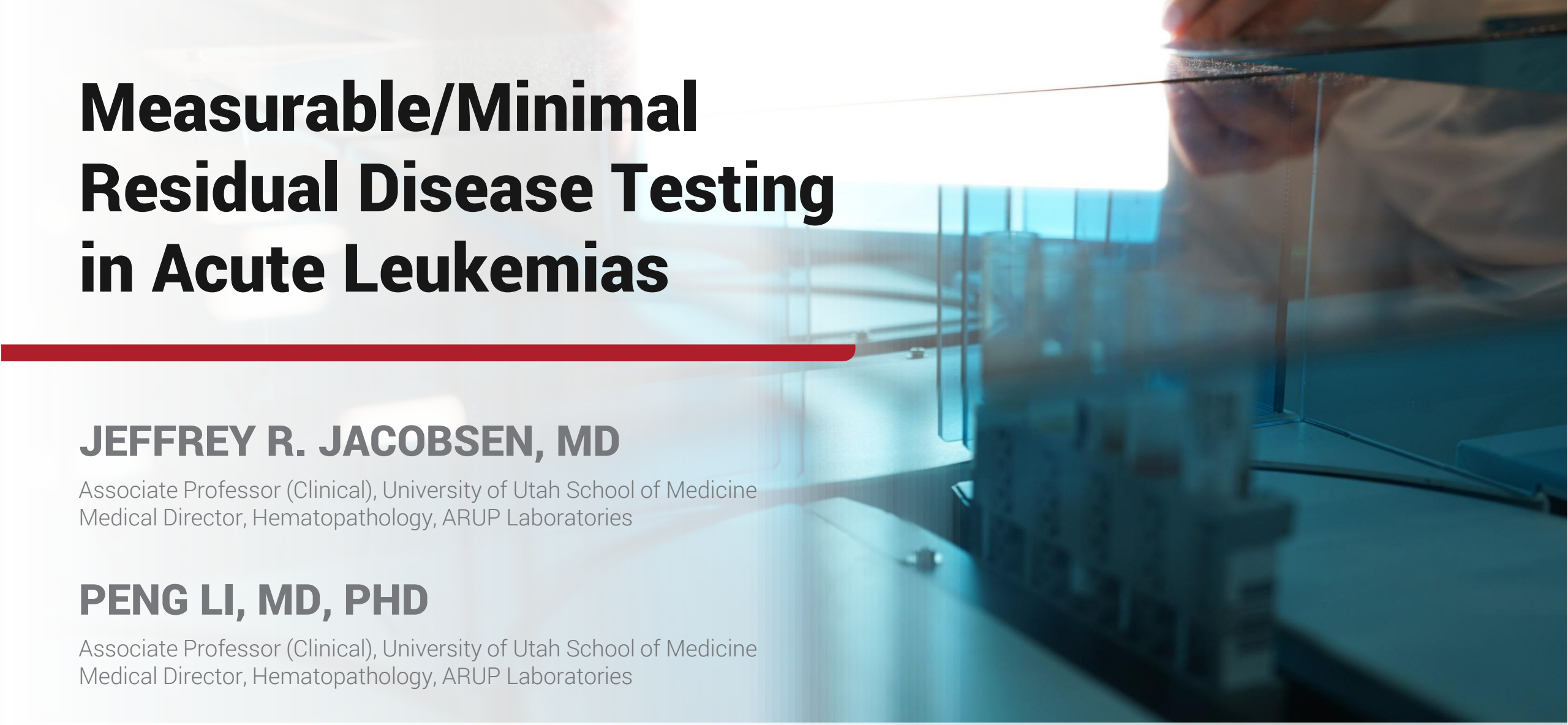
Measurable/Minimal Residual Disease Testing in Acute Leukemias



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Outline

- Learning objectives
- Clinical background of acute myeloid leukemia (AML)
- Define minimal (measurable) residual disease (MRD) in AML
- Clinical need and benefits of MRD testing in AML
- Available MRD testing methodologies
- Appropriate timing for MRD testing
- Future directions and innovations in AML MRD testing

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Learning Objectives

- Molecular and flow cytometric MRD markers in AML
- MRD methods and flow cytometric panels
- Clinical significance of AML MRD testing
- Future developments in AML MRD testing

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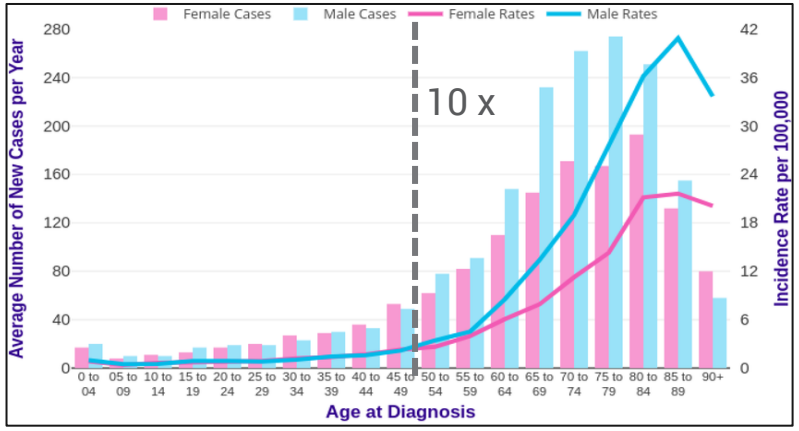
Background of AML

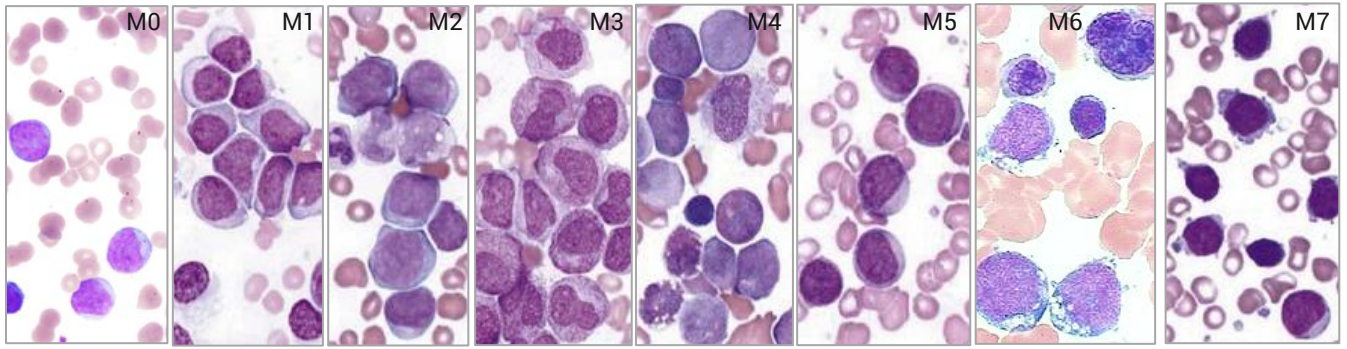
- A heterogenous group of diseases
  - » Clinical heterogeneity
  - » Phenotypic heterogeneity
  - » Molecular heterogeneity
- A subclone disease
  - » A group of lethal disease
  - » Curable in select cases

Heterogeneity in AML

AML Incidence



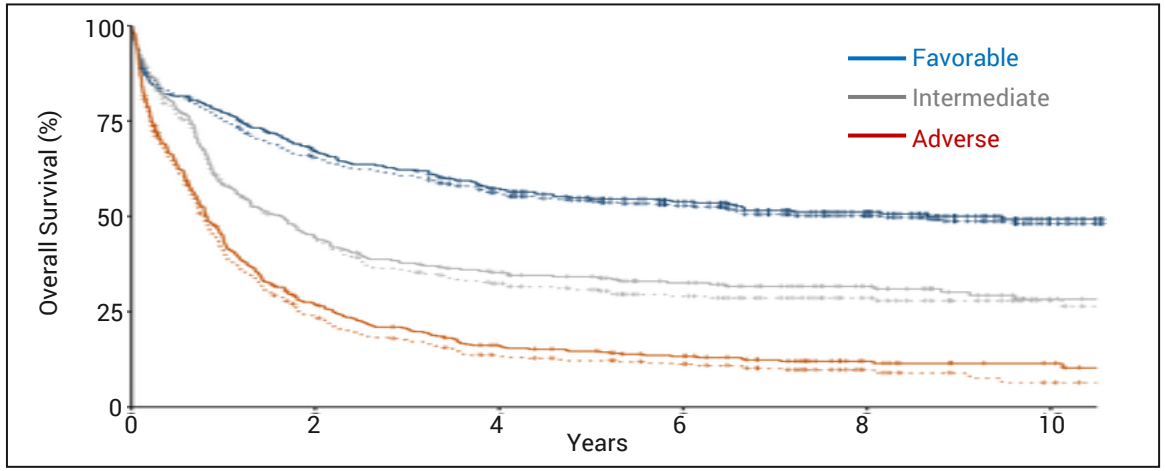
AML - FAB



AML with defining genetic abnormalities

AML with RUNX1::RUNX1T1 fusion
AML with CBFB::MYH11 fusion
Acute promyelocytic leukemia (APL) with PML::RARA fusion
AML with KMT2A rearrangement
AML with DEK::NUP214 fusion
AML with MECOM rearrangement
AML with RBM15::MRTFA fusion
AML with BCR::ABL1 fusion*
AML with NUP98 rearrangement
AML with other defined genetic alterations
AML with NPM1 mutation
AML with CEBPA mutation*
AML, myelodysplasia-related
AML with other defined genetic alterations
AML defined by differentiation

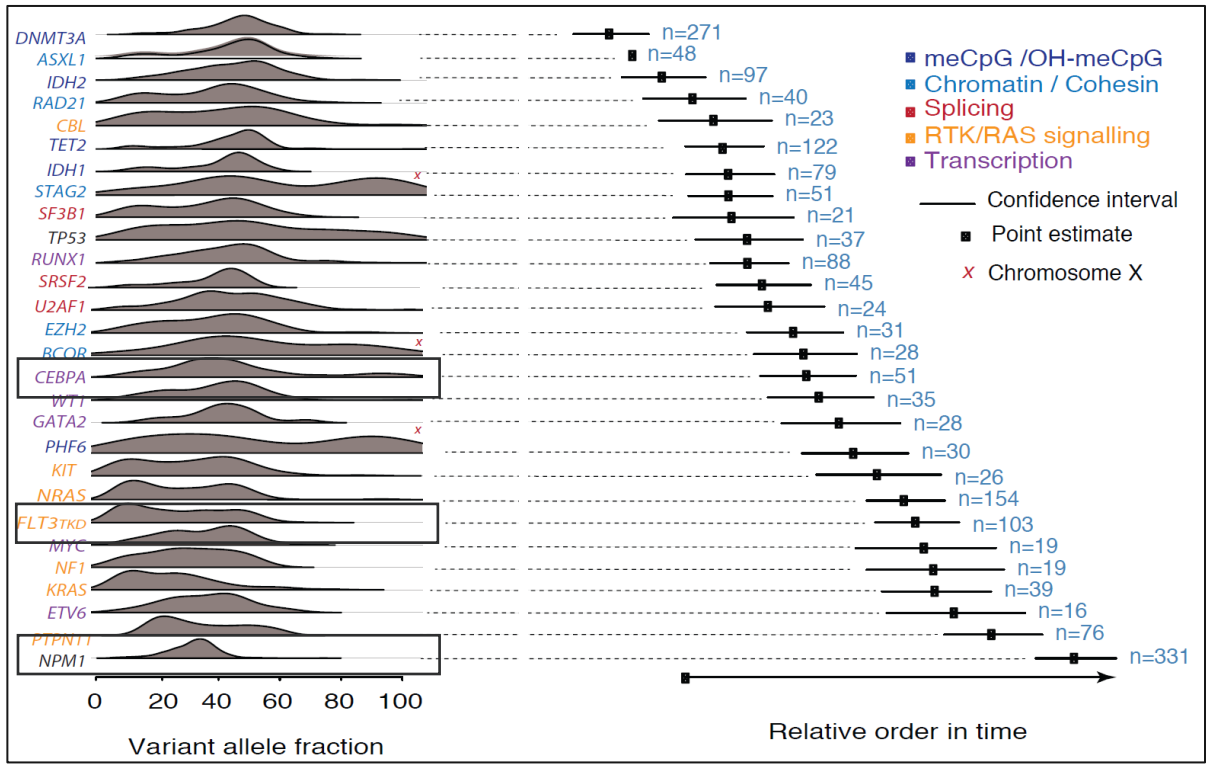
Outcome per ELN risk classification



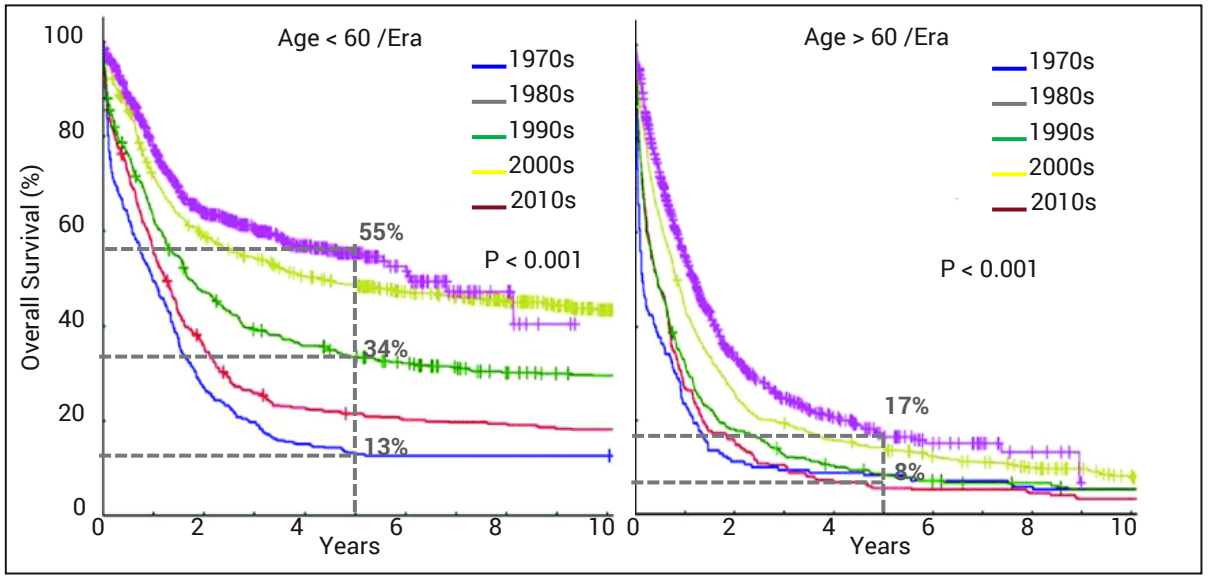
Döhner, et al, Blood, 2022

Sub-clonal disease – lethal but curable

Relative order of mutation acquisition in AML



Improved overall survival for AML over decades



Papaemmanuil, et al, NEJM, 2016; Sasaki, et al, Cancer, 2021; Döhner, et al, Blood, 2022

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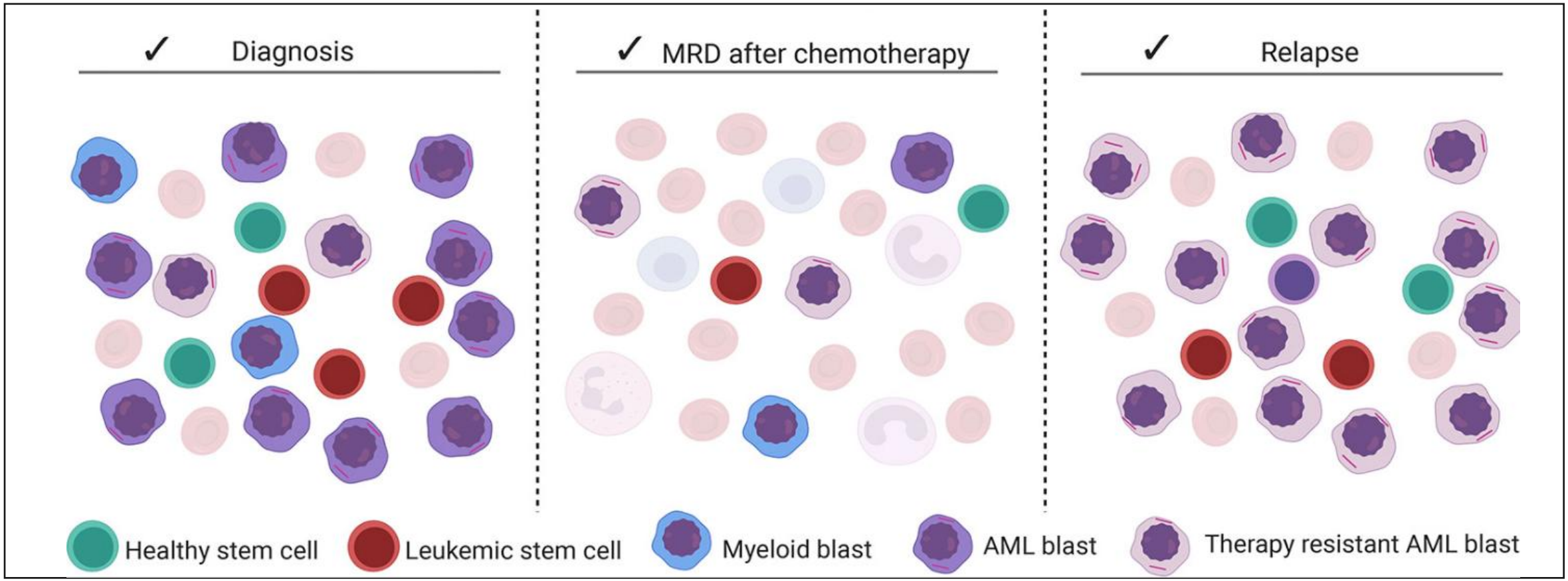
Clonal Evolution & Phenotypic Switch



Norita, et al, Nature Communications, 2020

Outline

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- Clinical background of acute myeloid leukemia (AML)
- Define minimal (measurable) residual disease (MRD) in AML
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- Available MRD testing methodologies
- Appropriate timing for MRD testing
- Determine which patient populations should be tested
- Future directions and innovations in AML MRD testing

Clinical Need and Benefits

- Provide actional information to help patients, with the goal of improving clinical outcomes
- Offer better guidance for ongoing management and identifying the next best step
- Enable enrollment in clinical trials for MRD positive patients prior to BMT
- Facilitate early detection of relapse following BMT, allowing early intervention

- Challenges…
  - » MRD tests for AML are not standardized
  - » AML drugs are not perfect
  - » Treatment regimens are not optimized

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AML MRD Methodology

- Multiparametric flow cytometry
  - » Leukemia-associated immunophenotype and difference from normal
  - » Sampling and preanalytical phase: technical requirements
  - » Gating strategies and calculations for MFC-MRD
  - » Advantages and limitations
- Molecular MRD tests
  - » qPCR and digital PCR based MRD assays
  - » NGS-based molecular MRD assessment
  - » Selection of MRD markers for NGS-MRD

MFC-MRD testing

- Evaluation of antigen expression patterns in leukocyte populations to differentiate neoplastic cells from normal hematopoietic elements
- Phenotypic aberrancies come in two primary flavors
  - » Lineage infidelity: expression by neoplastic cells of a B-cell or T-cell associated antigen not normally expressed on myeloid progenitors (e.g. CD19 or CD5)
  - » Maturational dyssynchrony: Decoupling of antigens typically co-expressed at defined maturational stages (e.g. expression of CD34 without CD44) or coupling of antigens normally expressed at different maturational stages on a single population (e.g. co-expression of CD34 and CD14)
- Requires extensive familiarity with conserved patterns of maturation in hematopoietic progenitors both in normal conditions and in post-therapeutic marrow regeneration
  - » Example: dim CD7 and dim CD11b expression may be seen in regenerative CD34+ myeloblasts and do not represent lineage infidelity or maturational dyssynchrony respectively in a regenerative marrow

MFC analysis: Difference from normal (DFN) and Leukemia associated Immunophenotype (LAIP)

- Difference from normal (DfN)
  - » Any instance where expression patterns of leukemic cells differ from those of normal hematopoietic maturation patterns
- Leukemia associated immunophenotype (LAIP)
  - » Broadly defined: the expression profile of the leukemic population for all antigens evaluated
  - » Narrowly defined: the subset of leukemic expression patterns that differ from normal in a given patient
- When described broadly, LAIP is an invalid approach to MRD assessment
- When described narrowly, LAIP is an extension of DfN evaluation

MFC analysis: Difference from normal (DFN) and Leukemia associated Immunophenotype (LAIP)

- Limitations of LAIP evaluation
  - » Requires pretreatment (baseline) immunophenotyping
  - » Is subject to antigen shifts (loss of some or all defining aberrancies present at diagnosis)
  - » Often requires custom patient-specific antibody/tube configurations
- Benefits of LAIP evaluation
  - » Many phenotypic aberrancies remain stable over time
  - » When LAIP aberrancies are retained, one or more aberrancy is often unambiguous and easy to interpret
- Combined approach (recommended) 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party | Blood | American Society of Hematology
  - » If LAIP is known, evaluate for LAIP aberrancies first; many positive cases can be identified rapidly and objectively
  - » If LAIP is unknown or if no abnormal population is identified using LAIP markers, proceed with comprehensive DfN evaluation

MFC analysis: Factors that Determine Sensitivity

THE OFFICIAL JOURNAL OF THE INTERNATIONAL CLINICAL CYTOMETRY SOCIETY
CLINICAL CYTOMETRY
ORIGINAL ARTICLE | Free Access

High-sensitivity flow cytometric assays: Considerations for design control and analytical validation for identification of Rare events

Ulrike Sommer, Steven Eck, Laura Marszalek, Jennifer J. Stewart, Jolene Bradford, Thomas W. McCloskey, Cherie Green, Alessandra Vitaliti, Teri Oldaker, Virginia Litwin

First published: 17 September 2020 | https://doi.org/10.1002/cyto.b.21949 | Citations: 40

- Limit of blank: highest signal in the absence of the measurand (background)
- Limit of detection: lowest signal above background which can be detected (minimum event number)
- Sensitivity is thereby a function of the number of events collected as well as the ability to resolve abnormal populations/events from background signal
- In AML MRD, often only a fraction of abnormal leukocytes can be resolved from background hematopoiesis, functionally reducing the expected sensitivity

Desired CV (%)		1	5	10	20	40
Cells of interest [a], [r]		10,000	400	100	25	6.25
When occurring at a frequency of		Total number of cells that must be analyzed [b]				
%	1:*n* cells					
10	10	10^5	4×10^3	10^3	2.5×10^2	6.25×10^1
1	100	10^6	4×10^4	10^4	2.5×10^3	6.25×10^2
0.1	1,000	10^7	4×10^5	10^5	2.5×10^4	6.25×10^3
0.01	10,000	10^8	4×10^6	10^6	2.5×10^5	6.25×10^4
0.001	100,000	10^9	4×10^7	10^7	2.5×10^6	6.25×10^5
0.0001	1,000,000	10^{10}	4×10^8	10^8	2.5×10^7	6.25×10^6
0.00001	10,000,000	10^{11}	4×10^9	10^9	2.5×10^8	6.25×10^7

FMC-MRD Implementation: ST JUDE AML02 multicenter trial

- 2002-2008
- 232 patients with de novo acute myeloid leukemia (206), therapy or MDS-related AML (12), or mixed lineage acute leukemia (14)
- MRD assessment
  - » Four-color assay
  - » Baseline phenotype assessment at diagnosis
  - » Patient-specific panel performed at follow-up assessment (combined LAIP and DFN approach)
  - » Detection threshold 0.1%

FMC-MRD Implementation: ST JUDE AML02 multicenter trial, Baseline Evaluation

- Twenty-nine 4-color tubes performed at diagnosis to establish LAIP
- Sensitivity (LAIP detected) for each tube ranged from 41.2% of leukemias for CD13, CD133, CD34, CD33 to 0.5% for CD235a, CD13, CD34, CD33
- No LAIP/DfN was identified in 11/201 AML baseline evaluations (5.47%) Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial - The Lancet Oncology

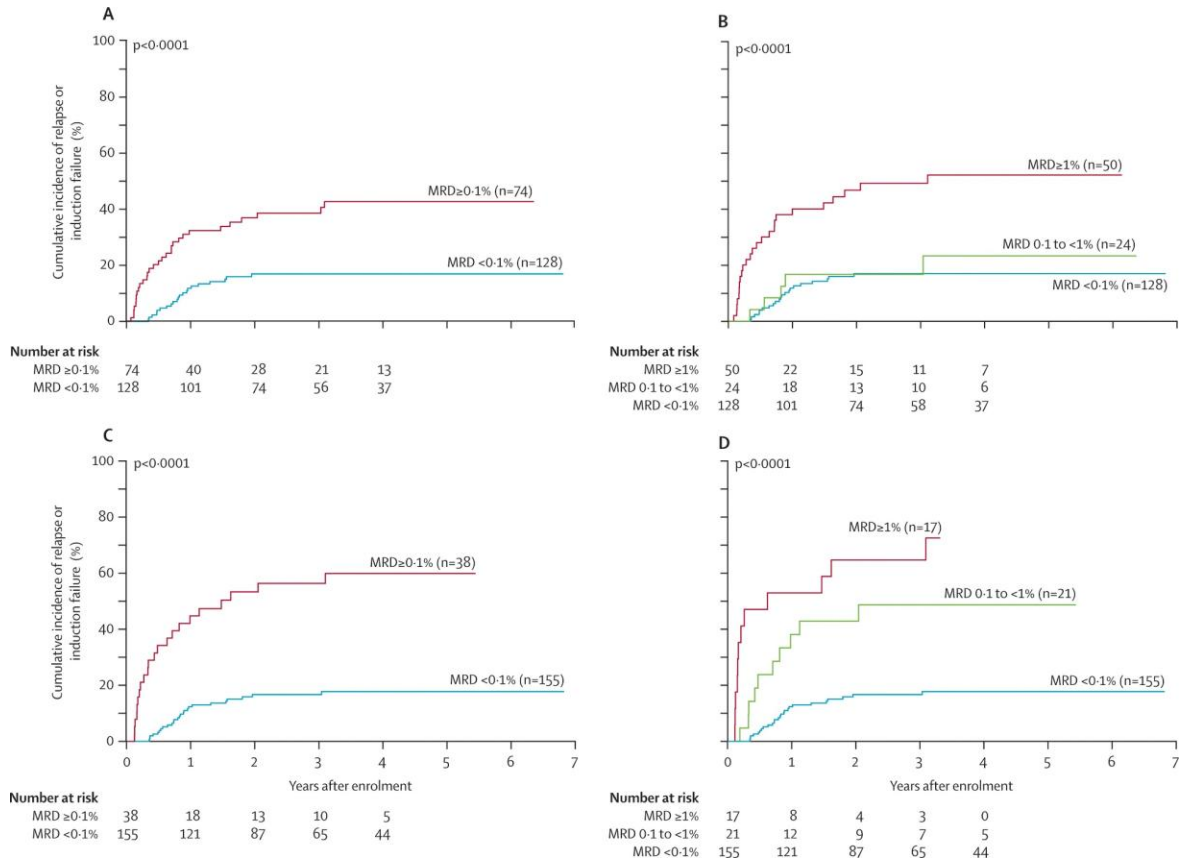
Table S1. Set of markers used to monitor MRD in AML02 and number of patients studied with each set

Marker combination[1]	Number of Patients Studied (%)[2]
CD13 / CD133 / CD34 / CD33	84 (41.2)
CD13 / CD117 / CD34 / CD33	67 (32.8)
CD15 / CD117 / CD34 / CD33	62 (30.4)
CD38 / CD13 / CD34 / CD33	60 (29.4)
CD15 / CD13 / CD34 / CD33	55 (27.0)
CD13 / CD56 / CD34 / CD33	52 (25.5)
HLA-Dr / CD117 / CD34 / CD33	47 (23.0)
HLA-Dr / CD13 / CD34 / CD33	30 (14.7)
CD13 / anti-NG2 / CD34 / CD33	28 (13.7)
CD11b / CD13 / CD34 / CD33	20 (9.8)
CD11b / CD117 / CD34 / CD33	20 (9.8)
CD38 / CD117 / CD34 / CD33	20 (9.8)
CD13 / CD123 / CD34 / CD33	18 (8.8)
CD7 / CD13 / CD34 / CD33	15 (7.4)
CD11b / CD133/ CD34/ CD33	15 (7.4)
CD65 / CD117 / CD34 / CD33	13 (6.4)
CD33 / CD13 / CD34 / CD4	13 (6.4)
CD13 / CD56 / CD4 / CD33	10 (4.9)
CD33 / CD117 / CD34 / CD4	10 (4.9)
CD65 / CD13 / CD34 / CD33	10 (4.9)
CD7 / CD117 / CD34 / CD33	10 (4.9)
CD45 / CD13 / CD34 / CD33	8 (3.9)
CD19 / CD13 / CD34 / CD33	8 (3.9)
CD41 / CD13 / CD34 / CD33	6 (2.9)
CD41 / CD117 / CD34 / CD33	5 (2.5)
CD41 / CD38 / CD45 / CD33	4 (2.0)
CD41 / CD38 / CD45 / HLA-Dr	4 (2.0)
CD2 / CD13 / CD34 / CD33	3 (1.5)
CD235a / CD13 / CD34 / CD33	1 (0.5)

[1]The order of the individual antibodies in each set corresponds to the fluorochrome to which they were conjugated, i.e., fluorescein isothiocyanate / phycoerythrin / peridinin chlorophyll protein / allophycocyanin. The source of the antibodies was BD Biosciences (San Jose, CA), Beckman Coulter (Miami, FL), Dako (Carpinteria, CA) and Miltenyi Biotec (Auburn, CA).

[2]Number of patients in whom the set of markers listed allowed a sensitivity of MRD detection of at least 0.1% among the 204 patients with leukemia-associated immunophenoptypes identified at diagnosis. The immunophenotypes were identified with 7 sets of markers in 4 patients, with 6 sets in 8 patients, with 5 sets in 37 patients, with 4 sets in 39 patients, with 3 sets in 55 patients, with 2 sets in 55 patients and with one set in 6.

FMC-MRD Implementation: ST JUDE AML02 multicenter trial: Outcomes



Recent advances in FMC-MRD testing: Leukemic Stem Cell evaluation

- HOVON-SAKK132 trial: Prospective validation of the prognostic relevance of CD34+CD38- AML stem cell frequency in the HOVON-SAKK132 trial
- Three-tier initial risk stratification
  - CD34 negative (10%): <1% CD34+ blasts, no LAIP within CD34+ blast fraction, no LSC population
  - LSC low (57%): <0.03% CD34+CD38-LSC+ cells
  - LSC high (34%): >0.03% CD34+CD38-LSC+ cells

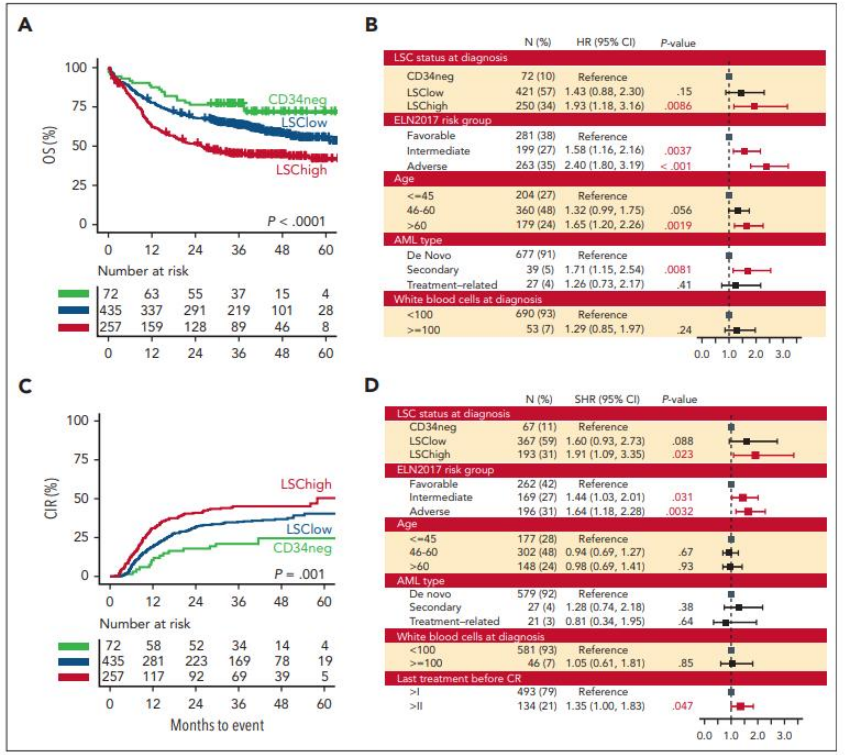


Figure 1. Prognostic value of LSC burden at diagnosis. (A) Kaplan-Meier curve for OS. (C) CIR. The overall group (A,C) is divided into $CD34^{neg}$, LSC^{low}, and LSC^{high}, with the cut-off of 0.03% $CD34^{+}CD38^{-}LSC^{+}$ population of WBCs. (B,D) Multivariate analysis adjusted for age, AML type, ELN2017 risk group, WBCs at diagnosis, and last treatment before reaching CR (only for CIR) of (B) OS (Cox regression) and (D) CIR (Fine and Gray regression).

Recent advances in FMC-MRD testing: Leukemic Stem Cell evaluation

» Continued prognostic significance after 2 cycles of chemotherapy

Prospective validation of the prognostic relevance of $CD34^{+}CD38^{-}$ AML stem cell frequency in the HOVON-SAKK132 trial

Lok Lam Ngai,[1,2] Diana Hanekamp,[1-3] Fleur Janssen,[1,2] Jannemieke Carbaat-Ham,[1,2] Maaike A. M. A. Hofland,[1,2] Mona M. H. E Fayed,[1,2] Angèle Kelder,[1,2] Laura Oudshoorn-van Marsbergen,[1,2] Willemijn J. Scholten,[1,2] Alexander N. Snel,[1,2] Costa Bachas,[1,2] Jesse M. Tettero,[1,2] Dimitri A. Breems,[4] Thomas Fischer,[5] Bjørn T. Gjertsen,[6] Laimonas Griškevičius,[7] Gunnar Juliusson,[8] Arjan A. van de Loosdrecht,[1,2] Johan A. Maertens,[9] Markus G. Manz,[10,11] Thomas Pabst,[11,12] Jakob R. Passweg,[11,13] Kimmo Porkka,[14] Peter J. M. Valk,[3] Patrycja Gradowska,[15] Bob Löwenberg,[3] David C. de Leeuw,[1,2] Jeroen J. W. M. Janssen,[1,2,16] Gert J. Ossenkoppele,[1,2] and Jacqueline Cloos[1,2]

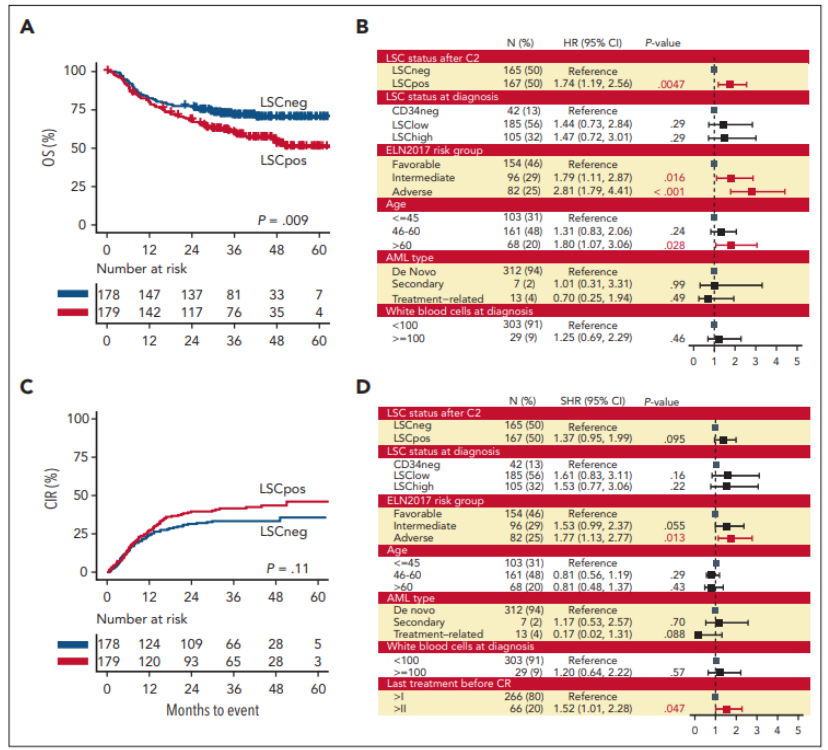


Figure 2. Prognostic value of LSC burden after 2 cycles of chemotherapy. Kaplan-Meier curves of (A) OS, (C) CIR after C2. The overall group in A and C is divided into patients with LSC^{pos} and LSC^{neg} with the cut-off of 0.00000% $CD34^{+}CD38^{-}LSC^{+}$ population. B and D show multivariate analysis adjusted for LSC status at diagnosis, age, AML type, ELN2017 risk group, WBCs at diagnosis, and last treatment before reaching CR (only CIR) of (B) OS (Cox regression) and (D) CIR (Fine and Gray subdistribution hazard regression).

FMC-MRD testing: Room for Growth/Spectral Flow Cytometry

» No longer a choice between standardization, LAIP evaluation, and sensitivity due to expansive single tube assays

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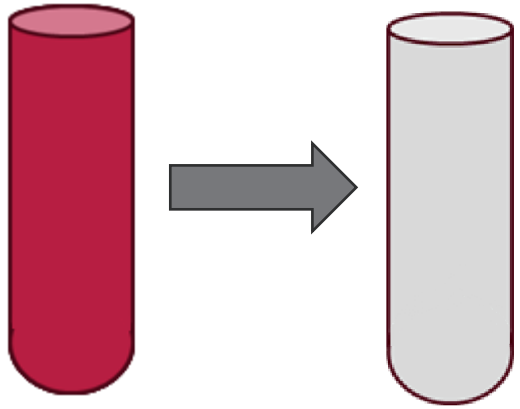
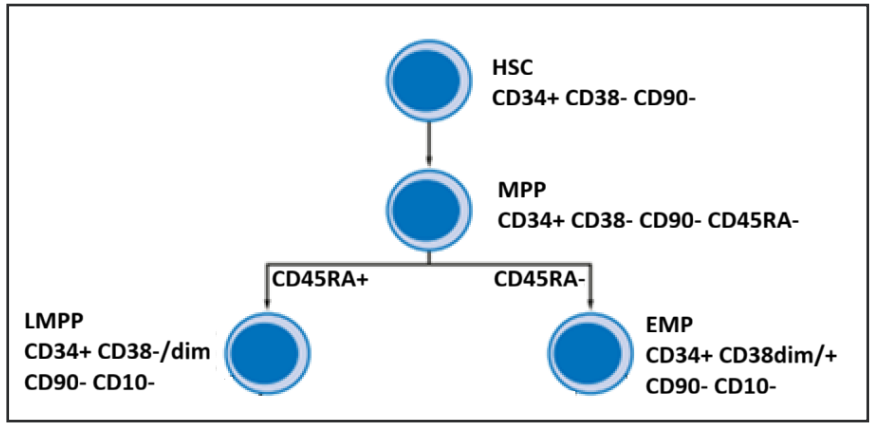
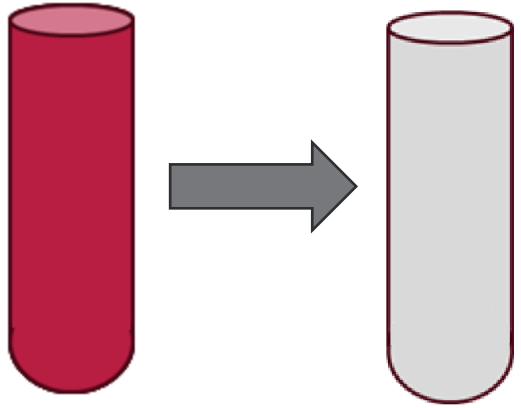


CD2	CD13	CD34	CD56	CD235a
CD4	CD15	CD38	CD65	HLADR
CD7	CD19	CD41	CD117	NG2
CD11b	CD33	CD45	CD133	

FMC-MRD testing: Room for Growth/Spectral Flow Cytometry

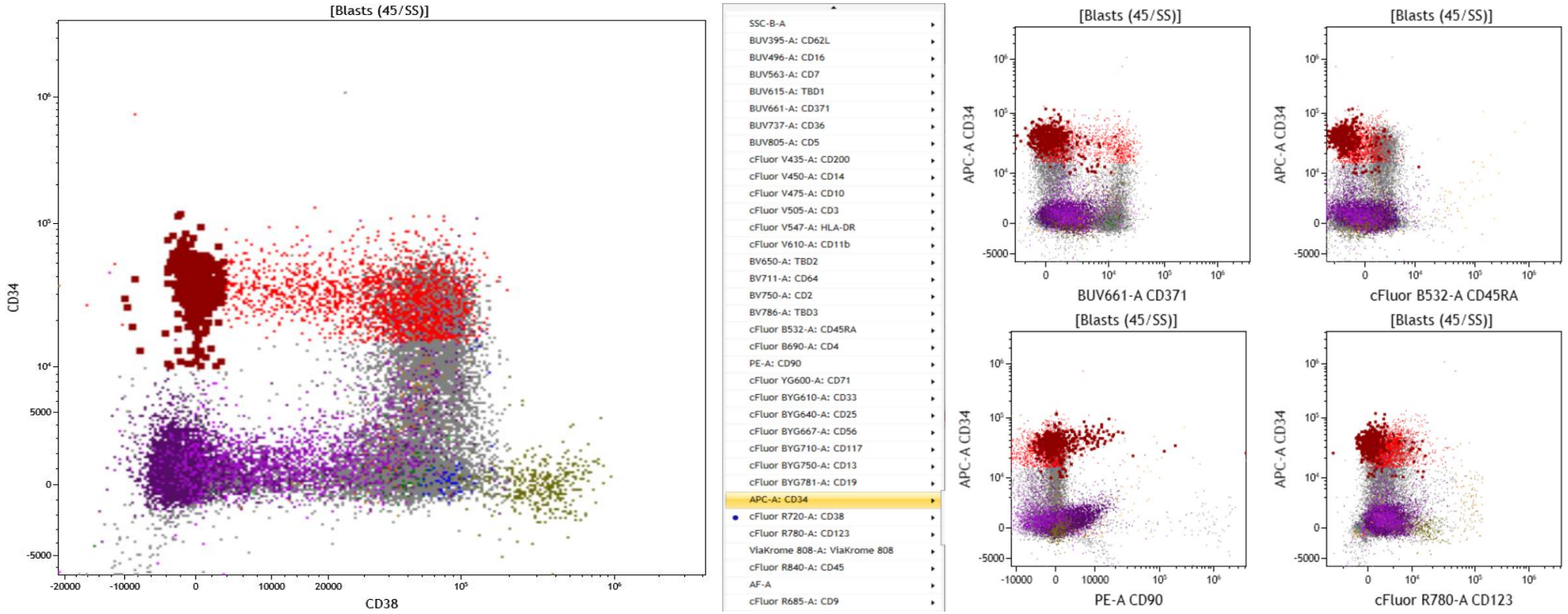
» Integration of LSC markers into existing AML MRD panels





CD2	CD13	CD34	CD56	CD235a
CD4	CD15	CD38	CD65	HLADR
CD7	CD19	CD41	CD117	NG2
CD11b	CD33	CD45	CD133	CD371
CD44	CD45RA	CD90	CD123	CD366

FMC-MRD testing: Room for Growth/Spectral Flow Cytometry



MFC analysis: Standardization

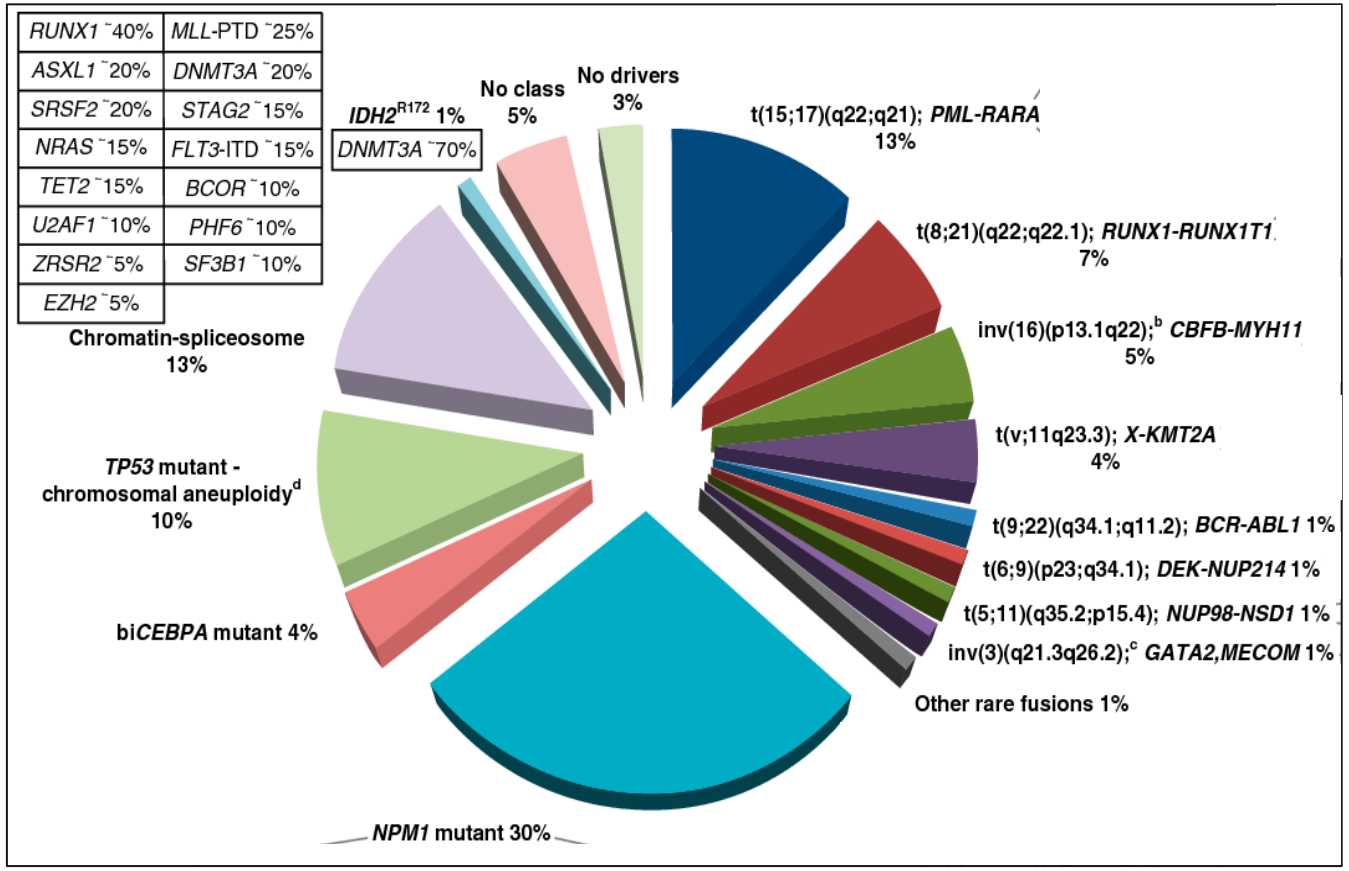
2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party

Michael Heuser, Sylvie D. Freeman, Gert J. Ossenkoppele, Francesco Buccisano, Christopher S. Hourigan, Lok Lam Ngai, Jesse M. Tettero, Costa Bachas, Constance Baer, Marie-Christine Béné, Veit Bücklein, 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 Philippe, 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

- Consensus guidelines such as those developed by the European LeukemiaNet Working Party utilizing standard MFC have been issued, however, there is currently rapid growth and development in AML MRD detection by FCM with the adoption of spectral flow cytometry platforms
- As new technology is implemented, additional data must emerge concerning populations of interest (leukemia stems cells), optimal gating strategies, and knowledge of unusual populations encountered in regenerative/post-treatment samples

Molecular AML MRD tests

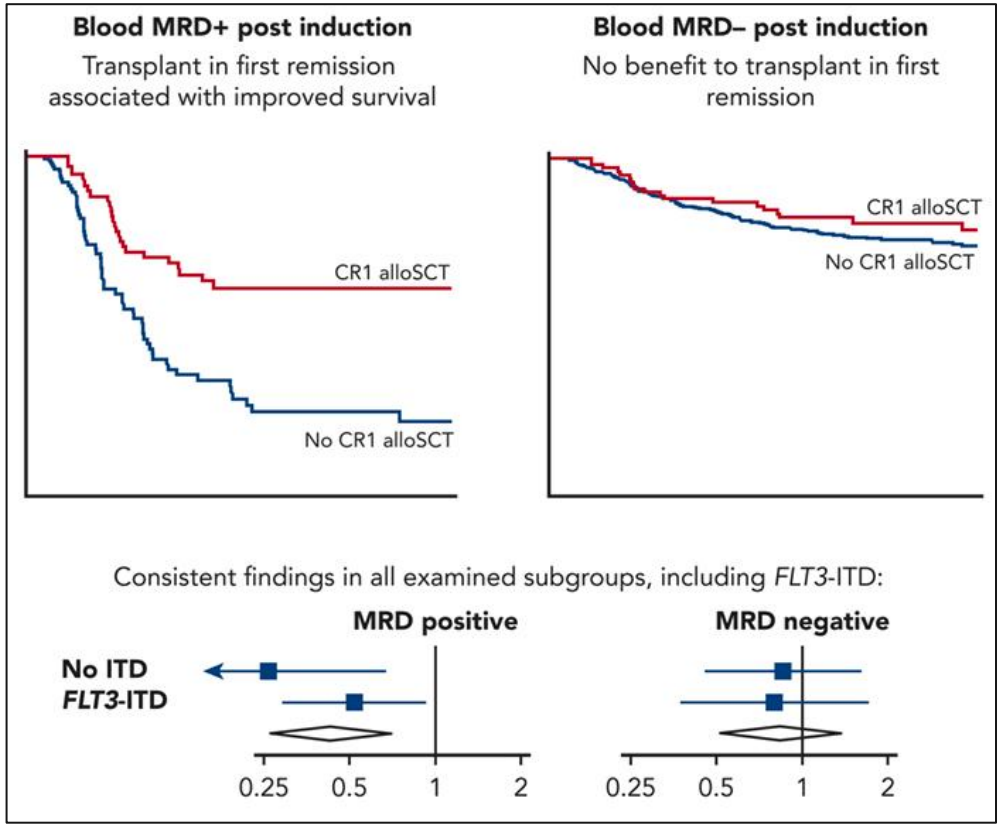
- AML MRD molecular markers
  - » Driver mutations (60-65%), preferred
    - ▪ Fusion transcripts (30-35%)
      - › RUNX1-RUNXT1 (7%)
      - › CBFB-MYH11 (5%)
      - › PML-RARA (13%)
      - › KMT2A (4%)
      - › NUP98% (4%)
      - › BCR-ABL1 (1%)
      - › DEK-NUP214 (1%)
      - › Others…
    - ▪ NPM1 (30%)
  - » Subclones (15-30%), low negative predictive values
    - ▪ FLT3-ITD
    - ▪ RAS, KIT …
  - » Therapeutic targets (>50%)
    - ▪ FLT3-ITD
    - ▪ IDH1/2
    - ▪ KMT2a fusions/NPM1
- Germline and CHIP variants should be excluded



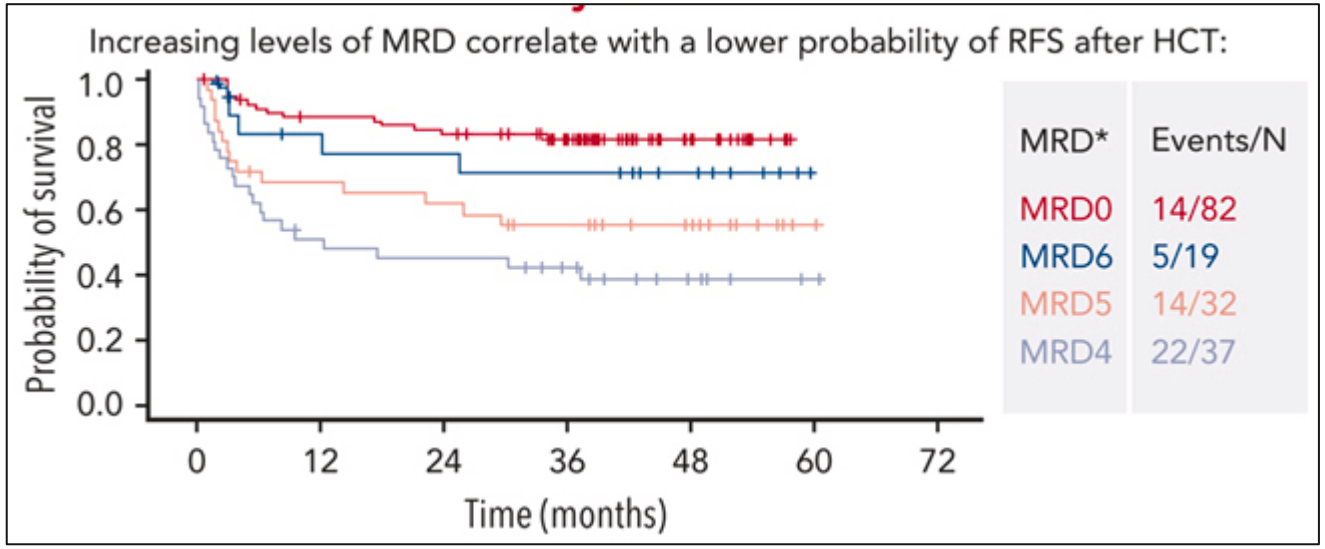
Heuser, et al, Blood, 2021

Clinical Need and Benefits

NPM1 mutated AML, NPM1 MRD by RT-qPCR in blood



FLT3-ITD positive AML, FLT3-ITD MRD by NGS in bone marrow

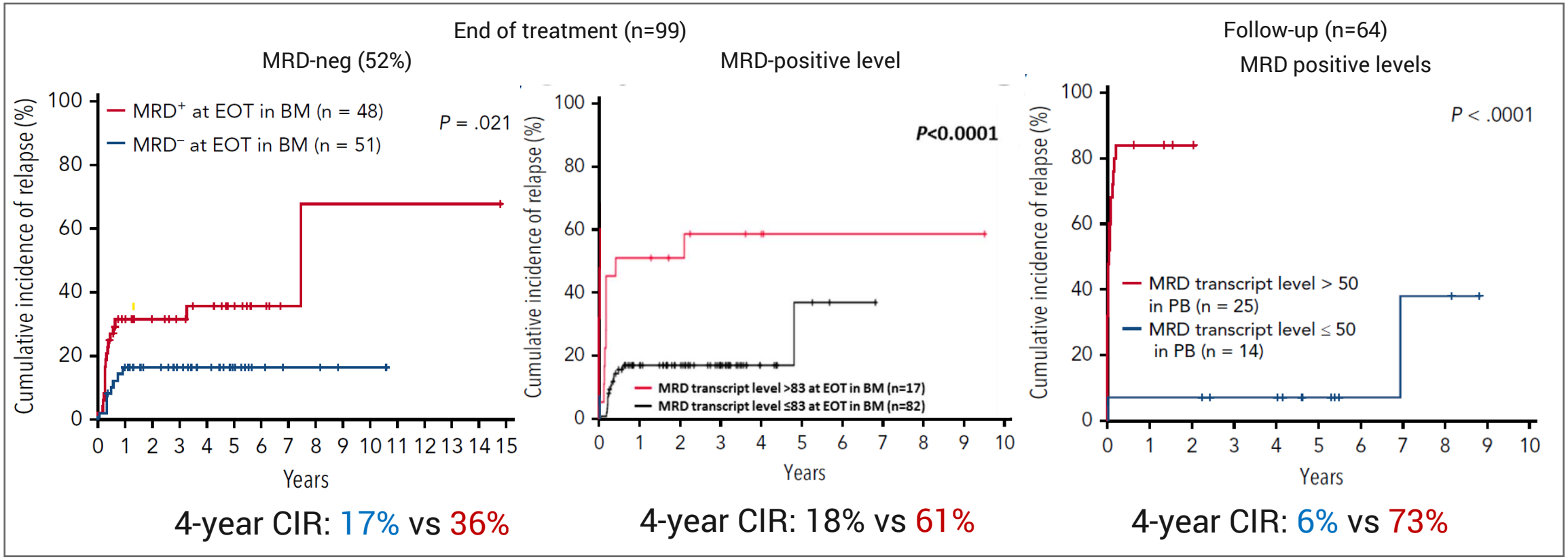


- NPM1 MRD negativity in blood after induction show no survival benefit from SCT
- FLT3-ITD MRD levels correlate with RFS post SCT with gilteritinib maintenance

Othman, et al, Blood, 2024; Levis, et al, Blood 2024

Clinical Need and Benefits

t(8;21) AML, RUNX1-RUNX1T1 MRD by RT-qPCR in blood



Rücker, et al, Blood, 2019

Molecular techniques for AML MRD tests

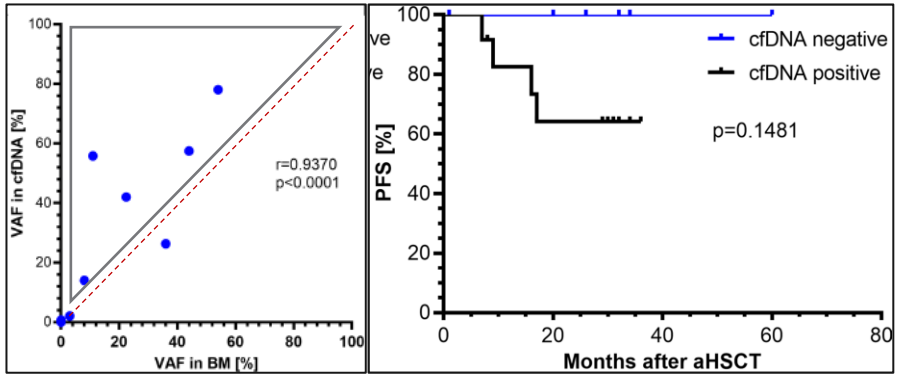
- RT-qPCR
- Digital PCR
  - » Droplet digital PCR
  - » Nanoplate digital PCR
  - » Others
- Error-corrected NGS (reduce background noise)

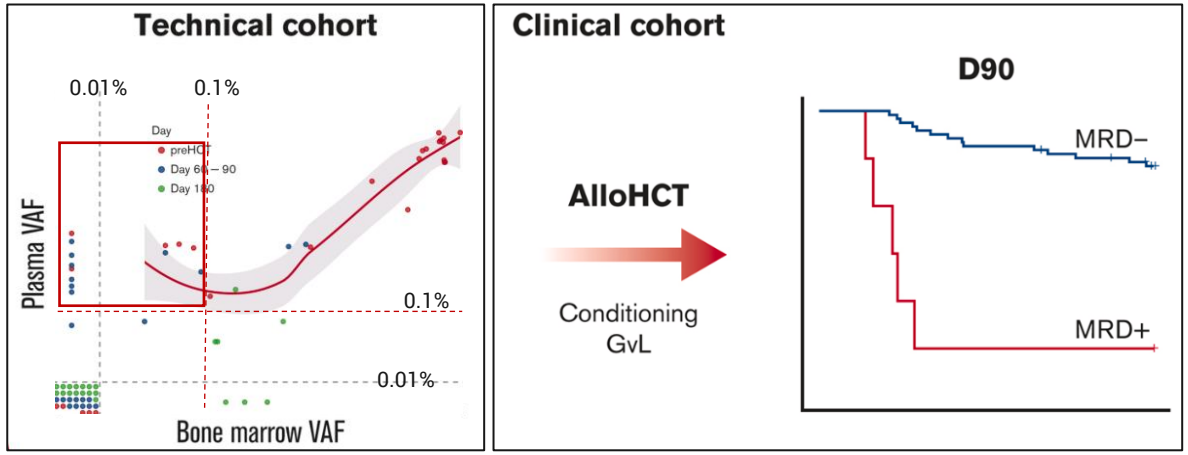
Sensitivity/specificity, Pros and cons

Feature	RT-qPCR	Digital PCR (such as ddPCR)	Error-Corrected NGS
Sensitivity	~10^{-4} to 10^{-5}	~10^{-4} to 10^{-5}	~10^{-4} to 10^{-6}
Specificity	High for known fusion transcripts	Very high for known mutations	High, especially with error correction
Targets per assay	Limited to known variants (e.g., NPM1, CBF)	1–2 mutations per patient	Multiple variants cross all AML genotypes
Quantification	Relative quantification	Absolute quantification	Quantification (VAF)
Turnaround time	Fast (hours)	Moderate (1–2 days)	Longer (several days)
Cost	Low	Moderate	High
Clinical utility	Established for NPM1, CBF fusions	Emerging, prognostic value confirmed	Predictive of relapse-free survival (RFS)
Advantages	Widely available, standardized	High sensitivity, absolute quantification	Tracks clonal evolution, multiple mutations
Limitations	Not applicable to all AML genotypes	Limited to specific mutations	Requires high-quality DNA, complex bioinformatics

Samples for AML MRD tests

- Bone marrow aspirate, preferred
  - » Only 5 mL of BM aspirate should be used for molecular MRD
  - » First pull in EDTA or heparin
  - » The method of cell/DNA isolation should be kept consistent
- Peripheral blood
  - » Not inferior to bone marrow aspirate for long term monitor
- Cell free DNA (when VAF < 0.2%)
  - » Has the potential to outperform bone marrow aspirate
    - Sampling issue – better represents the tumor burden
    - Neoplastic cell enriched – faster turnover
    - Extramedullary disease



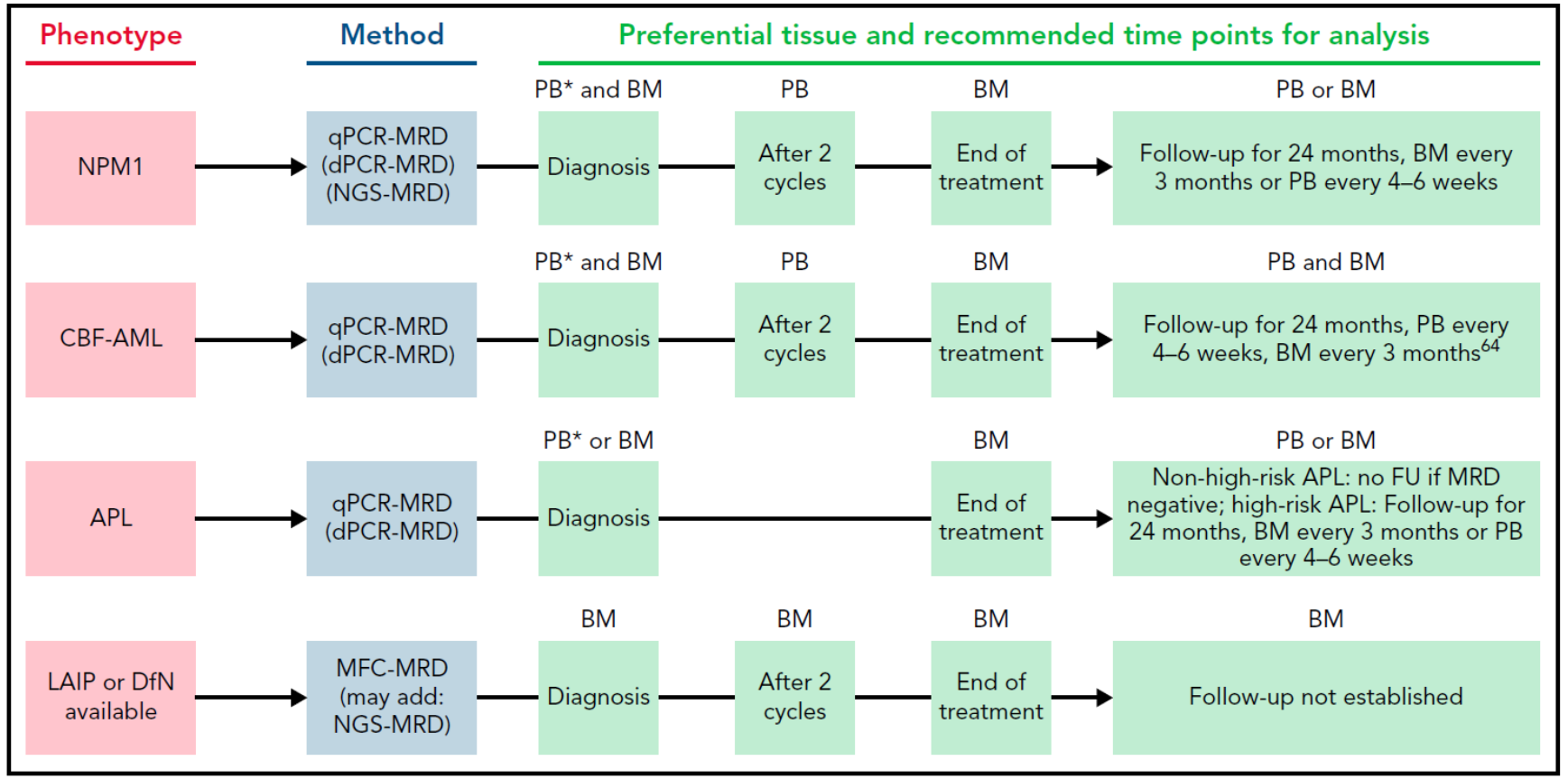


Pasca, et al, Blood Advances, 2023; Sommer, Scientific Reports, 2025

Outline

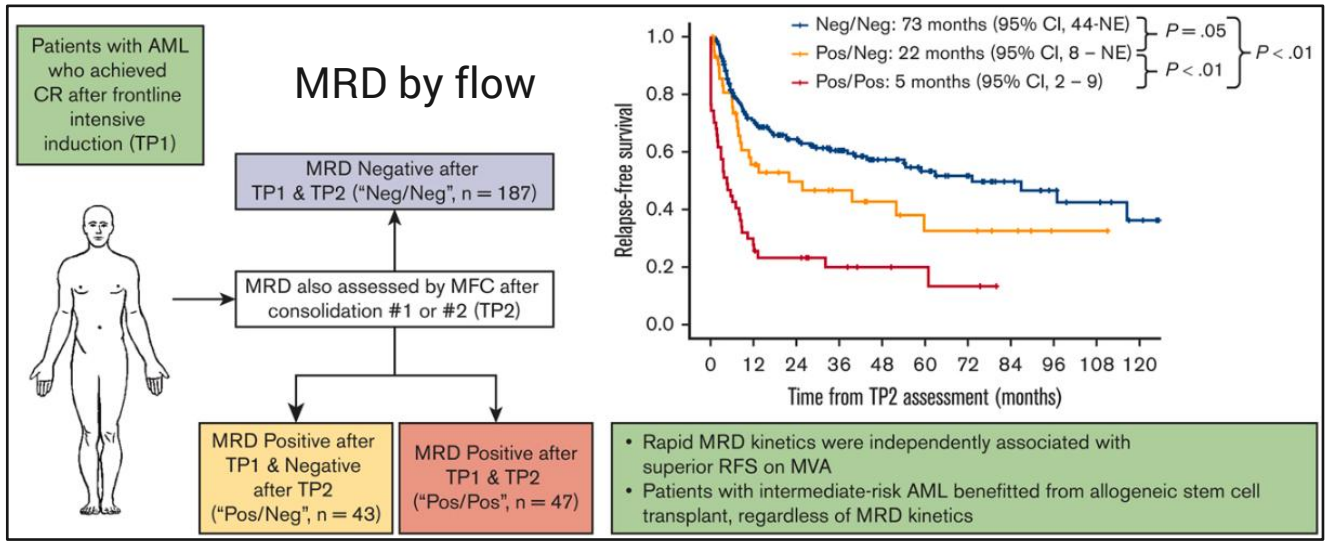
- Learning objectives
- Clinical background of acute myeloid leukemia (AML)
- Define minimal (measurable) residual disease (MRD) in AML
- Clinical need and benefits of MRD testing in AML
- Available MRD testing methodologies
- Appropriate timing for MRD testing
- Determine which patient populations should be tested
- Future directions and innovations in AML MRD testing

When to Test


Phenotype
Method
Preferential tissue and recommended time points for analysis
PB* and BM
PB
BM
PB or BM
NPM1
qPCR-MRD (dPCR-MRD) (NGS-MRD)
Diagnosis
After 2 cycles
End of treatment
Follow-up for 24 months, BM every 3 months or PB every 4–6 weeks
PB* and BM
PB
BM
PB and BM
CBF-AML
qPCR-MRD (dPCR-MRD)
Diagnosis
After 2 cycles
End of treatment
Follow-up for 24 months, PB every 4–6 weeks, BM every 3 months[64]
PB* or BM
BM
PB or BM
APL
qPCR-MRD (dPCR-MRD)
Diagnosis
End of treatment
Non-high-risk APL: no FU if MRD negative; high-risk APL: Follow-up for 24 months, BM every 3 months or PB every 4–6 weeks
BM
BM
BM
BM
LAIP or DfN available
MFC-MRD (may add: NGS-MRD)
Diagnosis
After 2 cycles
End of treatment
Follow-up not established

When to Test

- Apply MRD tests at baseline to acquire the MRD signature
- Incorporate MRD/kinetics to risk assessment for treatment decisions
- Monitoring tool for relapse
- Surrogate endpoint for clinical trials
- First remission prior to transplant
- Utility of MRD testing in secondary AML



Jen, et al, Blood Advances, 2025

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Future directions

- New technology
  - » Increase test sensitivity and specificity
  - » Less invasive monitoring
    - Sample type: cell free DNA
    - Digital PCR and NGS based testing
    - Combine flow and molecular testing
- Standardized test platform and threshold
- MRD-guided therapy

Future directions – Standardization

- Standardized test thresholds
- Reference materials – BCR-ABL1 for CML patients as a gold standard
  - » Peripheral blood
  - » Bone marrow
  - » Cell free DNA
- Harmonize the molecular platforms

Future directions – MRD guided therapy

- MRD-guided therapy
  - » Monitoring subclinical disease
  - » Detection of early relapse - pre-emptive interventions
  - » Consolidation strategies
  - » Transplant conditioning

Panel Discussion

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