MRD applications in hemato-oncology: ELN 2025 guidelines

Workshop 3.2. MB&C26 meeting, UCLL

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ELN-DAVID

ELN Foundation
European LeukemiaNet

MRD ASSESSMENT and VALIDATION in AML

distinct applications? **systematically combined?**

Review Article

blood

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

Hartmut Döhner,[1] Elihu Estey,[2] David Grimwade,[3] Sergio Amadori,[4] Frederick R. Appelbaum,[2] Thomas Büchner,[5] Hervé Dombret,[6] Benjamin L. Ebert,[7] Pierre Fenaux,[8] Richard A. Larson,[9] Ross L. Levine,[10] Francesco Lo-Coco,[4] Tomoki Naoe,[11] Dietger Niederwieser,[12] Gert J. Ossenkoppele,[13] Miguel Sanz,[14] Jorge Sierra,[15] Martin S. Tallman,[10] Hwei-Fang Tien,[16] Andrew H. Wei,[17,18] Bob Löwenberg,[19] and Clara D. Bloomfield[20]

blood

Special Report

Check for updates

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

Michael Heuser,[1] Sylvie D. Freeman,[2] Gert J. Ossenkoppele,[3] Francesco Buccisano,[4] Christopher S. Hourigan,[5] Lok Lam Ngai,[3] Jesse M. Tettero,[3] Costa Bachas,[3] Constance Baer,[6] Marie-Christine Béné,[7] Veit Bücklein,[8] Anna Czyz,[9] Barbara Denys,[10] Richard Dillon,[11] Michaela Feuring-Buske,[12] Monica L. Guzman,[13] Torsten Haferlach,[6] Lina Han,[14] Julia K. Herzig,[12] Jeffrey L. Jorgensen,[15] Wolfgang Kern,[6] Marina Y. Konopleva,[14] Francis Lacombe,[16] Marta Libura,[17] Agata Majchrzak,[18] Luca Maurillo,[4] Yishai Ofran,[19] Jan Philippe,[10] Adriana Plesa,[20] Claude Preudhomme,[21] Farhad Ravandi,[14] Christophe Roumier,[21] Marion Subklewe,[8] Felicitas Thol,[1] Arjan A. van de Loosdrecht,[3] Bert A. van der Reijden,[22] Adriano Venditti,[4] Agnieszka Wierzbowska,[23] Peter J. M. Valk,[24] Brent L. Wood,[25] Roland B. Walter,[26] Christian Thiede,[27,28] Konstanze Döhner,[12] Gail J. Roboz,[13] and Jacqueline Cloos[3]

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MRD ASSESSMENT and VALIDATION in AML

Process optimization

Blood. 2025 Dec 15:blood.2025031480. doi: 10.1182/blood.2025031480. Online ahead of print.

2025 Update on MRD in Acute Myeloid Leukemia: A Consensus Document from the ELN-DAVID MRD Working Party

Jacqueline Cloos [1], Peter J M Valk [2], Christian Thiede [3], Konstanze Döhner [4], Gail J Roboz [5], Brent L Wood [6], Roland B Walter [7], Sa A Wang [8], Agnieszka Wierzbowska [9], Andrew H Wei [10], David Wu [10], Francois Vergez [11], Adriano Venditti [12], Bert A van der Reijden [13], Arjan A van de Loosdrecht [14], Ing Soo Tiong [15], Felicitas R Thol [16], Marion Subklewe [17], Christophe Roumier [18], Tom Reuvekamp [19], Farhad Ravandi [20], Claude Preudhomme [21], Adriana Plesa [22], Jad Othman [23], Gert J Ossenkoppele [24], Yishai Ofran [25], Aguirre Mimoun [26], Luca Maurillo [27], Agata Majchrzak [28], David C de Leeuw [29], Wolfgang Kern [30], Dennis Dong Hwan Dong Hwan Kim [31], Maura Rosane Valério Ikoma-Colturato [32], Lukas H Haaksma [24], Monica L Guzman [5], Michaela Feuring [33], Barbara Depreter [34], Anna Czyz [35], Veit L Bücklein [17], Constance Baer [36], Costa Bachas [37], Sylvie D Freeman [38], Francesco Buccisano [39], Christopher S Hourigan [40], Richard James Dillon [41], Michael Heuser [42]

Introduction

Key updates of the revised AML MRD recommendations

2025 Update on MRD in Acute Myeloid Leukemia: A Consensus Document from the ELN–DAVID MRD Working Party

56 recommendations
- 22 on clinical implementation
- 15 on MFC-MRD
- 19 on molecular MRD
- /

Only six recommendations remain unchanged from 2021

Aligns MRD assessment explicitly with the ELN 2022 genetic risk classification, moving beyond methodological standardisation

Integrated clinical framework by strongly embedding MRD into treatment decision-making

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

59 recommendations
- 29 on clinical implementation (8 and 8a)
- 12 on MFC-MRD
- 14 on molecular MRD
- 4 on future improvement

Methodological and technology-centred consensus on MRD

No concrete decision pathways based on MRD

Key updates of the revised AML MRD recommendations

1) Detailed MRD guidance is tailored to each (genetic) subgroup, aligned with the **ELN 2022 risk classification**, for each treatment-deciding timepoint in adults receiving intensive chemotherapy

! Does **not** include guidance on MRD assessment in **non-intensively** treated patients

Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN

Hartmut Döhner,[1] Andrew H. Wei,[2] Frederick R. Appelbaum,[3] Charles Craddock,[4] Courtney D. DiNardo,[5] Hervé Dombret,[6] Benjamin L. Ebert,[7] Pierre Fenaux,[8] Lucy A. Godley,[9] Robert P. Hasserjian,[10] Richard A. Larson,[11] Ross L. Levine,[12] Yasushi Miyazaki,[13] Dietger Niederwieser,[14] Gert Ossenkoppele,[15] Christoph Röllig,[16] Jorge Sierra,[17] Eytan M. Stein,[18] Martin S. Tallman,[18] Hwei-Fang Tien,[19] Jianxiang Wang,[20] Agnieszka Wierzbowska,[21] and Bob Löwenberg[22]

Genetic risk classification for adults with AML receiving less-intensive therapies: the 2024 ELN recommendations

Hartmut Döhner,[1] Courtney D. DiNardo,[2] Frederick R. Appelbaum,[3] Charles Craddock,[4] Hervé Dombret,[5] Benjamin L. Ebert,[6] Pierre Fenaux,[7] Lucy A. Godley,[8] Robert P. Hasserjian,[9] Richard A. Larson,[10] Ross L. Levine,[11] Yasushi Miyazaki,[12] Dietger Niederwieser,[13] Gert Ossenkoppele,[14] Christoph Röllig,[15] Jorge Sierra,[16] Eytan M. Stein,[11] Martin S. Tallman,[11] Hwei-Fang Tien,[17] Jianxiang Wang,[18] Agnieszka Wierzbowska,[19] Andrew H. Wei,[20] and Bob Löwenberg[21]

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RESEARCH ARTICLE

AJH WILEY

Measurable residual disease monitoring in patients with acute myeloid leukemia treated with lower-intensity therapy: Roadmap from an ELN-DAVID expert panel

Key updates of the revised AML MRD recommendations

ELN 2022 favorable risk

Risk Category	Genetic Abnormality
Favorable	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 bZIP in-frame mutated *CEBPA*

ELN risk	Subgroup	After 2 cycles induction or pre-alloHCT	End of treatment (EOT: after consolidation/after alloHCT)	Follow-up
Favorable	mut*NPM1* without *FLT3*-ITD	PB	BM	PB or BM[2]
	RUNX1::RUNX1T1	BM	BM	PB or BM[4]
	CBFB::MYH11	BM	BM	PB or BM[4]
	PML::RARA	Not recommended	BM[5]	Only if MRD-positive at EOT, and in chemotherapy-treated high-risk APL even when MRD-negative; BM[1,5]
	CEBPA bZIP in-frame	Some evidence	Future research needed	Future research needed

Adapted version of Table 2. of the original ELN 2025 update: Recommended MRD assay per ELN risk group and per mutational status. Cloos, J., et al. (2025). Blood.

Key updates of the revised AML MRD recommendations

ELN 2022 intermediate risk

Intermediate	Mutated *NPM1* with *FLT3*-ITD Wild-type *NPM1* with *FLT3*-ITD t (9;11) (p21.3;q23.3); *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favorable or adverse

ELN risk	Subgroup	After 2 cycles induction or pre-alloHCT	End of treatment (EOT: after consolidation/after alloHCT)	Follow-up
Intermediate	*FLT3*-ITD and *NPM1*wt	*FLT3*-ITD UHS-NGS: BM>PB	*FLT3*-ITD UHS-NGS[4]: BM>PB	*FLT3*-ITD UHS-NGS[6]: PB or BM[1]
		MFC: BM	MFC: BM	MFC: BM (q3 months for 12 months)
	FLT3-ITD and mut*NPM1*	mut*NPM1*-qPCR: PB	mut*NPM1*-qPCR: BM	mut*NPM1*-qPCR: PB or BM[1]
		FLT3-ITD UHS-NGS:BM>PB	*FLT3*-ITD UHS-NGS[6] BM>PB	*FLT3*-ITD UHS-NGS[6]: PB or BM[1]
	KMT2A::MLLT3, other fusion genes[7]	qPCR and MFC: BM	qPCR and MFC: BM	qPCR: PB or BM[1]
				MFC: BM (q3 months for 12 months)
	Other (with LAIP/DfN)	BM	BM	BM (q3 months for 12 months)
	Other (with gene mutation)	Cut-offs, time points and sample matrices for UHS-NGS of gene mutations should be further validated gene by gene		After alloHCT (only validated genes)[8] BM>PB[1,9]

Adapted version of Table 2. of the original ELN 2025 update: Recommended MRD assay per ELN risk group and per mutational status. Cloos, J., et al. (2025). Blood.

Key updates of the revised AML MRD recommendations

ELN 2022 adverse risk

Adverse	t (6;9) (p23;q34.1); *DEK-NUP214* t (v;11q23.3); *KMT2A rearranged* t (9;22) (q34.1;q11.2); *BCR-ABL1* inv(3) (q21.3q26.2) or t (3;3) (q21.3;q26.2); *GATA2, MECOM(EVI1)* t (3q26.2;v); *MECOM (EVI1)*-rearranged −5 or del (5q); −7; −17/abn (17p) Complex karyotype, monosomal karyotype Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1*, or *ZRSR2* Mutated *TP53*

ELN risk	Subgroup	After 2 cycles induction or pre-alloHCT	End of treatment (EOT: after consolidation/after alloHCT)	Follow-up
Adverse	Fusion genes (e.g. *KMT2A::X*)	qPCR and MFC: BM	qPCR and MFC: BM	qPCR: see section on future research
				MFC: BM (q3 months for 12 months)
	Other (with LAIP/DfN)	BM	BM	BM (q3 months for 12 months)
	Other (with gene mutation)[10]	Cut-offs, time points and sample matrices for UHS-NGS of gene mutations should be further validated gene by gene		After alloHCT (only validated genes)[8] BM>PB[1,9]

Adapted version of Table 2. of the original ELN 2025 update: Recommended MRD assay per ELN risk group and per mutational status. Cloos, J., et al. (2025). Blood.

Key updates of the revised AML MRD recommendations

2) Every **MRD answer**, regardless of the technique, should be **three-tiered**:
   - MRD%
   - MRD burden (categoric variable)
     - Negative
     - Low-level positive (MRD-LL): detectable, prognostic?
     - Positive
   - Qualitative MRD response (contextual)
     ! CML model
     - Optimal
     - Warning
     - High risk of treatment failure
       or
     - MRD relapse

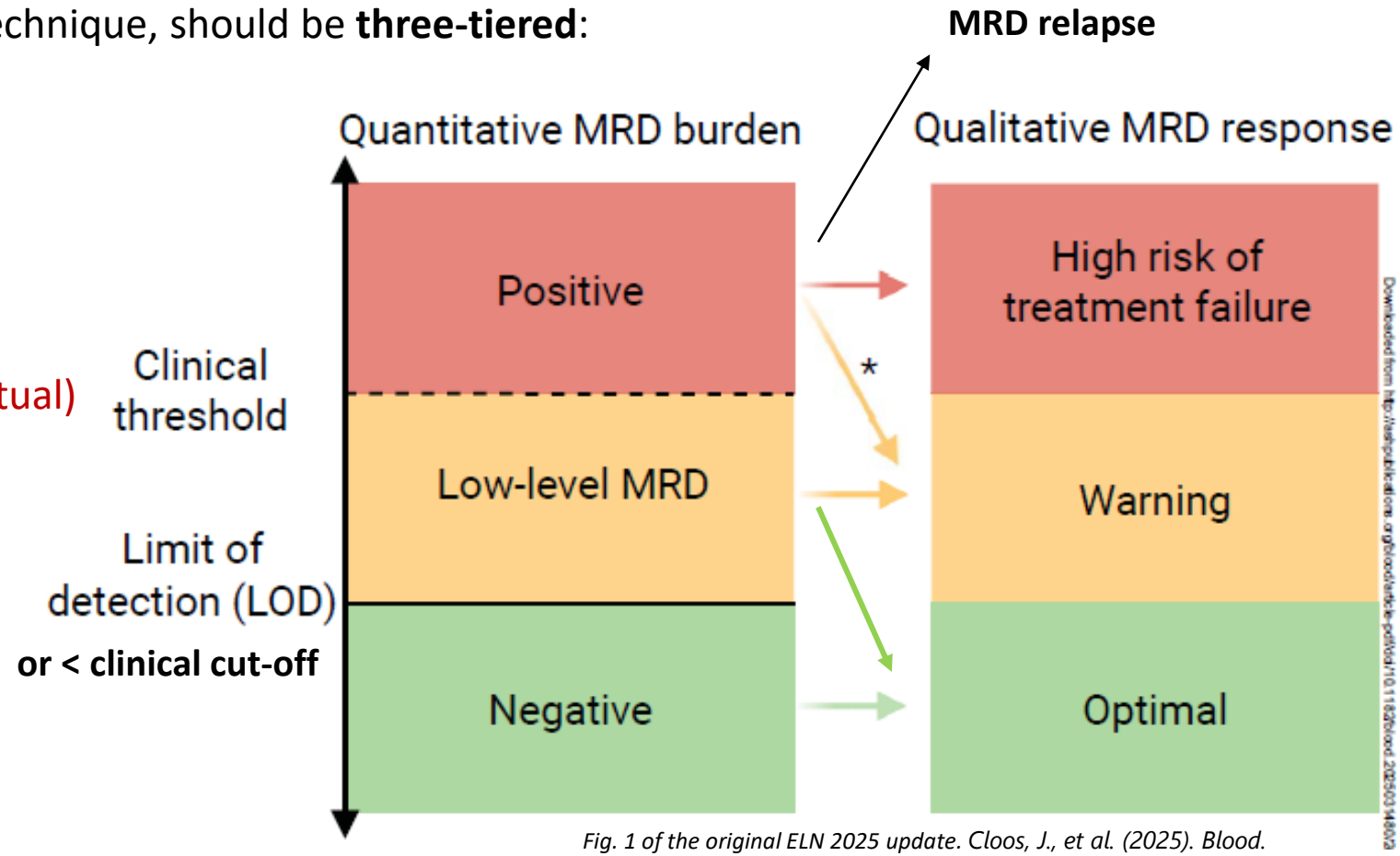

Fig. 1 of the original ELN 2025 update. Cloos, J., et al. (2025). Blood.

High flexibility in interpretation based on AML subtype, timepoint, and sample matrix

Key updates of the revised AML MRD recommendations

3) No one-fits-all-principle: **thresholds for MRD positivity** and **LOD** differ between **targets, assays, sample matrices and timepoints**

- Timepoints:
  - After induction/pre-alloHCT
  - After consolidation/after alloHCT
  - Follow-up
- Sample matrix
  - PB
  - BM
  - PB or BM
- Assay
  - Multicolour flowcytometry (MFC)
  - Quantitative PCR (qPCR)
  - Ultrahigh-sensitivity (UHS) NGS
- Targets
  - mut*NPM1* AML
  - CBF AML
  - APL
  - *CEBPA* bZIP in-frame
  - *FLT3*-ITD/mutNPM1
  - *FLT3*-ITD/*NPM1*wt
  - *KMT2A::MLLT3* and other fusion transcript
  - Other with LAIP/DfN
  - Other with gene mutations

Key updates of the revised AML MRD recommendations

4) **MRD Relapse** is defined by the conversion from undetectable to detectable MRD combined with **AML (genetic) subtype-specific cut-offs** (and sample matrix specific cut-offs in case of mut*NPM1*)*

- CBF AML qPCR: PB or BM: ≥0.1% — **Always**
- LAIP/DfN: BM: ≥0.1% — **Only if no high diagnostic LAIP/DfN certainty**
- *NPM1* qPCR — **Only if *NPM1/ABL1* <1% in BM**
  - PB: ≥0.01%
  - BM: ≥0.1%
- Gene mutations UHS-NGS: PB or BM ≥0.01% — **Always**
- *PML::RARA* qPCR: BM: ≥0.001% — **Always**
- *FLT3*-ITD UHS-NGS: PB or BM: ≥LOD — **Only if MRD ≥LOD but <0.01% VAF**

? Repeat sample to confirm MRD relapse (within 4 weeks, preferably both in PB and BM)

*detectable signal (CT<40 in ≥2/3 replicates)

*In patients who have never tested MRD-, a ≥1 $\log_{10}$ increase from the measured nadir within the same tissue regardless of threshold is required

Learning quiz part 1

30sec/question max
double points for right answer

Quiz part 1

NPM1 monitoring: which statement is correct?

A. PB is a suitable matrix in follow-up to detect relapse
B. PB is a suitable matrix during treatment to determine MRD
C. Only BM may be used in follow-up
D. Only BM may be used during treatment

Quiz part 1

NPM1 monitoring: which statement is correct?

A. PB is a suitable matrix in follow-up to detect relapse
B. PB is a suitable matrix during treatment to determine MRD
C. Only BM may be used in follow-up
D. Only BM may be used during treatment

NPM1 monitoring

- All frequent (A,B and D) and rare subtypes
- Diagnostic BM not needed
- No choice in sample matrix before EOT, only after
  - PB after induction/pre-alloHCT
  - BM after consolidation/after alloHCT
  - PB OR BM during follow-up
- Monitoring by qPCR for 24 months using either BM (every three months) or PB (every 4-6 weeks)

NPM1 monitoring

Time point	Tissue	MRD %	MRD burden	Qualitative MRD response
*qPCR monitoring by mut**NPM1** using cDNA as mut**NPM1**/ABL1 copies (%)*				
2 cycles of intensive chemotherapy or pre-alloHCT	PB	<0.001% OR undetectable[1]	Negative	Optimal
		≥0.001% to <0.01% AND detectable[2]	Low-level positive	Warning
		≥0.01% AND detectable[2]	Positive	Warning
End of treatment (after consolidation/after alloHCT)	BM	<0.001% OR undetectable[1]	Negative	Optimal
		≥0.001% to <0.1% AND detectable[2]	Low-level positive	Warning
		≥0.1% AND detectable[2]	Positive	High risk of treatment failure
Follow-up	PB	<0.001% OR undetectable[1]	Negative	Optimal
		≥0.001% to <0.01% AND detectable[2]	Low-level positive	Warning[3]
		≥0.01% AND detectable[2,4,5]	MRD relapse	MRD relapse
Follow-up	BM	<0.001% OR undetectable[1]	Negative	Optimal
		≥0.001% to <0.1% AND detectable[2]	Low-level positive	Warning[3]
		≥0.1% AND detectable[2,4,5]	MRD relapse	MRD relapse

Cut-offs before and after stop therapy are the same within one matrix

The same MRD can have a different MRD burden/qualitative MRD response

Same cut-off for negative results

Different cut-offs for positivity between PB and BM

Adapted version of Table 3. of the original ELN 2025 update: Cloos, J., et al. (2025). Blood.

Quiz part 1

Patient diagnosed with **inv(16) AML** in Hospital A with 40% blasts in peripheral blood and 65% blasts in bone marrow. Decides to go to Hospital B for treatment. Both Hospital A and B use RNAseq for fusion transcript analysis at diagnosis and qPCR for monitoring. Hematologist from Hospital B calls the clinical biologist to ask if state-of-the-art MRD monitoring is possible.

A. Yes
B. No
C. Maybe

Quiz part 1

Patient diagnosed with **inv(16) AML** in Hospital A with 40% blasts in peripheral blood and 65% blasts in bone marrow. Decides to go to Hospital B for treatment. Both Hospital A and B use RNAseq for fusion transcript analysis at diagnosis and qPCR for monitoring. Hematologist from Hospital B calls the clinical biologist to ask if state-of-the-art MRD monitoring is possible.

A. Yes
B. No
C. Maybe

Core-binding factor (CBF) AML

- Sole entity where diagnostic BM is indispensable: MRD burden calculated based on the $\log_{10}$ reduction
- Only BM before EOT
- Implementation of MRD-LL
- Monitoring by qPCR for 24 months: PB 4-6 weeks > q3 month BM
- ‘high’ clinical cut-off for MRD relapse: weak positivity during follow-up without relapse < confounding non-leukemic mast cells?

Core-binding factor (CBF) AML

qPCR monitoring RUNX1::RUNX1T1 or CBFB::MYH11 (CBF AML) as CBF mutant/ABL1 copies (%)				
Baseline	BM	Quantify baseline transcript levels for log reduction	-	-
2 cycles of intensive chemotherapy or pre-alloHCT	BM	≥3-log_{10} reduction from diagnostic levels	Low-level positive /negative	Optimal
		<3-log_{10} reduction from diagnostic levels	Positive	Warning
End of treatment (after consolidation/ after alloHCT)	BM	<0.001% OR undetectable[1]	Negative	Optimal
		≥3-log_{10} reduction from diagnostic levels	Low-level positive	Warning[3]
		<3-log_{10} reduction from diagnostic levels	Positive	High risk of treatment failure
Follow-up	PB or BM	<0.001% OR undetectable[1]	Negative	Optimal
		Detectable[2] AND ≥3-log_{10} reduction from diagnostic levels OR detectable[2] AND <0.1%	Low-level positive	Warning[3]
		Conversion from undetectable[1] to detectable[2] AND ≥0.1%[4,5]	MRD relapse	MRD relapse

Adapted version of Table 3. of the original ELN 2025 update: Cloos, J., et al. (2025). Blood.

Only BM during therapy

11-52%

Kinetics!

Introduction of MRD-LL

41%

Quiz part 1

Patient diagnosed with **APL** in Hospital A with 40% blasts in peripheral blood and 65% blasts in bone marrow. Decides to go to Hospital B for treatment. Both Hospital A and B use RNAseq for fusion transcript analysis at diagnosis and qPCR for monitoring. Hematologist from Hospital B calls the clinical biologist to ask if state-of-the-art MRD monitoring is possible.

A. Yes
B. No
C. Maybe

Quiz part 1

Patient diagnosed with **APL** in Hospital A with 40% blasts in peripheral blood and 65% blasts in bone marrow. Decides to go to Hospital B for treatment. Both Hospital A and B use RNAseq for fusion transcript analysis at diagnosis and qPCR for monitoring. Hematologist from Hospital B calls the clinical biologist to ask if state-of-the-art MRD monitoring is possible.

A. Yes
B. No
C. Maybe

Acute promyelocytic leukaemia (APL)

- Only one sample matrix (BM) and cut-off
- Limited monitoring:
  - Goal = *PML::RARA* negativity post-consolidation
  - HR APL treated with ATRA-**chemotherapy** during follow-up: every q3 months
  - **Arsenic trioxide (ATO)**/ATRA-treated patients who are MRD+ at EOT
- 'low' clinical cut-off for MRD relapse: no safe stable MRD levels

Acute promyelocytic leukaemia (APL)

qPCR monitoring PML::RARA (APL) as PML::RARA/ABL1 copies (%)				
End of treatment (after consolidation/ after alloHCT)	BM	<0.001%[7] OR undetectable[1]	Negative	Optimal
		≥0.001%[7] AND detectable[2]	MRD positive	Warning
Follow-up	BM	<0.001[10]% OR undetectable[1]	Negative	Optimal
		≥0.001% AND detectable[2,4,6]	MRD relapse	MRD relapse

No HRTF

No warning

Adapted version of Table 3. of the original ELN 2025 update: Cloos, J., et al. (2025). Blood.

Quiz part 1

FLT3-ITD monitoring: which statement is correct?

A. PB is not appropriate during therapy for MRD assessment.
B. PB is appropriate during follow-up to detect relapse.
C. Only BM may be used during follow-up.
D. Only BM may be used during therapy.
E. Both matrices are equally suitable if sample matrix specific cut-offs are used, regardless of the time point

Quiz part 1

FLT3-ITD monitoring: which statement is correct?

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D. Only BM may be used during therapy.
E. Both matrices are equally suitable if sample matrix specific cut-offs are used, regardless of the time point

FLT3-ITD AML

- (targeted) *FLT3*-ITD UHS-NGS
  - If UHS-NGS is unavailable → MFC
  - If possible: combine UHS-NGS + MFC
  - *FLT3*-ITD/mut*NPM1* comutated→ UHS-NGS/MFC and *NPM1* qPCR
- Timepoints
  - High level of evidence after intensive chemotherapy/before alloHCT
  - Further validation warranted (expert's experience): EOT and during follow-up
- MRD quantification: dominant vs cumulative clone reporting strategy
  (PRE-MEASURE: "highest" vs. "all" VAF definitions made little difference in terms of moving patients into different risk tiers)
- 'low' clinical cut-off for MRD relapse: no warning category

FLT3-ITD AML

2021 → From zero to hero → 2024

Mutations in signaling pathway genes (FLT3-ITD, FLT3-TKD, KIT, and RAS, among others) most likely represent residual AML when detected, but are often subclonal and have a low negative predictive value.
Not routinely used as MRD marker

Table 4. ELN 2021 clinical MRD recommendations based on a Delphi poll

No.	Clinical MRD recommendation
D4	NGS-MRD monitoring is useful to refine prognosis in addition to MFC but, to date, there are insufficient data to recommend NGS-MRD as a stand-alone technique

Increasing use of FLT3 inhibitors post-HCT
For patients with *FLT3*-ITD positive AML, MRD should be assessed by NGS at the specific treatment landmarks of remission assessment after intensive chemotherapy and before HSCT

No.	Molecular MRD recommendations
C7	Important considerations for UHS-NGS assays for *FLT3*-ITD-MRD include limit of detection, ability to detect *FLT3*-ITDs up to 200 bp length, input DNA required (usually at least 500 ng to confidently call MRD negativity), the bioinformatic pipeline, and minimum number of identical reads for a positive test.
C8	If multiple *FLT3*-ITD clones are present in UHS-NGS-based MRD testing, the VAF and sequence of each clone should be reported. As of now, the major clone should be used to determine the level of MRD. The panel suggests also to report the VAF summed up from all clones.
C9	UHS-NGS-based MRD testing for *FLT3*-ITD can identify patients at increased risk of relapse and death, but should not be used as the sole MRD test during follow-up monitoring when other MRD markers are available, as *FLT3*-ITD-negative relapses may occur.
C10	The prognostic impact of UHS-NGS MRD using *FLT3*-ITD at end of treatment and during follow-up should be further validated.
C11	UHS-NGS MRD for *FLT3*-ITD should be evaluated further regarding the impact of the ITD length, insertion site, evolution of *FLT3*-ITD clones, and the impact of considering the dominant clone vs considering all clones in patients with multiple ITD clones.

FLT3-ITD AML

Table 1. Studies assessing molecular MRD monitoring in *FLT3* mutated AML. the table summarizes the most important clinical studies assessing MRD in *FLT3* mutated AML and includes performed methods, sample source, the time point of monitoring, and the main conclusion of the study.

Methods	Source	n	Timepoint	Main findings	References
NGS DNA sequencing for *FLT3*-ITD and TKD	PB	339 + 412*	Pre allo-HSCT	Persistence of *FLT3*-ITD was associated with increased relapse rates and worse survival	[18]
PCR-NGS for *FLT3*-ITD	BM or PB	104	Pre allo-HSCT	*FLT3*-ITD MRD positivity was associated with increased risk of relapse and poorer overall survival	[17]
NGS-based *FLT3*-ITD	BM or PB	161	After two cycles of induction chemotherapy	NGS-based detection of *FLT3*-ITD MRD identified patients with profound risk of relapse and mortality post-transplant	[19]
NGS-based *FLT3*-ITD	BM or PB	321	After one or two cycles of induction chemotherapy	MRD negativity associated with better prognosis, but the FLT3 inhibitor quizartinib increased overall survival in MRD positive patients	[20]
NGS-based *FLT3*-ITD	BM or PB	142	After two cycles of induction chemotherapy	MRD negativity was the strongest independent favorable prognostic factor for relapse and overall survival	[21]
NGS-based *FLT3*-ITD	BM	356	Pre and post allo-HSCT	MRD negativity was associated with increased relapse free survival, but the FLT3 inhibitor gilteritinib increased relapse free survival in MRD positive patients	[22]

*Patients in discovery and validation cohort.

Abbreviation: FG, fusion genes; RT-qPCR, real time- quantitative polymerase chain reaction, NGS, next generation sequencing; BM, bone marrow; PB; peripheral blood; MRD; measurable residual disease, allo-HSCT, allogeneic hematopoietic stem cell transplantation.

Hakon Reikvam and Richard Dillon; expert review of hematology 2024

FLT3-ITD AML

Targeted FLT3-ITD UHS-NGS (FLT3-ITD positive/mutNPM1 or FLT3-ITD positive/NPM1 wild type) (LOD measured as VAF[8] %)				
2 cycles of intensive chemotherapy or pre-alloHCT	BM > PB	<LOD[9]	Negative	Optimal
		≥LOD[9]	Positive	High risk of treatment failure
End of Treatment (after consolidation/ after alloHCT)	BM > PB	<LOD[9]	Negative	Optimal
		≥LOD[9]	Positive	High risk of treatment failure
Follow-up	PB or BM	<LOD[9]	Negative	Optimal
		≥LOD[9,10]	MRD relapse	MRD relapse

BM and PB are interchangeable

No solid cut-off

No MRD-LL

No warning category

Adapted version of Table 3. of the original ELN 2025 update: Cloos, J., et al. (2025). Blood.

Quiz part 1

Which combination describes the ELN25 criterion for reporting **MFC-MRD negativity**?

A. <0.1% LAIP/DfN blasts and ≥500,000 $CD45^+$ cells acquired
B. <0.1% LAIP/DfN blasts, ≥500,000 $CD45^+$ cells, >100 events blast compartment ($CD45^+$weak/SSClow)
C. <0.01% LAIP/DfN blasts and ≥500,000 $CD45^+$ cells acquired
D. <0.01% LAIP/DfN blasts, ≥500,000 $CD45^+$ cells and ≥250 events blast compartment ($CD45^+$wk/SSClow)

Quiz part 1

Which combination describes the ELN25 criterion for reporting **MFC-MRD negativity**?

A. <0.1% LAIP/DfN blasts and ≥500,000 $CD45^+$ cells acquired
B. <0.1% LAIP/DfN blasts, ≥500,000 $CD45^+$ cells, >100 events blast compartment ($CD45^+$weak/SSClow)
C. <0.01% LAIP/DfN blasts and ≥500,000 $CD45^+$ cells acquired
D. <0.01% LAIP/DfN blasts, ≥500,000 $CD45^+$ cells and ≥250 events blast compartment ($CD45^+$wk/SSClow)

Other (genetic) AML subtypes with LAIP/DfN

- Compared to ELN 2021
  - = minimal 500 000 WBC event and check for hemodilution
  - ≠ No minimal events in the blast compartment (initially 100, no consensus on 300)

- Only BM during/after therapy
- Check hemodilution, report LOD and LOQ, ongoing validation by regular EQC
  - LOQ: minimal 0.01%
  - LOD: minimal 0.004%

- Panel: Table S5

8c ELN MRD CD45, CD33, CD13, CD34, CD117, HLADR, CD7, CD56

10c	12c	13-16c	monocytic
CD38			CD64
CD11b			CD14
	CD15		CD11b
	CD19		CD4
		CD123	CD123
		CD133	
		CD36	
		CD14	

Other (genetic) AML subtypes with LAIP/DfN

- MFC-MRD in ~90% of the AML patients
  - Regardless of the ELN 2022 risk stratification
  - Including *CEBPA*m[1,2] and *KMT2Ar* AML

- Combined LAIP+DfN approach: tracking diagnostic and emergent clones
- MRD reporting based on the **total sum** of **LAIP/DfN** positive cells
  - ! Tacit knowledge (ab)normal immunophenotypic expression patterns
  - ! Stressed and regenerating haematopoiesis[3]
  - ! Low risk MDS and clonal haematopoiesis of indeterminate potential (CHIP)[3]

- The same MRD burden and qualitative MRD response for each MRD%
- Implementation of MRD-LL
- Advised: q3 monitoring during follow-up for 1 year (instead of 2)

[1]Deng DX et al. Leuk. Lymphoma. 2019 ; [2]Vonck et al. HemaSphere. 2024;8(8):e141 ; [3]Ngai LL, et al. Blood. 2023;142(Supplement 1):1572–1572.

Other (genetic) AML subtypes with LAIP/DfN

Multiparameter flow cytometry MRD as LAIP+ or DfN+ blasts/CD45 expressing cells (%)				
Baseline	BM or PB[11]	≥10% of blasts	LAIP assessment	-
2 cycles of intensive chemotherapy or pre-alloHCT	BM	<0.01%[12] OR <LOD[12]	Negative	Optimal
		≥0.01% to <0.1% AND >LOD	Low-level positive	Warning
		≥0.1%	Positive	High risk of treatment failure
End of treatment (after consolidation/after alloHCT)	BM	<0.01%[12] OR <LOD[12]	Negative	Optimal
		≥0.01% to <0.1% AND >LOD	Low-level positive	Warning
		≥0.1%	Positive	High risk of treatment failure
Follow-up	BM	<0.01%[12] OR <LOD[12]	Negative	Optimal
		≥0.01% to <0.1% AND >LOD	Low-level positive	Warning[3]
		≥0.1%[13,14]	MRD relapse	MRD relapse

Adapted version of Table 3. of the original ELN 2025 update: Cloos, J., et al. (2025). Blood.

Quiz part 1

Multi-gene/agnostic UHS-NGS-MRD performed for a mut*NPM1* AML patient after alloHCT reveals a persisting mutation in *DNMT3A* and in *NPM1*, both increasing in serial measurements. Which interpretation aligns best with ELN2025?

A. Only *NPM1* is clinically relevant; CHIP-associated mutations have no prognostic significance post-alloHCT.

B. *NPM1* is clinically relevant, but serial multi-gene UHS-NGS, including CHIP-associated genes, may herald impending relapse and warrants further validation.

C. NPM1 not useful since gDNA analysis, CH-associated mutations should be disregarded; an increase only indicates donor-derived variants and is, by definition, MRD-false positive

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Other gene-mutated AML subtypes

- Jongen-Lavrencic et al. (2018, NEJM): Targeted NGS panel with **54 genen excluding DTA** measured in **CR**: **independently prognostic for higher 4y RR** (55% vs 32%)

- Thol et al. (2018, Blood): Targeted NGS panel with **54 genen including DTA** measured **pre-alloHCT**: **significant higher relapse (CIR 66% vs 17%)**

- Tsai et al. (2021, Blood Advances): TruSight Myeloid Sequencing Panel with **54 genes excluding DTA** measured **after induction** and **consolidation**: independent **poor prognostic factor for relapse (39% vs 57%), RFS and OS**

- Gui et al., ASH 2023: Residual **IDH2** variants in **blood pre-alloHCT** are predictive of relapse in except for *NPM1*/*FLT3*-ITD co-mutated

No.	Molecular MRD recommendations
C12	The prognostic impact of UHS-NGS for other mutations than *FLT3*-ITD has been demonstrated before and after chemotherapy consolidation/alloHCT and during follow-up. The cutoffs, time points and tissues should be further validated gene by gene following the framework in **Supplemental Table S7.**
C13	Mutations in genes associated with germline predisposition or age-related clonal hematopoiesis are not prognostic at the time of remission assessment after intensive chemotherapy and before alloHCT and should not be taken into account at these time points.
C14	UHS-NGSMRD monitoring using molecular markers present at diagnosis correlates with impending relapse when used for serial monitoring after alloHCT including genes associated with germline predisposition and clonal hematopoiesis. This approach should be further validated.
C15	Targeted UHS-NGS MRD using specific mutations identified at diagnosis vs agnostic panel approaches have different strengths and limitations and may be considered depending on sensitivity, turnaround time, resource use, setting (research, clinical trial, or clinical routine), and ability to standardize methodology and reporting.
C16	Emerging variants detected by UHS-NGS in remission that were not previously detected at diagnosis should be reported only if confidently detected above background noise and if donor origin has been excluded (e.g. by correlation with highly-sensitive chimerism analysis). The prognostic effect of emerging variants has not been sufficiently validated.

Other gene-mutated AML subtypes

- Targeted versus agnostic panel
  - no uniform bioinformatics pipeline/platform
  - “if you see it, you need to kill it”?
- Which diagnostic genes?
  - Validated in larger cohorts: *IDH2*
  - Not (yet) indicated:*TP53*, *IDH1*, bZIP-mutated *CEBPA* …
  - No CHIP (DTA) or germline mutations pre-alloHCT

  →gene-by-gene validation at different timepoint

  →Place for germline mutations post-alloHCT?
- Emerging clones?
  - Report only if donor origin is excluded
  - Prognostic effect not sufficiently validated

Test quiz part 2

+

hand-outs

20sec/question max
double points for right answer

Quiz part 2

In an **mut*NPM1* AML** patient, a **mutNPM1/ABL1 ratio of 0.005%** is detected in **peripheral blood** cDNA after **2 cycles of intensive chemotherapy**, clearly detectable in ≥2/3 replicates with CT<40 and 30,000 ABL1 copies.

According to ELN 2025 guidelines, what is the MRD burden and qualitative response at this timepoint?

A. MRD-negative, optimal response
B. Low-level MRD-positive, warning
C. MRD-positive, high risk of treatment failure
D. MRD-positive, warning

Quiz part 2

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Quiz part 2

A mutNPM1 AML patient has a **mutNPM1/ABL1 ratio** of **0.08%** in **BM** cDNA at **end of treatment**, detectable in ≥2/3 replicates with CT<40.

According to ELN 2025 guidelines, what is the MRD burden and qualitative response at this timepoint?

A. MRD-negative, optimal
B. Low-level MRD-positive, warning
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D. MRD-relapse

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Quiz part 2

A patient with **CBF AML (*RUNX1::RUNX1T1*)** exhibits a **2-log reduction from baseline** in **bone marrow** after **2 cycles of intensive chemotherapy**.

How is this interpreted according to ELN 2025 guidelines?

A. MRD-negative, optimal
B. MRD-LL positive, warning
C. MRD positive, warning
D. MRD-positive, high risk of treatment failure

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How is this interpreted according to ELN 2025 guidelines?

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C. MRD positive, warning
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Quiz part 2

Which statements are correct regarding **APL**: MRD monitoring is required if …
(>1 answer correct)

A. High-risk APL, chemotherapy and ATRA, MRD negative at EOT
B. High-risk APL, chemotherapy and ATRA, MRD positive at EOT
C. High-risk APL, ATO and ATRA, MRD negative at EOT
D. High-risk APL, ATO and ATRA, MRD positive at EOT
E. Low-risk APL, ATO and ATRA, MRD negative at EOT
F. Low-risk APL, ATO and ATRA, MRD positive at EOT

Quiz part 2

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C. High-risk APL, ATO and ATRA, MRD negative at EOT
D. High-risk APL, ATO and ATRA, MRD positive at EOT
E. Low-risk APL, ATO and ATRA, MRD negative at EOT
F. Low-risk APL, ATO and ATRA, MRD positive at EOT

Quiz part 2

In APL, a ***PML::RARA/ABL1*** **ratio** of **0.0005%** is found in **BM** cDNA at **EOT**. Which qualitative response corresponds according to ELN 2025?

A. MRD-negative, optimal
B. MRD-positive, warning
C. MRD-positive, high risk of treatment failure
D. MRD-relapse

Quiz part 2

In APL, a ***PML::RARA/ABL1*** **ratio** of **0.0005%** is found in **BM** cDNA at **EOT**. Which qualitative response corresponds according to ELN 2025?

A. MRD-negative, optimal
B. MRD-positive, warning
C. MRD-positive, high risk of treatment failure
D. MRD-relapse

Quiz part 2

An *FLT3*-ITD-mutated patient underwent 2 cycles of intensive chemotherapy; UHS-NGS on BM shows *FLT3*-ITD VAF of 0.008%: >LOD (0.005%) but <0.01%.

How does ELN 2025 classify this result at this timepoint?

A. MRD-negative, optimal
B. Low-level MRD-positive, warning
C. MRD-positive, high risk of treatment failure
D. MRD-relapse

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C. MRD-positive, high risk of treatment failure
D. MRD-relapse

Quiz part 2

A **mut*NPM1*/*FLT3*-ITD co-mutated** AML patient has **pre-alloHCT** *NPM1*-negative MRD, but UHS-NGS shows *FLT3*-ITD VAF >LOD.

What does ELN 2025 state regarding ***NPM1*- *FLT3*-ITD+ discrepancies** pre-alloHCT?

A. *NPM1* overrides *FLT3*-ITD; patient is low risk

B. Any *FLT3*-ITD positivity can be ignored if NPM1-negative

C. Persistent MRD via any of the two markers is associated with higher risk

D. Only *FLT3*-ITD determines risk, *NPM1* is irrelevant

Quiz part 2

A **mut*NPM1*/*FLT3*-ITD co-mutated** AML patient has **pre-alloHCT** *NPM1*-negative MRD, but UHS-NGS shows *FLT3*-ITD VAF >LOD.

What does ELN 2025 state regarding ***NPM1*- *FLT3*-ITD+ discrepancies** pre-alloHCT?

A. NPM1 overrides FLT3-ITD; patient is low risk

B. Any FLT3-ITD positivity can be ignored if NPM1-negative

C. Persistent MRD via any of the two markers is associated with higher risk

D. Only FLT3-ITD determines risk, NPM1 is irrelevant

Quiz part 2

According to ELN 2025, **MFC-MRD ≥0.1%** in **bone marrow during therapy** is classified as:

A. Low-level MRD-positive, warning

B. MRD-positive, high risk of treatment failure

C. MRD-relapse

D. Not interpretable; repeat after 4 weeks

Quiz part 2

According to ELN 2025, **MFC-MRD ≥0.1%** in **bone marrow during therapy** is classified as:

A. Low-level MRD-positive, warning

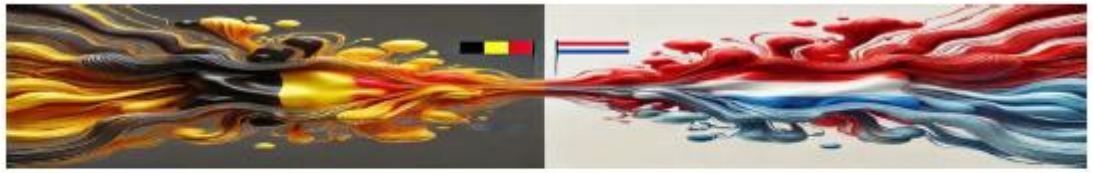
B. MRD-positive, high risk of treatment failure

C. MRD-relapse

D. Not interpretable; repeat after 4 weeks

Remarks

- The updated MRD recommendations are summarized in the AML MRD guideline App (https://eln-aml-mrd-2025.vercel.app/)
- Review BJH: practical must-to-knows (haematologist's point-of-view)
- More on flow MRD AML:



"Flow across borders"

16:00–17:30	**Session 3 – Monitoring** (Chair: Joyce van Beers)
16:00–16:30	CTC in MM patients (Mark van Duin, Rotterdam, NL)
16:30–17:00	MRD in AML: update ELN guidelines (Barbara Depreter, Roeselare, BE)
17:00–17:30	CAR-T monitoring (André Gothot, Liège, BE)