# Assessing Measurable Residual Disease as a Genetic Biomarker for Acute Myeloid Leukemia

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**Assessing measurable residual disease (MRD) in patients with acute myeloid leukemia (AML) has the potential to improve outcomes as it can allow physicians to predict relapse and determine the most appropriate treatments in a personalized way. However, current technologies for measuring MRD are limited by a lack of standardization, difficulties in interpretation, and the inability to detect multiple mutations in the same sample. In this article we will discuss the benefits of measuring MRD in AML and how some of the technological limitations are being overcome with the introduction of innovative next-generation sequencing (NGS) assays.**

ACUTE MYELOID LEUKEMIA is a rare malignancy that develops in myeloblasts (aka blasts) – the stem cell precursors of basophils, neutrophils, and eosinophils. In 2022, it is expected that there will be about 20,000 new AML cases in the USA, accounting for around 1% of all new cancer diagnoses.[1] Between 2012 and 2018 the five-year survival rate was 30.5%, with an estimated 11,500 deaths (1.9% of all cancer deaths) predicted in 2022.[1]

This heterogenous disease is characterized by the clonal expansion of immature blast cells that leads to ineffective hematopoiesis and ultimately bone marrow failure.[2] Its complex categorization comprises several diagnostic classification groups defined according to the presence of specific molecular and cytogenetic abnormalities.[3,4]

To establish an AML diagnosis, patients undergo immunophenotyping by multiparameter flow cytometry (MFC).[5] This measurement identifies disease-specific cell-surface and intracellular biomarkers, but the heterogeneity of the disease means that not all markers are expressed in all cases. A myeloid blast count in excess of 20% of total marrow cellularity represents a widely utilized threshold to diagnose acute leukemia and distinguish AML from lower grade disease states such as MDS; however, in accordance with recently updated guidelines, the presence of certain recurrent genomic abnormalities or gene fusions may also indicate diagnostic subclassification as AML in cases with fewer than 20% blasts.[3,4] Blast enumeration in routine clinical laboratories is traditionally confirmed by either morphologic review of a peripheral blood smear or marrow biopsy; additionally, the enumeration is also commonly based on conventional flow cytometric immunophenotypic analysis.

Cytogenetic karyotype analysis is also recommended in the evaluation of AML[5] as it provides important information regarding disease aggressiveness, likely response to treatment, and prognosis.[6] Complicating this analysis, however, is that 40–50% of patients have no detectable chromosomal rearrangements or structural abnormalities. Conventional chromosomal

karyotyping and Fluorescence in situ Hybridization (FISH) are also limited in their technical resolution for megabase or kilobase level abnormalities, respectively; molecular testing and genomic profiling are therefore critical to provide a deeper understanding of disease subclassification, which can inform risk stratification and ultimately therapy selection. This testing can be done using commercially available gene panel diagnostic tests or platforms that test for both mutations and rearrangements. Furthermore, and of significant importance, NGS methods are capable of single nucleotide level resolution. The results of these tests allow patients to be assigned to three genetic risk groups – favorable, intermediate, and high – based on the likelihood that they will experience disease relapse.

Most patients with AML will achieve remission following induction therapy[7] that typically involves anthracyclines and cytarabine. Unfortunately, relapse is common, is associated with worse long-term survival, and typically arises from a preexisting or closely genetically related clone.[7] Hence, being able to monitor for recurrence regularly may provide a safeguard against such poor outcomes.

## Monitoring measurable residual disease

One of the strongest predictors of relapse and clinical outcomes in AML is MRD, specifically the population of leukemia cells that survives treatment despite morphologic remission. It is therefore important to monitor patients throughout treatment for the presence of MRD as this can then guide post-remission therapy. A patient with favorable-risk AML may be reclassified as intermediate-risk depending on the absence or presence of MRD, respectively.[5]

### Methods to determine MRD
In the following paragraphs, we consider options for monitoring MRD cases. We first consider cytogenetic karyotyping analysis. This assessment plays an important role in AML diagnosis, but its limited sensitivity, slow turnaround time, and need

for a preexisting abnormal karyotype mean that it is not suitable for MRD assessment.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a common diagnostic methodology employed in clinical molecular laboratories, however RT-PCR is known to show relatively limited utility for monitoring MRD in AML.[5] Although this method is highly sensitive with a capability of assessing leukemic cellular burden with an analytic sensitivity as low as $1 \times 10^{-4}$ (0.01%), RT-PCR may fail to capture and track emerging leukemic cellular populations during clonal evolution or therapeutically selected subclones at the time of disease relapse. Furthermore, many AML subtypes lack characteristic chimeric gene fusions amenable to PCR amplification. RT-PCR primer design is often technically limited, failing to adequately account for the broad variety of potential partner genes involved in fusion events with genes known to be clinically significant in AML, such as *KMT2A* and *RARA*.

MFC has also been investigated for MRD monitoring in AML. While flow cytometry is inherently a fast method, the results can be difficult to standardize – cellular viability and sample quality may vary significantly, and data interpretation can be highly subjective, especially at lower levels of disease. Furthermore, cellular phenotype of AML can change over time so that an experienced, subspecialty trained hematopathologist is needed to interpret the data. Unfortunately, it is not uncommon for AML to lack immunophenotypically aberrant marker expression profiles, thereby limiting distinction of leukemic blasts from normal regenerative precursors following therapy. Lastly, post-therapy immunophenotypic drift significantly complicates serial monitoring in this setting, limiting the practical utility of tracking aberrant, leukemia-associated immunophenotypes which may have been notable at initial diagnosis.

More recently, NGS has emerged as a potentially useful diagnostic adjunct for monitoring MRD in AML. The 2022 update on MRD in AML from the European Leukemia Net (ELN) MRD Working Party[5] states that targeted NGS and gene panel-based approaches can both be considered to assess MRD status. Equally, these recommendations suggest that molecular methods of MRD assessment should reach a limit of detection of $10^{-3}$ (0.1%) or lower.

The NGS panel-based approaches allow clinicians to simultaneously examine a broad range of mutations across a variety of genes known to be recurrently mutated in AML, and can therefore potentially identify evidence of residual disease otherwise non-detectable by conventional

methodologies such as MFC or RT-PCR, significantly improving detection sensitivity, and ensuring that emerging subclones are not missed as can happen when only a single molecular target is followed through disease progression.[8] While NGS approaches have been well-established (many systems installed), are highly sensitive, and well-standardized, they still require error correction to overcome the inherently high false-positive and false-negative rates that arise when background mutations are introduced during preparation of DNA or cDNA libraries.

Besides the intrinsic limitations of the above-described technologies used to monitor MRD, it is equally important to highlight that the clinical interpretation of generated data remains a major challenge, no matter which approach is used. The high disease heterogeneity of AML combined with the appearance of confounding non-pathologic passenger mutations that might arise during follow-up further reinforces the need for additional research in the MRD field.[9]

A case in point: Mutations in genes such as DNMT3A and TET2 may be associated with benign age-related clonal hematopoiesis of indeterminate potential (CHIP), and identification of mutations in these genes following treatment may represent persistence of benign, pre-leukemic cellular subclones, as opposed to overt involvement by residual acute leukemia.[10,11] The challenge now is to build clear evidence for clinical relevance at different levels of detection, particularly as not all patients with MRD will relapse and not all of those without MRD will remain disease-free.

### The predictive value of measurable residual disease

MRD assessment in AML can be used as a prognostic or predictive biomarker to refine risk assessment, as a monitoring tool to evaluate the status of the disease, assess the risk of relapse, inform treatment decision-making, and also as a potential surrogate endpoint in clinical trials.

For prognostic risk assessment, the value of MRD has been demonstrated regardless of age, AML subtype, timing, and type of MRD assessment. A meta-analysis of 81 publications involving more than 11,000 patients showed that MRD negativity was associated with a significant 63% lower risk for disease progression or death compared to MRD positivity.[12]

The ELN recommends that MRD monitoring should be considered a standard of care procedure; by way of example, MRD assessed in bone marrow at diagnosis, after two cycles of induction chemotherapy, at the end of treatment, and then at 3-month intervals for 2 years post treatment.[9] If peripheral blood is being assessed, the follow-up

intervals are reduced to every 4–6 weeks. Monitoring MRD in this way allows clinicians to react quickly when they identify a patient who is likely to relapse on the basis of a positive MRD measurement.

The aim of induction therapy is complete remission (CR). After attaining CR, patients undergo consolidation therapy with a cytotoxic chemotherapy, such as intermediate-dose cytarabine either with or without allogenic hematopoietic stem cell transplant (HSCT). The decision to use allogenic HSCT is based on patient risk that is defined, in part, by the presence or absence of MRD. The procedure is recommended for patients with an estimated relapse risk of 35% to 40%, which includes individuals with adverse-risk disease regardless of MRD status and those with favorable – or intermediate-risk disease and MRD-persistence.[5]
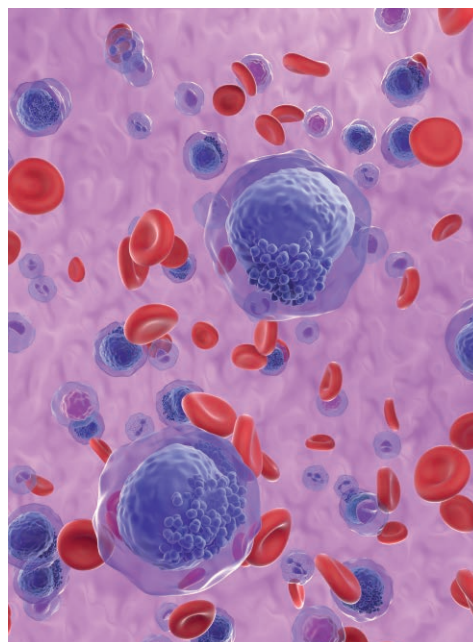


Following consolidation, patients are given maintenance therapy to reduce their risk for relapse. MRD monitoring during this phase may identify clonal evolution with the emergence of actionable therapeutic targets among patients that had no targetable mutations at diagnosis. If these patients subsequently relapse, they will then be eligible for targeted therapy. At present, targeted therapies for AML include FLT3 inhibitors such as midostaurin and gilteritinib, the IDH inhibitors ivosidenib and enasidenib, the CD-33 targeted agent gemtuzumab ozogamicin, the BCL-2 inhibitor venetoclax, and the hedgehog pathway inhibitor glasdegib.

The number of new drugs available for AML is increasing rapidly with many more in clinical trials launched or in progress. In line with this new wave

of drugs, the US Food and Drug Administration (FDA) has issued a guidance document regarding the regulatory considerations for the use of MRD in the development of therapeutic drugs and biological products.[12] Further, the FDA explains in this document how MRD data could serve as the basis for accelerated or traditional drug approval, depending on the strength of the evidence supporting surrogacy. Given that MRD negativity is strongly predictive of improved outcomes, it has been suggested that MRD could be used as a surrogate endpoint in such trials.[9] Since an MRD assay provides quantitative data on the number of residual leukemia cells present, it can be assumed that becoming MRD test negative is a biologically plausible surrogate for longer survival. Moreover, those patients achieving CR and without MRD has been shown to correlate with longer survival relative to CR with MRD.[13]

MRD is already used as a surrogate endpoint in acute lymphocytic leukemia clinical trials, and if adopted in AML, it could accelerate drug development, shorten clinical trials, reduce costs, and expose fewer patients to potentially toxic or ineffective treatments.[14]

### Progress in measurable residual disease monitoring

Although the benefits of monitoring MRD are clear, there is still a lack of well-standardized, sensitive assays that pick up multiple low-level residual clones. The good news is that solutions are becoming available to address some of these challenges. The Ion Torrent™ Oncomine™ Myeloid MRD Assays (RUO)* are the first NGS-based tests to support both DNA and RNA input, enabling a comprehensive and highly sensitive MRD assessment from blood and bone marrow samples. The Ion Torrent sequencing technology incorporates error-correcting unique molecular tags to reduce background noise and improve sensitivity. The DNA panel covers 33 genes, including key mutations in *NPM1, FLT3, DNMT3A* and full gene coverage for *CEBPA* and *TP53*, while the RNA panel detects 27 fusion driver genes, including *BCR-ABL1, RUNX1, CBFB-MYH11*, and *KTM2A*.[15] The broad fusion panel enables detection of over 990 unique fusion isoforms.

Thermo Fisher designed the assay for laboratories that need a streamlined method for detecting and tracking multiple mutations with high sensitivity and specificity for MRD assessment. It can detect variants down to 0.05% allele frequency, with a lower sample input than is required for MFC and PCR. Downstream analysis with software tools can help simplify data interpretation and automate the reporting of clinically significant findings, reducing the strain

on laboratory bioinformaticians and the need for deep informatics expertise. Overall, the Oncomine Myeloid MRD Assays (RUO) provide a simple sample-to-data workflow that minimizes user hands-on time while also minimizing turnaround time in a highly standardized manner.

The assay is being used in the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium Project that aims to validate new methods of detecting and quantifying MRD in patients with AML.[16] During the 4-year project, which launched at the beginning of 2022, Thermo Fisher will work with partners from the US National Cancer Institute, the National Heart Lung and Blood Institute, the FDA, the Dana-Farber Cancer Institute, the Fred Hutchinson Cancer Research Centre as well as other private sector businesses to establish a library of reference materials for researchers to use as a benchmark for MRD assay development and create a process and standards through which assays and new technologies can be developed and tested.

The project will also be involved with validating new assays, developing a publicly available MRD toolbox for researchers, and collecting data to support MRD as a validated surrogate endpoint in clinical trials. Achieving these goals should ultimately speed up development of new therapies for patients with AML, and in the long-term generate important molecular information to improve understanding of the disease and ultimately improve patient outcomes.[16]

### Future steps for MRD in AML

Broader efforts are under way to assess MRD as a potential biomarker in AML. Despite recently updated guidelines for disease subclassification and significant refinements in genetic subtyping of AML (some subtypes with clinical remission rates close to 80%), greater than half of adult AML patients will eventually experience disease relapse, largely due to the emergence of resistant clones after therapy. Utilization of NGS molecular barcodes, or unique molecular indices (UMIs) to facilitate error corrected NGS, represents a significant technical advancement that has dramatically improved analytic detection sensitivity to levels required for clinical MRD assessments in AML.[17] As the costs of sequencing and contemporary genomic profiling methodologies continue to decline, higher coverage depth NGS analysis exploiting UMIs will continue to push the limits of analytically sensitive and specific detection for clinically relevant, disease defining single nucleotide variants or insertion deletion events.

Despite the advances that are being made in understanding the significance of MRD in AML, many unanswered questions remain. The best

timepoints and frequencies for assessing MRD are unknown, as is the optimal specimen type (bone marrow vs blood). More work is also needed to understand whether preemptive treatment based on MRD findings leads to better outcomes than treatment at morphologic relapse and if further into the future MRD might be used to carry out refined molecular subgrouping, identifying more risk subgroups and developing treatments targeted to these subgroups.

Yet, just because we know how to detect MRD in AML does not mean we know how to eliminate it or if doing so will improve clinical outcomes either for all patients or specific subgroups. Similarly, it is still unclear why some MRD-positive patients do not always relapse and why some MRD-negative patients are not always cured. Further work to understand these questions should lead to improved outcomes for patients with AML. JoPM

## Summary Points

- Measurable residual disease in AML patients refers specifically to the population of leukemia cells that survive treatment despite morphologic remission.

- MRD can predict outcomes in patients with AML

- MRD can be measured by flow cytometry, PCR or NGS

- The methods for monitoring MRD have varying sensitivities and lack standardization

- Novel NGS assays, such as the Ion Torrent Oncomine Myeloid MRD Assays (RUO), may overcome some of the limitations of traditional MRD monitoring, with wider target range, improved sensitivity and good standardization

- More work is needed before the full impact of monitoring MRD in AML can be understood

### Dr. Bevan Tandon

Bevan is subspecialty certified by the American Board of Pathology in both Hematopathology and Molecular Pathology. His primary interests are in clinically relevant applications of next generation sequencing for hematologic neoplasms and solid tumors, as well as all aspects of traditional hematopathology practice, ranging from lymph node and bone marrow biopsy interpretation to flow cytometry data review.

### Luca Quagliata

Luca holds a PhD in Vascular Medicine and is a Board-Certified Medical Affairs Specialist (BCMAS), and works to advance Precision Medicine through cutting-edge sequencing technology and solutions for research and clinical applications through his role at Thermo Fisher Scientific. Before joining Thermo Fisher, he worked as Senior Director for the R&D Unit as well as Leader of the Molecular Diagnostics Team at the Institute of Medical Genetics and Pathology, care of the University Hospital Basel, Switzerland.

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**Footnote**

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