

# Towards Further Harmonization on Technical Aspects of Flow Cytometry-based MRD Quantification in AML: Experience of the ELN MRD Working Party

Jesse Tettero, 22th September 2022

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ESCCA  
European Society  
for Clinical Cell Analysis



**No disclosures**

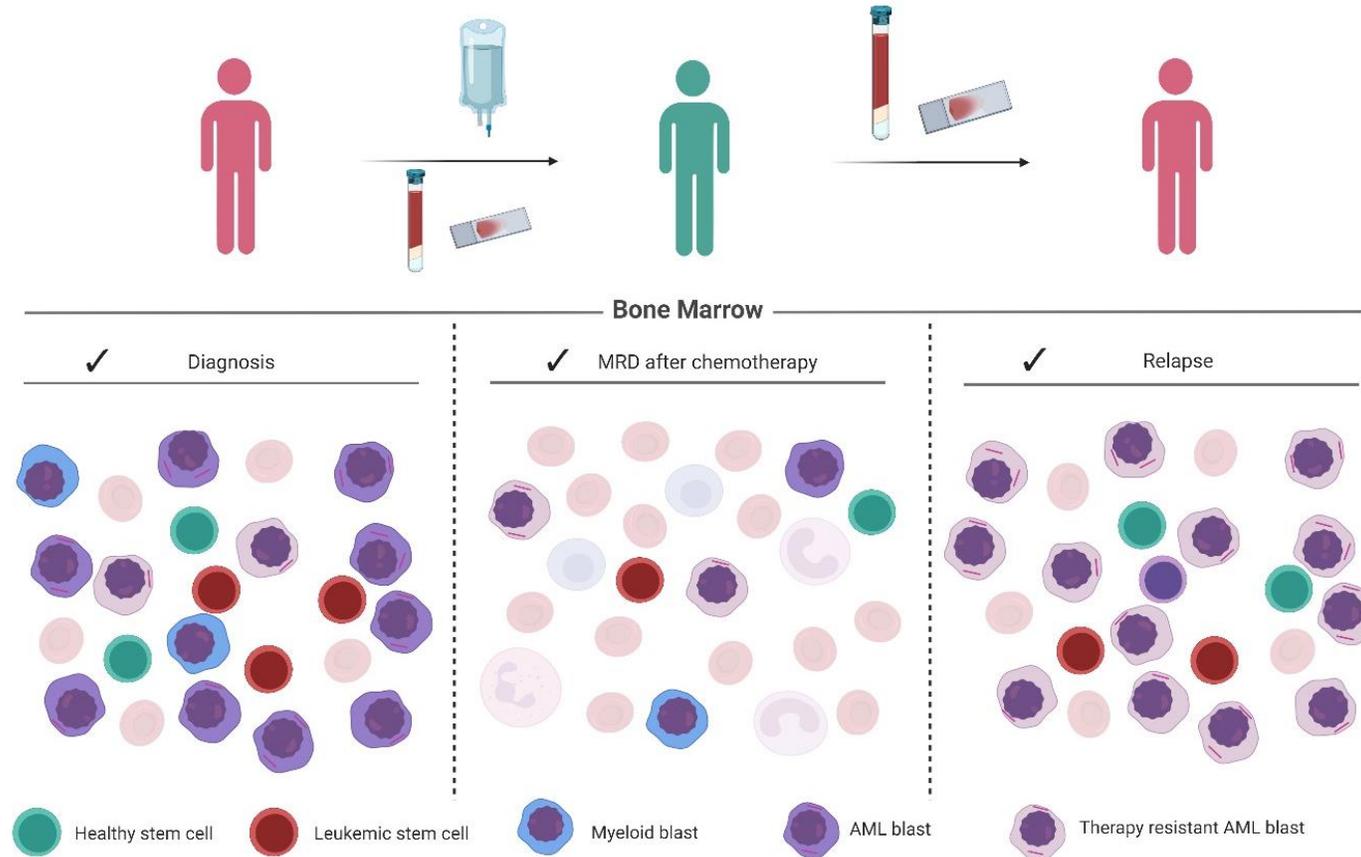


# Overview





# Measurable residual disease in AML





# MRD techniques

| Method                                  | Availability | Sensitivity                         |
|---|--------------|-------------------------------------|
| Morphology                              | 100%         | $5 \times 10^{-2}$ (5%)             |
| Cytogenetics                            | 70%          | $1-5 \times 10^{-2}$                |
| FISH                                    | 40%          | $1 \times 10^{-2}$                  |
| (Real-time) RT-PCR*                     | 20-40%       | $1 \times 10^{-3}-1 \times 10^{-6}$ |
| Next generation sequencing*             | 80-90%       | $1 \times 10^{-3}-1 \times 10^{-4}$ |
| Flow cytometry  <br>(Immunophenotyping) | 80-90%       | $1 \times 10^{-4}-1 \times 10^{-5}$ |

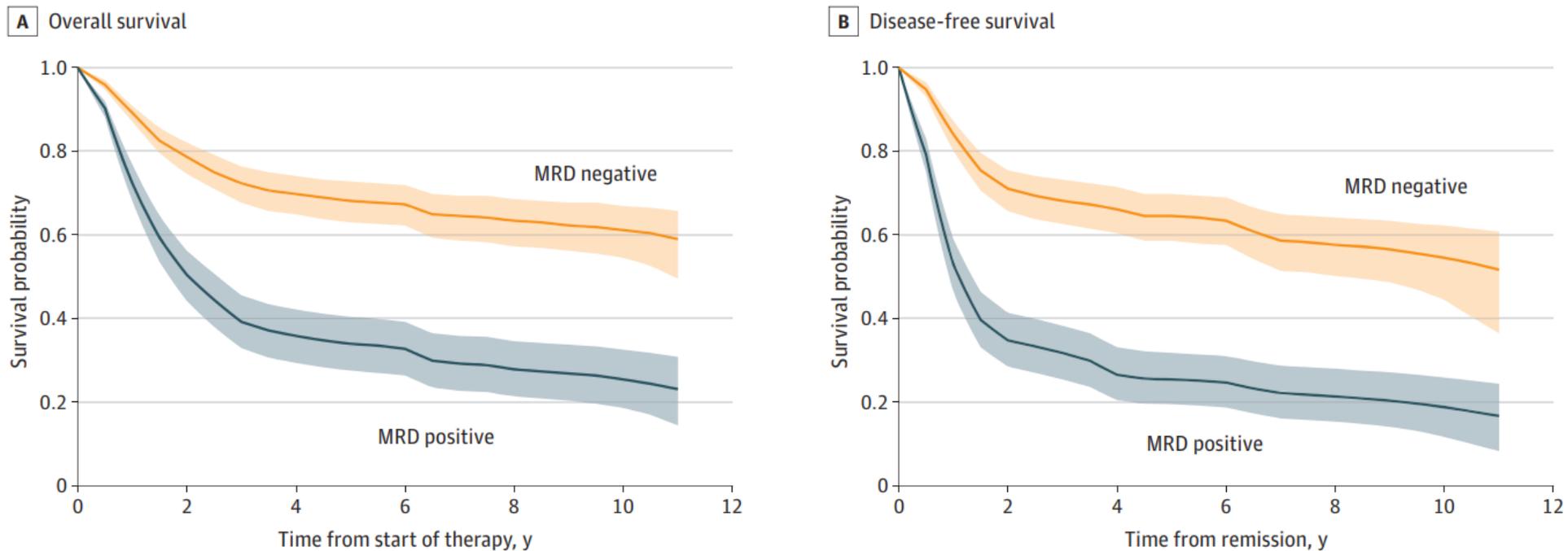
*FISH, fluorescent in situ hybridization; RT-PCR, reverse transcription-polymerase chain reaction.*

*\*Sensitivity dependent on gene.*



# Prognostic relevance of MRD

Figure 2. Estimated Survival Curves, Stratified by Measurable Residual Disease (MRD) Status



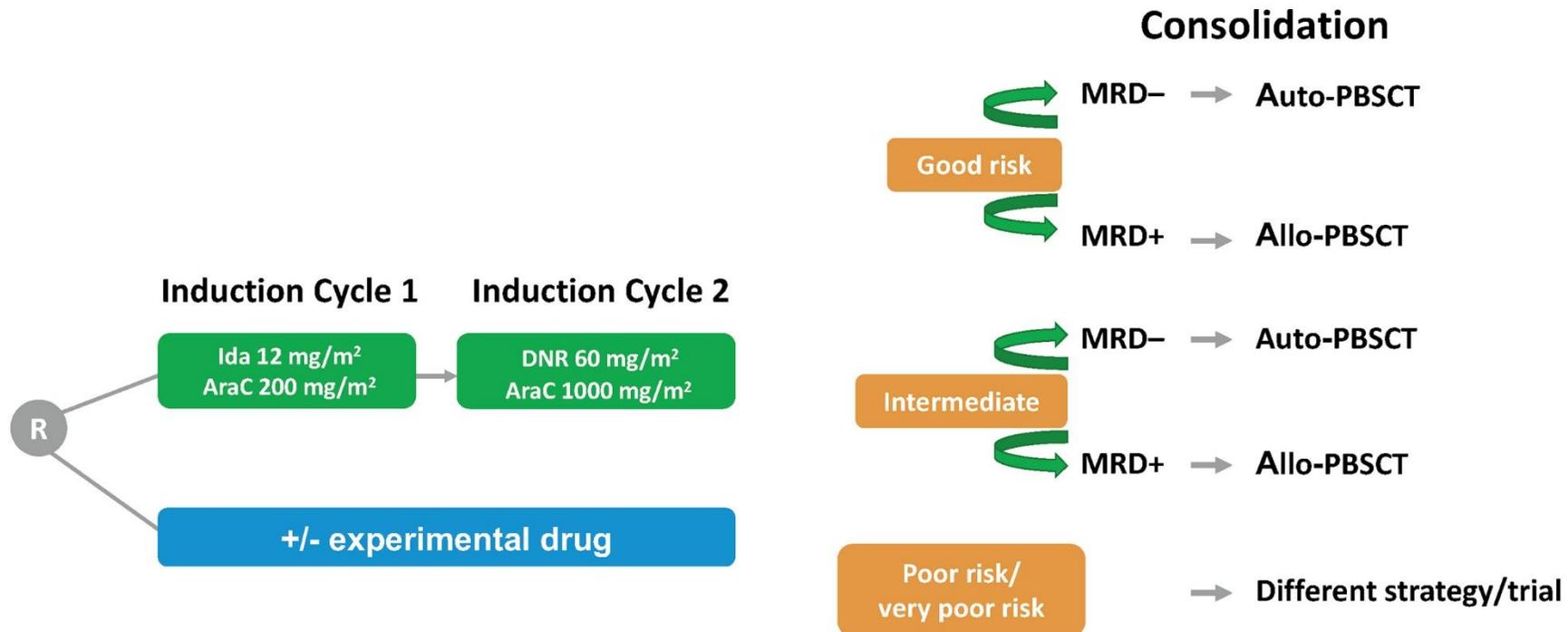
## Meta-analysis:

- 81 publications
- 64% reduction in risk of death for MRD-negative patients
- Combination of MRD techniques → Multicolor Flow cytometry (MFC)



# Other use of MRD in AML

## MRD-guided treatment in HOVON trials



Allo-PBSCT, allogeneic peripheral blood stem cell transplantation; AraC, cytarabine; auto-PBSCT, autologous peripheral blood stem cell transplantation; DNR, daunorubicin; HOVON, Haemato Oncology Foundation for Adults in the Netherlands; Ida, idarubicin; MRD, minimal residual disease



# How to ensure harmonised flow data

jove Journal of Visualized Experiments

www.jove.com

Video Article

## Comprehensive Protocol to Sample and Process Bone Marrow for Measuring Measurable Residual Disease and Leukemic Stem Cells in Acute Myeloid Leukemia

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REVIEW ARTICLE | NOVEMBER 1, 2021

## 2021 Update Measurable Residual Disease in Acute Myeloid Leukemia: European LeukemiaNet Working Party Consensus Document

Michael Heuser, Sylvie D Freeman, Gert J Ossenkoppele, Francesco Buccisano, Christopher S Hourigan, Lok Lam Ngai, Jesse Marc Tettero, Costa Bachas, Constance Baer, Marie C Béné, Veit Buecklein, Anna Czyz, Barbara Denys, Richard Dillon, Michaela Feuring-Buske, Monica L Guzman, Torsten Haferlach, Lina Han, Julia K Herzig, Jeffrey L Jorgensen, Wolfgang Kern, Marina Y. Konopleva, Francis Lacombe, Marta Libura, Agata Majchrzak, Luca Maurillo, Yishai Ofran, Jan Philippé, Adriana Plesa, Claude Preudhomme, Farhad Ravandi, Christophe Roumier, Marion Subklewe, Felicitas Thol, Arjan A van de Loosdrecht, Bert A. van der Reijden, Adriano Venditti, Agnieszka Wierzbowska, Peter J.M. Valk, Brent L. Wood, Roland B Walter, Christian Thiede, Konstanze Döhner, Gail J. Roboz, Jacqueline Cloos ✉



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Article history

## Technical Aspects of Flow Cytometry-based Measurable Residual Disease Quantification in Acute Myeloid Leukemia: Experience of the European LeukemiaNet MRD Working Party

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Cloos et al, 2018. *J Vis Exp*,  
Tettero et al, 2021. *Hemasphere*,  
Heuser et al, 2021. *Blood*.

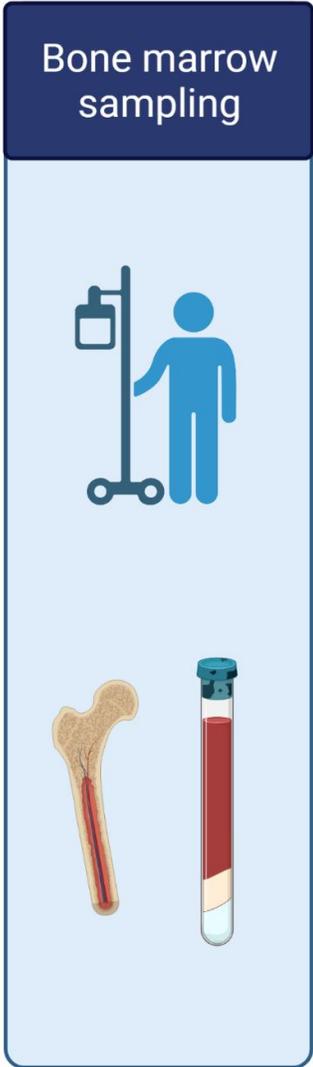


# Standardisation/Harmonisation and Qualification

Questionnaire → live discussions → Delphi poll

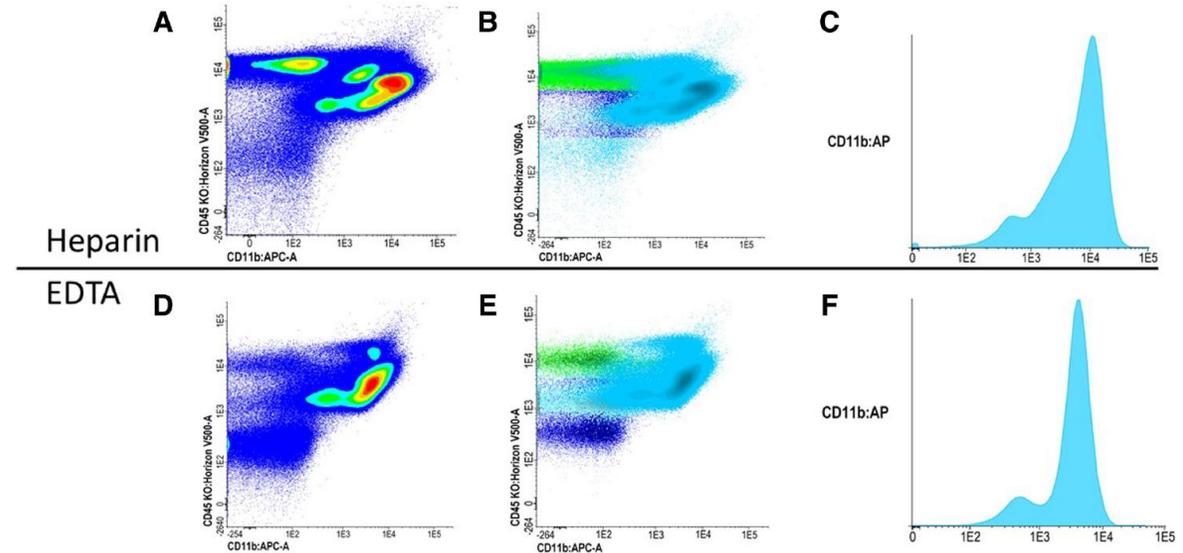
- What must be standardized?
- What can be harmonized?





## Bone marrow sampling

- Bone marrow aspirate
  - Which anticoagulant
  - Hemodilution
    - Small volume (<5 ml)
    - *Estimate peripheral blood contamination*



| Formula for Detecting Hemodilution   | Additional Requirements                                  |
|--|--|
| $\text{Bone marrow purity} = [1 - (\text{erythrocytes BM} / \text{erythrocytes PB}) \times (\text{leukocytes PB} / \text{leukocytes BM})] \times 100\%$                                  | Matched PB <sup>16</sup>                                 |
| $\text{PB contamination index} = -3.052 + 0.065 \times (\% \text{CD10}^+ \text{ neutrophils of granulocytes}) - 0.609 \times (\% \text{CD34}^+) - 2.008 \times (\% \text{plasma cells})$ | CD10, CD34 positive cells and plasma cells <sup>12</sup> |
| $\text{Normalized blast count} = (80\% / \% \text{dim CD16}) \times \text{blast count}$  | CD16 <sup>13</sup>                                       |
| $\text{Predicted bone marrow purity} = [1 - (\text{Lymphocytes FCM} / \text{Lymphocytes PB}) \times (\text{Leukocytes PB} / \text{Leukocytes FCM})] \times 100\%$                        | Matched PB <sup>14</sup>                                 |
| $\text{Suggested blood contamination if mast cell population (CD117}^+ \text{)} \leq 0.002\%$  | CD117 positive mast cells <sup>15</sup>                  |

BM = bone marrow; FCM = flow cytometry; PB = peripheral blood.

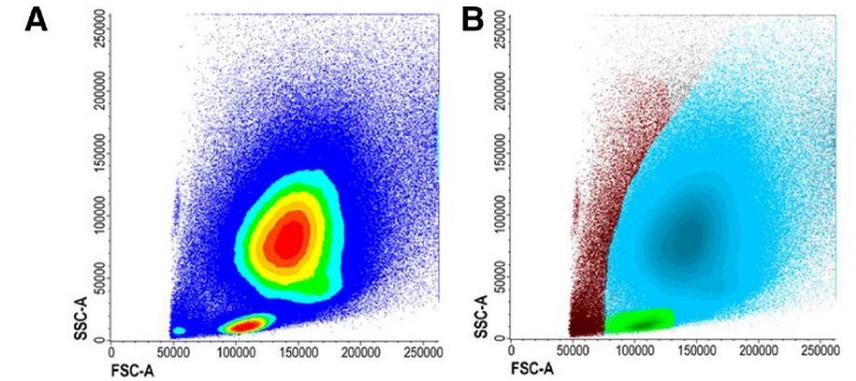


## Transport



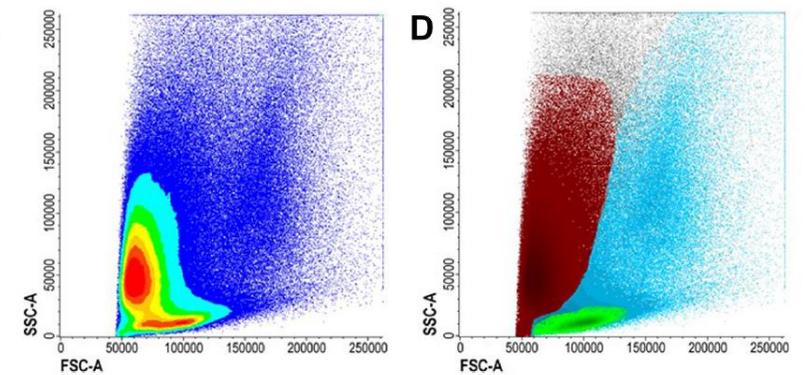
- Transport at room temperature
- Preferably <72 hours
- Assess viability and report →

Forward Scatter vs. Side Scatter



Control

>80% non-C  
viable

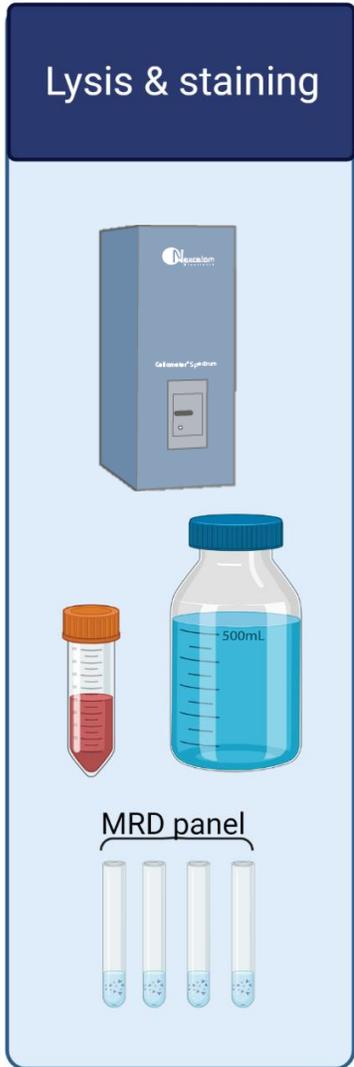




## Flow cytometer settings & quality control



- Setup based on Euroflow or Harmonemia instructions both on BD or BC machines
- Checks:
  - Daily CST beads run
  - Weekly check if spectral falls into target channels
  - Yearly maintenance of flow cytometer



- Reserve enough cells to stain for having a good and reliable result → 2,000,000 cells viable WBC per tube
- Stain-lyse-wash (SLW) and lyse-wash-stain-wash (LWSW) approach
  - No additional sample fixation → changes in morphology
  - Maintain optimal FSC and SSC properties
- IVDR validated assay

| Tube | FITC | PE    | PerCP-CY5.5 | PeCy7 | APC   | APC-H7 | BV421 | HV500c |
|------|------|-------|-------------|-------|-------|--------|-------|--------|
| 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   |



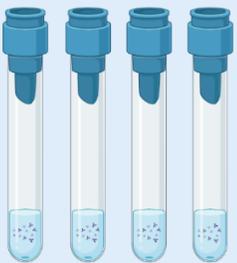
## Acquisition



Events/s

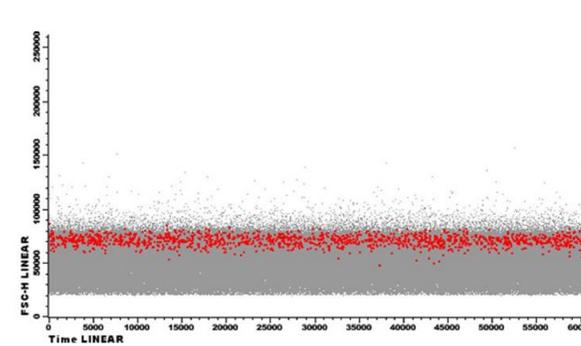
Flow rate

Regular cleaning

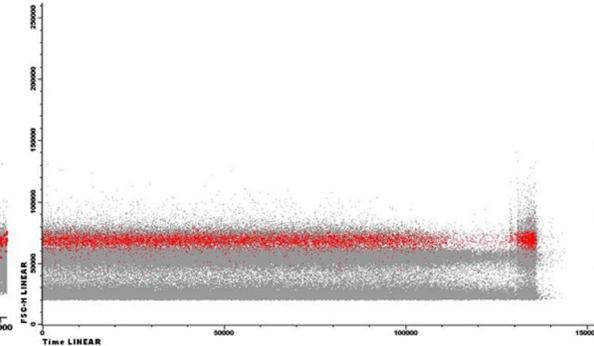


- Stable event rate per second
- Flow rate is the same for all tubes
- Enough cells to acquire
  - Diagnosis: min 50,000 in blast gate
  - Follow up: min 500,000 to 1,000,000 CD45-expressing events

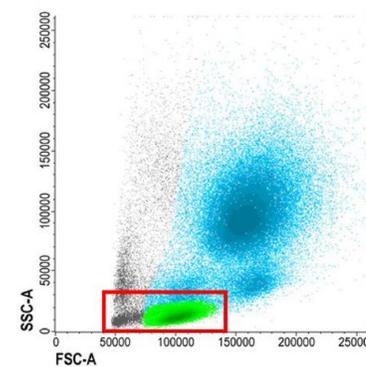
**A** Normal time plot



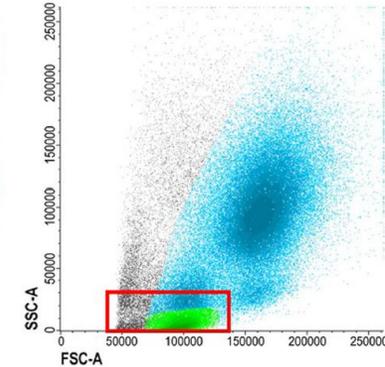
**B** Fluidic alteration over time



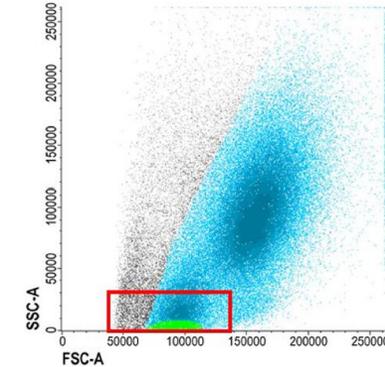
**A** Low event rate

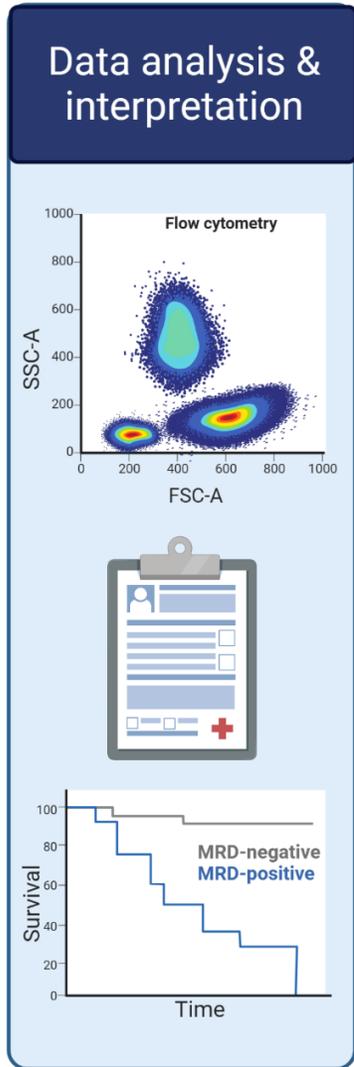


**B** Medium event rate

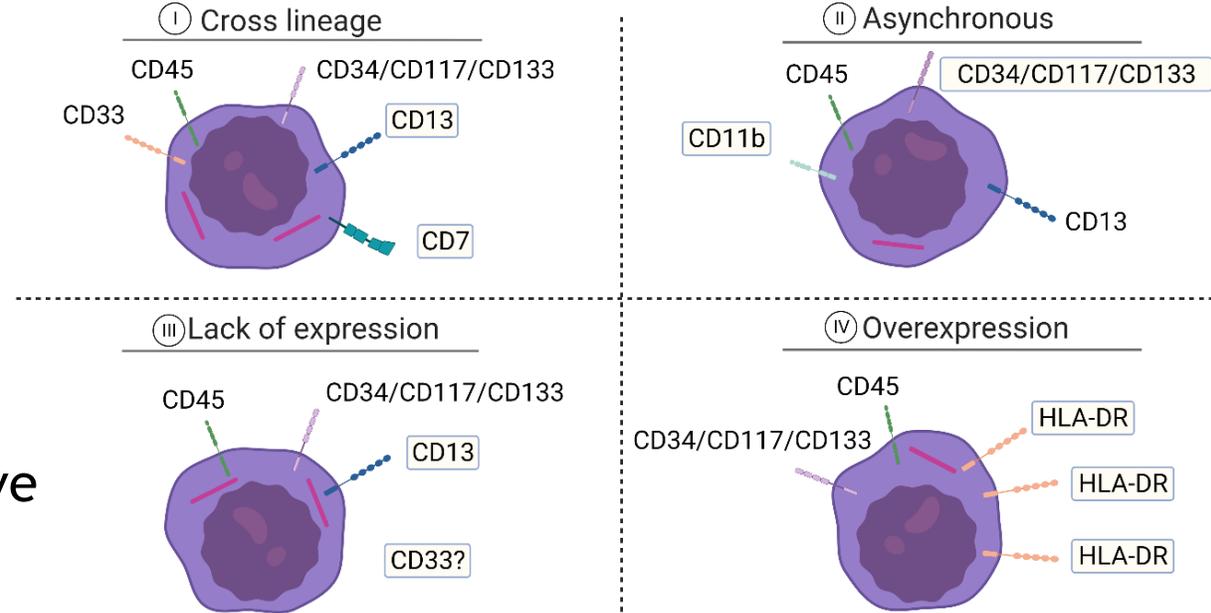


**C** High event rate



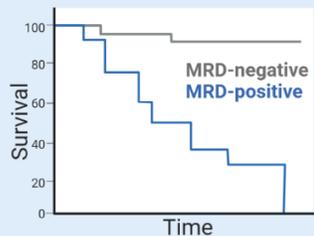
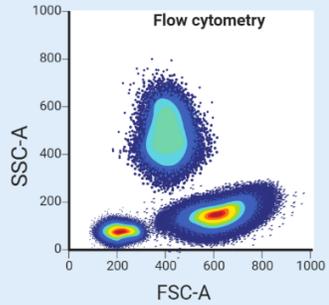


- Standardized gating strategy
  - Exclude aggregates
  - CD45 (WBC marker)
  - CD34/CD117/CD133 (primitive marker)
  - CD13/CD33 (myeloid marker)
  - Find Leukemia Associated Immuno-Phenotype (LAIP)

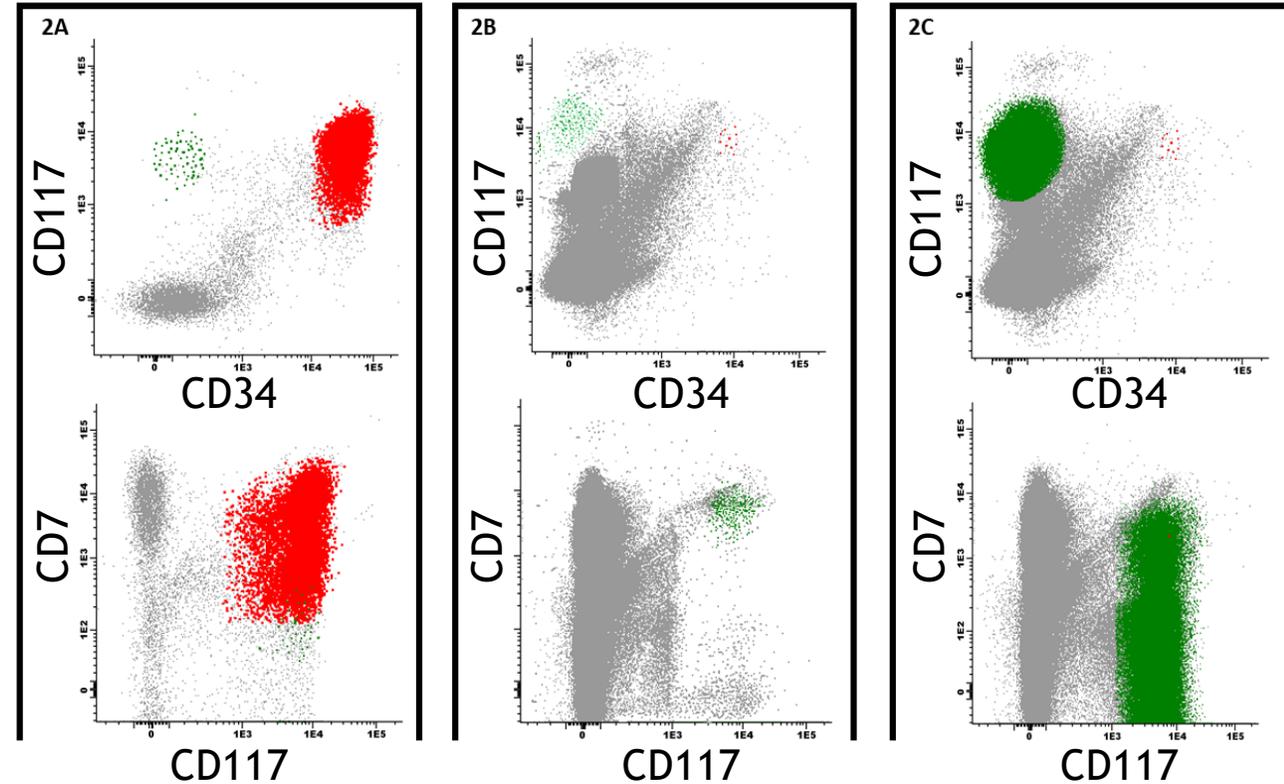




## Data analysis & interpretation

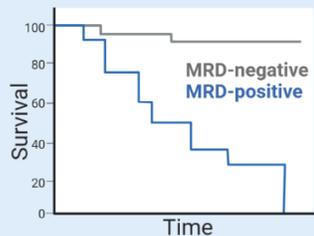
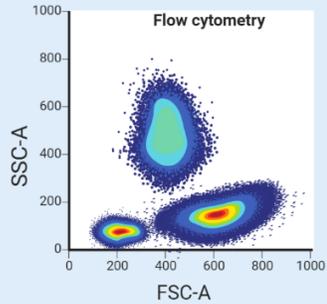


- Approach
  1. LAIP
  2. Different from normal (DfN)
- Age-matched “control” BM and regenerating BM samples
- Cut-off 0.1% LAIP from WBC (prognostic after 2 cycles of chemotherapy)



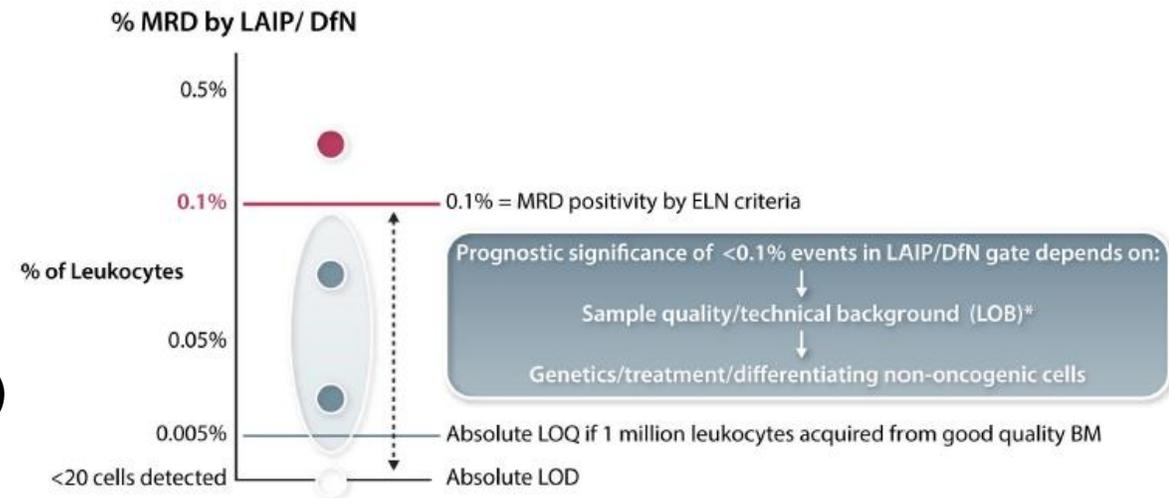


## Data analysis & interpretation



## Reporting

- MRD-negative or MRD-positive
- “Technical MRD” below 0.1% threshold
  - Accurate knowledge of assay performance:
    - Limit of blank (LOB)
    - Limit of detection (LOD)
    - Limit of quantification(LOQ)
- Monitoring with short follow-up

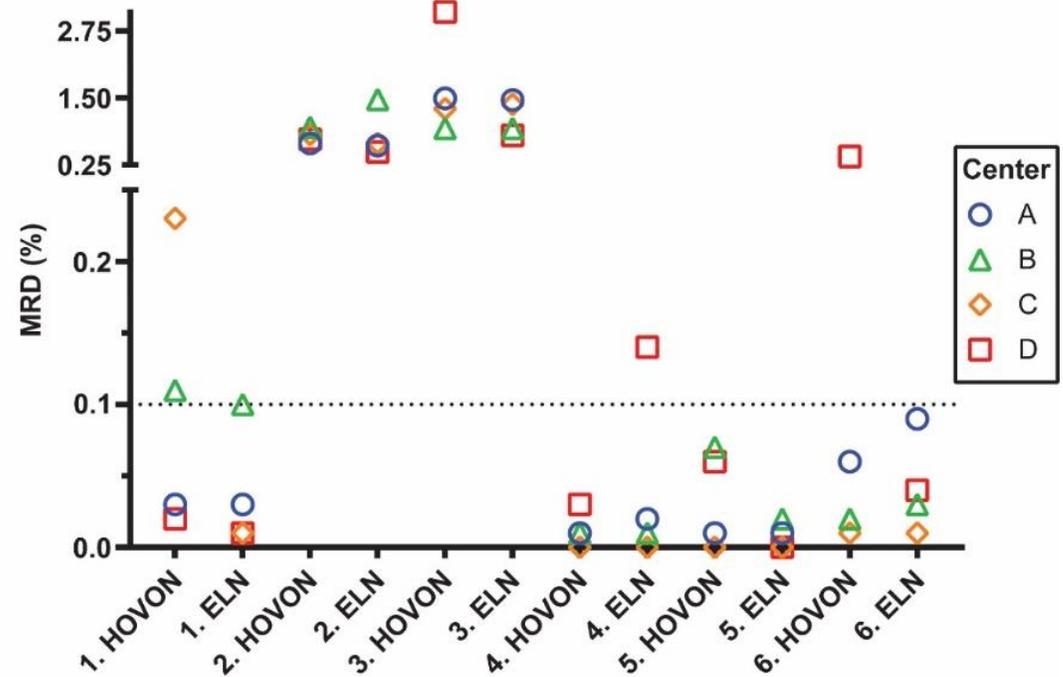




# Inter-laboratory validation

6 samples measured in 4 centers using  
a consensus ELN tube

- Outliers revoked after discussion  
by adjusting the gates



| ELN Tube     |        |        |             |            |          |                |         |        |        |
|--------------|--------|--------|-------------|------------|----------|----------------|---------|--------|--------|
| Fluorochrome | FITC   | PE     | PerCP-Cy5.5 | PECy7      | APC      | Alexa Fluor750 | PB      | V500   | KO     |
| Antigen      | CD34   | CD13   | CD7         | CD33       | CD56     | CD117          | HLA-DR  | CD45   |        |
| Clone        | 8G12   | L138   | M-T701      | D3HL60.251 | NCAM16.2 | 104D2D1        | Immu357 | HI30   | J33    |
| Manufacturer | BD     | BD     | BD          | BC         | BD       | BC             | BC      | BD     | BC     |
| Order No.    | 345801 | 347406 | 561602      | B92408     | 341027   | B92450         | B36291  | 560777 | B36294 |
| Amount       | 5 µl   | 5 µl   | 5 µl        | 5 µl       | 5 µl     | 5 µl           | 5 µl    | 5 µl   | 5 µl   |

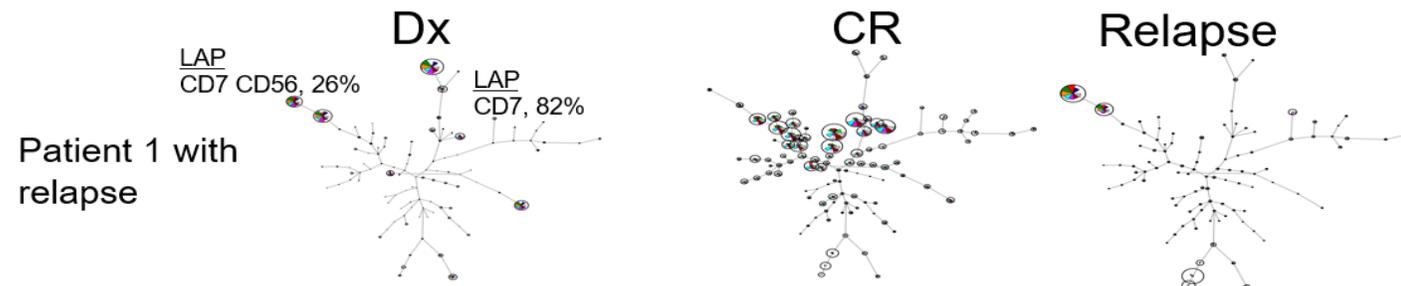
| Hovon P1 Tube |        |        |             |         |        |              |        |        |
|---------------|--------|--------|-------------|---------|--------|--------------|--------|--------|
| Fluorochrome  | FITC   | PE     | PerCP-Cy5.5 | PECy7   | APC    | APC-H7       | BV421  | KO     |
| Antigen       | CD7    | CD56   | CD34        | CD117   | CD33   | HLA-DR       | CD13   | CD45   |
| Clone         | M-T701 | MY31   | 8G12        | 104D2D1 | P67.6  | L243 (G46-6) | WM15   | J33    |
| Manufacturer  | BD     | BD     | BD          | BC      | BD     | BD           | BD     | BC     |
| Order No.     | 555360 | 345810 | 347222      | B49221  | 345800 | 641411       | 562596 | B36294 |
| Amount        | 5 µl   | 5 µl   | 5 µl        | 5 µl    | 5 µl   | 5 µl         | 5 µl   | 5 µl   |



# Future perspectives MRD

- Further harmonization
  - Better understanding of hemodilution
  - United Kingdom National External Quality Assessment Site to standardize MRD (UK-NEQAS)
- Optimal cut-off and time-point
  - Reduce false-negative results
  - LAIP specific LOB/LOD/LOQ
  - Implementing PB-MRD
- Computational (automated) analysis
  - Reduce subjectivity (requires standardised data)

**UK NEQAS**  
International Quality Expertise





# Take home messages

- Having MFC-MRD detected after induction therapy is a bad sign;
- It is extremely important to have comparable MRD-results among different laboratories and different clinical studies;
- Consensus approach shows which facets need to be standardized and which needs to be harmonized to achieve this.



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Karima Yahakoub

Maaïke Hofland

Bo Kuijk

Laura Oudshoorn

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Mona Fayet

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