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MRD as a Potential Predictor of Relapse in AML

Agenda

Section 1: Remission and Relapse in Acute Myeloid Leukemia (AML)

- AML overview
- Complete remission (CR)
- Response criteria
- Relapse factors

Section 2: Measurable Residual Disease (MRD)

- MRD defined
- The importance of MRD
- Mechanisms of relapse with MRD in CR
  - Clonal selection
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- MRD and rate of relapse
- Measuring and monitoring MRD
- MRD and the landscape of risk in AML

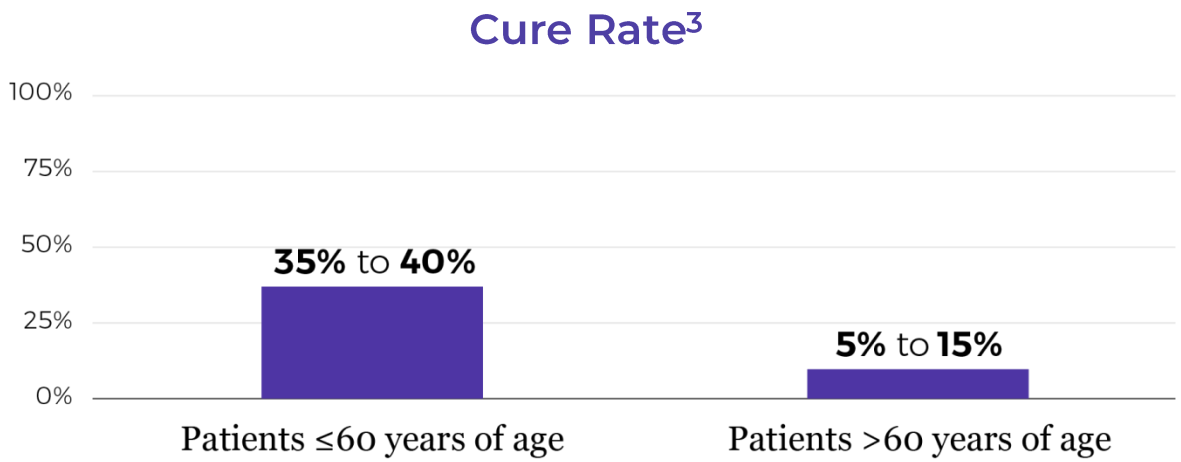
Summary

Section 1: AML

Remission and Relapse in Acute Myeloid Leukemia

AML is a generally fatal malignancy

- Acute Myeloid Leukemia (AML) is a biologically complex,[1] clinically and genetically heterogeneous disease with a poor prognosis[2]
- AML is fatal for ~80% of patients[2]
- Relapse represents the major cause of treatment failure[3]

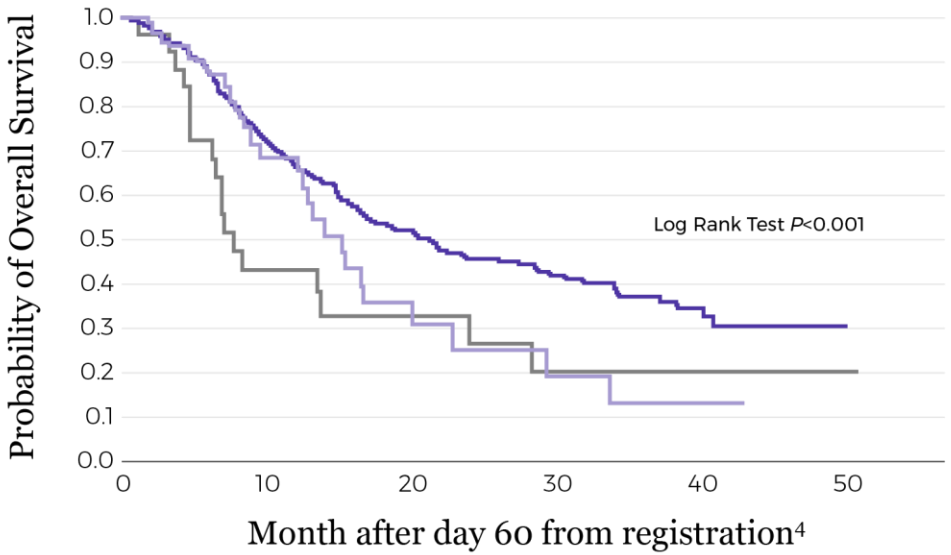


References: 1. Ossenkoppele G, et al. *Hematology Am Soc Hematol Educ Program.* 2016;2016(1):356-365. **2.** Watts J, et al. *F1000Res.*2018;7. pii: F1000 Faculty Rev-1196. **3.** Döhner H, et al. *N Engl J Med.* 2015;373:1136-1152.

CR is the initial goal in AML

The initial goal of therapy for AML is to achieve a complete remission (CR); CR is requisite, although not sufficient, for cure.[1,2]

"There is no cure without CR."[3]



A retrospective analysis of 727 patients receiving intensive therapy confirmed that achieving CR in AML significantly improved survival outcomes. This confirms the prognostic advantage of true CR, regardless of the number of induction cycles required to achieve it.[4]

Induction Response	Total	Median OS
CR	280	20.1
CRI	31	14.9
LFS	26	7.4

The importance of achieving CR was confirmed in a retrospective analysis of 6,283 patients with newly diagnosed AML treated on ECOG and SWOG protocols or at M.D. Anderson Cancer Center. Patients achieving CR had longer OS than those achieving CR without platelet recovery (CRp) or who were resistant to induction therapy.[5]

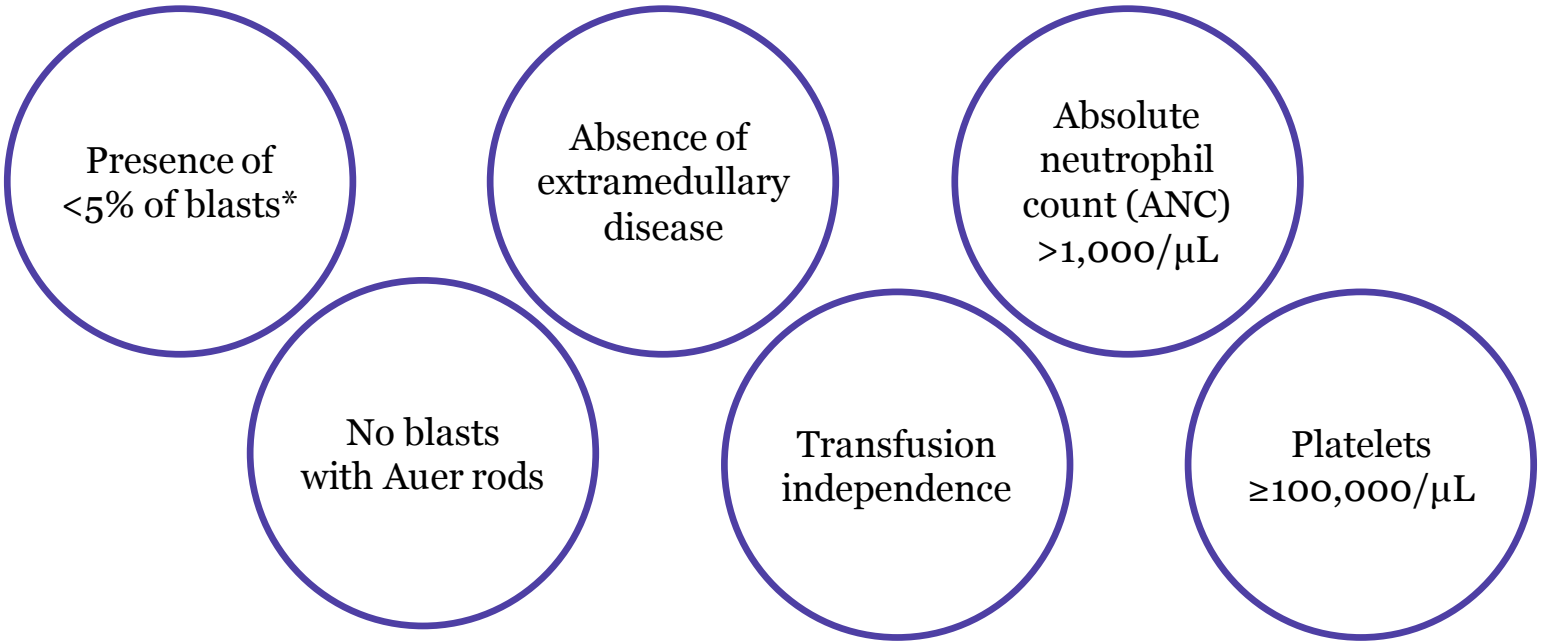
Adapted from Foran JM, et al. *Blood*. 2016;128(22):1-7.

CRi, CR with incomplete hematologic recovery; ECOG, Eastern Cooperative Oncology Group; LFS, leukemia-free state (also called MLFS, morphologic leukemia-free state); SWOG, Southwest Oncology Group.

References: 1. Cheson BD, et al. *J Clin Oncol*. 2003;21:4642-4649. **2.** Luskin MR, et al. *J Oncol Pract*. 2017;13(8):471-480. **3.** Ossenkoppele G, et al. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):356-365. **4.** Foran JM, et al. *Blood*. 2016;128(22):1-7. **5.** Walter RB, et al, *J Clin Oncol*. 2010;28:1766-1771.

Characterization of CR

CR (known as morphologic CR or mCR) is defined as[1]:



*In an aspirate sample with spicules and a count of at least 200 nucleated cells.

Reference: 1. Cheson BD, et al. *J Clin Oncol.* 2003;21:4642-4649.

Most patients achieve CR but many will relapse

- Historically, CR has been achieved in 60% to 85% of adults ≤60 years of age, and 40% to 60% in patients >60 years of age following induction therapy[1]
- However, published reviews suggest that most patients with AML relapse within 3 years after diagnosis[1]

In a pooled analysis of 4,601 patients with newly diagnosed AML, most (79%) achieved CR but relapsed (or died) at a median of 15 months[2]

Parameter[2]*	N=4,601
CR after 1–2 courses of induction chemotherapy	79%
Relapse-free survival, median	15 months

*Pooled retrospective data from adults with newly diagnosed AML as based on WHO 2008 classification criteria treated with curative intent on 6 trials conducted by the U.K. Medical Research Council/National Cancer Research Institute (MRC/NCRI), 6 by the Dutch-Belgian Cooperative Trial Group for Hematology/Oncology and the Swiss Group for Clinical Cancer Research (HOVON/SAKK), 4 by the U.S. cooperative group SWOG, and various MD Anderson Cancer Center protocols[2]

References: 1. Döhner H, et al. *N Engl J Med.* 2015;373:1136-1152. **2.** Walter RB, et al. *Leukemia.* 2015;29(2):312-320.

Response criteria in AML

Response criteria[1,2]	Definition	Neutrophils (µL)	Platelets (µL)	BM blasts (%)	Other
Morphologic CR (mCR)*	No circulating blasts or blasts with Auer rods; no extramedullary disease	≥1,000	≥100,000	<5	Transfusion independence Absence of EMD
CR with incomplete hematologic recovery (CRi)	All CR criteria except for residual neutropenia	<1,000 or	<100,000	<5	Cytogentics—normal, EMD
Morphologic leukemia-free state	No blasts with Auer rods; no extramedullary disease; hematologic recovery not required	NA	NA	<5	Flow cytometry EMD
Partial Response (PR)	All hematologic criteria of CR	>1,000	>100,000	Decrease of ≥50% or decrease to 5%-25%	Blasts <5% if Auer rod positive

Adapted from Cheson BD, et al. *J Clin Oncol.* 2003;21:4642-4649 and Döhner H, et al. *Blood.* 2017;129:424-447.

The presence of <5% blasts has been the benchmark of CR and has not changed in the past 60 years[3]

*All criteria should be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules.[1]
BM, bone marrow; CR, complete remission; EMD, extramedullary disease.

References: 1. Cheson BD, et al. *J Clin Oncol.* 2003;21:4642-4649. **2.** Döhner H, et al. *Blood.* 2017;129(4):424-447. **3.** Bisel HF. *Blood.* 1956;11:676-681.

Major factors predicting relapse

- Prognostic factors for relapse can be subdivided into those that are related to the patient and those that are related to the disease[1]

PATIENT-RELATED FACTORS[1,2]

- Increasing age
- Comorbid conditions
- Poor performance status
- Prior myeloid disorder
- Prior cytotoxic therapy
- Prior allogeneic hematopoietic cell transplantation

DISEASE- AND TREATMENT-RELATED FACTORS[1-3]

- Late onset and/or short duration of first CR
- White blood cell count
- Genetic abnormalities
  - Driver gene mutations
  - Leukemic-cell genetic changes
  - Complex karyotype

Emerging predictor: measurable residual disease (MRD)

References: 1. Döhner H, et al. *N Engl J Med*. 2015;373:1136-1152. **2**. Döhner H, et al. *Blood*. 2017;129:424-447. **3**. Thomas X, et al. *Leuk Res*. 2012;36(9):1112-1118.

Section 2: MRD

Measurable (formerly Minimal) Residual Disease

Measurable residual disease

- Definition: the persistence of leukemic cells below the threshold of <5% blasts once morphological complete remission has been achieved[1,2]
- Independent prognostic information:
  - MRD can predict relapse[3]
  - MRD confers a negative prognosis consistent with persisting leukemia[1]
- Potential in risk stratification[4]

Achieving CR (<5% blasts) does not imply MRD negativity (CR_{MRD-}). A patient may achieve mCR but still harbor as many as 10^{10} leukemic cells, equivalent to a 2-cubic-centimeter solid tumor mass[5]

References: 1. Buccisano F, et al. *Expert Rev Hematol.* 2018;11(4):307-313. **2.** DeAngelo DJ, et al. *Am Soc Clin Oncol Educ Book.* 2016;35:e302-e312. **3.** Brinda B, et al. *J Cell Mol Med.* 2018;22(3):1411-1427. **4.** Schuurhuis GJ, et al. *Blood.* 2018;131(12):1275-1291. **5.** Hourigan CS, et al. *Nat Rev Clin Oncol.* 2013;10(8):460-471.

The importance and potential of MRD

1

MRD after different cycles of therapy may reflect the sum of all diagnosis and post-diagnosis resistance mechanisms/factors[1]

2

MRD can establish the presence of leukemia cells down to levels of 1:1000 to 1:1000000 WBCs, compared with 1:20 for morphologic CR[1]

3

Given the prognostic significance of MRD, the 2017 European LeukemiaNet (ELN) recommendations for response criteria now include CR without MRD (CR_{MRD-}) in AML[2]

4

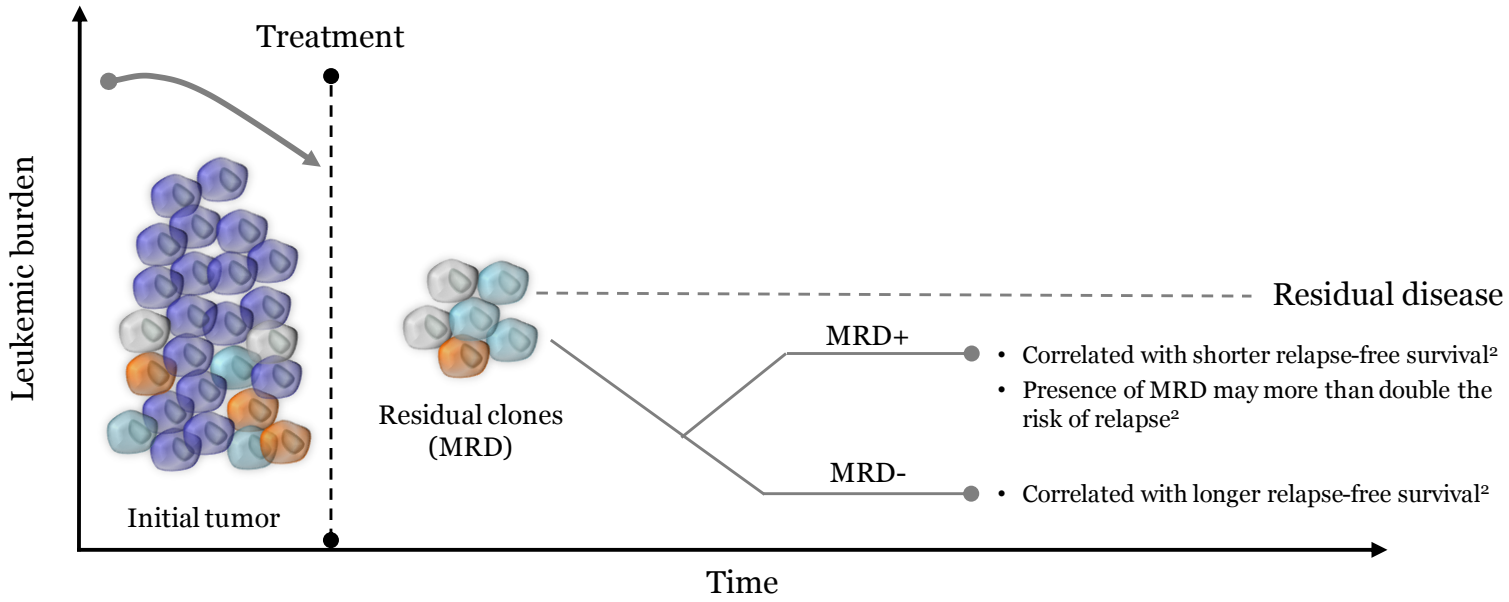
NCCN Clinical Practice Guidelines In Oncology (NCCN Guidelines®) acknowledge existing data that have demonstrated/support a correlation between MRD and risk for relapse[3]

References: 1. Ossenkoppele G, et al. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):356-365. **2.** Döhner H, et al. *Blood*. 2017;129(4):424-447. **3.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Acute Myeloid Leukemia V.1.2019.

Mechanism of relapse with MRD in CR:
1. Clonal selection

Eradicating the major clone may select for a minor, resistant clone of MRD which proliferates, causing relapse[1]

AML cells at relapse are often descendants of some present at diagnosis[1]

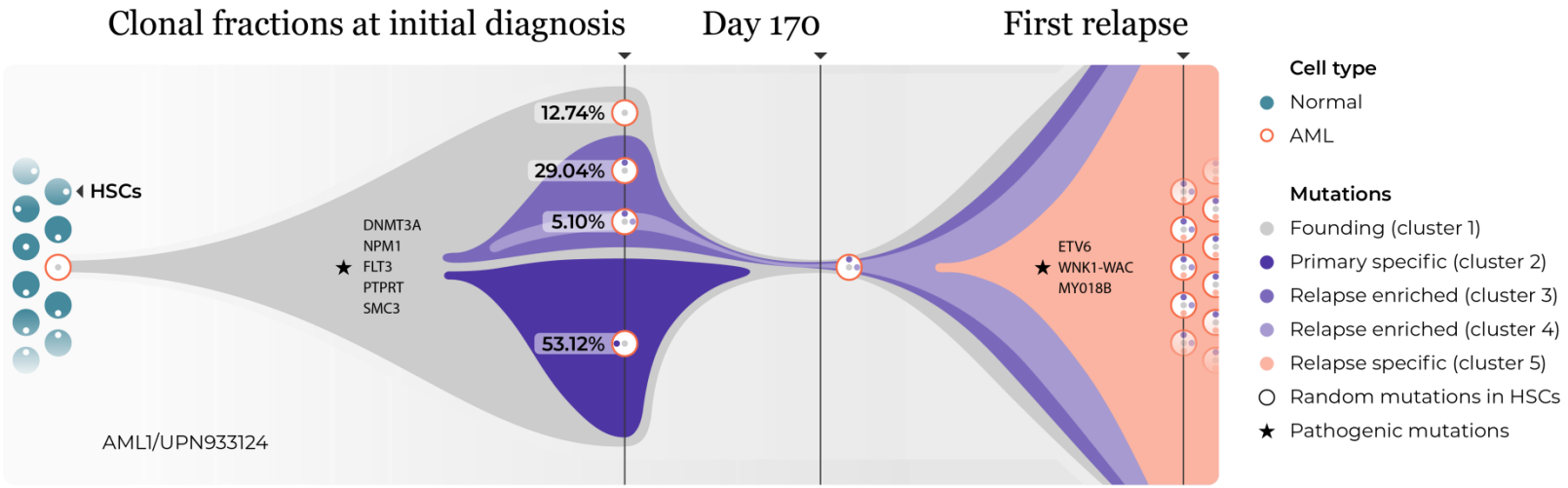


Pre-leukemic and leukemic stem and progenitor cells are both present during AML, persist in CR, and may cause relapse[1]

References: 1. Brinda B, et al. *J Cell Mol Med.* 2018;22(3):1411-1427. **2.** Chen X, et al. *J Clin Oncol.* 2015;33(11):1258-1264.

Mechanism of relapse with MRD in CR:
2. Clonal evolution

MRD may also cause relapse in AML by clonal evolution, in which an otherwise treatment-sensitive clone acquires additional mutations, allowing it to survive and become the dominant clone at relapse[1]



Even if dominant leukemic clones are suppressed or eliminated, leading to CR, genetically related but distinct clones may arise, contributing to relapse[2]

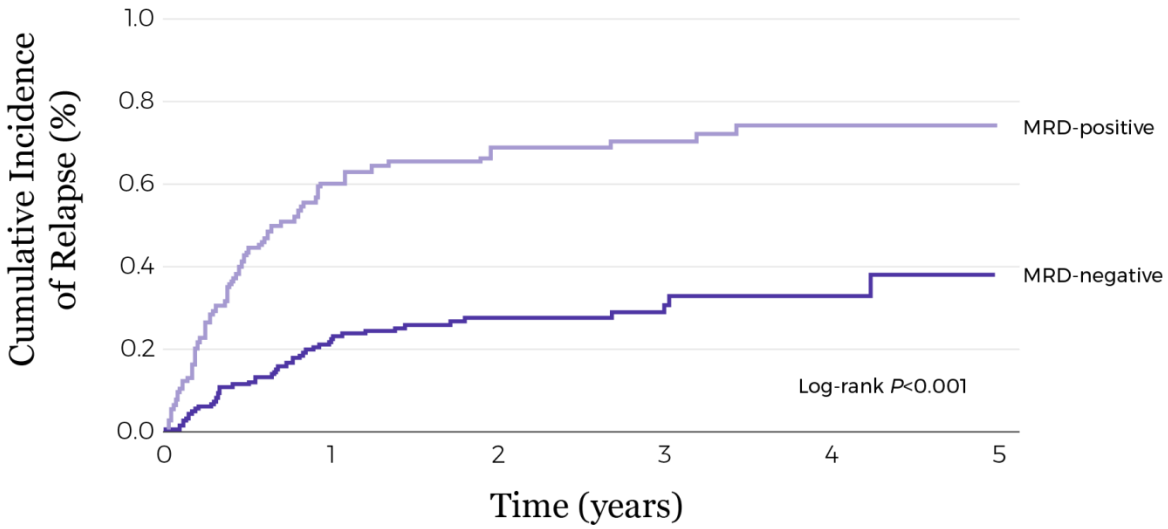
Adapted from Ding L, et al. *Nature*. 2012;481(7382):506-510.

References: 1. Ding L, et al. *Nature*. 2012;481(7382):506-510. **2.** Brinda B, et al. *J Cell Mol Med*. 2018;22(3):1411-1427.

Presence of MRD increases rate of relapse

In a retrospective analysis of 245 adults with AML who achieved CR, CRp, or CRi after induction therapy, MRD was significantly predictive of relapse[1-4]

MRD is an independent prognostic factor for relapse in AML[1]



Cumulative incidence of relapse in all patients[1]

Adapted from Chen X, et al. *J Clin Oncol.* 2015;33(11):1258-1264.
MRD was defined according to IWG criteria including <5% blasts, assessed by 10-color MFC.[1]

References: 1. Chen X, et al. *J Clin Oncol.* 2015;33(11):1258-1264. **2.** Jongen-Lavrencic M, et al. *N Engl J Med.* 2018;378(13):1189-1199. **3.** Terwijn M, et al. *J Clin Oncol.* 2013;31(31):3889-3897. **4.** Ravandi F, et al. *Cancer.* 2017;123(3):426–435.

Investigational MRD detection techniques vary in sensitivity

1

Multiparameter flow cytometry (MFC)

Sensitivity: ~10^{-4} to 10^{-5}

detects antigens expressing leukemia-associated immunophenotypes (LAIPs) or manifesting a "different" immunophenotypic maturation profile[1]

2

Next-generation sequencing (NGS)

Sensitivity: ~10^{-3} to 10^{-5}

allows multiple molecular biomarkers to be monitored simultaneously[1]

3

Real-time quantitative PCR (RT-qPCR)

Sensitivity: ~10^{-3} to 10^{-5}

measures molecular mutations and leukemia-associated fusion transcripts[1]

4

Fluorescence in situ hybridization (FISH)

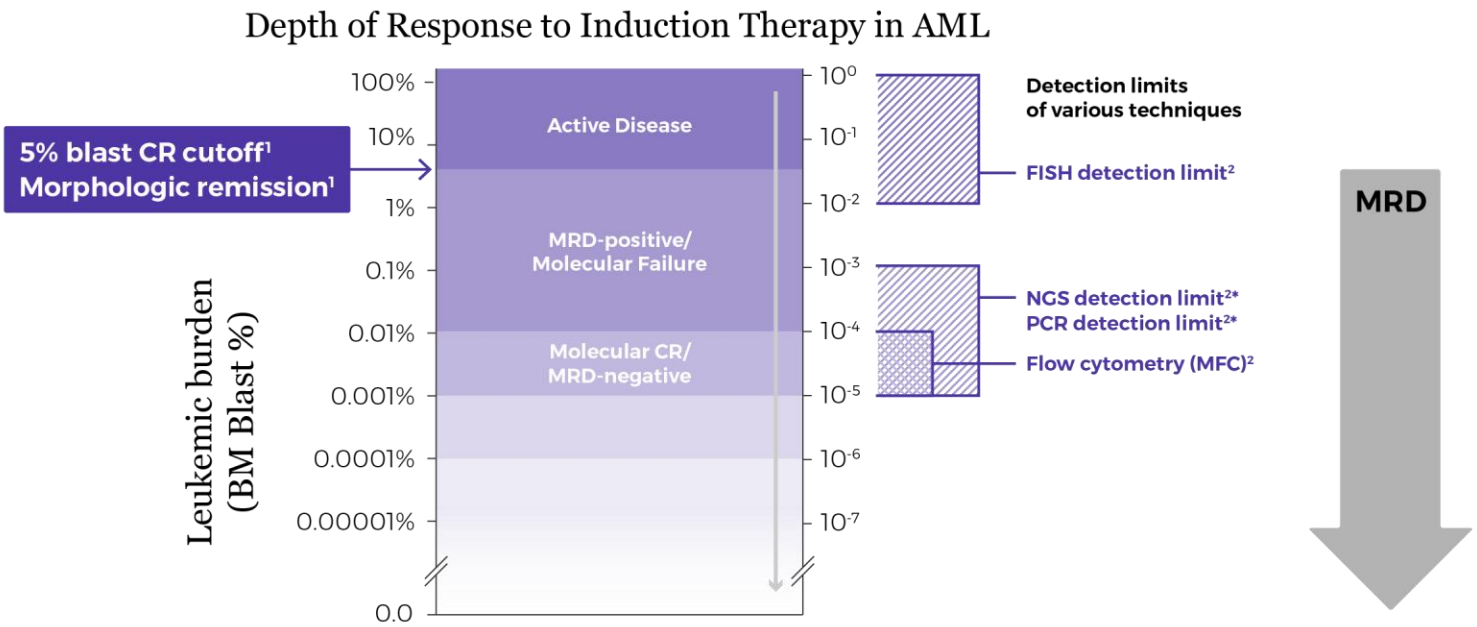
Sensitivity: ~1 to 10^{-2}

detects gene rearrangements, gene fusions, or loss of chromosome material[2]

References: 1. Rivandi F, et al. *Blood Adv*. 2018;2(11):1356-1366. **2.** Döhner H, et al. *Blood*. 2010;115:453-474.

MRD can define depth of response in AML

Various techniques are under investigation to measure the depth of response to treatment, with different sensitivities for detecting MRD[1]



mCR by light microscopy is defined as <5% blasts (1 leukemic cell in 20); investigational techniques can detect 1 leukemic cell in 1,000,000[3]

*Sensitivity of response assessment varies by method used and by marker tested.[4]

NGS, next-generation sequencing; PCR, polymerase chain reaction.

References: 1. Schuurhuis GJ, et al. *Blood.* 2018;131(12):1275-1291. **2.** Rivandi F, et al. *Blood Adv.* 2018;2(11):1356-1366. **3.** Ossenkoppele G, et al. *Hematology Am Soc Hematol Educ Program.* 2016;2016(1):356-365. **4.** Döhner H, et al. *Blood* 2017;129:424-447.

Investigational MRD detection techniques: features and considerations

Method[1]	Advantages	Disadvantages
MFC	• Wide applicability (>90%) • Relatively quick (results ≤1 day) • Single result interpretable • High specificity when using defined LAIP • Can detect cells with leukemia-stem cell phenotype • Can distinguish between live and dead cells • Ease of data storage • Provides information about whole sample cellularity	• Challenging and somewhat subjective interpretation requires experienced pathologist • Sensitivity dependent on antibody panel used • Limited harmonization and standardization across laboratories • Leukemic phenotype not necessarily stable over time (eg, initial LAIP may not identify subclones leading to relapse)
NGS	• Relatively easy to perform • Sensitive • Applicable to specific subgroups	• Limited standardization • Error rate leads to low-sensitivity of mutated sequences • Mutated genes can be detected in healthy people without hematologic abnormalities • Persistence of some genetic abnormalities in patients in long-term remission • Risk of contamination
RT-qPCR	• Wide applicability • May be run by any certified laboratory with RT-qPCR capacity • High sensitivity (≥MFC) • Well standardized • Quality assurance routinely incorporated	• Results may take multiple days • Expensive (computationally demanding and time-consuming) • Requires high-level expertise • Requires setting of threshold limits • Interpretation often requires trend of results • Different mutations have different biological consequences in AML • Molecular targets applicable to only ~50% of all AML cases and <35% in older patients
FISH	• Superior to PCR-based assays for detection of numeric cytogenetic abnormalities (gains and losses of whole chromosomes or deletions / duplications)	• Considerably less sensitive than PCR or MFC • Not useful for patients with normal karyotype • Technique is labor intensive

Adapted from Rivandi F, et al. *Blood Adv*. 2018;2(11):1356-1366.

Reference: 1. Rivandi F, et al. *Blood Adv*. 2018;2(11):1356-1366.

When to monitor MRD

2017 European LeukemiaNet recommendations[1]

Post-induction therapy:

to assess remission status and determine kinetics of disease response

Post-consolidation therapy:

to assess remission status and determine kinetics of disease response

Post-HSCT:

to inform post-HSCT measures aiming to avoid frank relapse

Serial monitoring of MRD during CR:

beyond consolidation to detect impending morphologic relapse[1]

HSCT, hematopoietic stem cell transplantation.

Reference: 1. Döhner H, et al. *Blood*. 2017;129:424-447.

When to monitor MRD

2019 NCCN Guidelines® suggest monitoring MRD[1]

Post-induction therapy

Before allogeneic transplantation

Additional time points should be guided by regimen used

Acceptance of MRD

Investigations suggest that CR_{MRD}- may emerge as a better definition of leukemic burden than mCR[1]

MRD can be detected down to levels of 1:1000 to 1:1000000 WBCs, compared with 1:20 for mCR[1]

Based on the importance of MRD in AML, a new response category has been established: CR without minimal residual disease (CR_{MRD-})[2]

2017 ELN guidelines actively recommend monitoring for MRD[2]

NCCN Guidelines state that MRD should be evaluated over the course of sequential therapy[3]

References: 1. Ossenkoppele G, et al. *Hematology Am Soc Hematol Educ Program.* 2016;2016(1):356-365. **2.** Döhner H, et al. *Blood.* 2017;129(4):424-447. **3.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Acute Myeloid Leukemia V.1.2019. © National Comprehensive Cancer Network, Inc. 2019. All rights reserved. Accessed January 18, 2019. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

Summary

In summary

- CR is requisite, although not sufficient, to achieve a cure in AML[1,2]
- Most patients achieve an initial CR,[3] but many will relapse and succumb to progressive disease[4]
- Patients with AML could relapse for a variety of reasons, but the depth of remission, reflected by MRD, could be the primary factor[5]
- MRD provides independent prognostic information, conferring a negative prognosis consistent with persistent leukemia[6]
- The possibility of detecting MRD far below the level of 5% blast cells is changing the landscape of risk classification[5]

References: 1. Cheson BD, et al. *J Clin Oncol.* 2003;21:4642-4649. **2.** Luskin MR, et al. *J Oncol Pract.* 2017;13(80):471-481. **3.** Döhner H, et al. *N Engl J Med.* 2015;373:1136-1152. **4.** Ding L, et al. *Nature.* 2012;481(7382):506-510. **5.** Ossenkoppele G, et al. *Hematology Am Soc Hematol Educ Program.* 2016;2016(1):356-365. **6.** Buccisano F, et al. *J Clin Oncol.* 2012;30:3625-3632.

Appendix

Response criteria in AML, per 2017 ELN criteria: defining treatment failure and relapse

Treatment failure	Definition	Comment
Primary refractory disease	No CR or CRi after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	Regimens containing higher doses of cytarabine are generally considered to be the best option for patients not responding to a first cycle of 7+3; the likelihood of responding to such regimens is lower after failure of a first induction
Death in aplasia	Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic BM obtained within 7 days of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no BM examination available	

Relapse	Definition	Comment
Hematologic relapse (after CR_{MRD-}, CR, CRi)	BM blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by quantitative RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cut-off values must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

Adapted from Döhner H, et al. *Blood*. 2017;129:424-447.

Reference: 1. Döhner H, et al. *Blood*. 2017;129:424-447.