



Francesco Buccisano

MEASURABLE RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA, CONSENSUS DOCUMENT OF EUROPEAN LEUKEMIANET

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Conflict of Interest Disclosure

In accordance with criterion 24 of document UEMS 2012/30 "Accreditation of Live Educational Events by the EACCME®" we herewith declare to have submitted a Conflict of Interest Disclosure Form to ESCCA.

This COI Disclosure Form can be viewed at the ESCCA 2019 Conference website www.escca.eu/norway2019 - Programme section / Accreditation page

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European LeukemiaNet recommendations for AML

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Diagnosis and management of adult AML: 2017 ELN recommendations by international expert panel

Category	Definition	Comment
Response		
CR1 without minimal residual disease (CR1 _{MRD} -)	If studied protocol, CR1 with negativity for a genetic marker by RT-qPCR or CR1 with negativity by MRD	Complete remission (CR) by marker testing, not by method MRD methods, test used and sensitivity of the assay should be reported. CR1s should be done Immunophenotypic (immunofluorescence) diagnosis
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC $\geq 3 \times 10^9/L$ (1000/L); platelet count $\geq 100 \times 10^9/L$ (100000/L)	MRD ¹ or unknown

ELN Recommendations for AML, treatment, Dohner H, Blood 2017



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ELN MRD recommendation expert panel

Flow MRD

- Gerrit Jan Schuurhuis
- Paresh Vyas
- Brent Wood
- Wolfgang Kern
- Luca Maurillo
- Claude Preud'homme
- Francesco Buccisano
- Jeffrey Jorgensen
- Jacqueline Cloos
- Marie-Christine Bene
- Sylvie Freeman

Molecular MRD

- Christian Thiede
- Bert van de Reijden
- Michael Heuser
- Konstanze Döhner
- Torsten Haferlach

Clinical MRD

- Gert Ossenkoppele
- Robert Hills
- Gail Roboz
- Roland Walter
- Adriano Venditti

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Standardization or Harmonization?

- **Standardization:** is the process of implementing and developing technical standards based on the consensus of different parties (firms, users, interest groups, governments, standards organizations). **Output is a SOP or a guideline.**
- **Harmonization:** is the process of coordinating different systems by eliminating major differences and creating minimum requirements of standards. **Output is a recommendation.**

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Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party

Gerrit J. Schuurhuis,¹ Michael Heuser,^{2*} Sylvie Freeman,^{3*} Marie-Christine Béné,⁴ Francesco Buccisano,⁵ Jacqueline Cloos,^{1,6} David Grimwade,⁷ Torsten Haflerlach,⁸ Robert K. Hills,⁹ Christopher S. Hourigan,¹⁰ Jeffrey L. Jorgensen,¹¹ Wolfgang Kern,⁷ Francis Lacombe,¹² Luca Mailliard,¹³ Claude Preussmann,¹⁴ Bert A. van der Reijden,¹⁵ Christian Theede,¹⁶ Adriano Venditti,¹⁷ Paweł Wjś,¹⁸ Brent L. Wood,^{19,20} Roland B. Walter,^{21,22} Konstanze Döhner,^{23,24} Gail J. Roboz,²⁵ and Gert J. Ossenkoppele¹

- There are several reasons to apply MRD detection in AML
 - To provide an objective methodology to establish a deeper remission status,
 - To refine outcome prediction and inform post-remission treatment,
 - To identify impending relapse and enable early intervention in the post-treatment (e.g. post-transplant) phase,
 - To use as a surrogate endpoint to accelerate drug testing and approval.

Schuurhuis GJ, Blood 2018 

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General principles for clinical practice

- Should be monitored using RT-PCR
 - Acute Promyelocytic Leukemia
 - Core-binding factor AML
 - AML with NPM1 mutation
- Use flow cytometry for MRD assessment
 - AML subgroups NOT including APL, CBF AML, and AML with NPM1 mutation

Schuurhuis GJ, Blood 2018 

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Outline

- Technical requirements
- Approach for MRD detection
 - Markers selection
 - Gating strategies (Next presentation)
 - Timepoints, thresholds
 - Rare events analysis requirements (B. Brando, PAR08, this morning)
- MRD reporting
- Clinical studies and surrogacy

Technical requirements

- Bone marrow (BM) sampling
 - It is strongly recommend to **submit the first BM pull for MRD analysis**
 - It is recommended to **estimate the possible contamination with PB**
- BM transport
 - Transport at controlled room temperature.
 - **Up to 3 days storage is allowed**, without the need for a viability marker, provided BM is stored undiluted.
- Flow cytometers
 - **Harmonization of instrument settings** is of high value for interlaboratory comparison of results.
- Preparation of samples
 - **Stain/lyse/wash (or no wash)** has the advantage of reducing cell losses;
 - **Bulk lysis** followed by washing and staining (and washing) has the advantage of having all tubes prepared in a similar way for the different staining steps.
 - Both approaches are in use for AML MRD assays.

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Estimate of the possible contamination with PB

- It is recommended to estimate the possible contamination with PB, the presence of >90% mature neutrophils in a BM sample indicating significant hemodilution.

1. Lukens MR, Chu S-C, Fritsche W, Kahooski M, Wells DA. Normalization of bone marrow aspirates for hemodilution in flow cytometric analyses. *Cytometry B Clin Cytom.* 2009; 76B(1):27-36.
2. Delgado JA, Giller N, Gómez F, Moreno C, et al. A simple flow-cytometry method to evaluate peripheral blood contamination of bone marrow aspirates. *J Immunol Methods.* 2017; 442:54-68.
3. Brostrom RA, Kiran J, van Putten W, Cornelissen JJ, Lo Verberg B, Galatone JW. Flow cytometric differential of leukocyte populations in normal bone marrow: influence of peripheral blood contamination. *Cytometry B Clin Cytom.* 2009; 76B(1):18-26.
4. Aldamood AM, Kinkade Z, Rosado FG, Egan CA, Gibson LF, Vora JA. A novel method to assess bone marrow purity is useful in determining blast percentage by flow cytometry in acute myeloid leukemia and myelodysplasia. *Ann Hematol Oncol.* 2015; 10:1038.
5. Nombela-Arieta C, Manz MG. Quantification and three-dimensional microanatomical organization of the bone marrow. *Blood Adv.* 2017; 1(6):1407-1416.

Schuurhuis GJ, Blood 2018 

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Defining a Sample as UNSUITABLE for MRD Analysis

- When an excess peripheral blood contamination is observed (i.e. if CD16+ mature myeloid cells are >20%, or if the presence of more than 90% mature neutrophils)
- When red cell precursors are overall <10-15 %.
- When B-Lymphoid progenitors are absent or <1-2%.
- When normal PCs are undetectable
- When mast cells $\leq 0.002\%$

J Flores-Montero Leukemia [2017] 31, 2094–2103

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Markers for MRD assessment

- **Number and nature of fluorochromes**
 - Taking advantage of **extensive validation studies** as done, for example, by the Euroflow consortium
- **Panel content**
 - **Gating** on CD45, sideward scatter (SSC), forward scatter (FSC), a primitive marker (CD34, CD117), and abnormal expression of marker(s) or abnormal combination(s) of marker expression.
 - **Monocytic combination**, including CD64, CD11b and CD4 is proposed
 - **Leukemic Stem Cell analysis** requires a dedicated approach

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About LAIPs

- Types of LAIPs
 - With stem cell markers
 - Cross lineage
 - Asynchronous (including under-expression)
 - Without stem cell markers
- Quality of LAIPs
 - Sensitivity (% coverage of blast compartment)
 - Specificity (LAP expression in normal and regenerating bone marrow)
 - Stability (immunophenotypic shifts)

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LAIP absent or present at very low frequency in normal or post-chemotherapy BM

- Multiparameter four- and six-color flow cytometry test
 - normal individuals (n° 20)
 - patients receiving chemotherapy for acute lymphoblastic leukemia (n° 20)
 - Patients with AML (n° 53)
- In six-color panel
 - 47 phenotypes were totally absent
 - 41 phenotypes were identified in less than 0.05% of blast cells
- In four-color panel
 - 30 phenotypes present at a frequency <0.05%.

Olaru et al., Cytometry 2008

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Consensus on markers in ELN

- 7/7 for 7 markers: **CD7, CD13, CD15, CD33, CD34, CD45, CD117**
- 6/7: for 4 markers: **CD14, CD19, CD56, HLA-DR**
- 4/7: for 5 markers: **CD2, CD4, CD11b, CD38, CD64**
- 2/7 for 4 markers: **CD16, CD36, CD123, CD133**
- 1/7: for 5 marker: **CD5, CD22, CD25, CD65, CD71**

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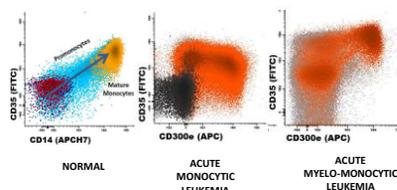
FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Horizon V450	Horizon V500
CD64	CD11b	CD14	CD4	CD34	HLADR	CD33	CD45

	Normal BM	Post-CT BM median (range)
14+ DR+ 4+ 11B+ 64-	<0.01	<0.01
14+ DR+ 4- 11B- 64-	<0.01	<0.01 (0-0.03)
14+ DR+ 4- 11B- 64-	<0.01	<0.01 (0-0.02)
14+ DR- 4+ 11B+ 64+	<0.01	<0.01
14+ DR- 4+ 11B+ 64-	<0.01	<0.01
14+ DR- 4- 11B- 64-	<0.01	<0.01
14+ DR- 4- 11B- 64-	<0.01	<0.01
14- DR+ 4+ 11B+ 64+	<0.01	<0.01 (0-0.03)
14- DR+ 4+ 11B+ 64+	<0.01	<0.01
14- DR- 4+ 11B+ 64+	<0.01	<0.01
14- DR- 4+ 11B+ 64-	<0.01	<0.01

Olaru et al., Cytometry 2008

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Patterns of monocytic aberrancies



ESCCA/CCS/ELN MonoCon committee kick-off in Bergen

Matarraz S, Cytometry Part B, 2017

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Gating strategies for MFC MRD assessment (LAIP vs different from normal)

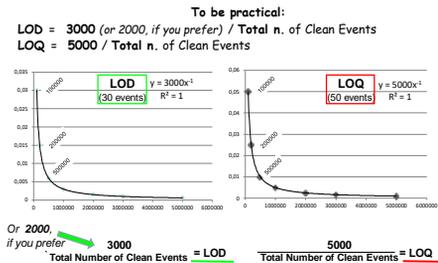
- LAIPs are DfN abnormalities in the vast majority of cases, and the difference between these two approaches is likely to disappear if an adapted, sufficiently large panel of antibodies (preferably ≥ 8 colors) is utilized.
- We recommend that the advantages of both approaches be combined to best define MFC MRD burden, allowing detection of new aberrancies emerging at follow-up, and monitoring patients when there is an absence of diagnostic information.
- New definition of "LAIP-based DfN approach"

Focus on gating strategies coming on

Timepoints, thresholds and denominator cell number target for MRD assessment

- MRD is to be used for risk analysis at an early time point (after 1-2 cycles),
- The suggested threshold to assess a positive/negative status is 0.1% (10⁻³),
- The minimum number of cells needed for accurate reporting of MRD is 500,000 – 1,000,000, excluding all CD45-negative cells and debris,
 - Lower cell numbers may still suffice if the level of MRD is relatively high,
 - Higher numbers enable to assess possible MRD below the level of 0.1%.

Estimated LOQ and LOQ According to the Total Number of Cells Acquired

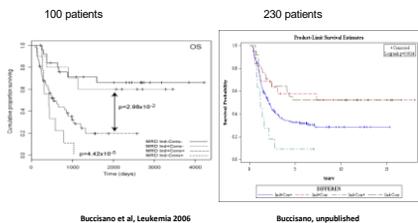


Courtesy of Bruno Brando

Timepoint determination in MRD studies

- Early Timepoints
 - Fast treatment allocation (e.g. allogeneic transplant for high-risk patients)
 - Caveat:
 - Overtreatment of slow responders
- Late Timepoints
 - No over-treatment of slow responders
 - Caveat:
 - Longer time to transplant delivery (e.g. alternative donors)
 - Relapses before transplant delivery

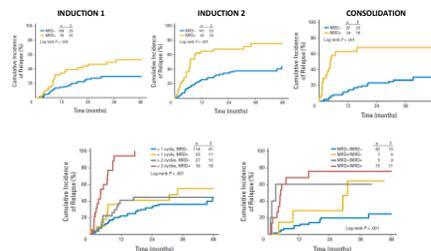
Optimal time-points are those functioning to the global treatment strategy



Buccisano et al, Leukemia 2006

Buccisano, unpublished

HOVON/SAKK 42a

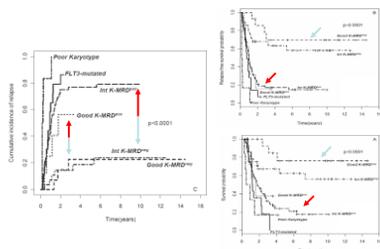


Terwijn M, JCO 2013

Threshold determination in MRD studies

- Threshold selection is affected by a number of technical and clinical factors
 - technical threshold vs. clinical threshold
- We recommend the use of a dedicated statistical approach
 - ROC analysis, Maximally selected log-rank statistics
- The reproducibility of that specific threshold throughout different laboratories still remains subject of debate
 - Particular cut-off which different laboratories can refer to
 - Should any laboratory set up its own?

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Threshold of positivity:
0.035% leukemic cells post consolidation cycle

Buccisano et al. Blood 2010

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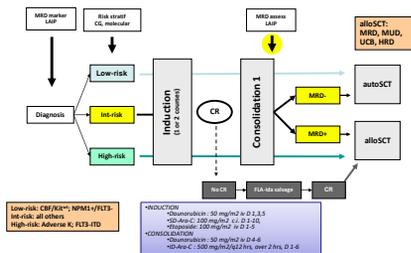
Validation of MRD-tailored therapy

- What do we need to tailor therapy on a biomarker (MRD):
 - Measurable biological or clinical characteristics
 - Well documented risk categories
 - Robust retrospective validation
 - Prospective randomized studies showing benefits of tailoring

INFORMED DECISIONS IN ACUTE MYELOID LEUKEMIA: BEYOND MOLECULAR AND CYTOGENETICS |
MRD in AML: does it already guide therapy decision-making?
 Gert Ossenkoppele and Gerrit Jan Schuurhuis
 Department of Hematology, KU University Medical Center Amsterdam, Amsterdam, The Netherlands

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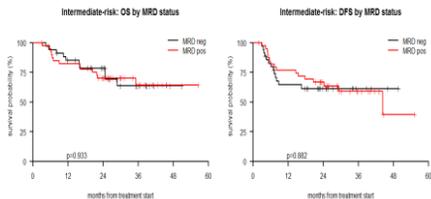
AML1310 – Schedule



AMERICAN SOCIETY OF CLONING

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AML1310: intermediate-risk OS and DFS by MRD status



Fondazione CIMEMA
 Comitato di Studio e Ricerca
 Centro Nazionale di Riferimento per le Leucemie Mieloidi Acute e Mielodisplasie

Venditti et al., Blood 2019

Key features of MRD report

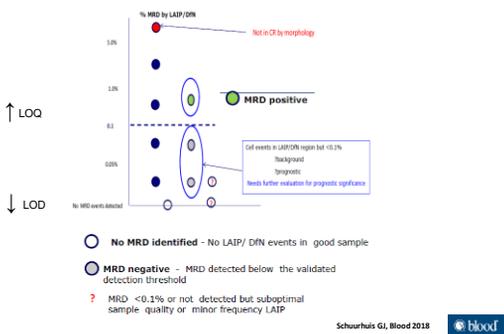
- Detection sensitivity threshold (LOD)
 - If known should be specified for the aberrancy used
- Denominator population (mononuclear cells, leukocytes, CD45 positive events, etc.)
- Comments on quality of the sample, including total blast percentage, poor viability, insufficient regeneration, and peripheral blood contamination
- Reports on MRD status should be constructed to allow the clinicians to draw clear conclusions about how to interpret the report
 - MRD positive vs. MRD negative, crude percentage, reference protocol.

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Design of MRD studies: multicenter vs single center approaches

- For multicenter studies samples may be processed by different centers applying the same MRD panels, according to the ELN recommendations.
 - With insufficient experience in MRD analysis, the final interpretation should be performed at a central institute or in a group workshop.
- Alternatively, samples may be sent under carefully controlled conditions to a central institute for workup and analysis.
- Single center studies without relevant experience are strongly discouraged.

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Use of MRD as a surrogate endpoint for survival to accelerate drug approval

- If MRD negativity is established as a surrogate endpoint for survival, it is likely to be helpful for the evaluation of new drugs,
 - possibly accelerating drug approval or,
 - stopping development of suboptimal drugs or treatment strategies
- This task will be accomplished only when prospective, randomized trials evaluating MRD driven therapy-interventions combined with survival end-points within homogeneous subgroups of non-APL AML, will be completed.

Schuurhuis GI, Blood 2018

Hourigan CS, Leukemia 2017

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FDA caveat on MRD in AML

- The molecular heterogeneity of AML poses substantial challenges to use of MRD as a biomarker.**
 - For the marker (e.g., cell surface or genetic mutation) selected to assess MRD, the sponsor should provide data showing that the marker reflects the leukemia and not underlying clonal hematopoiesis (false positive result).
 - The sponsor should also describe the false-negative rate that might result from relapse from a marker-negative clone.
 - If multiple markers and/or multiple platforms are used, the sponsor should provide an analysis of the risk of false-positive and false-negative results for each marker individually and for the panel as a whole.

<https://www.fda.gov/downloads/drugs/developmental/ucm070107.pdf>

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Conclusions

- MRD is a biomarker measuring the quality of morphological CR.
- Regardless of the technique employed, MRD confers a negative prognosis that is comparable to the one associated with morphological persisting leukemia.
- Multiparametric flow cytometry (MFC) and real-time quantitative PCR (RT-qPCR) are the leading techniques for MRD monitoring.
- MFC should be employed in AML subgroups NOT including APL, CBF AML, and AML with NPM1 mutation
- Standardization/harmonization process is underway.

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