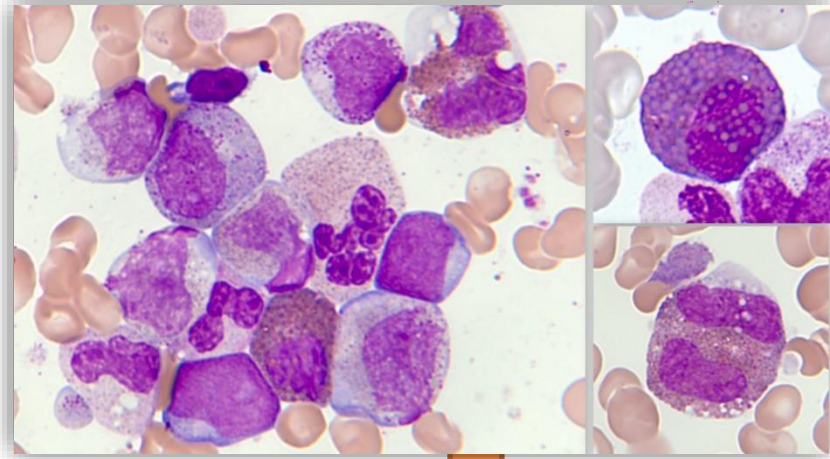
6° CURSO PRÁCTICO CITOMETRÍA DE FLUJO

**Enfermedad residual medible y seguimiento
inmunológico en LMA: ¿en dónde nos encontramos?**

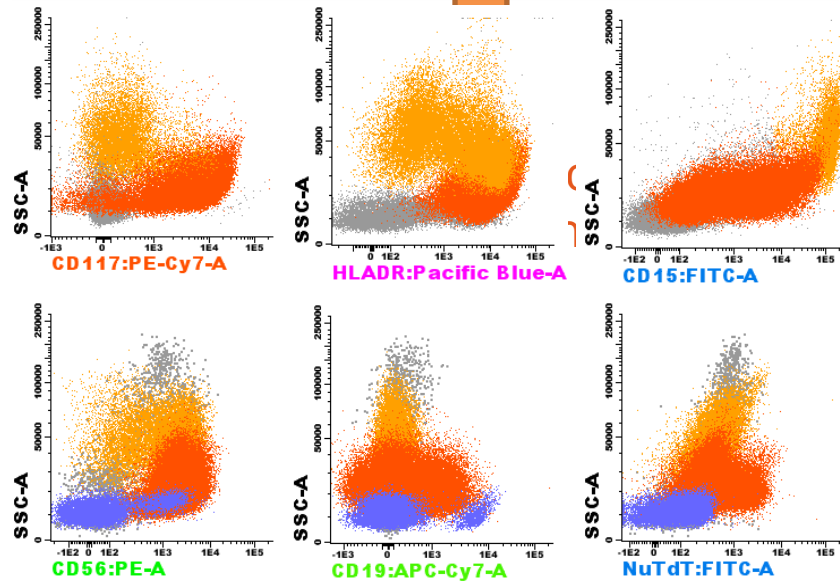
Teresa Caballero Velázquez
Hospital Universitario Virgen del Rocío

Acute Myeloid Leukemia

Morphology, flow cytometric, and molecular data

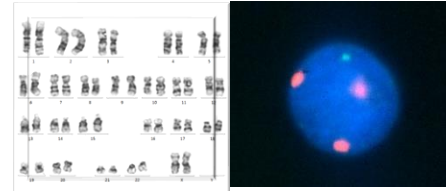
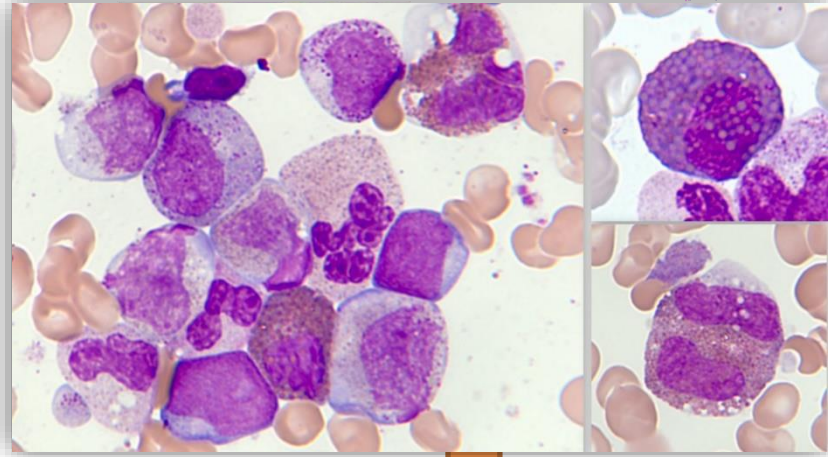


Biological characteristics



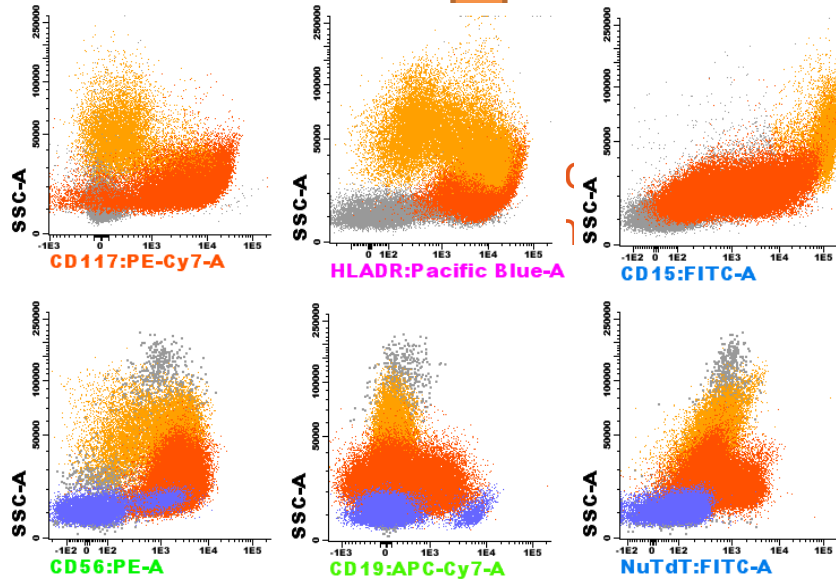
Acute Myeloid Leukemia

Morphology, flow cytometric, and molecular data



LMA con t(8;21)(q22;q22.1); *RUNX1::RUNX1T1*

Favorable category according 2022 ELN risk classification by genetics at initial diagnosis



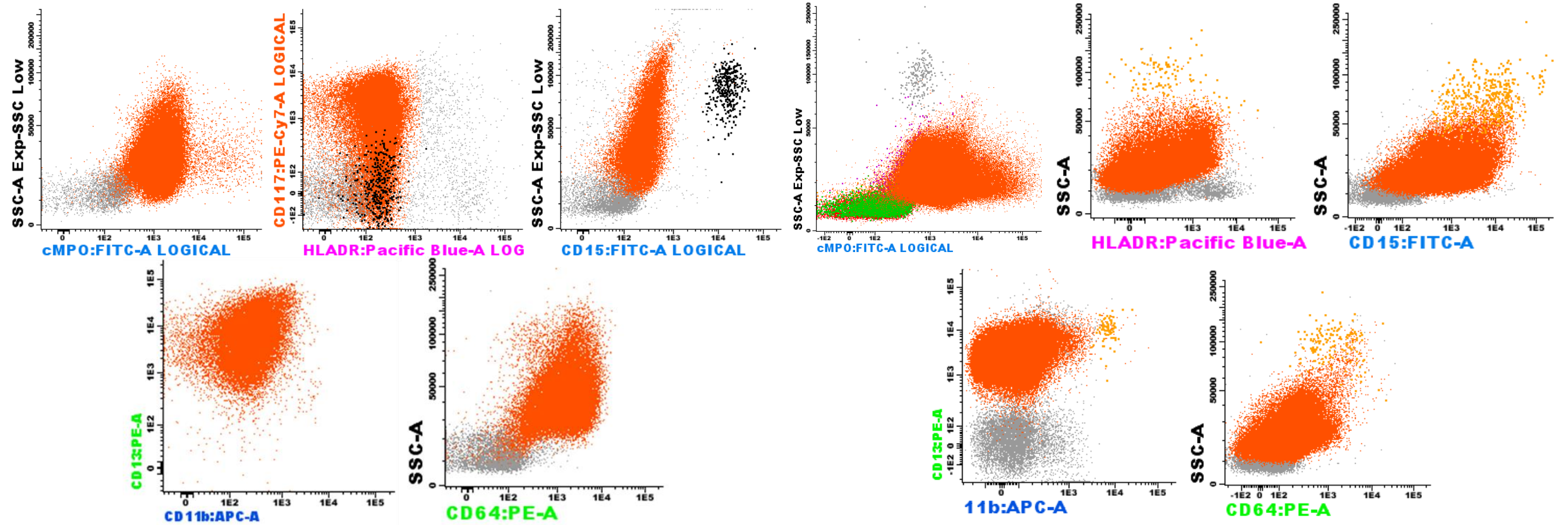
**t(8;21)(q22;q22.1); *RUNX1::RUNX1T1*
inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB::MYH11*
Mutated *NPM1* without *FLT3-ITD*
Biallelic mutated *CEBPA***

Acute Myeloid Leukemia

Morphology, flow cytometric, and molecular data

APL t(15;17)

NPM1+



APL showed significantly higher expression of CD64, CD2, and CD13 ¹

NPM1+ AML more leukemic cells were positive for CD11b, CD11c, CD15, CD36, and HLA-DR ¹

¹Gupta M, Radar plots facilitate differential diagnosis of acute promyelocytic leukemia and *NPM1*+ acute myeloid leukemia by flow cytometry. *Cytometry B Clin Cytom.* 2021

²Matarraz S, et al. Baseline immunophenotypic profile of bone marrow leukemia cells in acute myeloid leukemia with nucleophosmin-1 gene mutation: a EuroFlow study. *Blood Cancer J.* 2023

Acute Myeloid Leukemia

2022 ELN risk classification by genetics at initial diagnosis

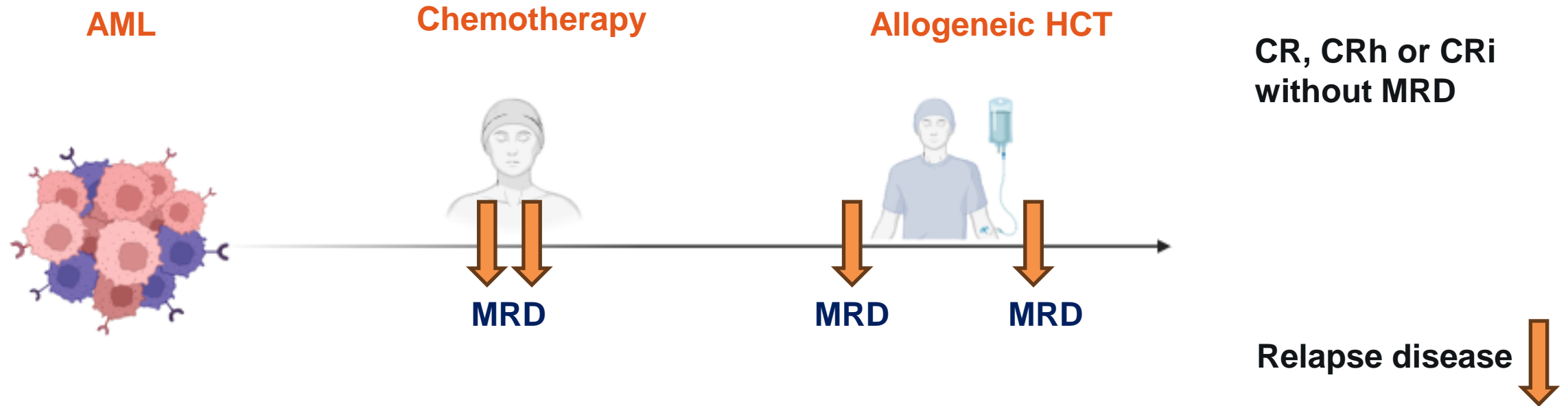
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Biallelic mutated *CEBPA*

Mutated *NPM1* and *FLT3-ITD*
Wild-type *NPM1* with *FLT3-ITD* (without adverse-risk genetic lesions)
t(9;11)(p21.3;q23.3); *MLLT3::KMT2A*
Cytogenetic/molecular abnormalities not classified as favorable or adverse

t(6;9)(p23;q34.1); *DEK::NUP214*
t(v;11q23.3); *KMT2A* rearranged
t(9;22)(q34.1;q11.2); *BCR::ABL1*
T(8;16)(p11,2;p13,3)/KAT6A::CREBBP
inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
-5 or del(5q); -7; -17/abn(17p)
Complex karyotype,^s monosomal karyotype
Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*,
U2AF1 y/o *ZRSR2*
Mutated *TP53*

Acute Myeloid Leukemia

Pronostic value of MRD detection

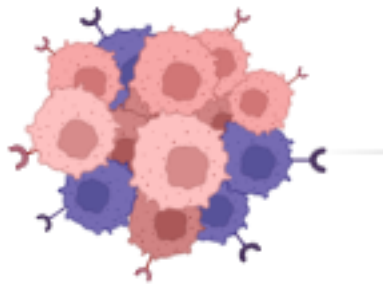


San Miguel Blood 2001 and Blood 1997; Venditti, Blood 2000; Kern, Haematologica. 2004; Kern, Blood 2004; Sievers, Blood 2003; Rubnitz, Lancet Oncol. 2010; van der Velden, Leukemia 2010; Prebet, Haematologica 2014; Walter, Leukemia 2015; Vidriales, Leuk Res. 2016; Zhou, Leukemia 2016; Araki, JCO 2016; Tierens, Br J Haematol. 2016; Balsat, JCO 2017; Buckley, Haematologica 2017; Willekens, Haematologica, 2016; Boddu, Leukemia 2018; Brodersen, Blood Adv 2020; Deng, Leuk Lymphoma 2019; Freeman, JCO 2018; Gao, Ann Hematol 2020; Guénot, Leukemia 2019; Hoffman, Bone Marrow Transpl. 2020; How, Am J Hematol. 2018; Jacobsohn, Biol Blood Marrow Transpl. 2018; Klyuchnikov, Eur J Haematol. 2021; Köhnke, Haematologica. 2019; Lacombe, Hematol. Oncol. 2018; Liu, Bone Marrow Transpl. 2019; Löwenberg, Blood Adv. 2021; Maiti A, Blood Adv. 2021; Minetto, Br J Haematol. 2019; Morsink, Leukemia. 2020; Morsink, Cancers 2020; Morsink, Leukemia 2020; Mushtaq, Leukemia. 2021; Patkar, Leukemia 2021; Rosso, Leuk Lymphoma. 2021. Rubnitz, J Clin Oncol. 2019. Shah, Biol Blood Marrow Trnspl. 2018. Shimomura, Leuk Lymphoma 2020; Caballero-Velázquez, Cancers 2023.....

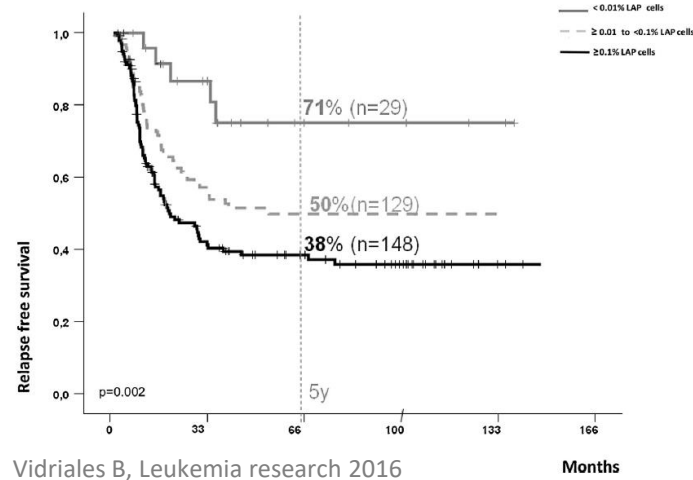
Acute Myeloid Leukemia

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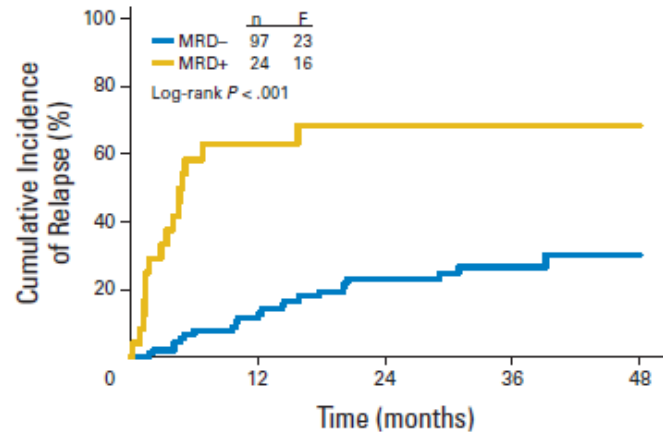
AML



Induction therapy

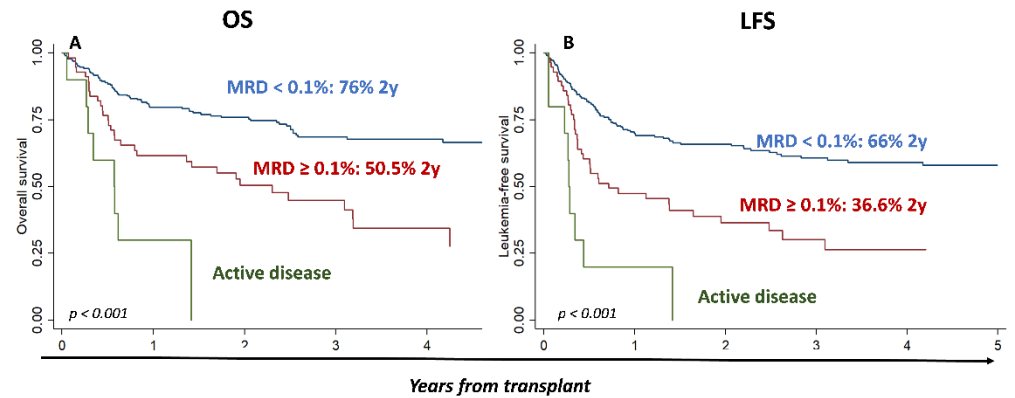


Consolidation therapy



Allogeneic HCT

Before transplantation

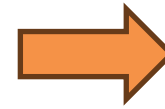


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San Miguel, Blood 2004; Feller, Leukemia 2004; Inaba, JCO 2012; Sievers, Blood 2003; Maurillo, Prebet, Haematologica 2014; Walter, Leukemia 2015; Vidriales, Leuk Res. 2016; Zhou, Haematologica 2017; Willekens, Haematologica, 2016; Boddu, Leukemia 2018; Brodersen, Leukemia 2019; Hoffman, Bone Marrow Transpl. 2020; How, Am J Hematol. 2019; Lacombe, Hematol. Oncol. 2018; Liu, Bone Marrow Transpl. 2020; Morsink, Leukemia. 2020; Morsink, Cancers 2020; Morsink, Leukemia 2020; Mushtaq, Blood 2019. Shah, Biol Blood Marrow Transpl. 2018. Shimomura, Leuk Lymphoma 2020;

Acute Myeloid Leukemia

2022 ELN risk classification by genetics at initial diagnosis



Refine relapse risk

MRD

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Mutated *TP53*

“(1) provide a quantitative methodology to establish a deeper remission status;

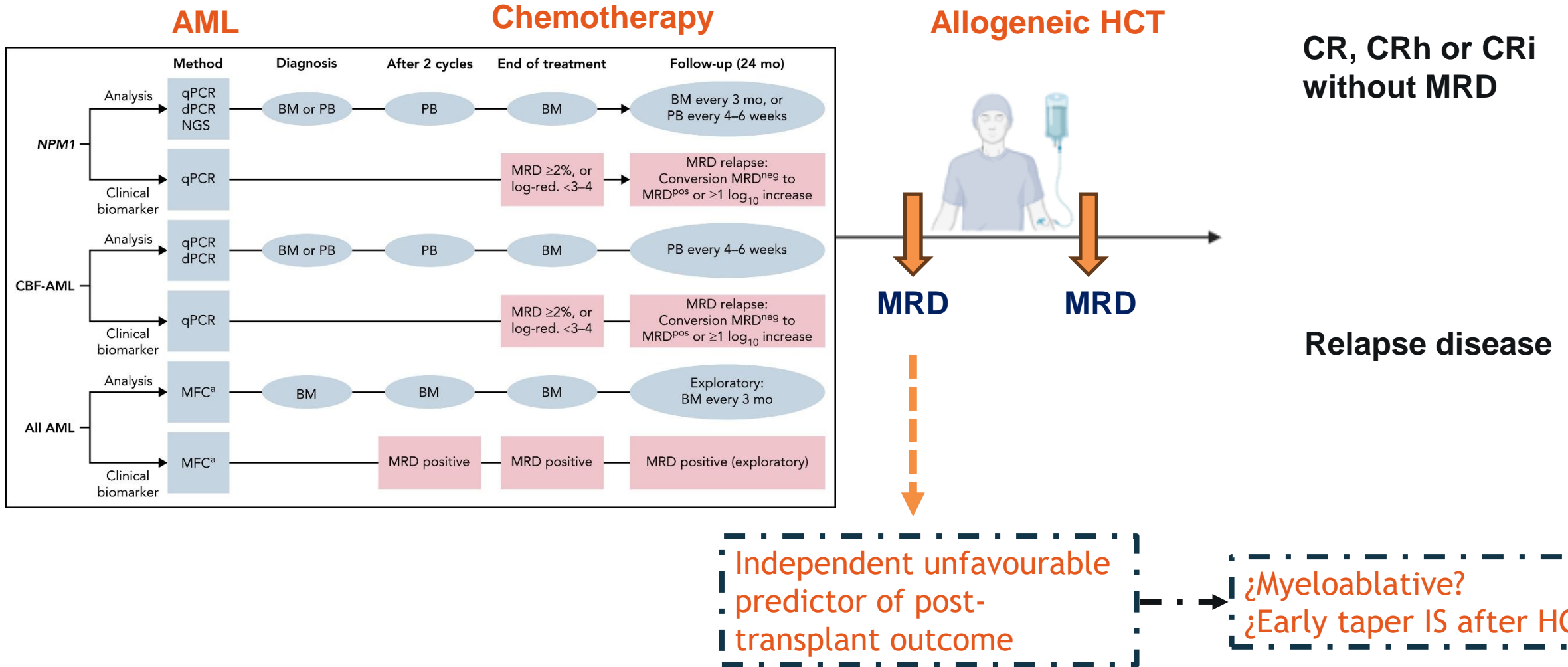
(2) refine postremission relapse risk assessment;

(3) identify impending relapse to enable early intervention;

(4) as a surrogate end point to accelerate drug testing and approval”¹

Acute Myeloid Leukemia

Pronostic value of MRD detection



Acute Myeloid Leukemia

Method for detection of MRD in AML

	Method	Target	Sensitivity	Applicable in % of AML	Turn-around time (days)	Limitations/problems
Established	Multi-parameter flow cytometry (MFC)	Leukemia-associated immunophenotype (LAIP) or different from normal (DfN)	10^{-3} to 10^{-4}	85-90	2	Less sensitive, more subjective analysis
Established	Real-time quantitative PCR (RT-qPCR)	Robust data: <i>NPM1</i> , <i>CBFB::MYH11</i> , <i>RUNX1::RUNX1T1</i> Less validated: <i>KMT2A::MLL3</i> , <i>DEK::NUP214</i> , <i>BCR::ABL1</i> , <i>WT1</i>	10^{-4} to 10^{-5}	40-50*	3-5	Limited applicability
Exploratory	Next-generation sequencing (NGS)†,‡	Potentially any somatic mutation†	10^{-2} to 10^{-4}	~100	5-10	Less sensitive, costly, technically challenging
Exploratory	Digital PCR (dPCR)	Specific targeted mutations	10^{-3} to 10^{-4}	~70	3-5	Specific assay necessary for every mutation, limited sensitivity

*Less frequent in elderly patients with AML.

†The NGS-MRD threshold has not been defined for individual mutations; NGS-MRD positivity is provisionally defined as $\geq 0.1\%$ variant allele frequency, excluding mutations related to clonal hematopoiesis and germline mutations.

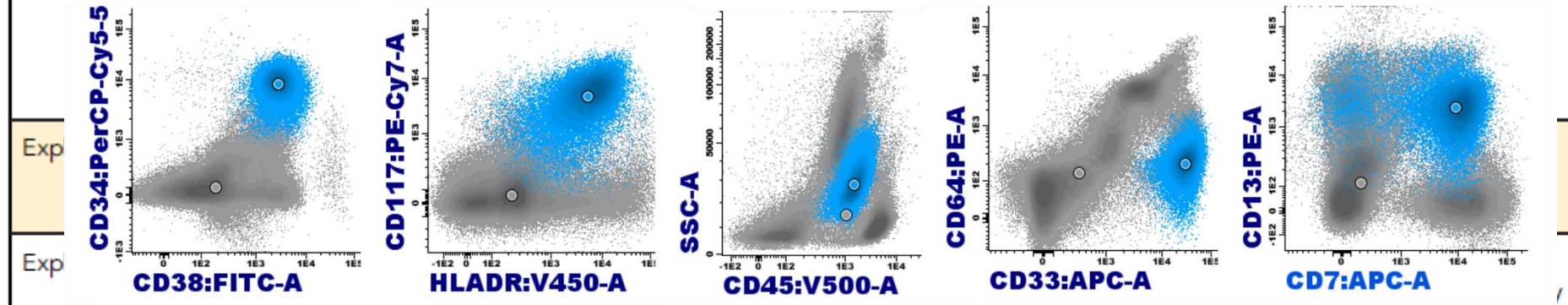
‡Common gene mutations consistent with pre-malignant clonal hematopoiesis such as *DNMT3A*, *TET2*, and *AXSL1* excluded; further study is required to determine which mutations are truly indicative of residual AML and not clonal hematopoiesis.

Acute Myeloid Leukemia

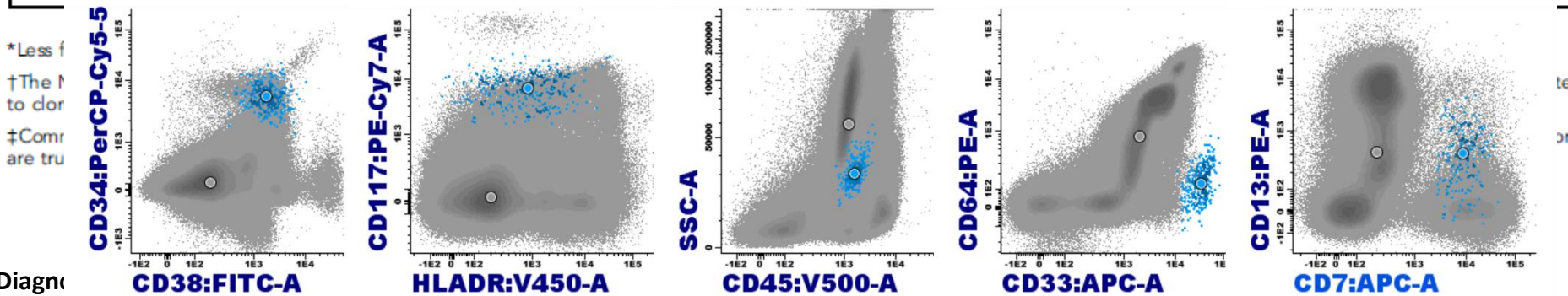
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Established	Real-time	Robust data: <i>NPM1</i> ,	10^{-4} to 10^{-5}	40-50*	3-5	Limited applicability

Relapse



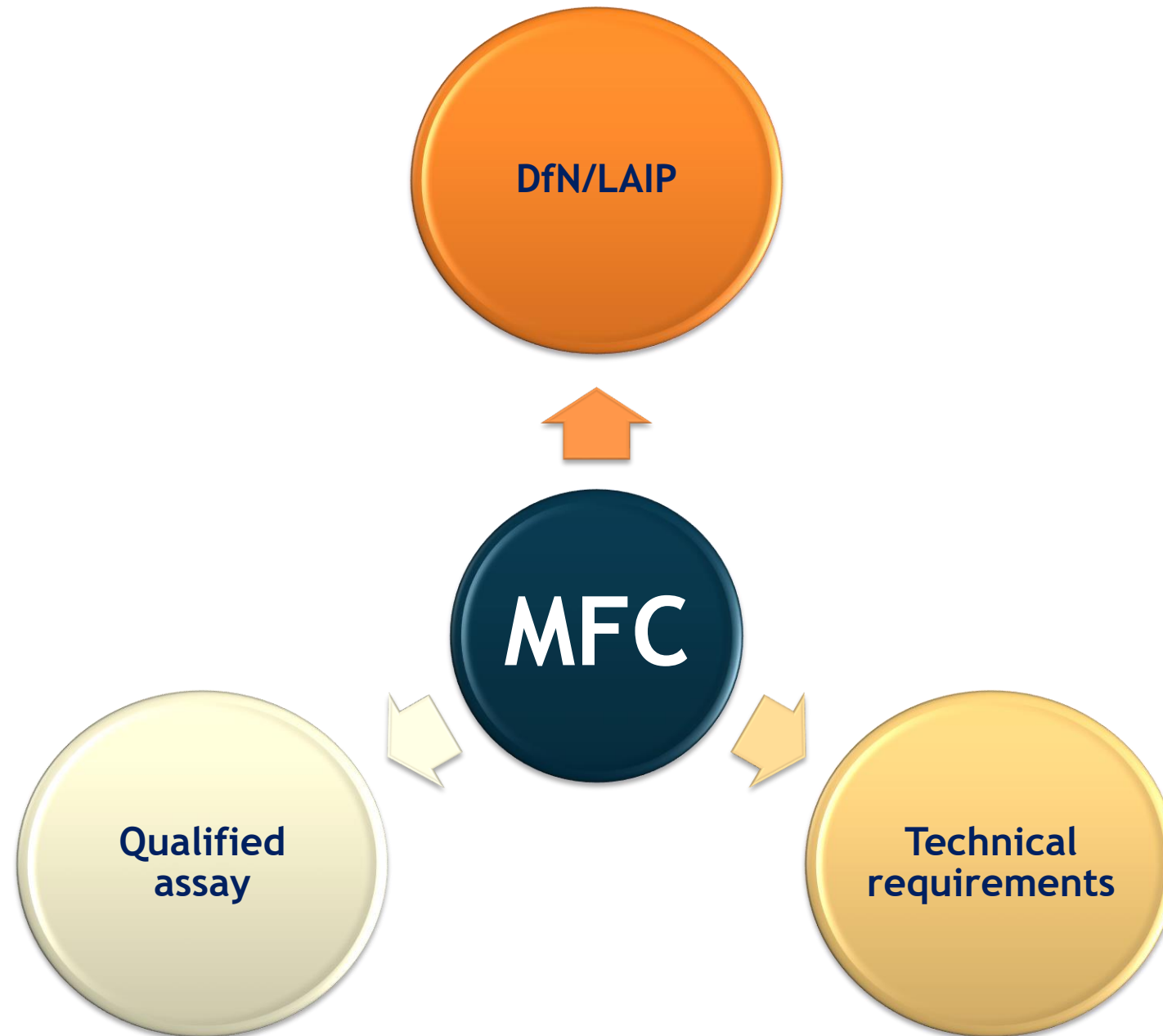
Before allo-HCT



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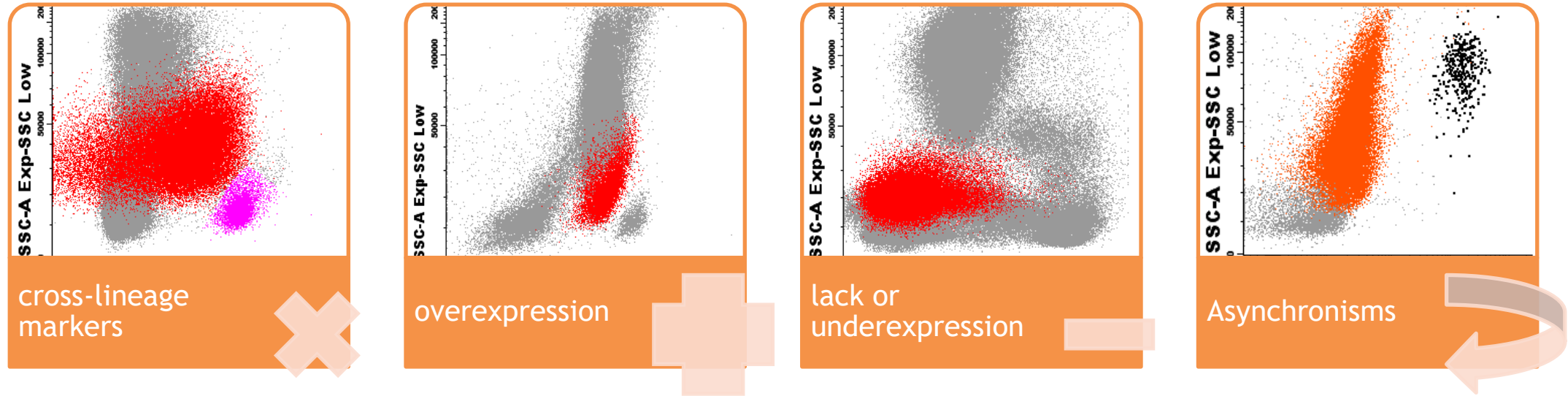
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Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML



Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML

LAIPs



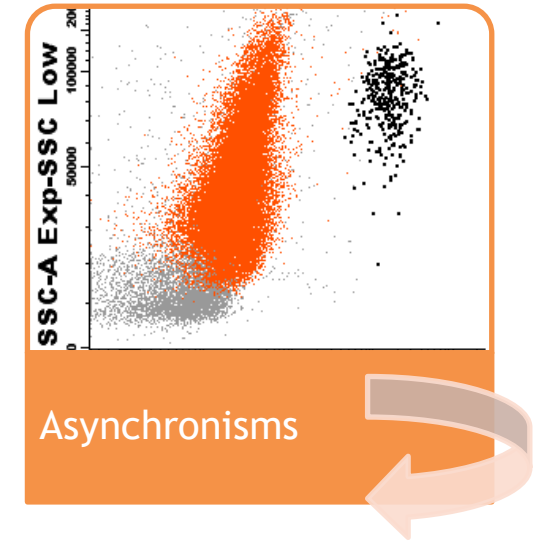
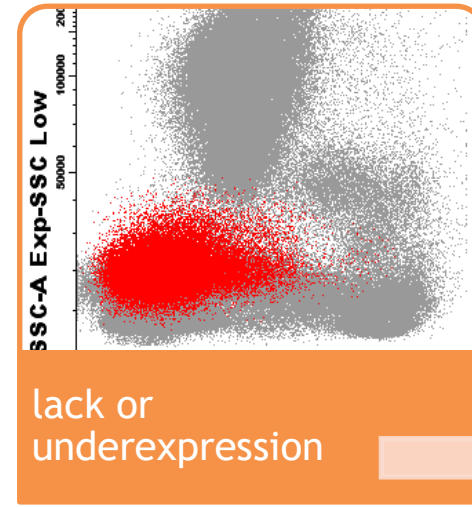
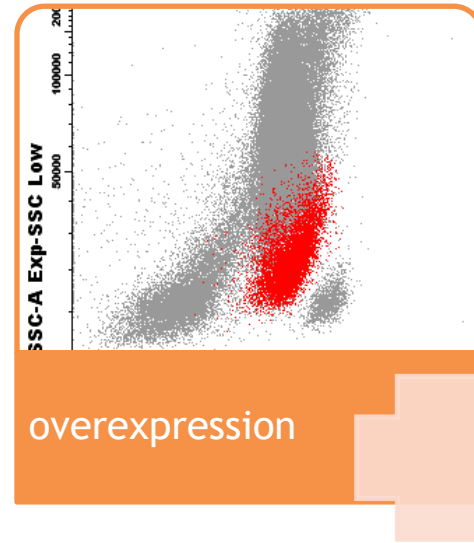
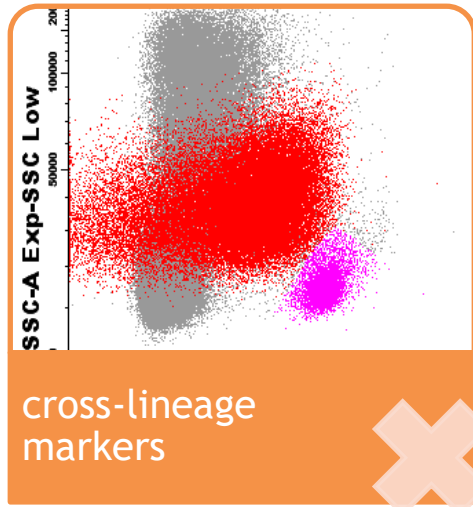
Lack of specific tumour antigens: immunophenotype patterns in more than 90% of AML

Phenotypic changes: Non-determinant, leukemic subpopulations, technical issues,.....

Training: automatic analysis

Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML

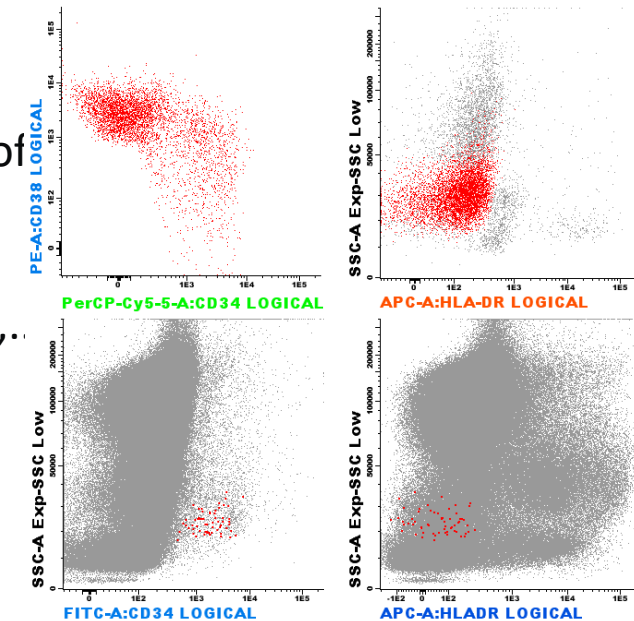
LAIPs



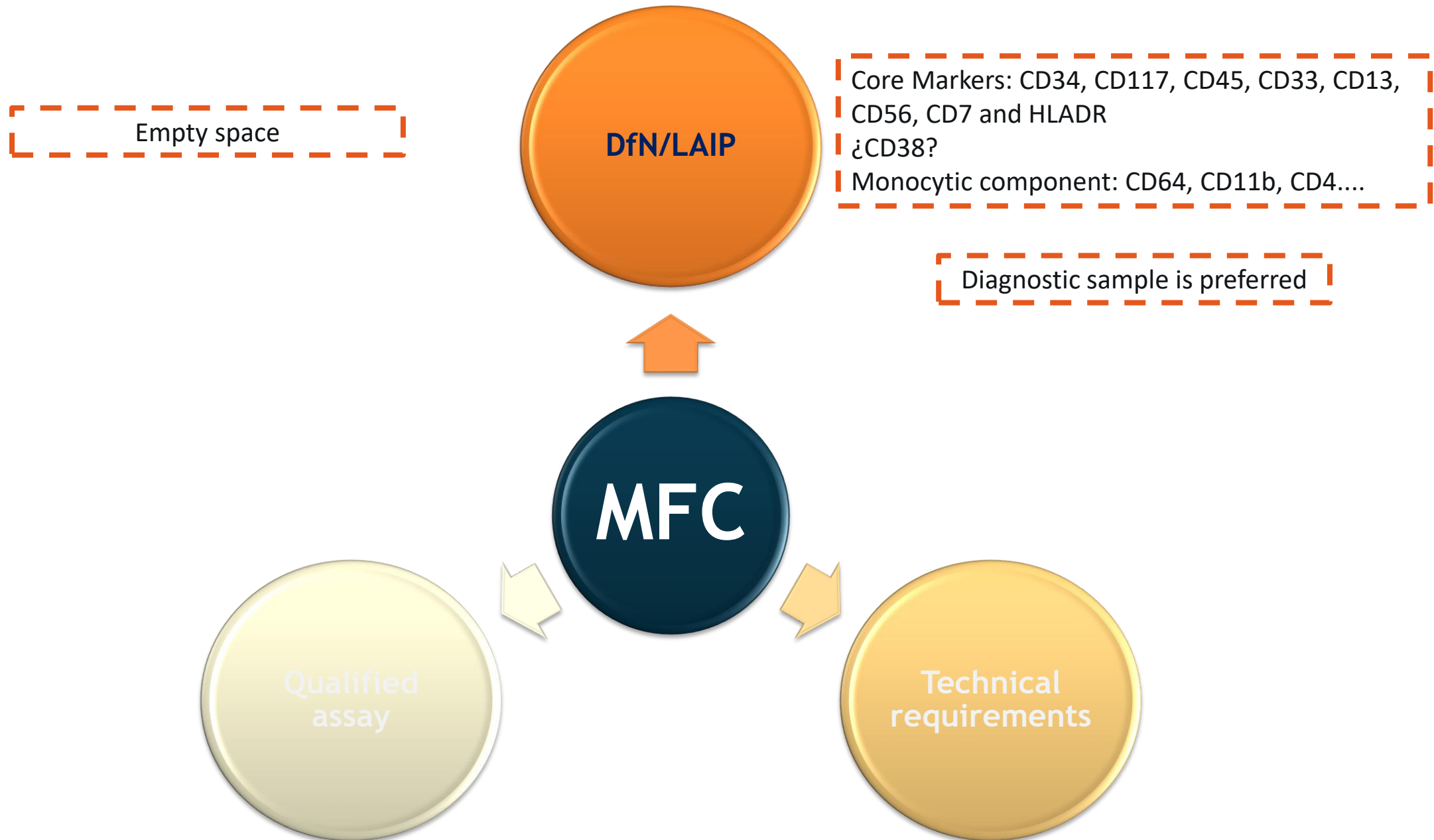
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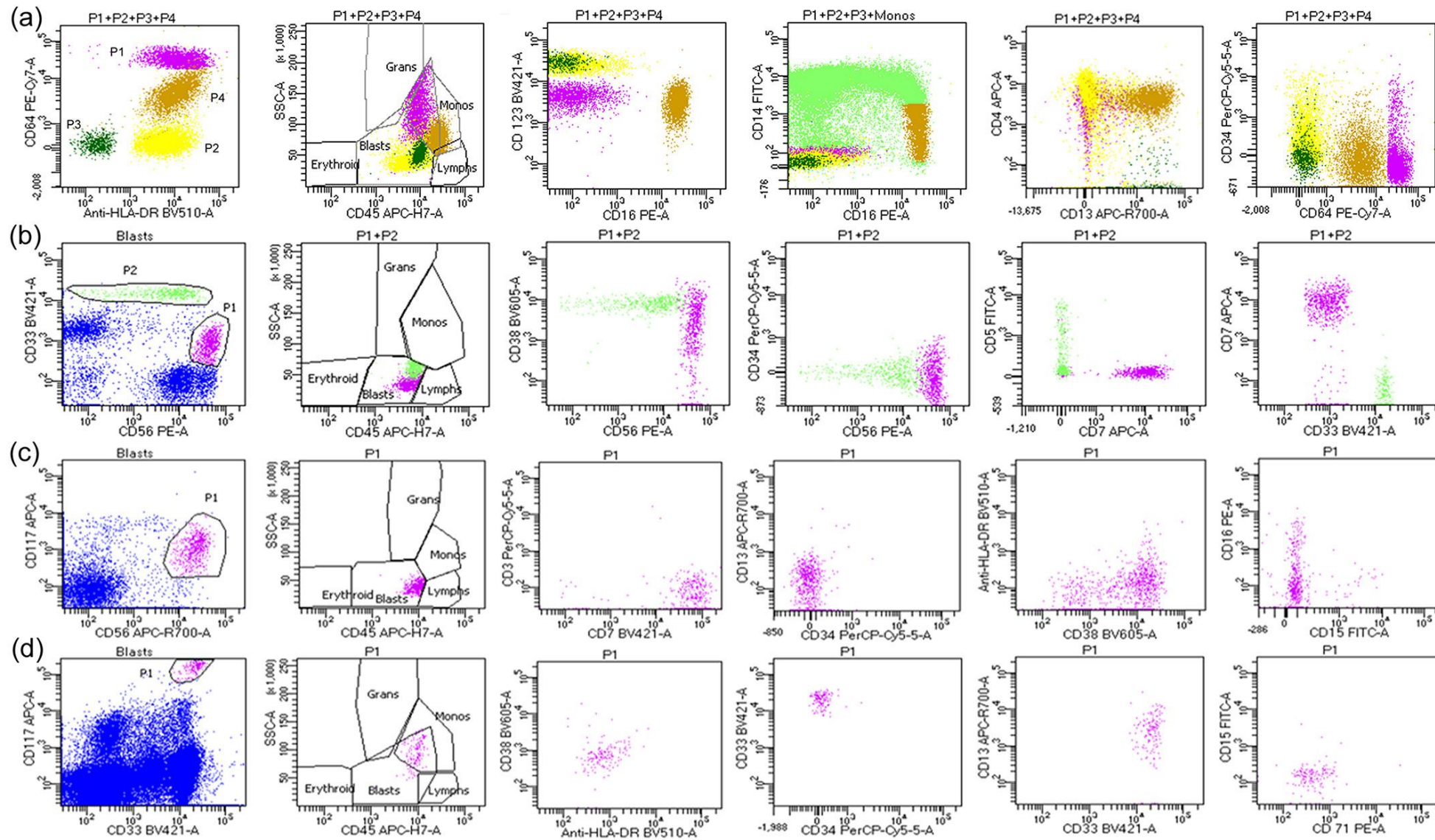


Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML

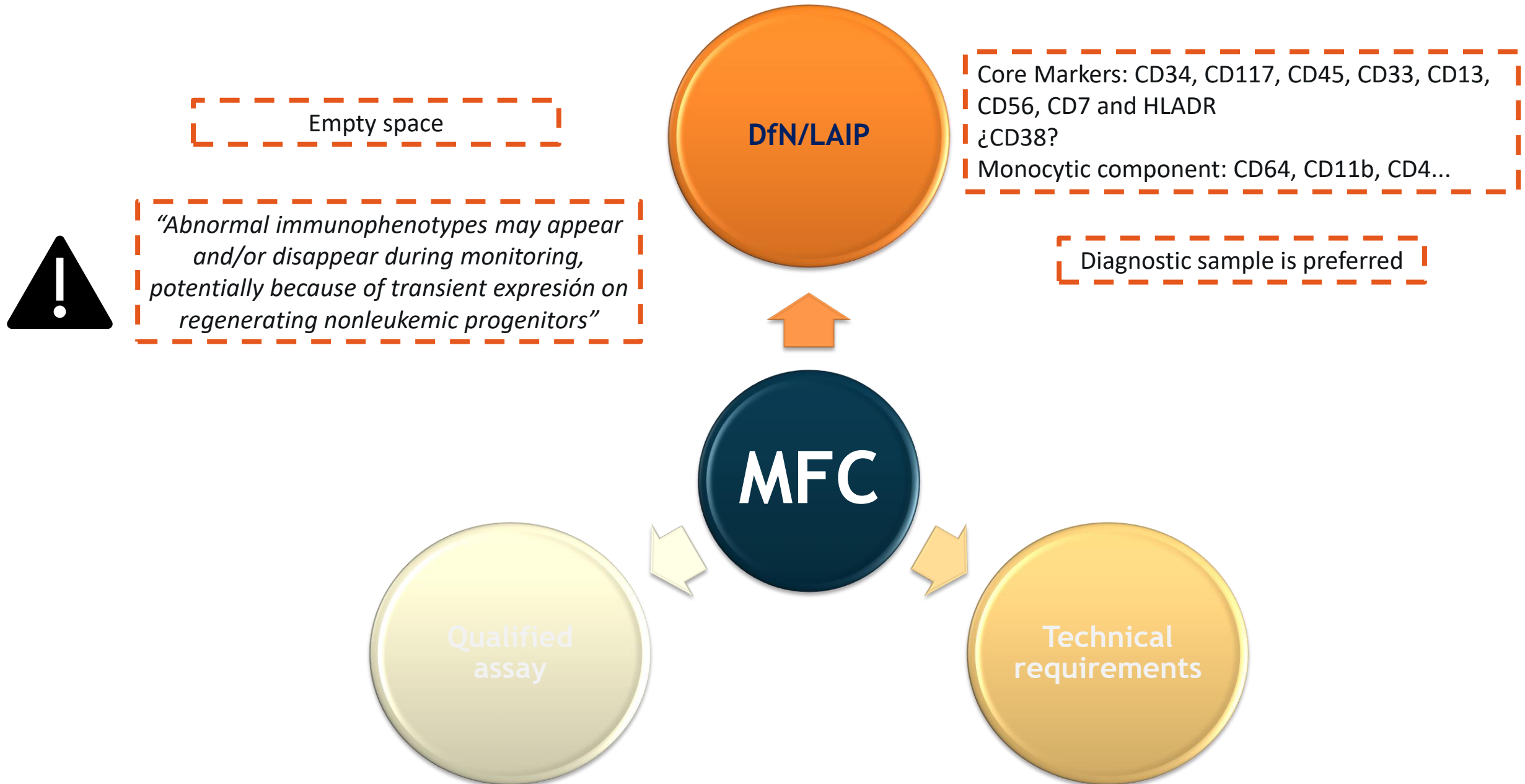


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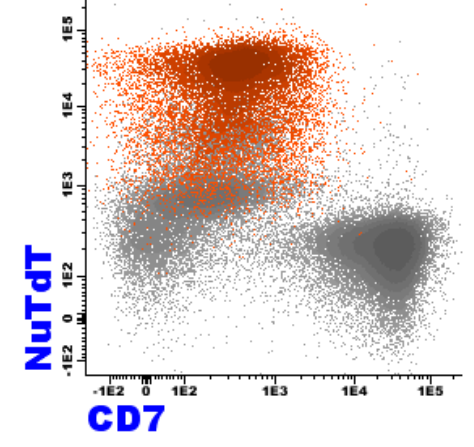
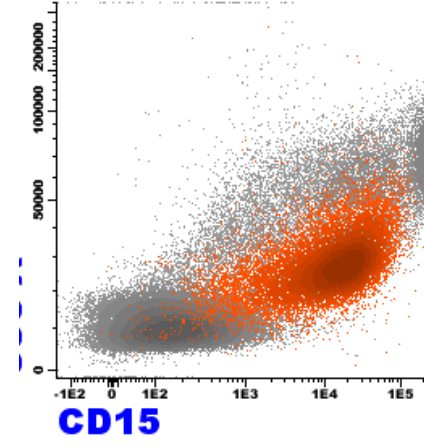
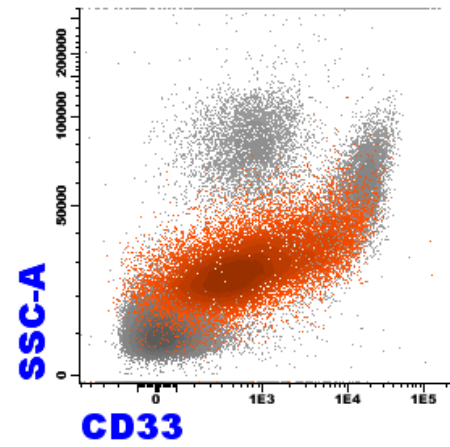
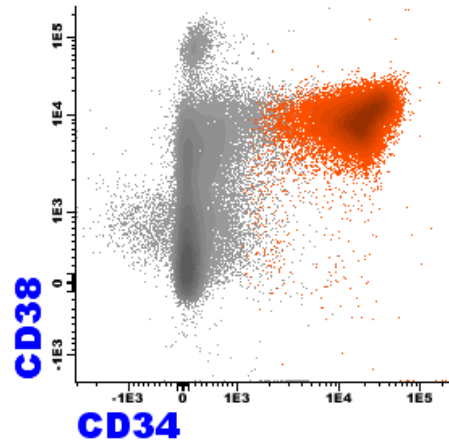
Normal or reactive minor cell populations in BM and PB mimic minimal residual leukemia by FCM



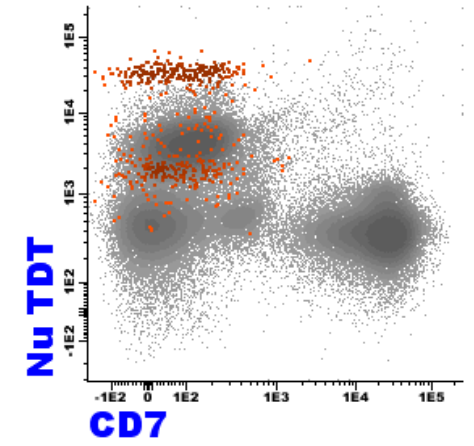
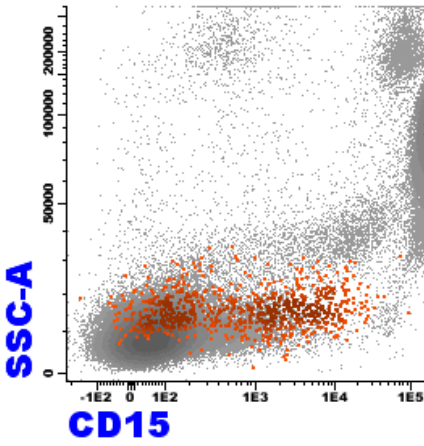
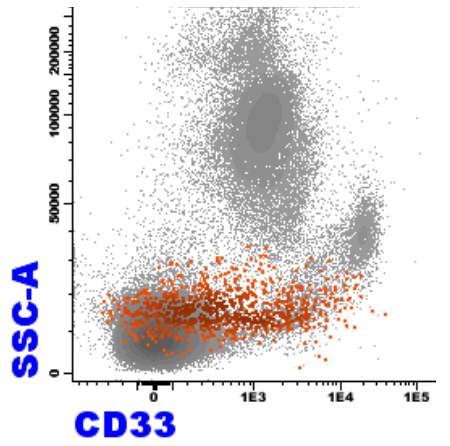
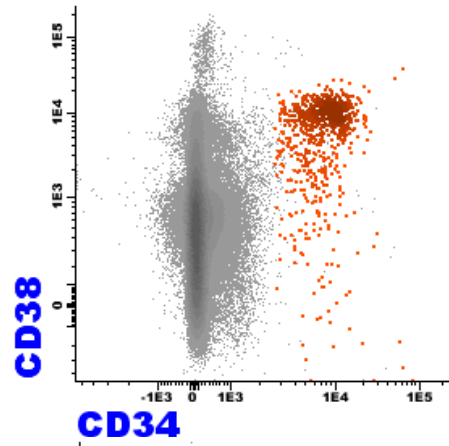
Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML



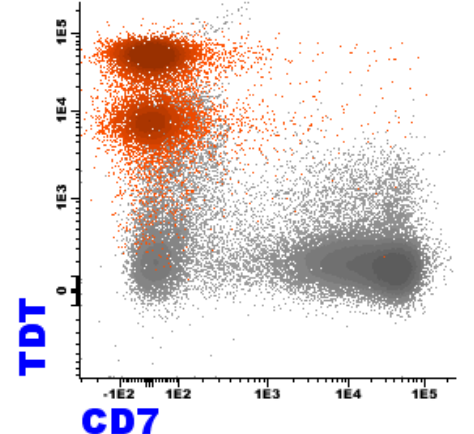
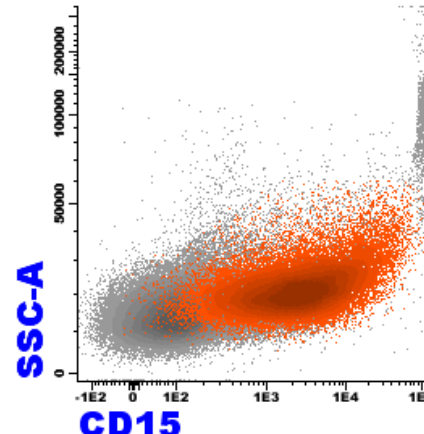
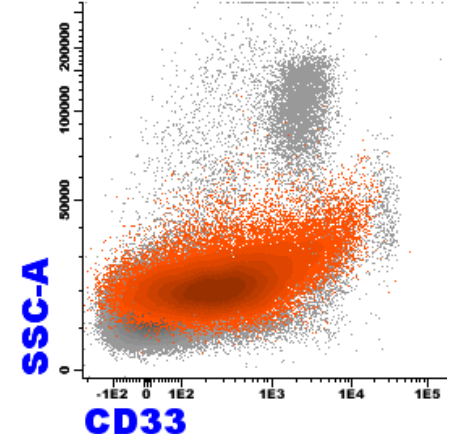
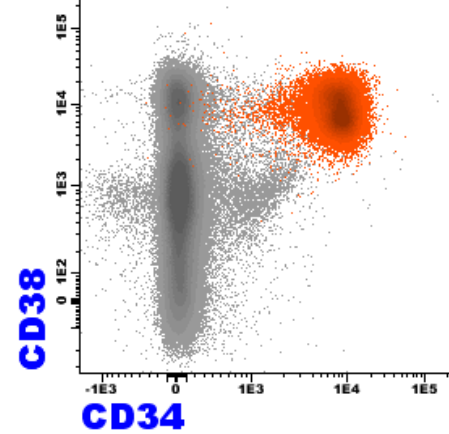
August 2019
Pancytopenia
Pneumonia
24% CD34+



September 2019
AB treatment
No chemotherapy
No cytopenia
1% CD34+



January 2020
AML
26% CD34+



Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML

Empty space



- Core Markers: CD34, CD117, CD45, CD33, CD13, CD56, CD7 and HLADR
- ¿CD38?
- Monocytic component: CD64, CD11b, CD4...

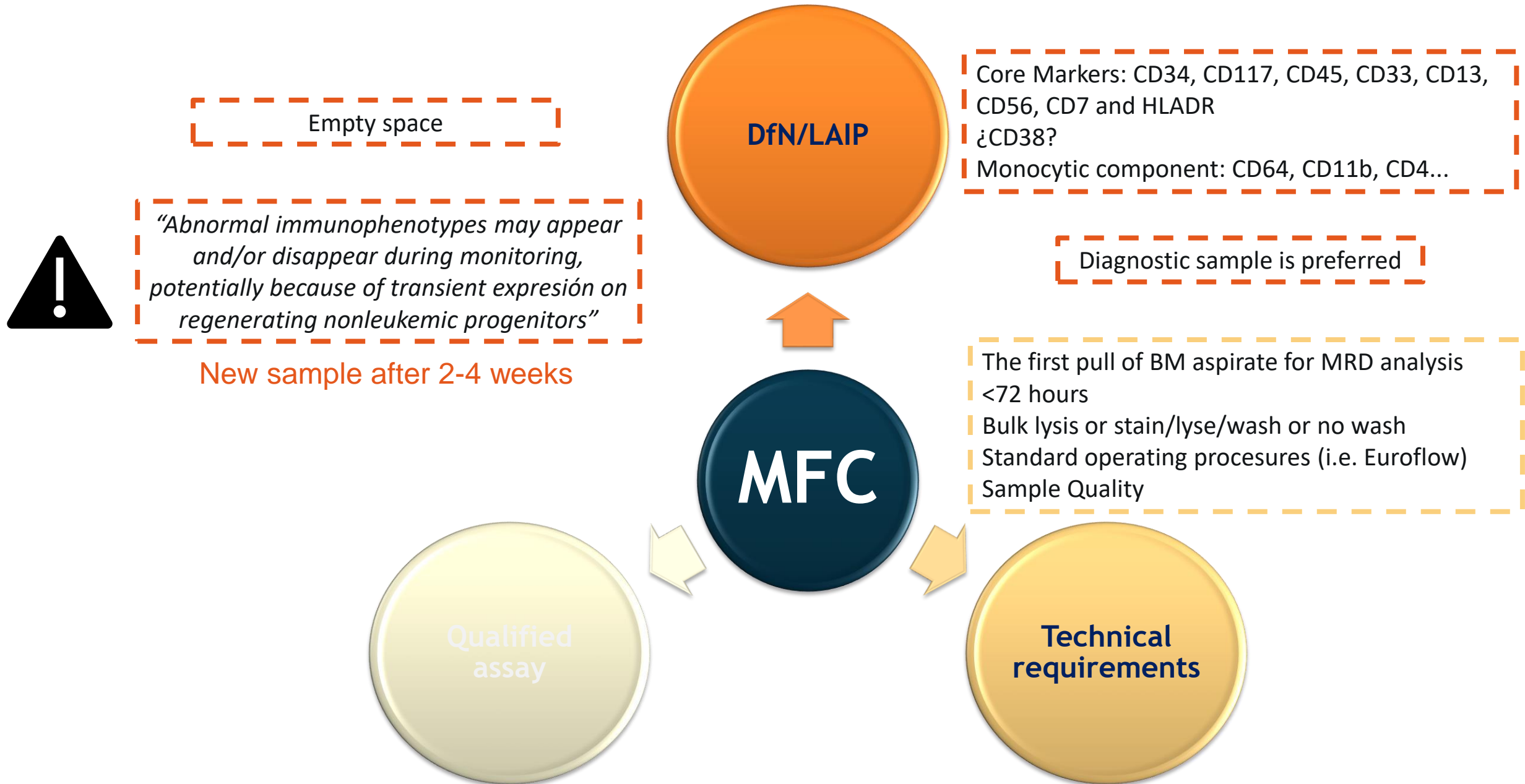
Diagnostic sample is preferred

“Abnormal immunophenotypes may appear and/or disappear during monitoring, potentially because of transient expresión on regenerating nonleukemic progenitors”

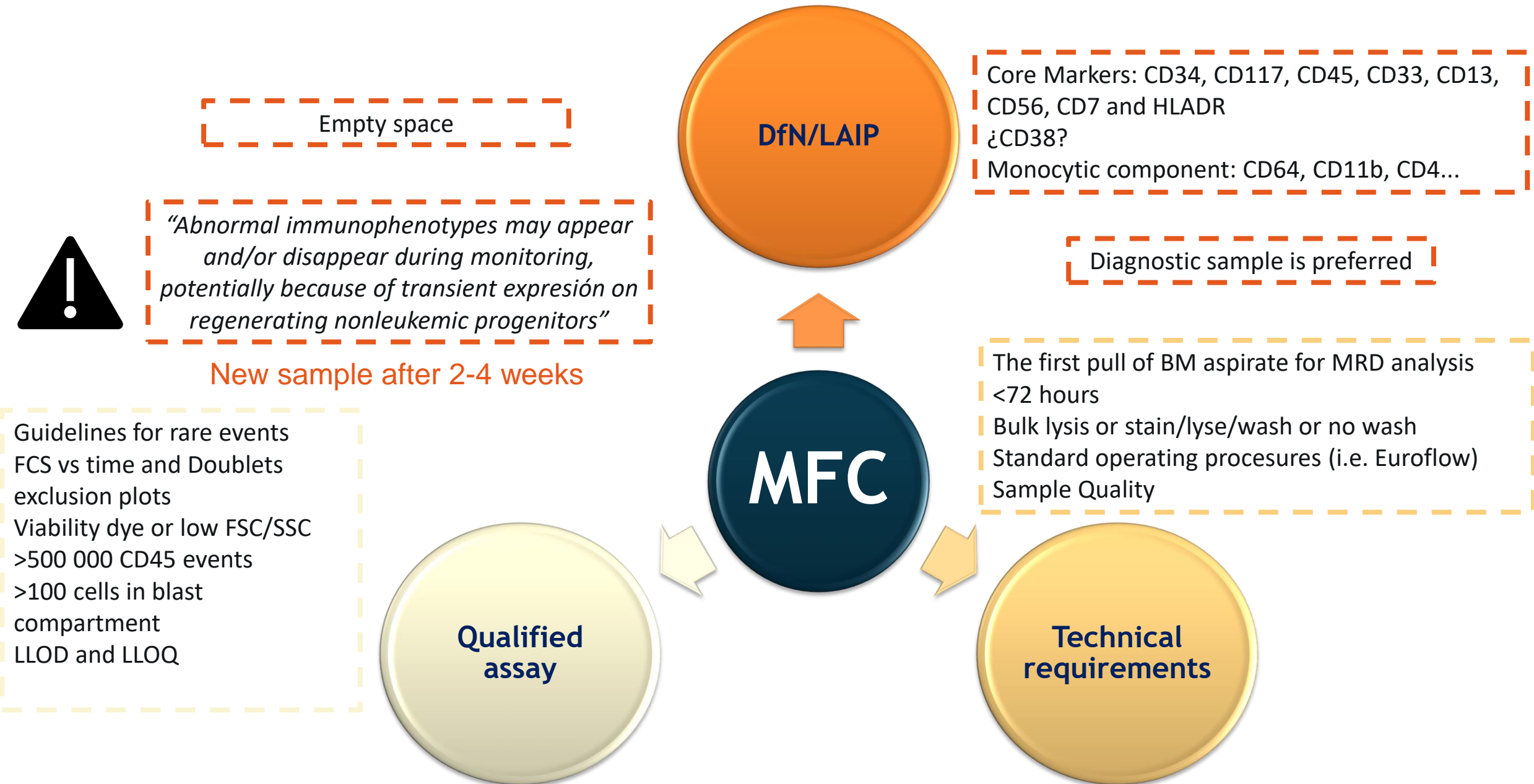
New sample after 2-4 weeks



Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML



Acute Myeloid Leukemia: Multiparameter flow cytometry MRD in AML



Caso: leukemic subpopulations

Follow up of *NPM1*+ AML

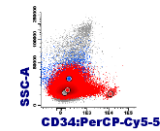
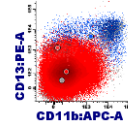
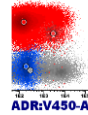
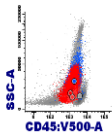
AML with extramedullary (palate) involvement with *NPM1*, *IDH1* and *GATA2* mutations (normal karyotype).

Treatment: PETHEMA LMA *NPM1* 2017 with negative *NPM1* MRD by qPCR after 1st consolidation.

Diagnosis

81,4% blasts

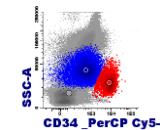
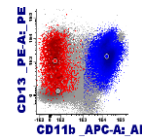
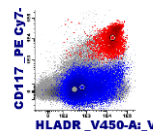
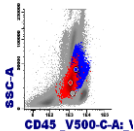
3,1% monocytes



At 10 months after autologous transplant (before *NPM* negative)

0% blast

6,7% monocyte



Caso: leukemic subpopulations

Follow up of *NPM1*+ AML

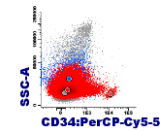
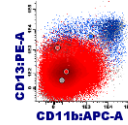
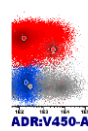
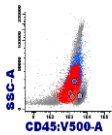
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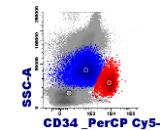
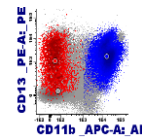
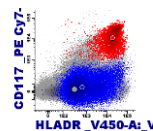
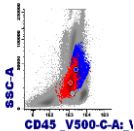
At 10 months after autologous transplant (before *NPM* negative)

IDH1 en NGS en BM

NPM1: PB+ BM-

0% blast

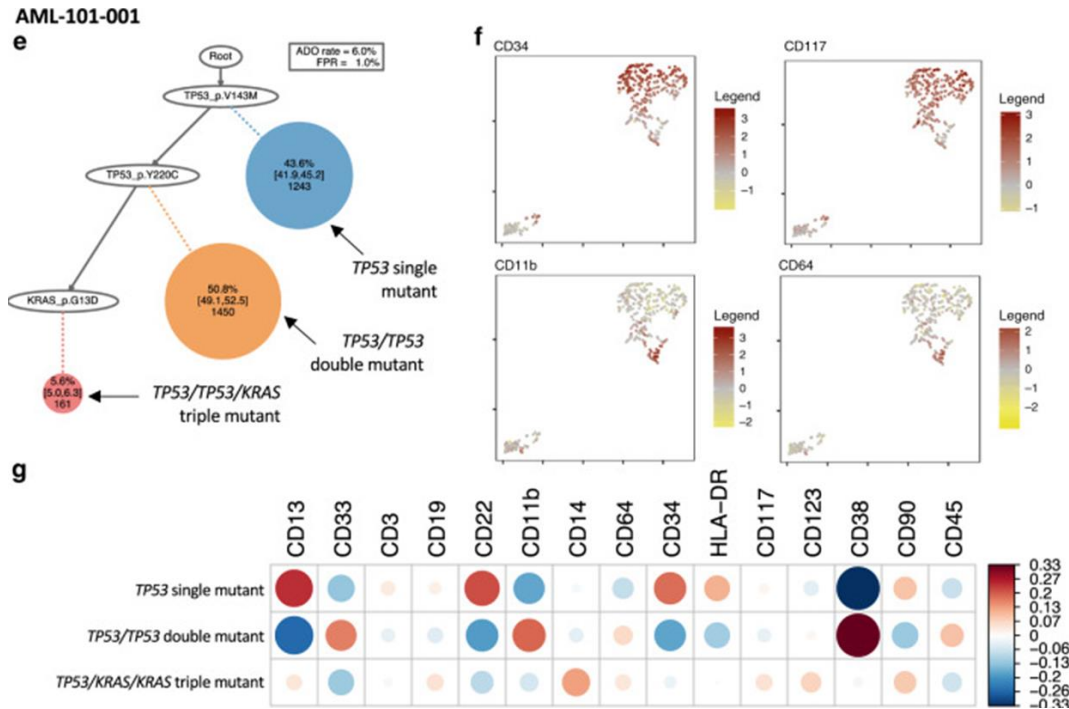
6,7% monocyte



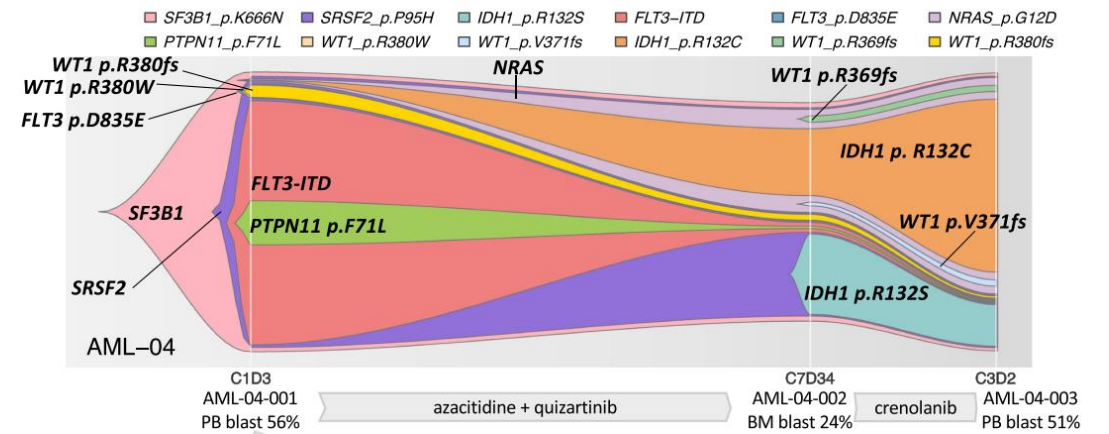
Acute Myeloid Leukemia

Clonal evolution

Genotype-phenotype correlation in single-cell studies (scDNA+protein-seq) in AML



Selection of IDH1/RAS clones during therapy containing a FLT3 inhibitor



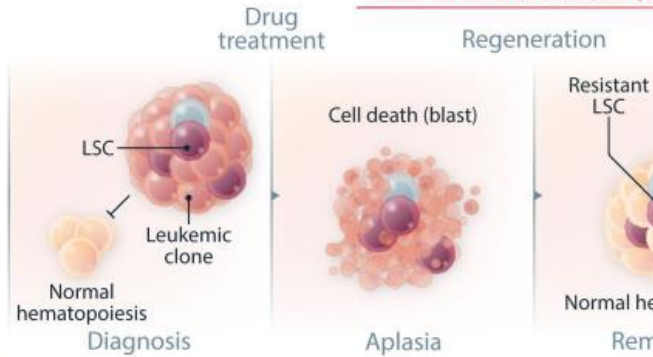
Other perspectives for monitoring

Stem cells leukemia

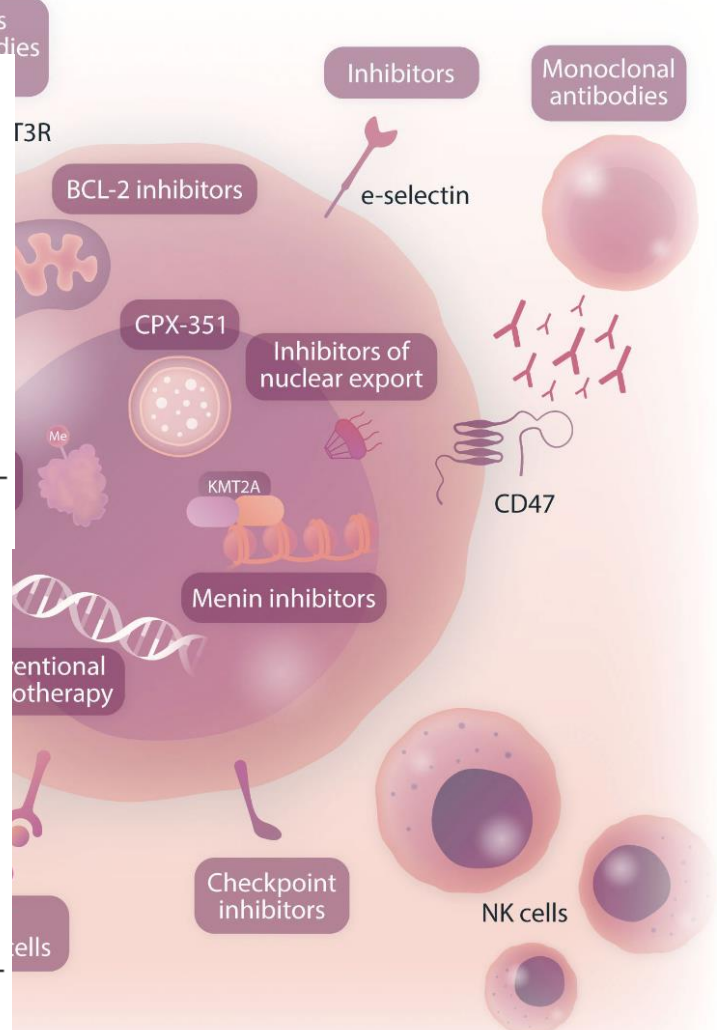
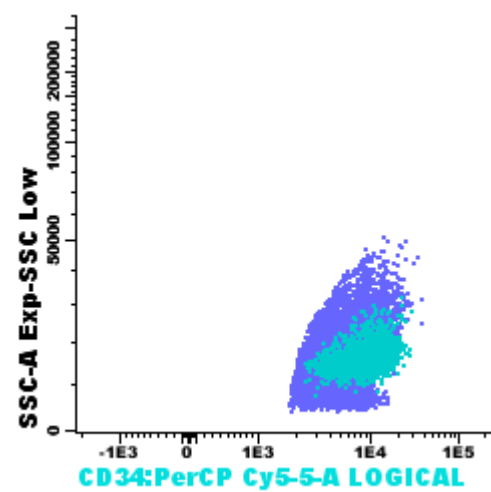
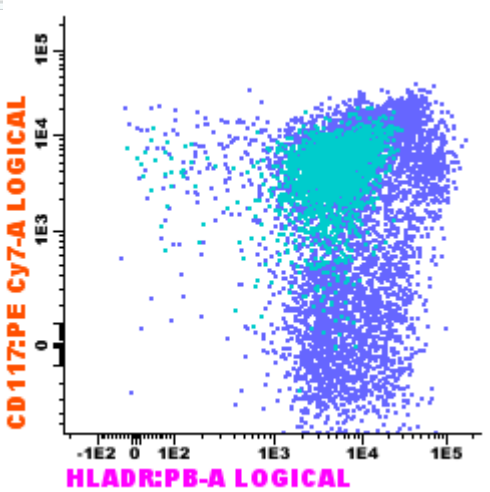
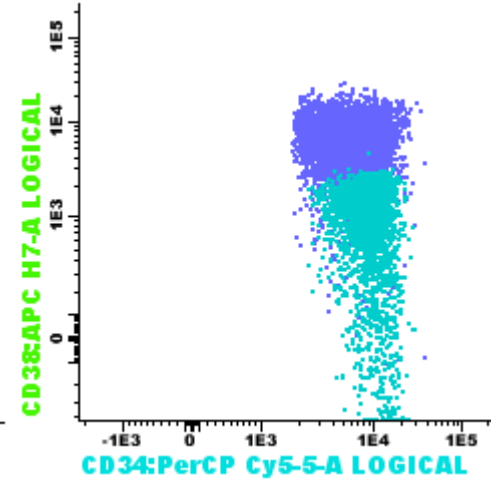
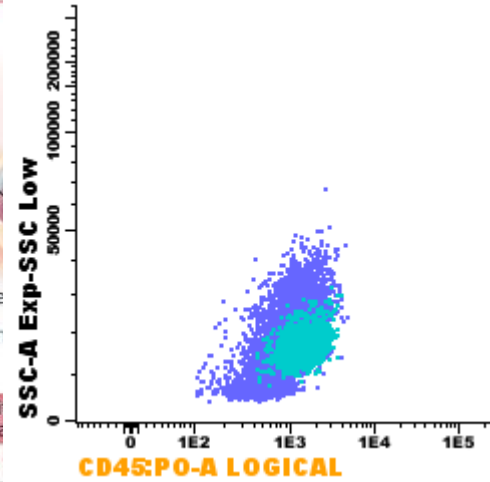
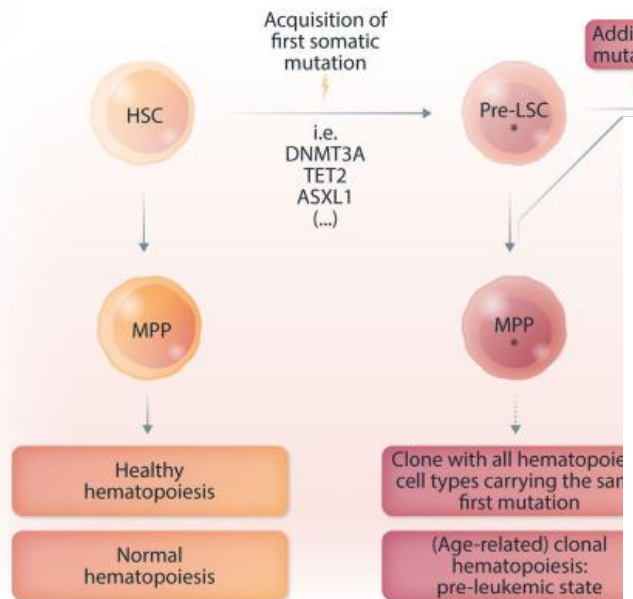
A

Therapy resistance and relapse

Minimal residual disease (MRD)



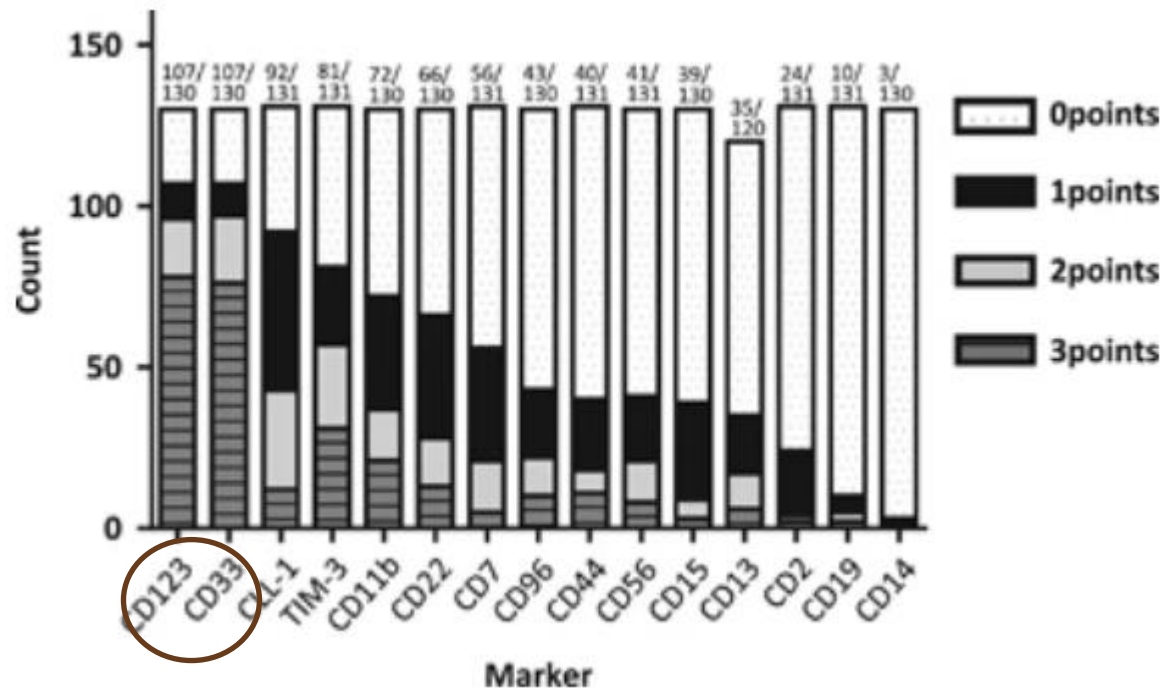
B



Stem cells leukemia

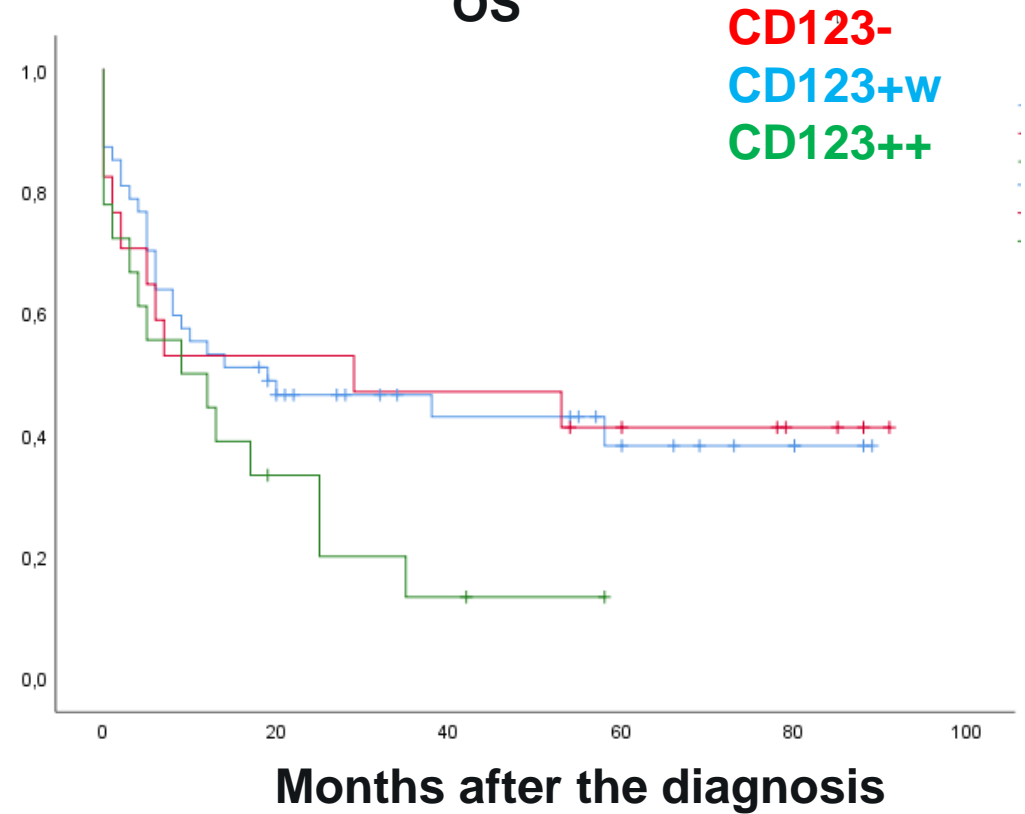
Discriminate HSCs and LSCs

SC vs LSC



Zeijlemaker W, et al. A simple one-tube assay for immunophenotypical quantification of leukemic stem cells in acute myeloid leukemia. *Leukemia*. 2016

OS



Stem cells leukemia

Follow up of LSCs

	<i>AML diagnosis BM</i>		<i>AML follow-up BM</i>		<i>NBM</i>		<i>Diagnosis vs f-up</i>	<i>Diagnosis vs NBM</i>	<i>Follow-up vs NBM</i>
	<i>Median (range)</i>	<i>n</i>	<i>Median (range)</i>	<i>n</i>	<i>Median (range)</i>	<i>n</i>	<i>P-value</i>	<i>P-value</i>	<i>P-value</i>
CLL-1	0.7 (0.1–2.1)	27	4.1 (0.3–14.0)	30	1.6 (0.3–3.0)	10	<0.001	0.02	0.001
TIM-3	0.6 (0.2–4.9)	32	2.2 (0.3–10.8)	28	0.8 (0.6–1.6)	9	<0.001	0.23	0.002
CD7	1.0 (0.4–3.1)	32	2.4 (0.4–5.9)	37	1.7 (0.2–3.6)	10	<0.001	0.05	0.26
CD11b	1.0 (0.2–2.7)	24	3.1 (0.5–5.9)	22	2.5 (0.7–5.4)	9	<0.001	0.001	0.65
CD22	2.0 (0.9–6.6)	12	3.5 (1.4–10.6)	9	5.4 (2.0–11.0)	9	0.13	0.02	0.34
CD33	2.1 (0.6–23.8)	30	5.5 (0.1–910.6)	35	19.4 (1.0–130.5)	21	<0.001	<0.001	0.09
CD56	1.1 (0.3–4.0)	25	4.3 (0.7–26.4)	24	2.5 (1.2–6.8)	9	<0.001	0.004	0.14
CD123	3.9 (0.4–17.7)	30	14.5 (1.3–40.1)	25	5.8 (0.5–86.3)	20	<0.001	0.06	0.006

Marker

MRD and LSC

Proposed panel

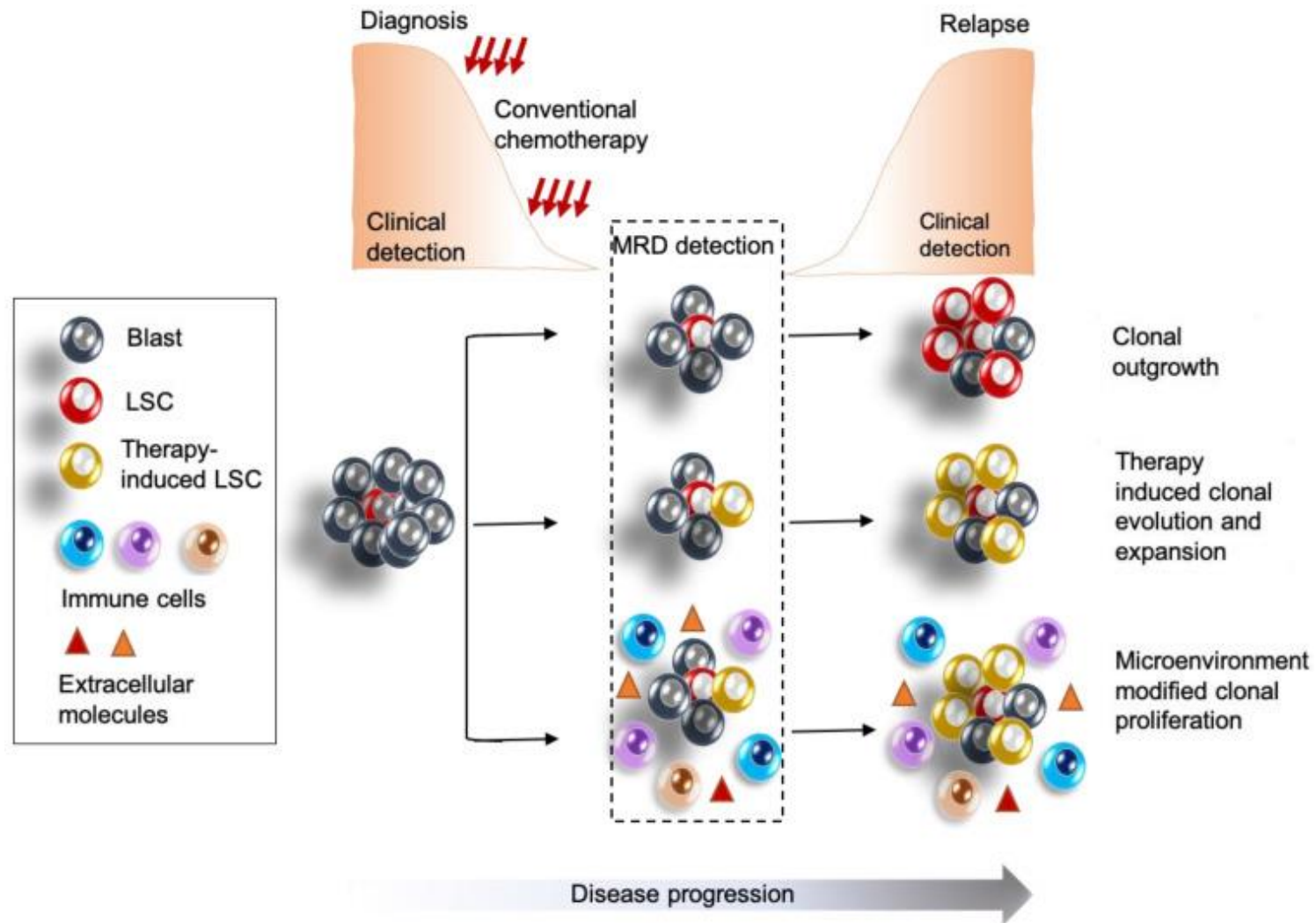
MRD

Tube	FITC	PE	PerCP-CY5.5	PC7	APC	APC-H7	BV421	KO
1	CD7	CD56	CD34	CD117	CD33	HLA-DR	CD13	CD45
2	CD15	CD22	CD34	CD117	CD19	HLADR	CD13	CD45
3	CD36	CD14	CD34	CD117	CD11b	HLADR	CD13	CD45
4	CD2	CD133	CD34	CD117	CD33	HLADR	CD13	CD45

LSC

Tube	FITC	PE	PerCP-CY5.5	PC7	APC	APC-H7	BV421	KO
1	CD45RA	Clec12a	CD123	CD34	CD38	CD44	CD33	CD45
		TIM-3						
		CD7						
		CD11b						
		CD22						
		CD56						

Other perspectives for monitoring

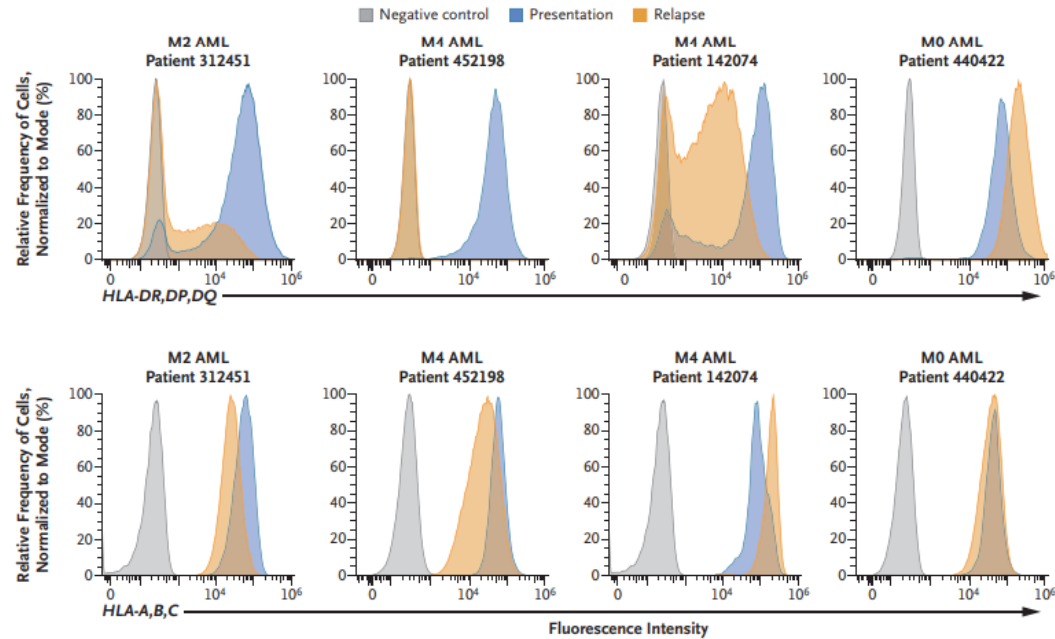


Defective anti-leukemic immune responses in AML patients

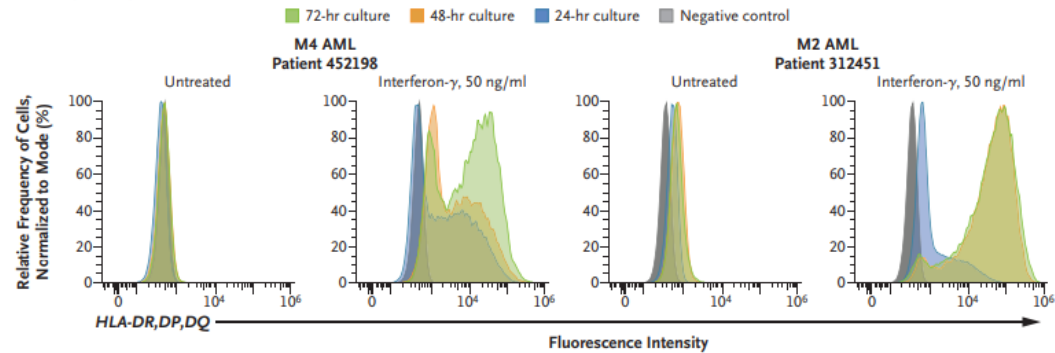
Deregulation of pathways involved in the innate/adaptive immune response

↑ co-inhibitory ligands in blasts and modifications in T cells

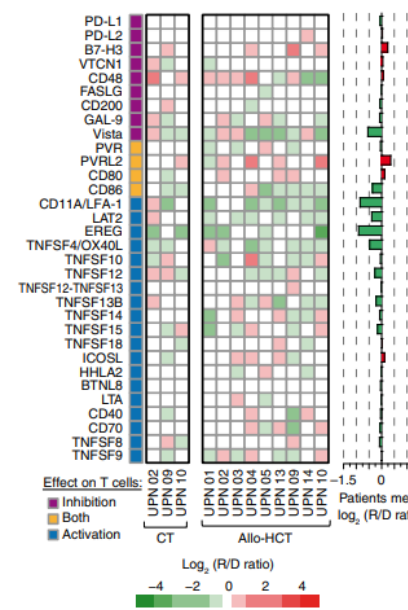
A Flow Cytometry



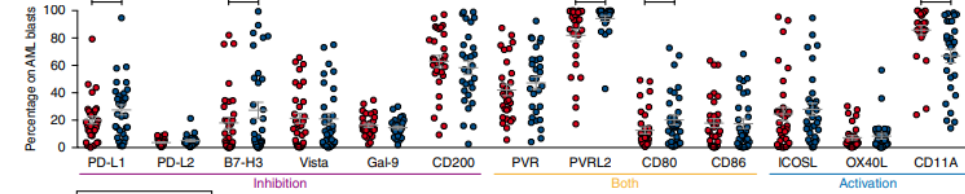
B Flow Cytometry after Treatment with Interferon- γ



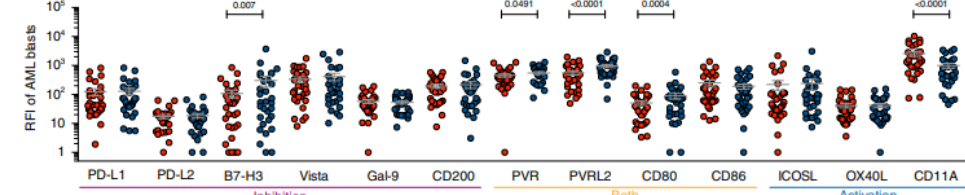
a



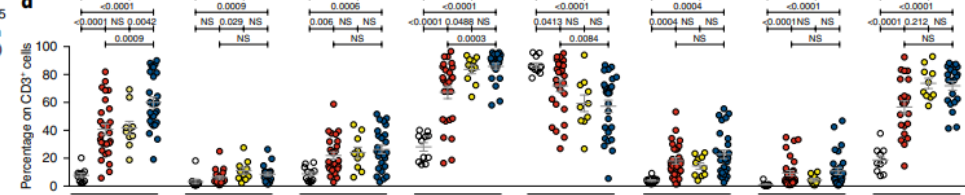
b



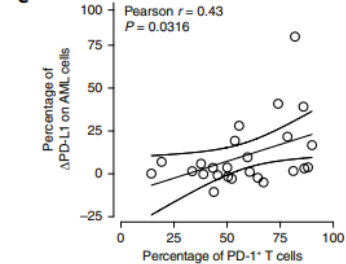
c



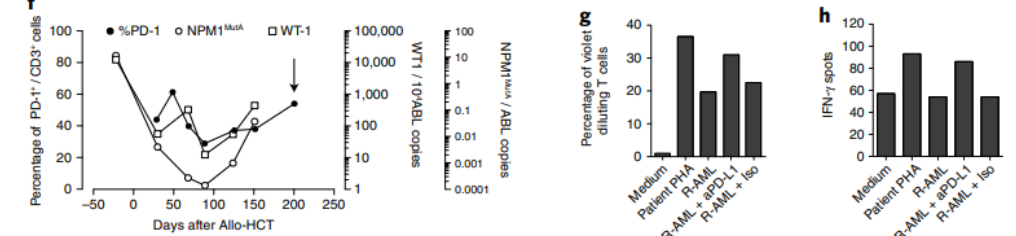
d



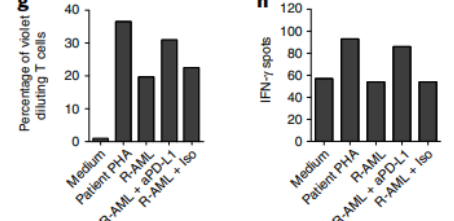
e



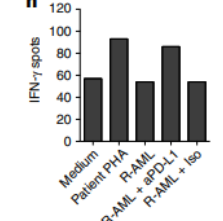
f



g



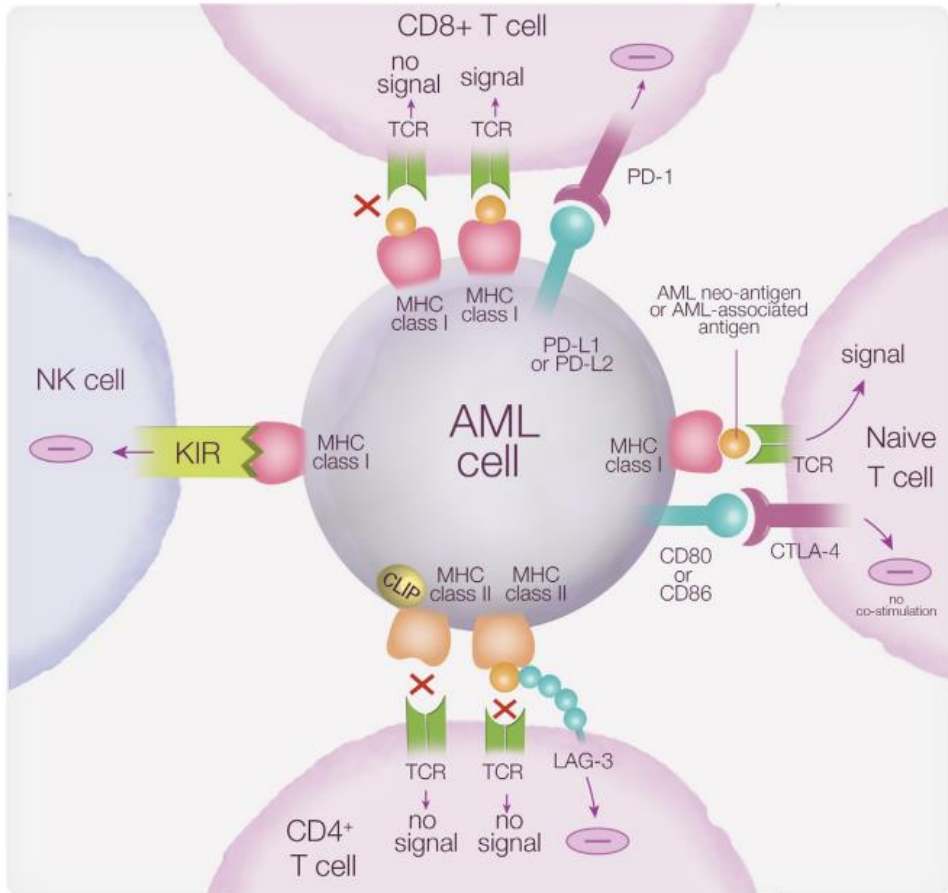
h



Defective anti-leukemic immune responses in AML patients

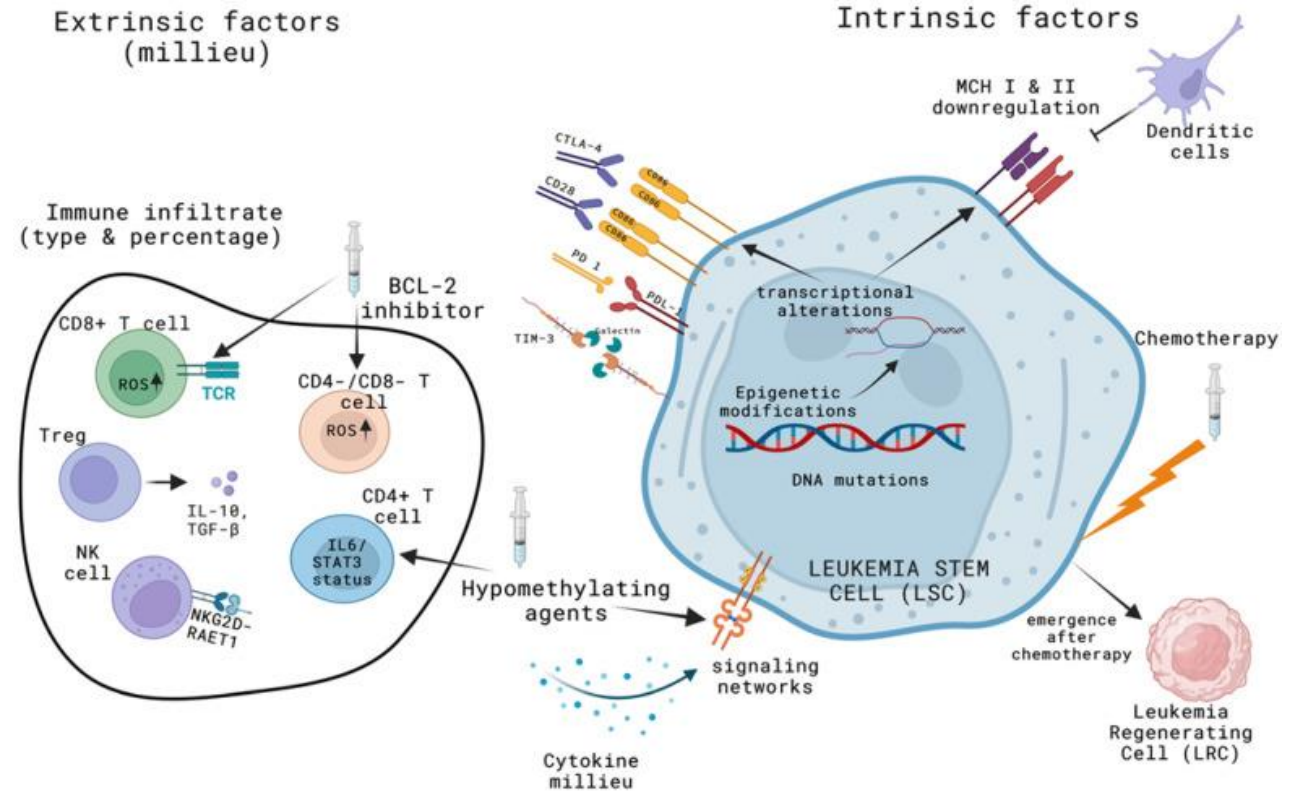
Immune system evasion mechanisms

Defective antigen presentation and immune activation in AML.



Austin R, et al. Harnessing the immune system in acute myeloid leukaemia. Crit Rev Oncol Hematol. 2016

New treatment

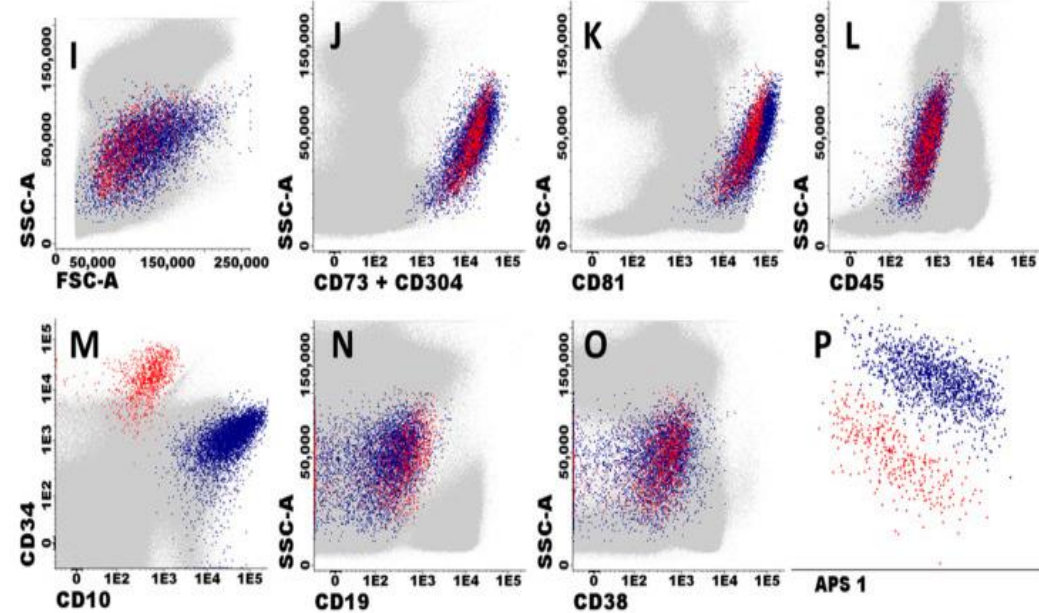


Pessach I, et al. MRD Monitoring by Multiparametric Flow Cytometry in AML: Is It Time to Incorporate Immune Parameters? Cancers (Basel). 2022

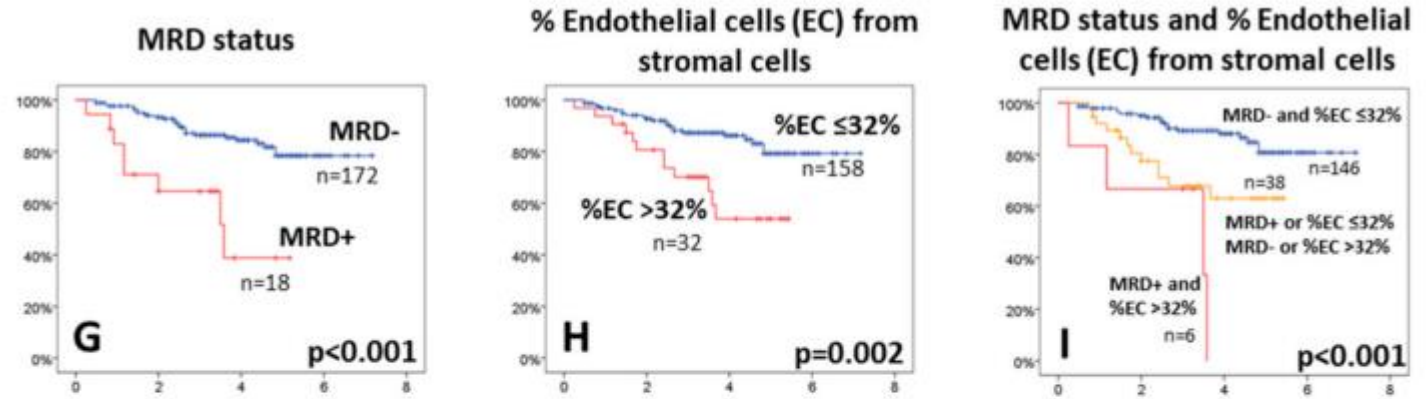
Immune system and MRD

Bone Marrow Stromal Cell Regeneration in B cell precursor ALL

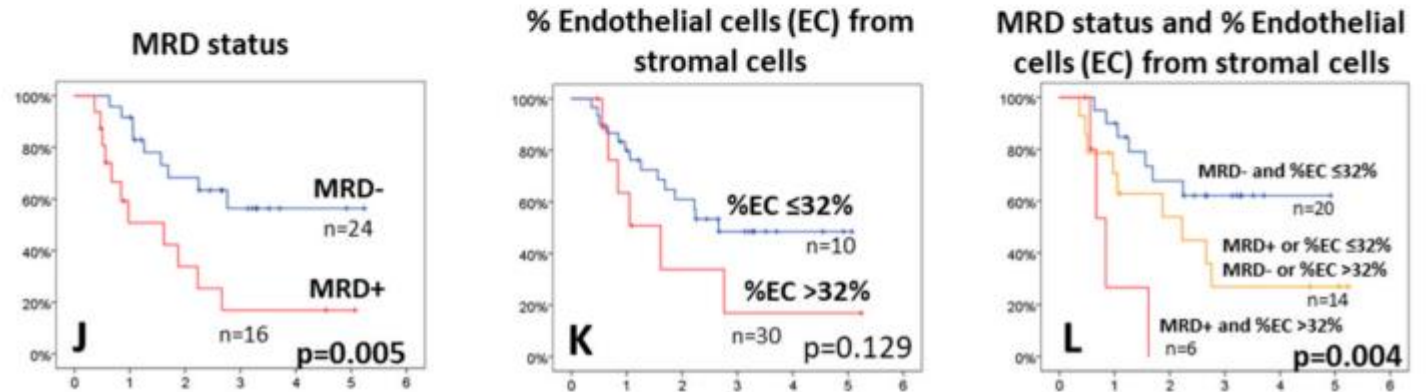
Endothelial vs MSC



Day +78 – Childhood BCP-ALL Discovery + Validation Cohort (n=190)



Day +78 – Adult BCP-ALL Validation Cohort (n=40)



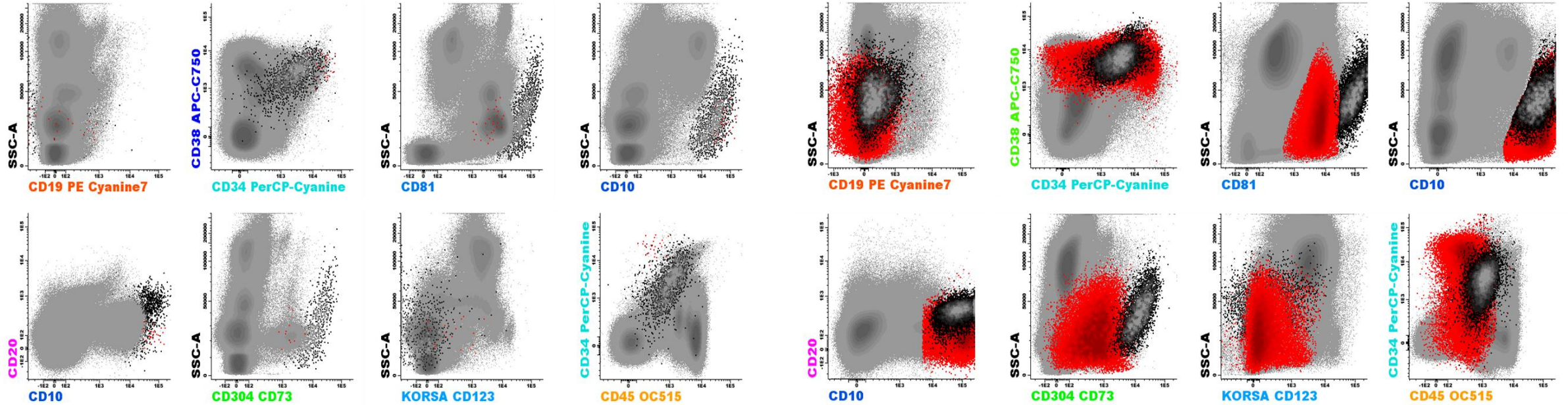
Immune system and MRD

Bone Marrow Stromal in MRD

B-Cell precursor ALL after CAR-T19

MRD

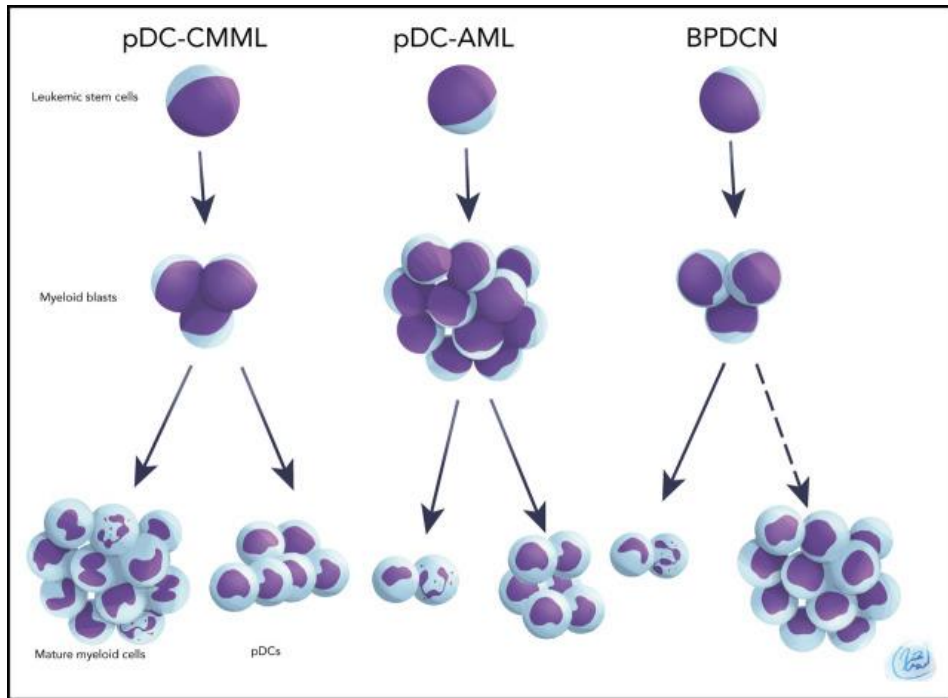
Relapse



HVR patient

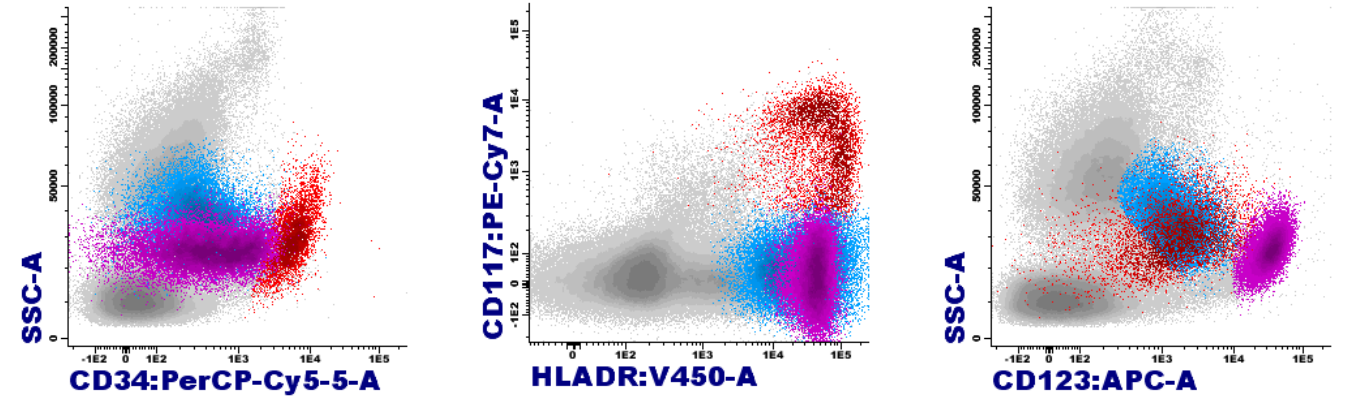
Immune system and MRD

Plasmacytoid dendritic cells

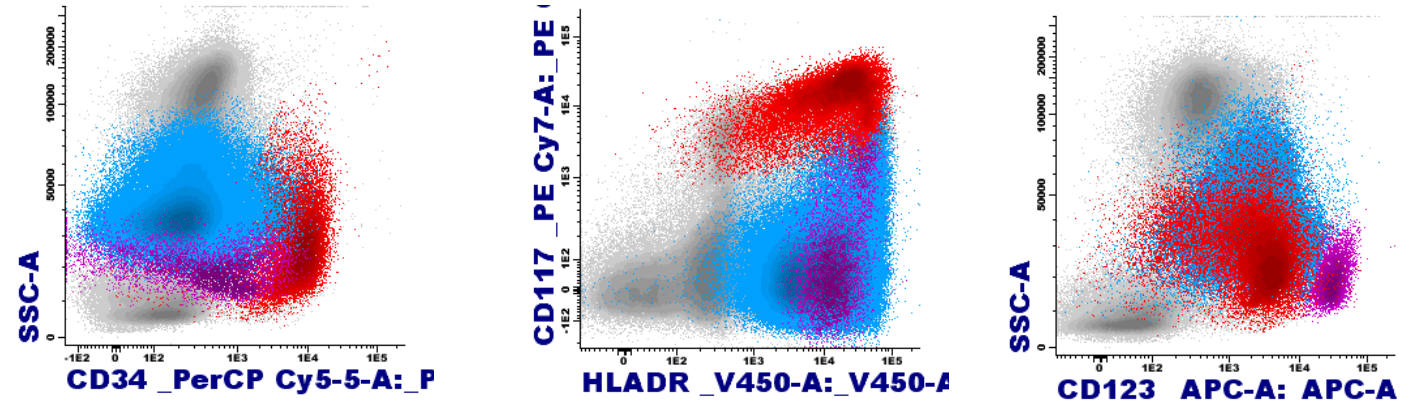


Xiao W, et al. Plasmacytoid dendritic cell expansion defines a distinct subset of RUNX1-mutated acute myeloid leukemia. *Blood*. 2021

pDC-LMMC

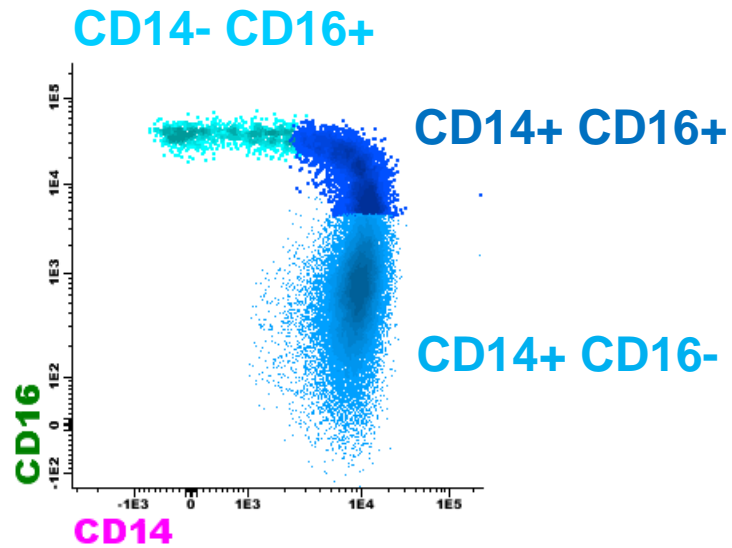


pDC-AML RUNX1

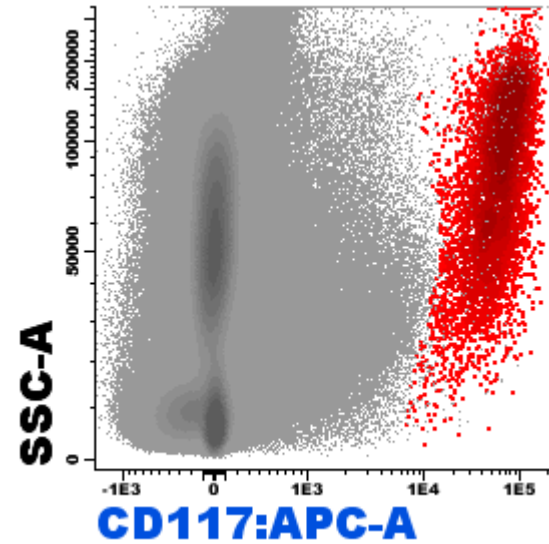


Immune system and MRD

Other populations

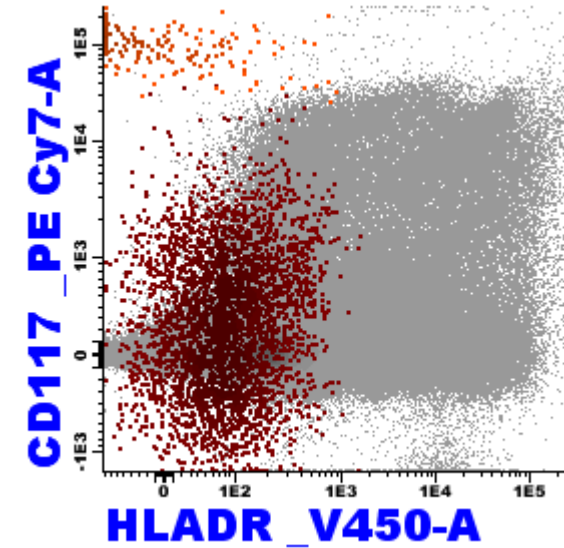


CD117+ 0,5%
Neutrophils 53%



Mast cells 0,14%

Basophils



Enfermedad residual medible y seguimiento inmunológico en LMA: ¿en dónde nos encontramos?

MRD.....

Citología/genética/molecular

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Eusebio Martín
M^a Teresa Vargas
Elena Soria
Estrella Carrillo
Javier Rojas

Unidad clínica pediátrica/adultos

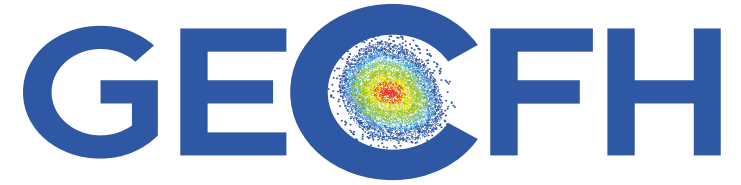
Águeda Molinos Quintana
Inma Pérez Soto
José María Pérez-Hurtado
Eduardo Rodríguez Arbolí
Cristina Blázquez
Francisco Martines
José González
Isabel Montero
José Falantes
Javi Delgado
Nancy Rodríguez
Fátima de la Cruz

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