XXV JORNADAS CIENTÍFICAS DE ANÁLISES CLÍNICAS E GENÉTICA HUMANA

Pesquisa da Doença Residual Mensurável em leucemias aguadas por citometria de fluxo

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Porto, 7 de fevereiro de 2026

Disclosures

- I have no COI to declare related to the content of this presentation

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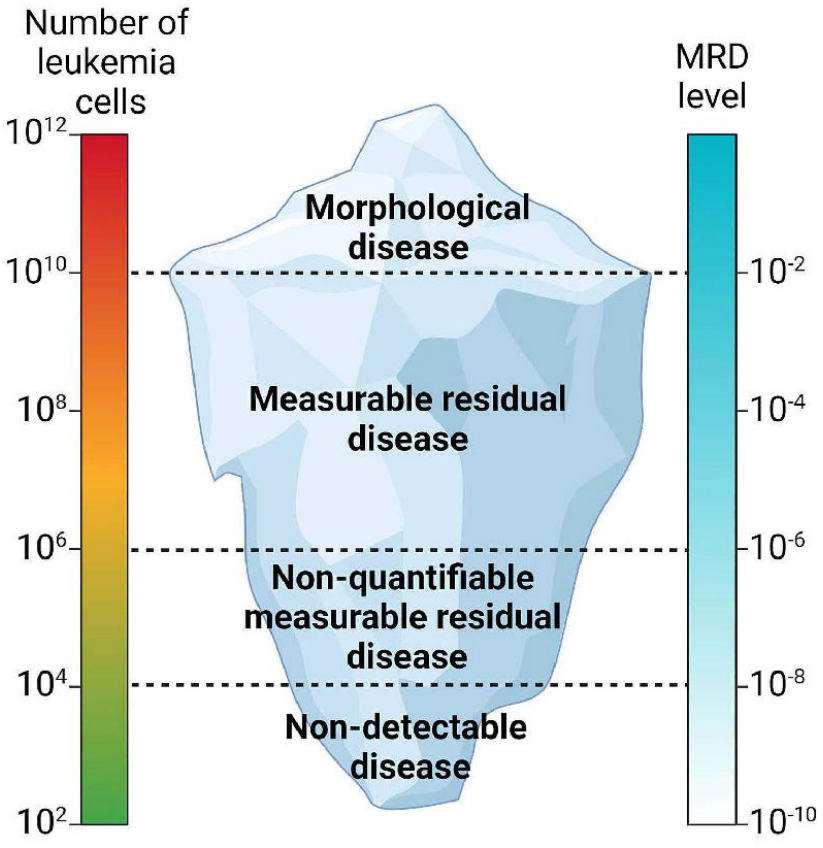
Why we investigate MRD is Acute Leukemias?

- Morphologic ***threshold*** for ***CR is 5%***
  - Many leukemic cells may remain
- MRD is ***the strongest independent predictor*** of relapse and survival *(since early 90's)*
- MRD is increasingly being used as ***Response Biomarker***, employed in ***Risk Stratification*** and ***Therapeutic Decision Making → Personalized Medicine***
- Actual ***clinical value depends*** on:
  - time point
  - Technique
  - Leukemia subtype



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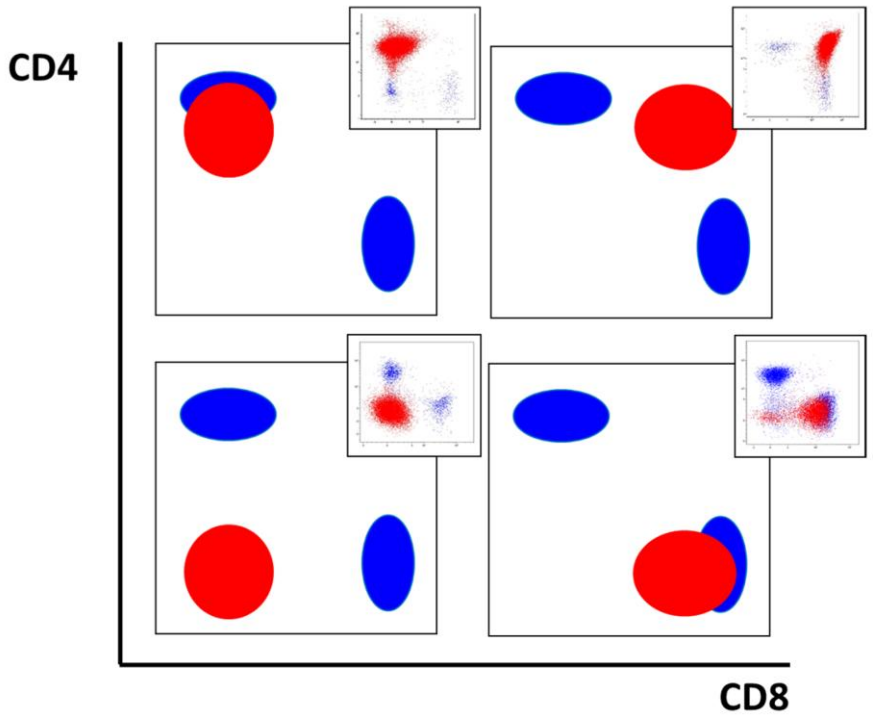
Kruse et al, Int J Mol Sci 2020; modified from Saygin et al, Haematologica; Cloos et al, Blood 2025

Available MRD assessment methodologies in Acute Leukemias

Method	MFC/NGF	RT-qPCR and dd-PCR for fusion genes	ASO-qPCR for IG/TR genes	NGS
Sensitivity	10^{-4} / 10^{-6}	10^{-5} – 10^{-6}	10^{-4} – 10^{-5}	10^{-6}
Applicability	>90%	40-50%	90-95%	>90%
Advantages	Results in hours Relatively inexpensive No need of Dx sample Detects therapy targets Assess sample quality Highly applicable Widely available	Specific Use of standardized primers Widely available Relatively inexpensive	Highly applicable Standardized	High applicability Clone-unbiased
Limitations	Requires expertise Use fresh samples Sensitive to immunophenotypic switches Partially standardized	Low applicability Time –consuming Labor-intensive Results in days	Time-consuming Expensive Requires pre-treatment samples	Expensive Long turn-around time Requires pre-treatment sample Requires high level of expertise Not standardize Affected by CHIP Occurrence of sequencing errors

Zhao et al, Ann of Hematol 2024; Gang et al, Cells 2025, Luo et al, Acta Haematol 2026

Strategies to investigate MRD using MFC



Different from normal (DfN) in T-ALL

- Independent from the diagnostic sample
- Compensates for immunophenotypic shifts

- Requires higher expertise
- Limited sensitivity

Semchenkova et al, Int J Mol Sci 2025

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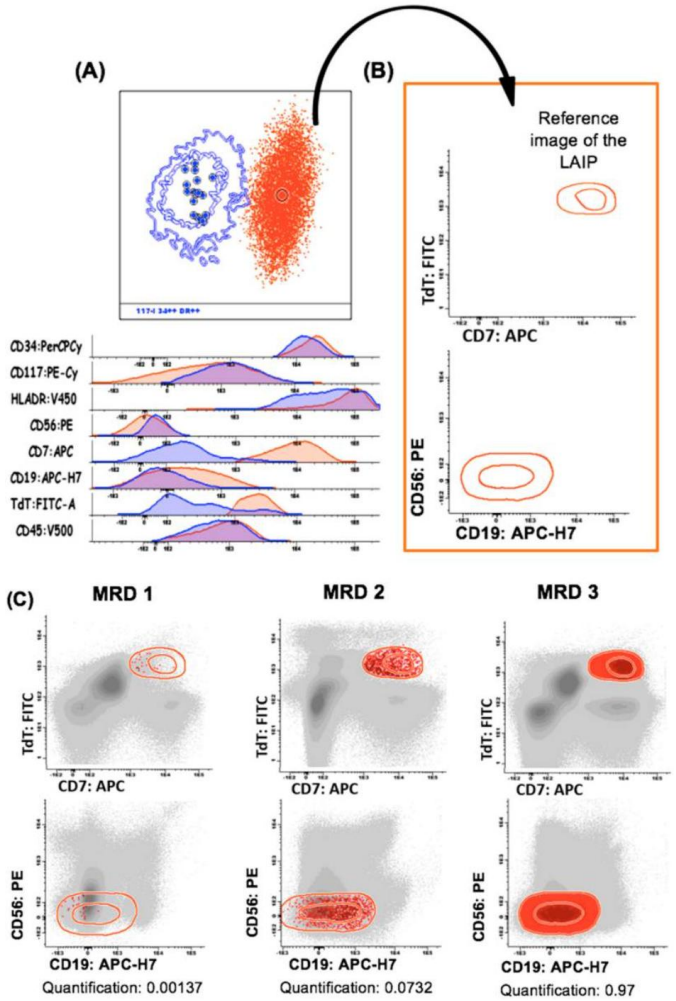
Strategies to investigate MRD using MFC



Leukemia associated immunophenotypes (LAIPs) in AML

- Simpler
- Higher sensitivity

- Subject to immunophenotypic switch and post-treatment antigen modulation

Figure from Piñero et al, Cancers 2022

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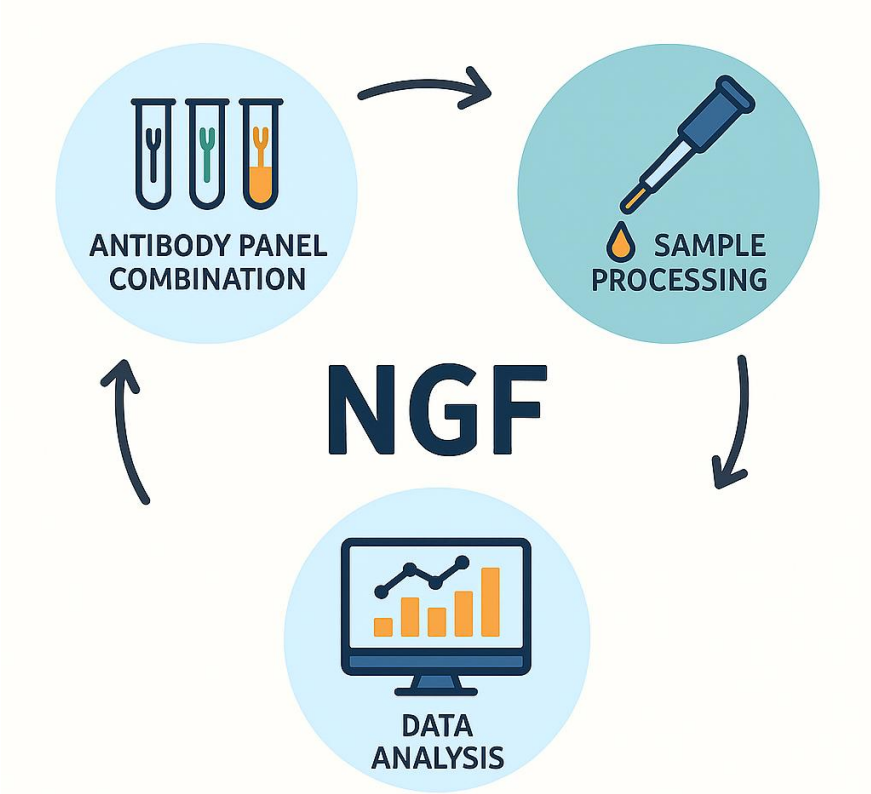
What is EuroFlow Next Generation Flow?

EuroFlow

- Advanced highly standardized flow cytometry strategy
- Prospectively developed to address conventional flow limitations
- Aims at higher efficiency and improved clinical impact



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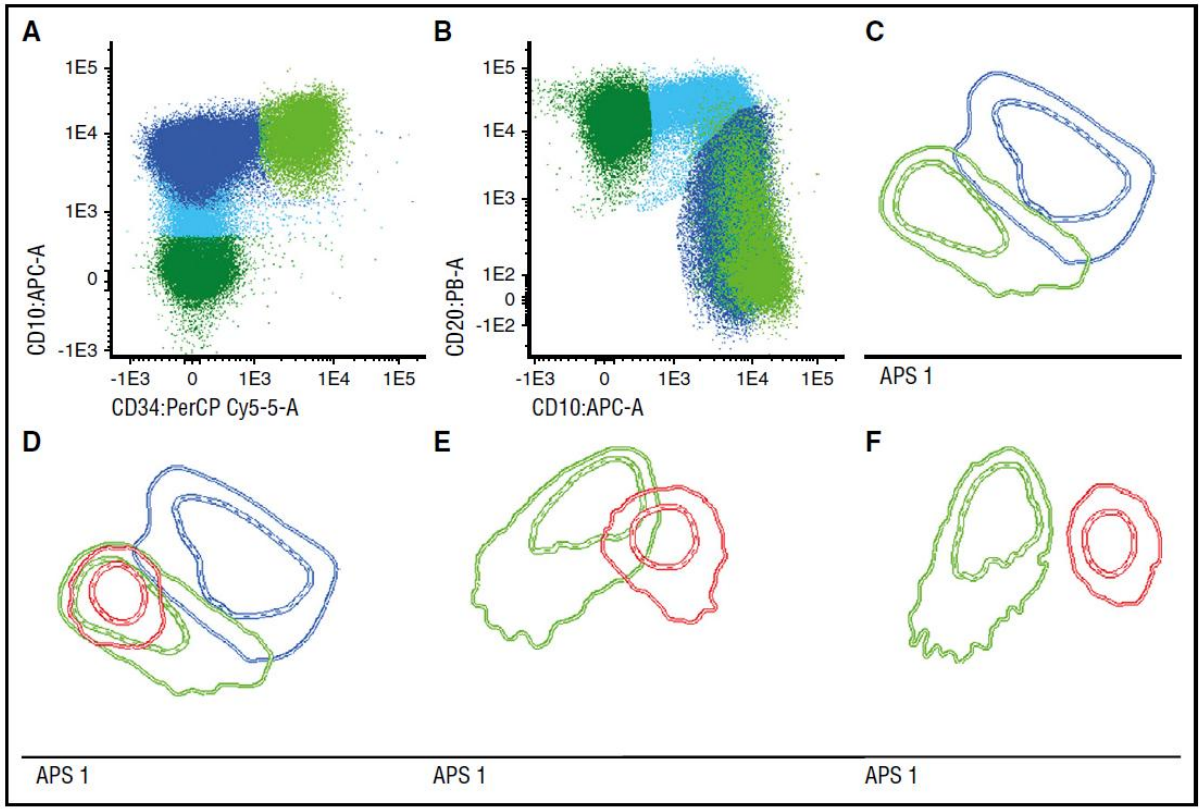
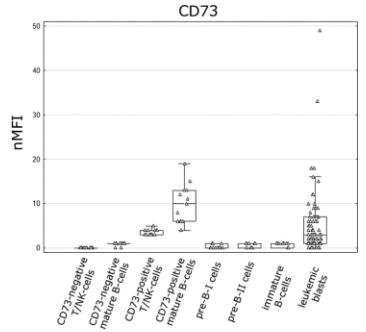
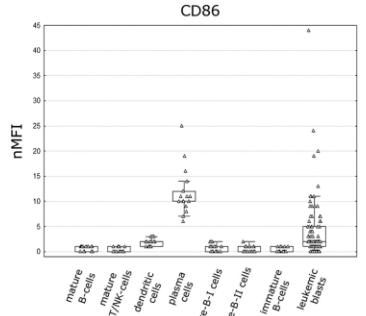
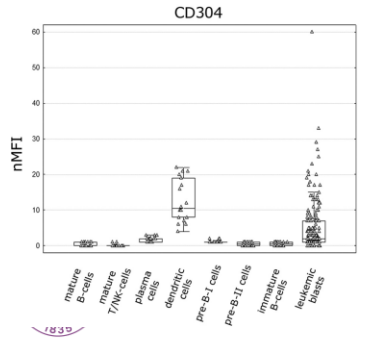
EuroFlow NGF for BCP-ALL MRD

- **Panel design:**
  - DfN^{+} + LAIPs









Theunissen et al, Blood 2017; Sedek et al, J Immunol Methods 2019

EuroFlow NGF for BCP-ALL MRD

- **Panel design:**
  - DfN^{+} + LAIPs
  - Panel optimization

Table 2. Development and design of the EuroFlow BCP-ALL MRD panel

Phase	Violet laser		Blue laser				Red laser	
Initial*	PB	PO	FITC	PE	PerCP Cy5.5	PECy7	APC	APC H7
	CD20	CD45	CD58	CD66c	CD34	CD19	CD10	CD38
	CD9	CD45	TdT	CD13	CD34	CD19	CD22	CD24
	CD21	CD45	CD15/CD65	NG2	CD34	CD19	CD123	CD81
1	PB	PO	FITC	PE	PerCP Cy5.5	PECy7	APC	APC H7
	CD20	CD45	CD58	CD66c	CD34	CD19	CD10	CD38
	CD20	CD45	TdT	SmIgK/L	CD34	CD19	CD10	CD24
	CD20	CD45	CD9	CD123	CD34	CD19	CD10	CD81
2	PB	PO	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
	CD20	CD45	CD9	**CD22**	CD34	CD19	CD10	CD38
	CD20	CD45	**CD123**	CD66c	CD34	CD19	CD10	CD24
3	PB	PO	FITC	PE	PerCP Cy5.5	PE Cy7	APC	**APC C750**
	CD20	CD45	**CD9 (ML13)**	**CD66c/CD123**	CD34	CD19	CD10	CD38
	CD20	CD45	**CD58**	**CD22**	CD34	CD19	CD10	**CD81**
4	PB	PO	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC C750
	CD20	CD45	**CD81**	CD66c/CD123	CD34	CD19	CD10	CD38
	CD20	CD45	**CD81**	**CD73/CD304**	CD34	CD19	CD10	CD38

Markers that were changed as compared with the previous panel are marked in boldface.

*Only tubes 1, 3, and 4 of the EuroFlow BCP-ALL diagnosis panel were evaluated, because most markers in tube 2 (Cyμ, SmIgK, SmIgL, SmIgM) were not expected to contribute to separation of normal and malignant BCP cells.

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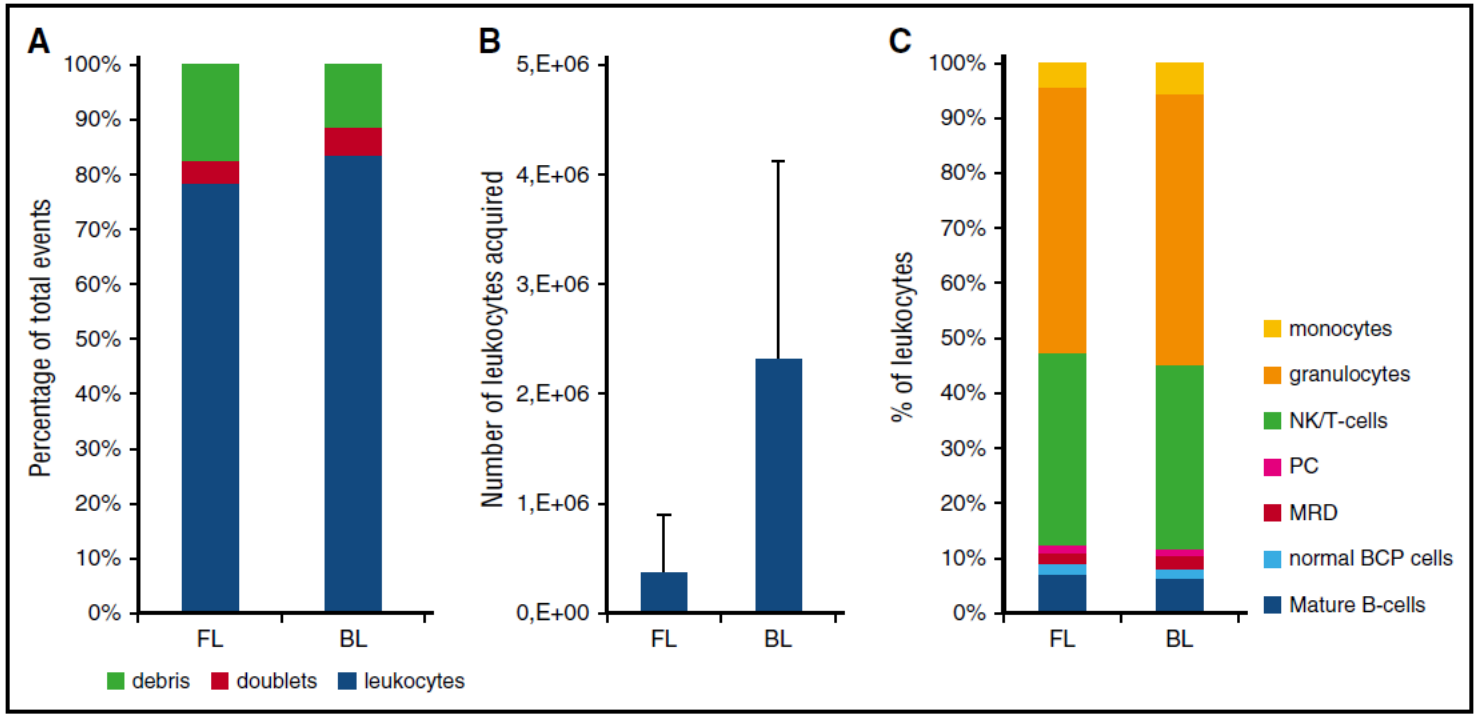
Theunissen et al, Blood 2017

EuroFlow NGF for BCP-ALL MRD

- **Panel design:**
  - DfN^{+} + LAIPs
  - Panel optimization
- **Sample Processing protocol**
  - Acquisition of 5 x10^{6} per tube
  - Sensitivity of 10^{-5}



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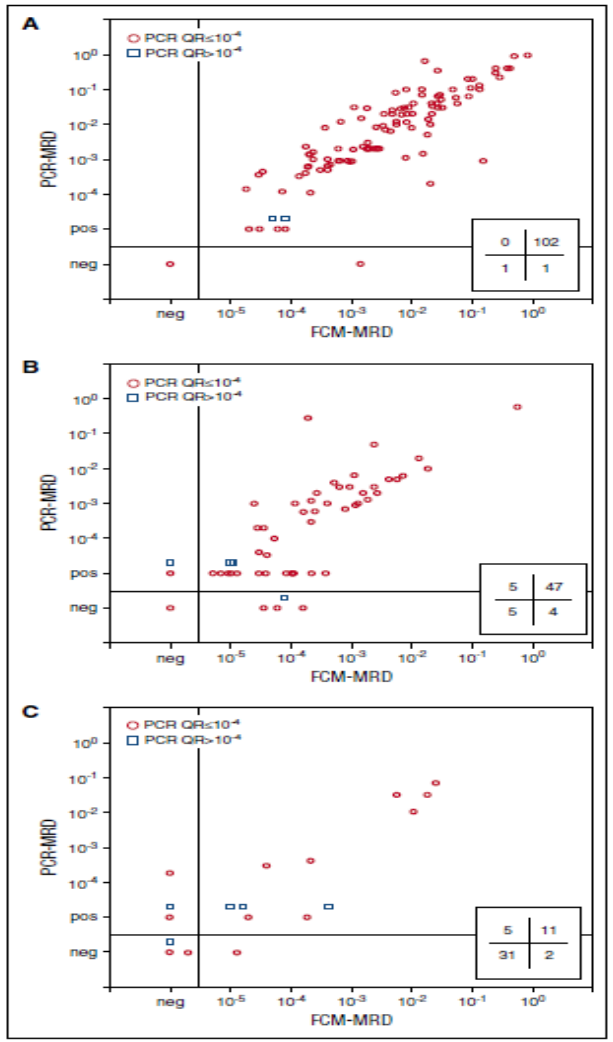
Theunissen et al, Blood 2017

EuroFlow NGF for BCP-ALL MRD

- **Panel design:**
  - DfN^{+} + LAIPs
  - Panel optimization
- **Sample Processing protocol**
  - Acquisition of 5 x10^{6}
  - Sensitivity of 10^{-5}
- **Data analysis and evaluation of results**
  - Excellent correlation with PCR



Theunissen et al, Blood 2017

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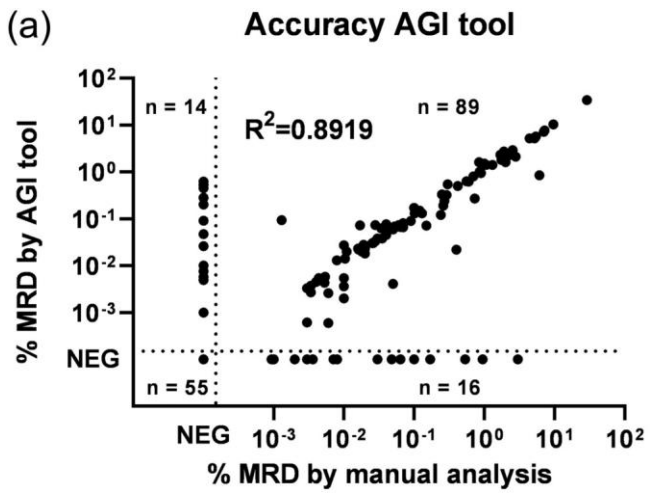
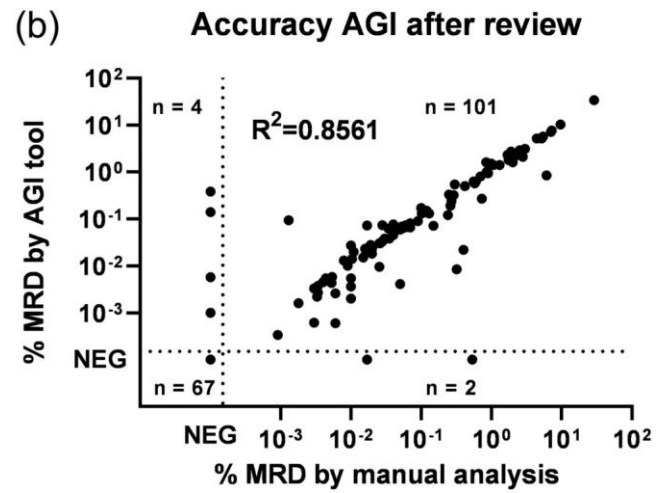
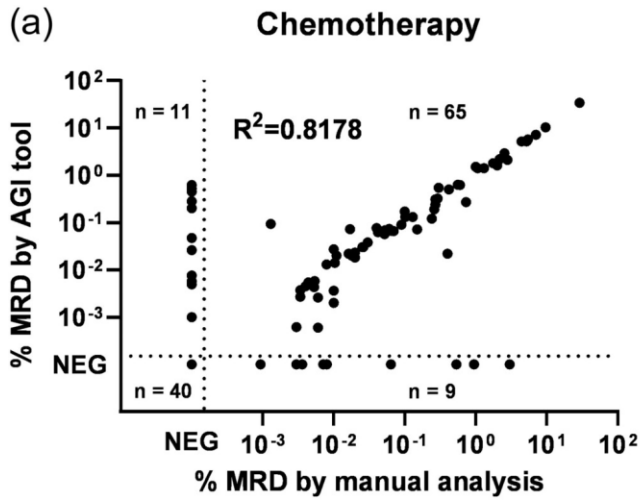
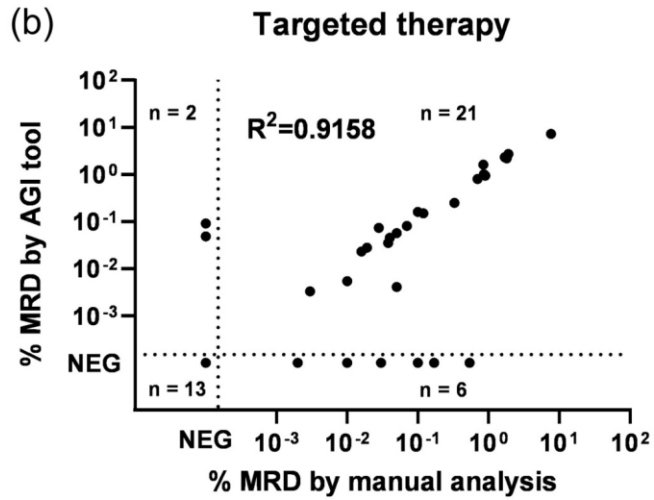
EuroFlow NGF for BCP-ALL MRD

- **Panel design:**
  - DfN^{+} + LAIPs
  - Panel optimization
- **Sample Processing protocol**
  - Acquisition of 5 x10^{6}
  - Sensitivity of 10^{-5}
- **Data analysis and evaluation of results**
  - Excellent correlation with PCR
  - Database assisted analysis





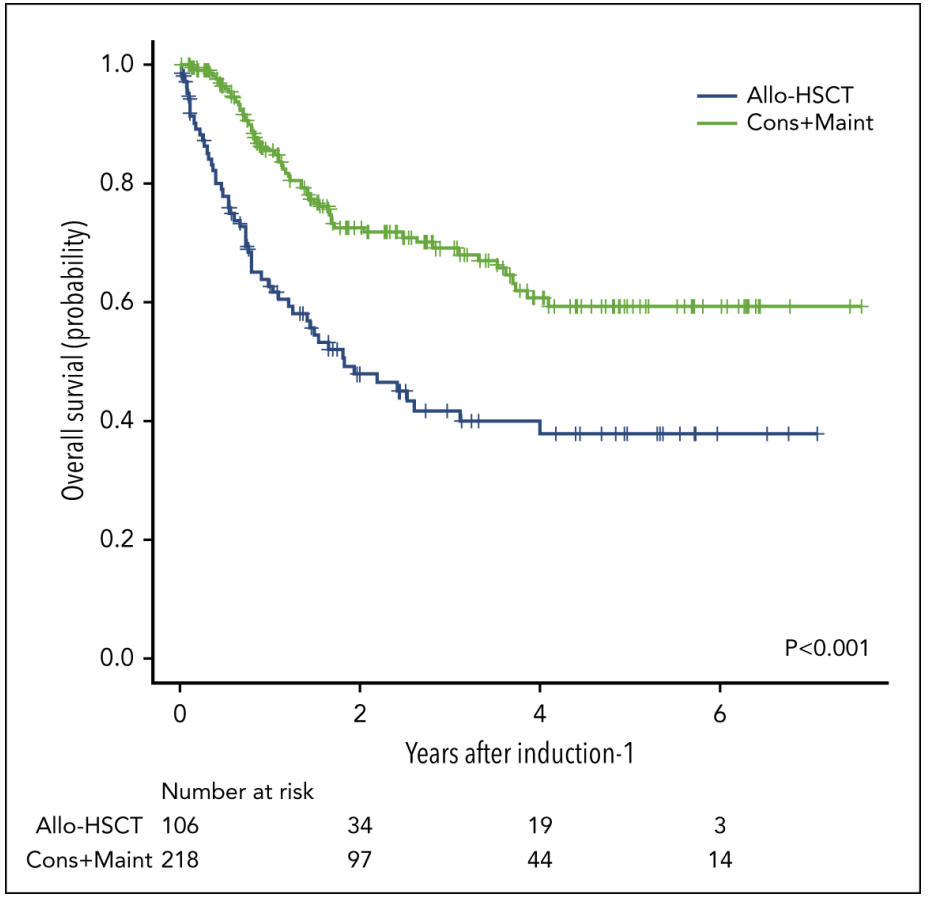




Theunissen et al, Blood 2017

EuroFlow NGF for BCP-ALL MRD

- **Clinical validation within PETHEMA:**
  - Adults with HR, Ph^- ALL, and good MRD clearance after induction and early consolidation show promising outcomes without allo-HSCT.



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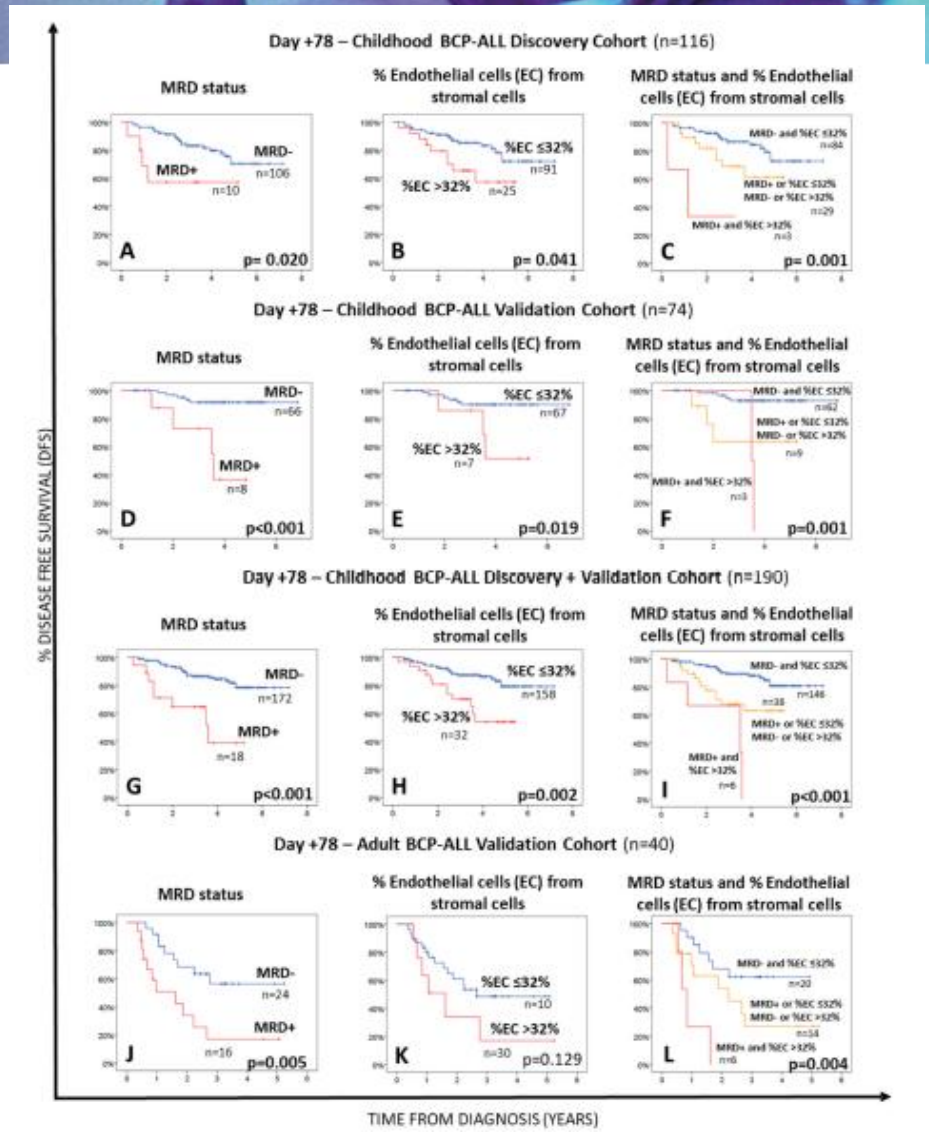
Ribera et al, Blood 2021

EuroFlow NGF for BCP-ALL MRD

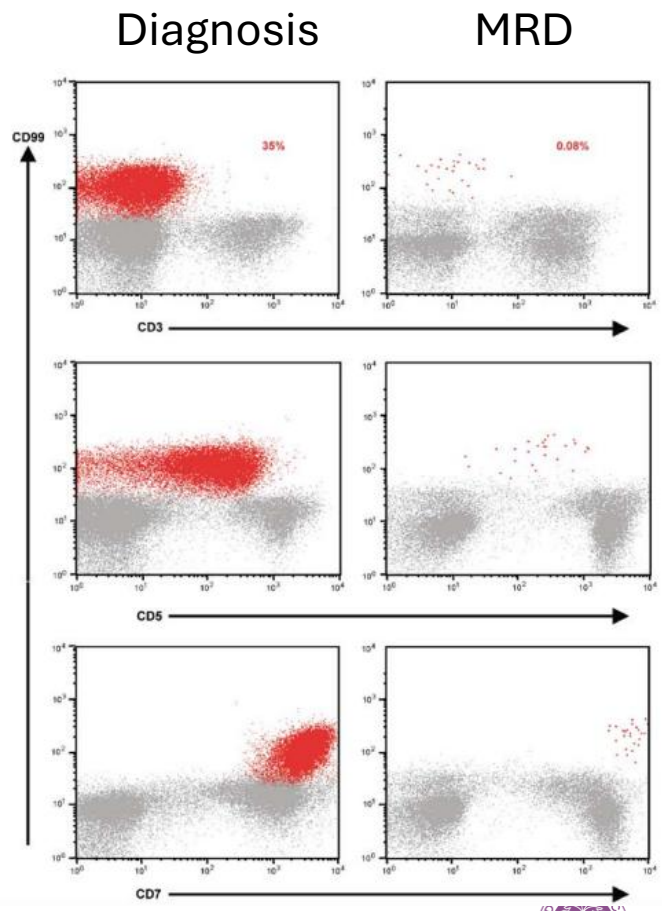
- **Complemental information:**
  - increase of endothelial cells (>32%) at day +78 of therapy predicts significantly shorter disease-free survival, regardless of MRD status.



Oliveira et al, Cancers 20222

Flow MRD-based in T-ALL

- **MRD in T ALL:**
  - **Good overall survival, but relapses has dismal prognosis**
  - **Absence of clonal rearrangements in ~25%**
  - **Limited applicability of IG/TR ASO-qPCR**



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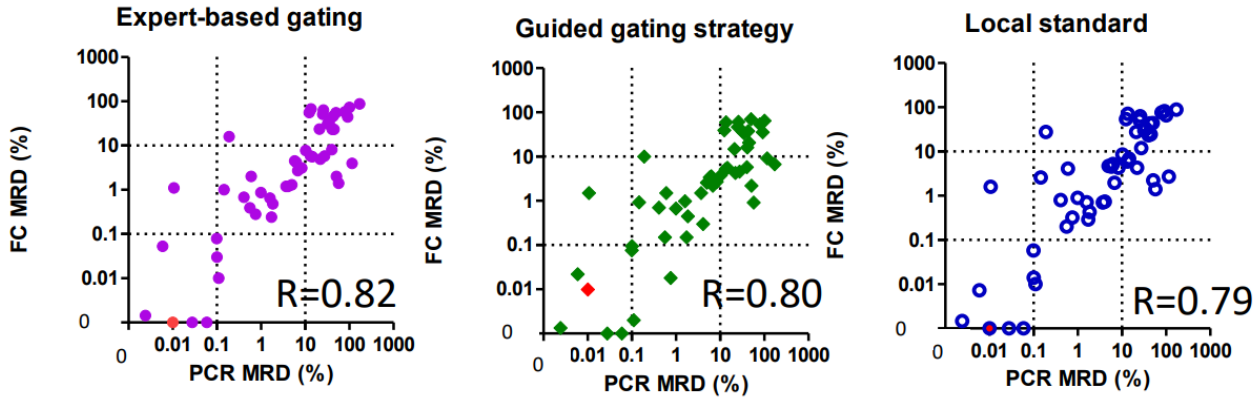
Vega-García et al, Front Pediatr 2021; Reiterová et al, Clin Chem Lab Med 2025

Flow MRD-based in T-ALL

- **AIEOP-BMF recommendations:**
  - Proposed two 8-color and one 12-color tubes
  - Stablished and tested two gating strategies (expert vs guided)
  - Acquisition of ~500.000 nucleated cells in MRD samples → 10^{-4}
  - Good correlation with molecular MRD
  - Questions the value of cyCD3



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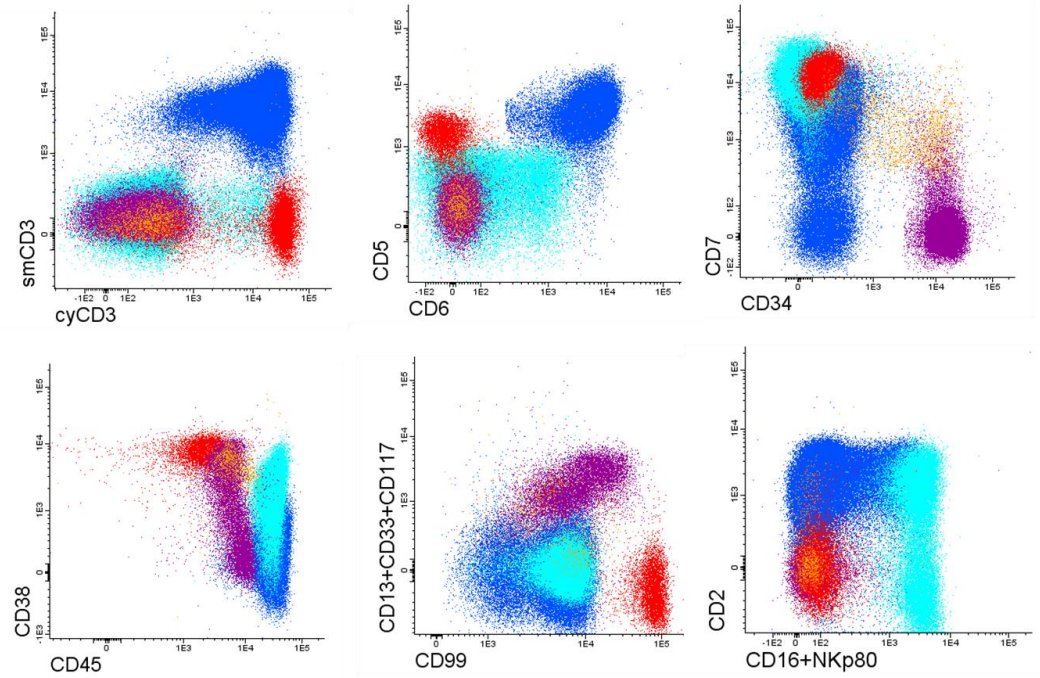
Reiterová et al, Clin Chem Lab Med 2025

EuroFlow MRD in T-ALL

EuroFlow

- **EuroFlow approach:**
  - **Two-tube 12-colors Next Generation Flow approach**
  - **LOD ≤ 10^{-5} (0,001%)**
  - **Excellent correlation with PCR reference method**



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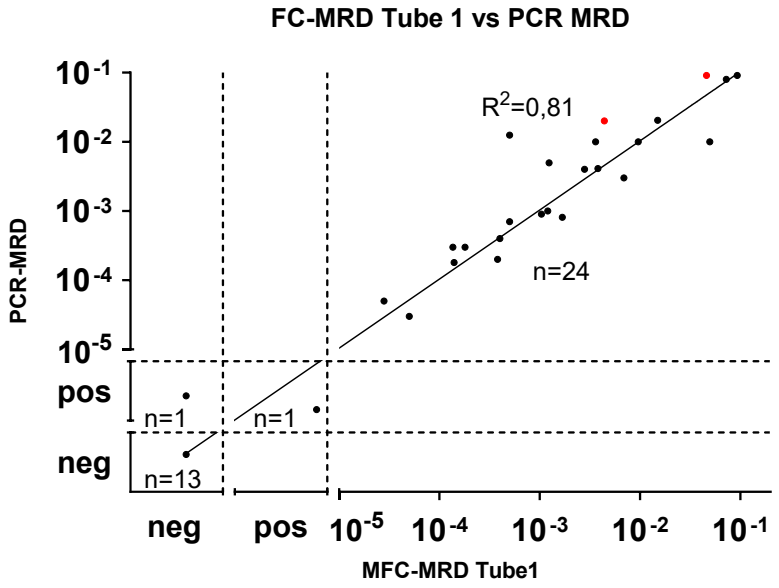
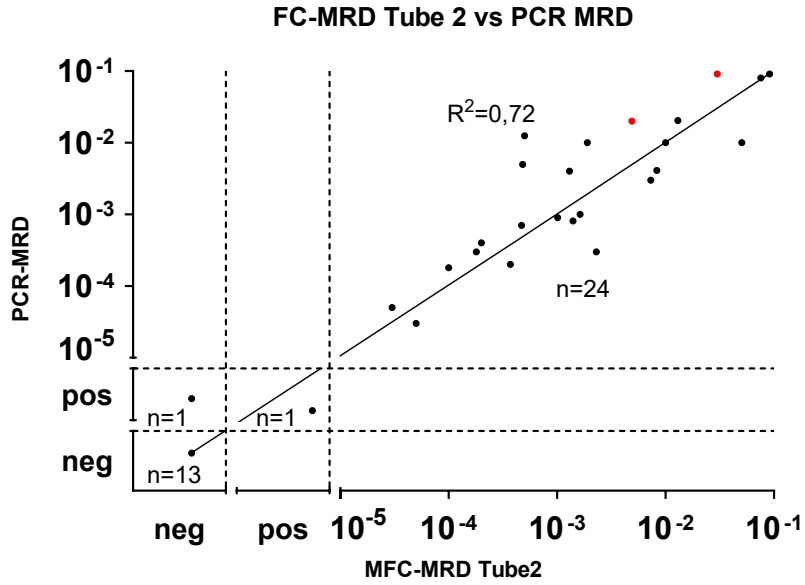
Responsible scientists L Sedek. Sedek et al, Blood 2024

EuroFlow MRD in T-ALL

EuroFlow NGF for T-ALL MRD vs PCR-based MRD





	NGF Positive	NGF Negative	Total
PCR Positive	25 / 39 (64.1%)	1 / 39 (2.6%)	26 / 39 (66.7%)
PCR Negative	0 / 39 (0%)	13 / 39 (33.3%)	13 / 39 (33.3%)
Total	25 / 29 (64.1%)	14 / 39 (35.9%)	39 / 39 (100%)

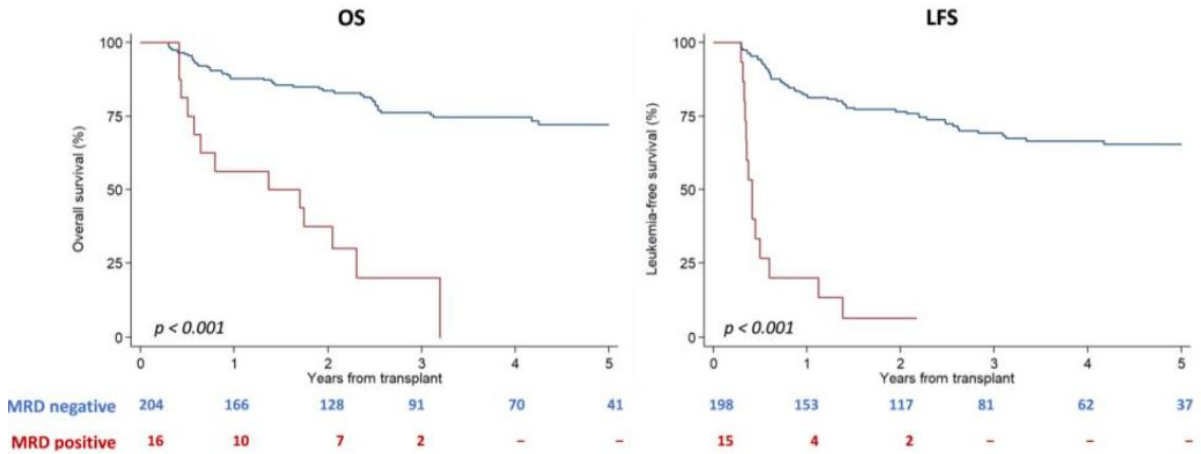
97.4% concordance

Responsible scientists L Sedek. Sedek et al, Blood 2024

Flow MRD-based in AML
(recommendations from ELN-DAVID MRD working party)

- **Applicability ~90%** vs 50%-60% by highly standardize molecular methods.
- **Strong prognostic impact**
  - After intensive chemotherapy
  - Peri / post -transplant
- Some-times **stronger predictor** (e.g., *CEBPA* b*ZIP* AML) **others → complemental** (e.g., NPM1)
- Search for **Leukemic Stem Cells** (LSC)
  - Prognostically relevant
  - Enhanced sensitivity

Impact of MRD on day +100 after transplantation at 2 years (MRD ≥ 0.1 on day +100).



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Cloos et al, Blood 2025; Caballero-Velázquez et al, Cancers 2023

Flow MRD-based in AML
(recommendations from ELN-DAVID MRD working party)

- **Operational definitions:**
- **Analyte:** % of $LAIP^+$ or DfN^+ from BM $CD45^+$ cells
- **Sensitivity:** 10^{-4} (LOD 0.004% / LOQ 0.01%)
  - Analyze ≥ 500 000 $CD45^+$ events
- **Sample:**
  - Bone Marrow
- **When:**
  - After 2 cycles of intensive chemotherapy / pre- alloHCT
  - End of treatment
  - Follow-up / surveillance
    - Every 3 months for a year, and then molecular

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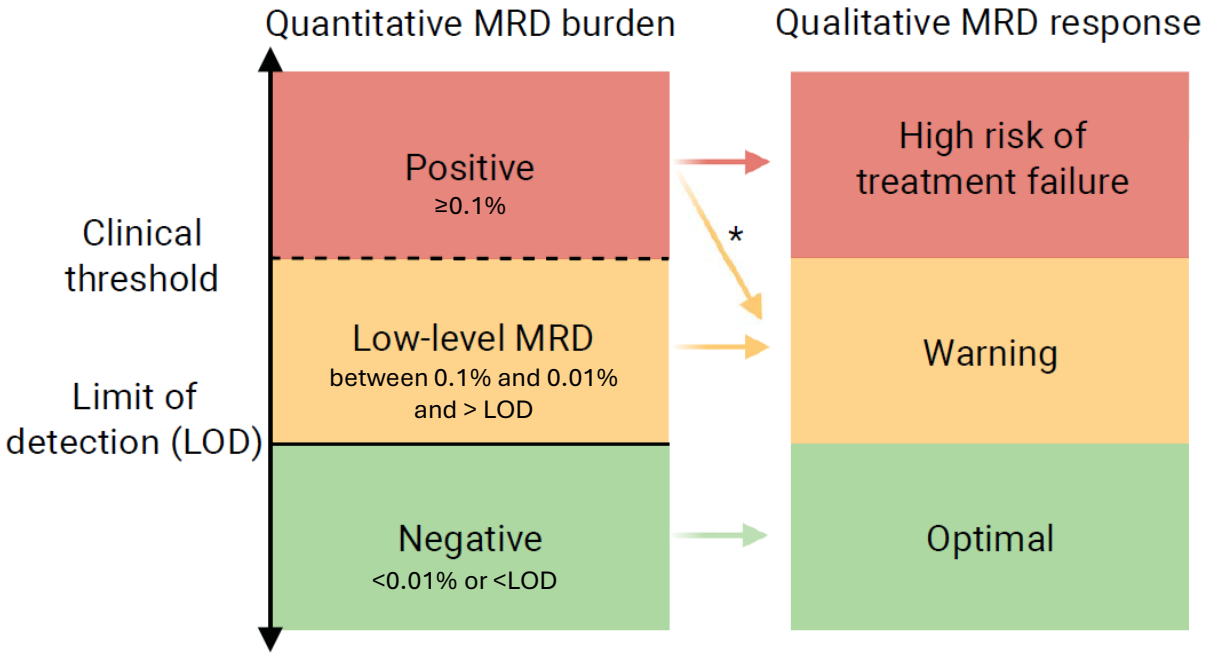
Cloos et al, Blood 2025

Flow MRD-based in AML
(recommendations from ELN-DAVID MRD working party)

Response categories:



- **FLOW-MRD Clinical impact:**
  - **MRD-positive or relapse:**
    - Urgent therapeutic action
  - **MRD-LL:**
    - Continue as planned (consolidation/allHCT) but intensify monitoring (e.g., monthly)
  - **MRD-negative:**
    - Continue according to baseline risk definition
    - For intermediate risk- may consider to defer allHCT

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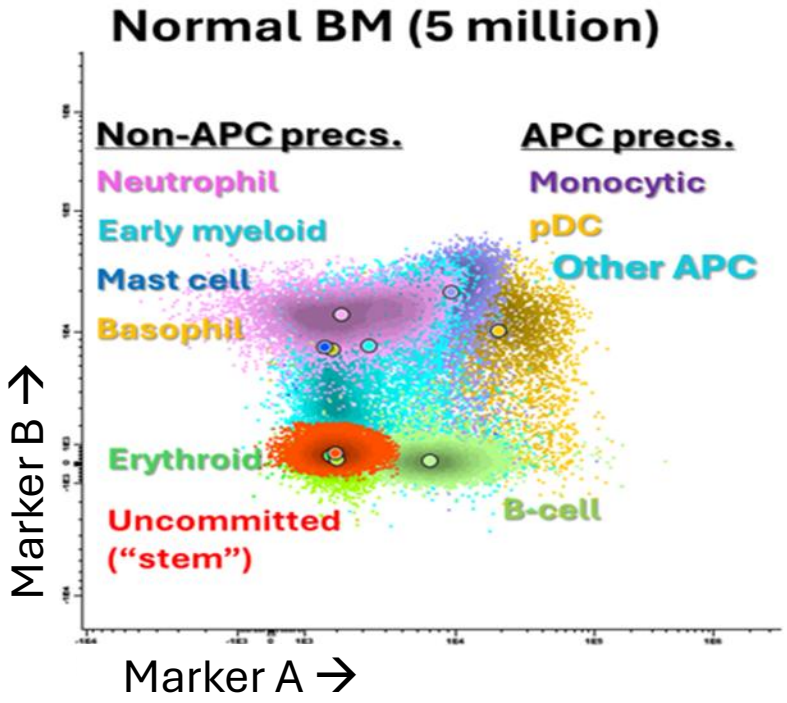
Cloos et al, Blood 2025

Flow MRD-based in AML

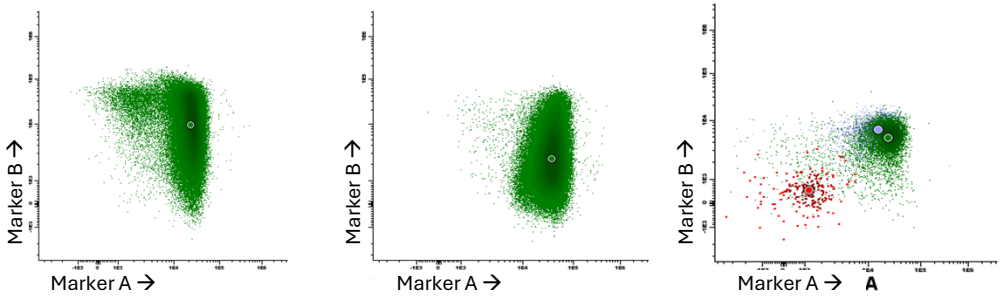
What's new? The EuroFlow strategy

EuroFlow

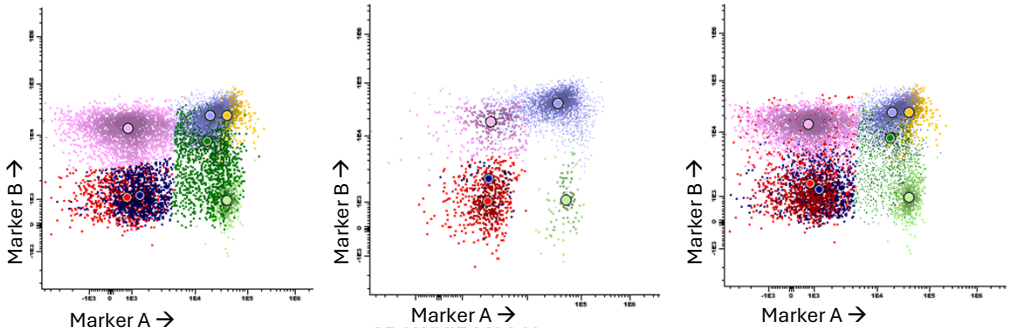


NPM1-MRD^{neg} AML with altered marker expression show early relapse

BM CD34+ HPC distribution of relapsing cases



BM CD34+ HPC distribution of non relapsing cases



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Responsible scientists S. Matarraz & A. Orfao

In summary

- **MRD is central** to **risk assessment and** a **key biomarker**
- **Flow MRD** offers **broad applicability**, **speed** and **cost-effectiveness**
- **Flow** is **independent** of the **underlaying molecular abnormality**
- **High sensitive and standardized assay rival the sensitivity of molecular methods**
- A **careful definition** of **time point**, a standardized **methodology**, **analysis and reporting** are required to **exploit** the **full potential** of **MRD evaluation**

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