

Facts About Measurable Residual Disease (MRD)

Introduction

Patients who achieve complete hematologic remission after treatment for blood cancer often harbor residual cancer cells in the bone marrow or peripheral blood that can result in relapse. These cells can be present at levels so low they are undetectable by conventional cytomorphology. The ability to detect low levels of residual cells, referred to as measurable residual disease (sometimes called minimal residual disease, or MRD) has vastly improved in recent years. Technology available today can detect the presence of 1 cancer cell in 10,000-1,000,000 nucleated cells, compared to 1 cancer cell in 20 nucleated cells for conventional cytomorphology. Due to advancing technology in MRD assessment, “remission” in acute lymphoblastic leukemia (ALL) has been redefined and new response categories in acute myeloid leukemia (AML) and multiple myeloma (MM) have been determined.

It is important for clinicians to understand the different methods available to assess MRD, how samples should be obtained, and how to interpret the results to best inform risk assessment and make treatment decisions. This Fact Sheet will explain the methods currently used for MRD assessment, how and when testing should occur for different hematologic malignancies, and how the resulting information can inform prognosis and decisions about care.

Highlights

- Methods used for MRD assessment include multiparameter flow cytometry (MFC), real-time quantitative polymerase chain reaction (RQ-PCR) and next-generation sequencing (NGS)-based assays.
- MFC can detect 1 cancer cell in 10,000 - 100,000 nucleated cells. It relies on the detection of antigens on neoplastic cells as compared to normal cells.
- RQ-PCR is able to detect 1 cancer cell in 100,000 nucleated cells. It is widely used for patients harboring well-defined genetic aberrations, like the *BCR-ABL1* fusion gene.
- Some newer NGS-based assays developed to address some of the limitations of RQ-PCR can detect 1 cancer cell in 1 million nucleated cells.
- The ClonoSEQ® assay is an NGS-based assay that is FDA-cleared for MRD analysis in B-cell ALL [B-ALL], chronic lymphocytic leukemia (CLL) and multiple myeloma (MM).
- MRD assessment is standard clinical practice in both adult and pediatric ALL to predict outcomes and guide therapy.
- MRD monitoring is used in AML as a prognostic indicator, to identify impending relapse and allow for robust post-transplant surveillance
- In MM, MRD is used to measure depth of response at each stage of treatment to inform prognosis
- MRD analysis in chronic myeloid leukemia (CML) is used to gauge response to therapy, inform prognosis and identify patients in deep remission who might discontinue therapy

MRD Basics: Sensitivity and methods of assessment

The sensitivity, or detection thresholds, of different MRD techniques can be expressed in several ways, reflecting the number of cancer cells that can be detected in a sample per the number nucleated cells. This is shown in **Table 1**.

Table 1. MRD detection sensitivity thresholds

Maximum sensitivity (no. cancer cells per no. nucleated cells)	Percentage	Sensitivity threshold
1 in 20	5%	
1 in 1,000	0.1%	10 ⁻³
1 in 10,000	0.01%	10 ⁻⁴
1 in 100,000	0.001%	10 ⁻⁵
1 in 1,000,000	0.0001%	10 ⁻⁶

The modalities used for MRD assessment in hematologic malignancies include multiparameter flow cytometry (MFC) and molecular methods, including real-time quantitative polymerase chain reaction (RQ-PCR) and next-generation sequencing (NGS)-based assays. These modalities differ in their sensitivity thresholds and clinical applications.

Sampling for MRD assessment

For MRD assessment of bone marrow (BM) samples, the quality of the sample is very important. In order to avoid false negative results that can be caused by hemodilution, a sample of 2-5 mL resulting from the first pull is recommended. Dividing a large-volume pull is not appropriate for MRD assessment. If an interventional radiologist will be involved in the aspiration, it is important to communicate that the sample is for MRD analysis so that appropriate guidelines can be followed.¹⁻⁵

Multiparameter flow cytometry (MFC)

MFC relies on the detection of the expression of antigens on neoplastic cells compared to normal cells. MFC involves labeling cells in suspension with fluorochrome-linked antibodies specific to cancer cell antigens.⁶

There are 2 MFC-based methods for quantitating MRD⁷:

- 1. Leukemia-associated immunophenotypes (LAIPs).** This method relies on identification of the immunophenotype of leukemia blasts at the time of diagnosis that can then be followed over time. “MRD positive” refers to the presence of cells with the identified immunophenotype after treatment.
- 2. “Different from Normal” (DfN).** This approach relies on the differences between the immunophenotype of cells in the MRD sample compared to a stereotypical “normal” sample. A diagnostic sample is not necessarily needed for the DfN approach.

List of abbreviations

ALL: Acute lymphoblastic leukemia
AML: Acute myeloid leukemia
ASO: Allele-specific oligonucleotide
BiTE: Bispecific T-cell engager
BM: Bone marrow
B-ALL: B-cell ALL
CLL: Chronic lymphocytic leukemia
CML: Chronic myeloid leukemia
CR: Complete remission
DfN: “Different from Normal”
ELN: European LeukemiaNet
HSCT: Hematopoietic stem-cell transplantation
Ig: Immunoglobulin
IMWG: International Myeloma Working Group
IS: International Scale
LAIP: Leukemia-associated immunophenotype
MFC: Multiparameter flow cytometry
MM: Multiple myeloma
MMR: Major molecular response
MRD: Measurable (or minimal) residual disease
MRI: Magnetic resonance imaging
NGF: Next-generation flow cytometry
NGS: Next-generation sequencing
PB: Peripheral blood
PET: Positron emission tomography
PET-CT: PET-computed tomography
Ph+: Philadelphia chromosome positive
RQ-PCR: Real-time quantitative polymerase chain reaction
TCR: T-cell receptor
T-ALL: T-cell ALL
WBC: White blood cell

MFC is widely available and has a sensitivity down to 10^{-4} , or 10^{-5} with next-generation technology. The sensitivity increases with increased numbers of cellular events captured by the flow cytometer. MFC is applicable to nearly 100% of patients with AML, ALL, CLL and MM. Most laboratories use at least 6-color flow cytometry assays for MRD assessment, which can provide a detection sensitivity down to 10^{-4} .²

Sampling for MFC

A fresh sample is required for MRD analysis by MFC. As mentioned above, the detection threshold depends on the number of events captured by the flow cytometer. In order to reach a detection sensitivity of 10^{-4} , 200,000 – 500,000 cells must be sorted. A sensitivity of 10^{-5} requires that 2-5 million cells are sorted.¹

It is important for clinicians to communicate with the laboratory that an MRD assessment is being performed to ensure enough events are run and that the proper antibody panel is used. Without such guidance, clinical laboratories can fail to capture enough events to assure high sensitivity MRD assessments.¹ Because immunotherapy can affect the immunophenotype of the cells being monitored, it is important to inform the laboratory performing MRD analysis if the patient has received immunotherapy.²

In order to accurately interpret MRD assessments by MFC, clinicians should be aware of the antibody panels being used and the number of cellular events captured in the analysis. There is also a degree of operator subjectivity in the interpretation of MFC results, which should be considered when comparing analyses at different timepoints across different labs and pathologists

Molecular techniques

Genetic approaches to MRD assessment in hematologic malignancies rely on the following observations³:

1. More than 98% of lymphoid malignancies contain clonally rearranged immunoglobulin (Ig) and/or T-cell receptor (TCR) genes (“VDJ recombination”)
2. In 25%-35% of cases there are well-defined chromosome aberrations or mutations

Due to the clonal expansion of cancerous cells, the sequences of VDJ regions can be used to quantify disease.³ The most commonly used methods for genetic MRD assessment are (RQ-PCR)- and (NGS)-based assays.²

RQ-PCR

PCR is widely used for patients harboring well-defined genetic aberrations, like the *BCR-ABL1* fusion gene

in Philadelphia chromosome positive (Ph+) ALL and chronic myeloid leukemia (CML).^{2,7,11,12} RQPCR to quantitate MRD for these patients is simple, inexpensive, and widely applicable.^{7,12,13} RQ-PCR has a sensitivity threshold of approximately 10^{-5} .¹⁴

Allele-specific oligonucleotide (ASO)-based RQ-PCR can be used in malignancies that do not harbor well-defined genetic aberrations. It involves quantifying Ig or TCR rearrangements using primers and patient-specific PCR probes. In this approach, leukemia-specific Ig/TCR rearrangements must first be identified for each patient by sequencing these regions in a diagnostic sample. Patient-specific primers must then be generated so that MRD can be quantitated through RQ-PCR in a post-treatment sample.¹⁴ In patients with ALL, ASO-based RQ-PCR is a feasible approach in 90%-95% of cases.^{7,13} For multiple myeloma (MM), it is applicable in 60% to 70% of cases.^{15,16}

Because ASO-based RQ-PCR is labor intensive, it is also expensive. While used widely in Europe (the EuroMRD Consortium has developed guidelines for the interpretation of data), it is not typically used in the US.

Sampling for RQ-PCR

PCR approaches do not require a fresh sample – both fresh and stored material can be used. If an ASO-based RQ-PCR approach is used, a baseline sample with detectable disease is required in order to subsequently characterize the clones that will be analyzed.⁴

Next generation sequencing (NGS)

In order to address some of the limitations of RQ-PCR, MRD assessment technologies that combine PCR and NGS approaches have been developed. Instead of relying on unique, patient-specific PCR primers and probes, this technology relies on PCR “consensus primers” that allow the amplification of the complete set of Ig or TCR gene sequences in a patient sample.¹⁸

Once amplified, the collective samples are immobilized on a glass chip and sequenced simultaneously using NGS technology. The frequencies of different clonotypes in the baseline sample are determined, which can then be followed over time to measure disease burden.¹⁸ MRD is quantified using bioinformatic analysis.¹⁹

A high disease burden sample is required in order to identify the dominant clone.¹ There may be multiple MRD-relevant clones that can be identified through sequencing. NGS has a sensitivity threshold $>10^{-6}$ and has

been shown to be highly concordant with MFC and PCR techniques.⁷ This approach is available commercially as the ClonoSEQ[®] Assay, which has received FDA-clearance for MRD assessment in patients with B-ALL, CLL and MM.

Sampling for NGS

For NGS, a fresh sample is often preferred, but it may not always be possible. While it can be successfully performed on fewer than 1 million cells, higher numbers result in improved assay sensitivity.¹⁵ For ALL and MM, suitable tissues for NGS determination of MRD include^{1,15,16}:

- Fresh or frozen bone marrow
- Fresh or frozen peripheral blood (PB)
- Fresh or frozen tissue
- Bone marrow aspirate slides can be scraped to obtain material for NGS analysis
- Formalin-fixed paraffin-embedded bone marrow clot sections and tissue

While all of these sources are potentially suitable, their usability in a particular case may be limited by the fixative or the amount of DNA or cells obtained.

Decalcified bone marrow core biopsy material cannot be used. A summary comparing these 3 MRD assessment methodologies is found in **Table 2**.

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Table 2. Comparison of MRD assessment methods^{5-7,10,15,16,20}

	Multiparameter Flow Cytometry (MFC)	Molecular methods	
		RQ-PCR*	NGS (VDJ sequencing)
Availability	Widely available	Widely available	One platform/company available for MRD analysis (ClonoSEQ®)
Sensitivity	10 ⁻⁴ to 10 ⁻⁵	~10 ⁻⁵	~10 ⁻⁶
Applicability	Nearly 100%	Only in cases with well-defined translocation or mutation (e.g., <i>BCR-ABL1</i>)	FDA-cleared for use in ALL, MM and CLL
Sampling	Requires fresh sample (viable cells), analyzed within 24-48h of sampling	Both fresh and stored samples can be used	Both fresh and stored samples can be used
Diagnostic sample needed?	Yes for LAIP approach, not necessarily needed for DfN	No, but does require knowledge of the genetic aberration being tracked	Yes, requires sample with detectable disease to characterize clones for analysis
Other information provided?	Assessment of BM sample can get information about WBC subsets and distribution	No other information available	Provides information about Ig/TCR gene repertoire
Standardized?	No	For some malignancies	FDA-cleared NGS assay for MRD analysis in B-cell ALL [B-ALL], MM, and chronic lymphocytic leukemia (CLL)
Results available	Within a few hours	Usually within 1 week	Usually 2 weeks
Potential limitations	<ul style="list-style-type: none"> Lack of standardization Significant technical expertise required Risk of immunophenotypic shift can lead to false negatives in LAIP approach 	Limited to patients with well-defined genetic aberrations	Expensive

*Information provided here applies to standard RQ-PCR (not ASO-RQ-PCR)

DfN: "Different from normal"; LAIP: leukemia-associated immunophenotype

Putting MRD assessment into context

Because the various methods of MRD assessment differ in sensitivity, a status of “MRD negative” is not universal. Communication of MRD status should be qualified using sensitivity thresholds (e.g., “MRD less than 10⁻⁵”), particularly as more sensitive methods of MRD quantification reach routine clinical practice.¹ The method and timing of MRD assessment are also important factors to consider.

It is generally accepted that a finding of “MRD negativity” with a detection sensitivity of 10⁻⁴ accurately predicts outcomes in leukemia cases.^{5,21,22}

For multiple myeloma, current methods of MRD assessment provide a sensitivity of at least 10⁻⁵, and are fast approaching 10⁻⁶. The clinical utility of assessing MRD to a threshold of 10⁻⁶ is still being investigated, although several studies have shown the utility of this approach.^{41,42,43,44} The ability to detect 1 cancer cell in a million nucleated cells may allow for earlier detection of impending relapse, permitting salvage therapy before hematologic relapse is evident. It may also permit the use of PB for MRD assessment.^{1,16}

While MRD assessment to inform prognosis and treatment decisions has become incorporated into practice guidelines for some malignancies, its use is still being standardized for clinical decision making in others. Evaluation of the use of MRD as a surrogate endpoint for accelerated approval of new therapies is ongoing.

It is important to note that achievement of MRD negativity is not tantamount to a cure. Patients who achieve MRD negativity even by highly sensitive MRD methods can still relapse, due to persistence of disease below the level of detection.

MRD in ALL

After standard chemotherapy treatment, adults with ALL have rates of complete remission (CR) of nearly 90%, but relapses occur commonly. Up to 50% of ALL patients who achieve CR have measurable residual leukemic cells.⁷ In both pediatric and adult ALL patients (including both

B-ALL and T-cell ALL [T-ALL]), across all subtypes, achieving MRD negativity at a threshold of 10⁻⁴ has been shown to be the single best predictor of outcomes.^{7,14,23,24}

MFC is commonly used in Philadelphia chromosome-negative B-ALL and T-ALL, whereas RQ-PCR for *BCR::ABL1* is commonly used for Ph+ ALL and is superior to MFC in this subtype. NGS-based MRD can be used in any ALL subtype and may identify low levels of MRD that is not detectable with MFC or PCR for *BCR::ABL1*.

MRD assessment is now standard clinical practice in the care of patients with ALL, as reflected in the *National Comprehensive Care Network (NCCN) Clinical Practice Guidelines*, available [here](#) (please choose Treatment by Cancer Type in the Guidelines drop down menu in upper right corner). MRD assessments in ALL are carried out on bone marrow samples, and techniques used for quantitation include MFC, RQ-PCR and NGS.⁶

MRD assessment in patients with ALL is used to:

Predict outcomes

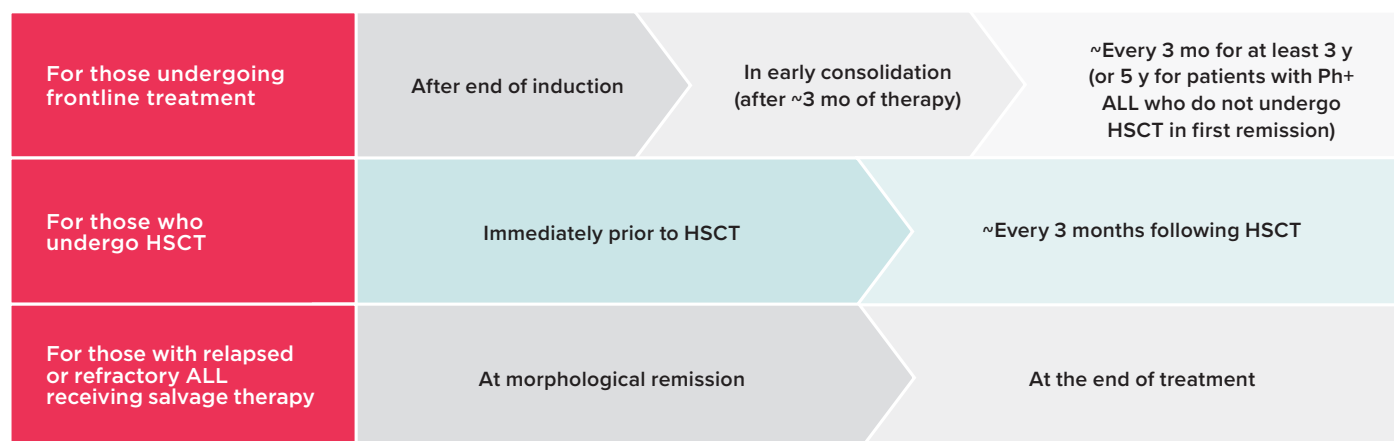
- Achievement of MRD negativity at a threshold of 10⁻⁴ is the best predictor of overall survival and leukemia-free survival in patients with ALL.¹⁴
- MRD negativity very early in induction therapy predicts an excellent outcome in both pediatric and adult ALL.⁶
- In both children and adults, MRD positivity both pre- and post-hematopoietic stem-cell transplantation (HSCT) is predictive of post-treatment relapse.⁷

Guide therapy

- Blinatumomab, a bispecific T-cell engager (BiTE), is approved for use in B-ALL patients in first or second remission with MRD ≥0.1% (10⁻³).²⁵
- MRD status can be used to optimize timing for HSCT and the type of conditioning regimen used.²¹

Recommendations for the timing of MRD assessment in adult ALL patients were published in a 2019 consensus document, and are shown in **Table 3**⁵.

Table 3. When to assess MRD in adult ALL (in BM samples)⁵



MRD in AML

AML is a heterogeneous, complex malignancy typically diagnosed in older adults. Standard induction chemotherapy results in CR in 50%-70% of patients, but relapse rates are high.²⁶ The percentage of patients surviving 5 years from 2010-2016 was 28.7%.²⁷

Measuring residual disease can be complicated in AML because leukemic clones are not stable over time – immunophenotypes or mutational profiles identified at diagnosis may not be those found at relapse.²⁸ MRD assessment is, however, critically important for prognosis. It is well established that MRD positivity after CR in a patient with AML is associated with a higher risk of relapse and shorter survival.^{29,30}

MRD in AML can be assessed using MFC and PCR approaches. PCR is applicable to the approximately 40% of AML patients harboring well-defined mutations. While NGS may be applicable to another 40%-50% of AML patients, its use is still being standardized and is not yet recommended outside of clinical trials.³⁰

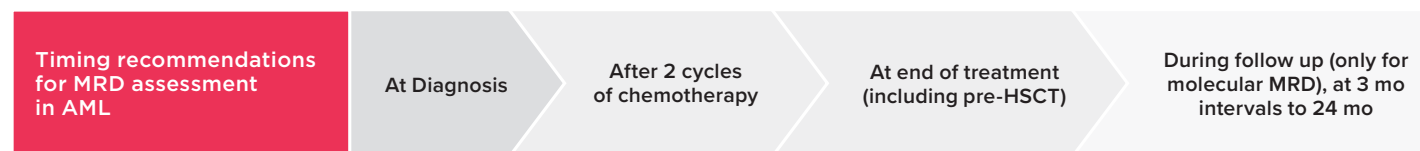
European LeukemiaNet Recommendations

In 2017 the European LeukemiaNet (ELN) recommended a new response category for AML based on MRD status: "CR without measurable residual disease, or CR^{MRD-}."³¹ This is defined as complete morphological remission accompanied by 2 successive MRD negative samples (with a sensitivity of at least 0.1%) obtained within an interval of ≥4 weeks.³⁰

While MRD assessment for AML may not firmly guide treatment decisions as in the case for ALL, the ELN makes the following recommendations^{29,30}:

- MRD monitoring should be considered standard of care.
- MRD should be assessed both before and after HSCT as a prognostic indicator. MRD positivity may impact monitoring decisions or prompt a recommendation to proceed to clinical trial. MRD positivity by MFC or PCR is predictive of inferior transplant outcomes.³²
- For MFC, 500,000 to 1 million nucleated cells should be analyzed. MRD positivity by MFC is highly prognostic.
- PCR should be used for patients harboring wellknown mutations like *RUNX1-RUNX1T1*, *CBFBMYH11*, *PML-RARA* and mutations in *NPM1*.
- Mutations in *NRAS*, *KRAS*, *DNMT3A*, *ASXL1*, *IDH1*, *IDH2*, *MLL-PTD*, and expression levels of *EVII* should not be used as single markers of MRD. They may be useful when combined with a second MRD marker, however.

ELN recommendations for the timing of MRD assessment in AML are found in **Table 4**.³⁰

Table 4. When to assess MRD in AML³⁰

MRD assessment in AML can inform prognosis, identify impending relapse to enable early intervention and allow for robust post-HSCT surveillance.³⁰ MRD assessment can also help to inform the decision of whether to recommend allogeneic stem cell transplantation in first remission, when considered along with a full assessment of patient- and disease-related factors. Given the poor outcomes associated with MRD-positive AML, enrollment of these patients into MRD-directed clinical trials is imperative.

MRD in MM

Over the past 15 years, refinement of multi-drug regimens for MM has resulted in greatly improved patient outcomes. Approximately 50% of patients achieve CR with approaches including immunomodulatory agents, proteasome inhibitors and monoclonal antibodies combined with autologous HSCT, post-transplant consolidation and prolonged maintenance therapy.^{15,20}

While the prognostic value of morphologic CR in MM is established, these improved outcomes have necessitated a more refined definition of CR. It has been shown that MRD negativity by MFC, NGS, magnetic resonance imaging (MRI) and positron emission tomography (PET) is highly prognostic for both progression-free survival and overall survival in MM.³³ The International Myeloma Working Group (IMWG) has defined new response categories of MRD negativity, with or without imaging-based absence of extramedullary disease.¹⁵ MRD in MM should be assessed first in the bone marrow by MFC or NGS.^{15,20}

Advanced next-generation flow (NGF) using 8-color 2-tube or 10-color 1-tube assays are widely used for MRD assessment in MM, as they have been shown to have superior sensitivity and prognostic value compared to conventional MFC.²⁰ NGF has a sensitivity of 10^{-5} if at least 2 million events are captured.

Because myeloma cells have Ig gene rearrangements that are stable over time, NGS can be used for MRD assessment.³⁴ NGS has high prognostic value in MM and shows high concordance with MFC and RQ-PCR.²⁰ In MM, the increased sensitivity provided by NGS ($>10^6$) appears to have clinical implications – patients achieving MRD negativity $<10^{-5}$ have better outcomes compared to those achieving MRD negative status $\geq 10^{-5}$.³⁵

Because MM is a hematologic malignancy with solid tumor features, imaging can be used to detect MRD outside the BM. Residual disease detected by MRI and positron emission tomography and computed tomography (PET-CT) has prognostic significance.^{16,33,36}

MRD negativity in MM is the strongest prognostic indicator, and has become a factor that can overcome negative prognostic implications of high-risk genetic markers (it is better to be high risk and achieve MRD negativity than have standard risk with MRD positivity).³⁸

While currently there is not enough evidence to alter treatment on the basis of MRD status in MM, measuring depth of response by MRD status is recommended at each stage of treatment to inform prognosis.³⁷

Whether to stop therapy in patients with sustained MRD negativity remains an unresolved question.

MRD in CML

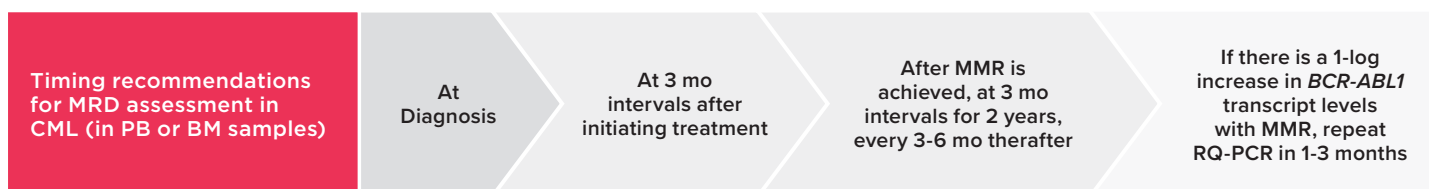
MRD assessment in CML is made easier by the fact that CML is driven by a well-characterized genetic abnormality. Patients with CML harbor a translocation between chromosomes 9 and 22 which generates the Philadelphia (Ph) chromosome, leading to the production of the BCRABL1 oncoprotein.

Treatment with targeted tyrosine kinase inhibitors directed toward BCR-ABL1 results in hematologic and molecular remission in 80%-90% of patients. MRD monitoring is necessary to gauge response to treatment, inform prognosis, and identify patients in deep remission who might discontinue therapy.¹²

The gold standard for MRD monitoring in CML remains the quantitation of *BCR-ABL1* transcripts by RQ-PCR.¹² An International Scale (IS) has been defined as the ratio of *BCR-ABL1* transcripts to a control transcript (e.g., *ABL1*). A “major molecular response” (MMR) is defined as a 1000-fold reduction in the *BCR-ABL1* transcript level compared to baseline.

MRD monitoring in CML can be done on PB or BM samples. NCCN Guidelines, available [here](#), recommend the timing outlined in **Table 5**⁴⁰.

Table 5. When to assess MRD in CML⁴⁰



MRD Laboratories

Flow cytometry is widely available in hospital hematopathology labs. A list of Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories that conduct MRD testing can be found [here](#).

Patient Education

The Leukemia & Lymphoma Society offers free educational material for your patients about MRD. It can be viewed and downloaded [here](#).

This publication is designed to provide accurate and authoritative information about the subject matter covered. It is distributed as a public service by The Leukemia & Lymphoma Society (LLS), with the understanding that LLS is not engaged in rendering medical or other professional services.

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