Next Generation Sequencing for the Assessment of Measurable Residual Disease (MRD)

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DESCRIPTION

Measurable residual disease (MRD), also known as minimal residual disease, refers to residual clonal cells in blood or bone marrow following treatment for hematologic malignancies. MRD is typically assessed by flow cytometry (FC) or polymerase chain reaction (PCR), which can detect one clonal cell in 100, 000 cells. It is proposed that next generation sequencing (NGS), which can detect one residual clonal sequencing out of 1,000,000 cells will improve health outcomes in patients who have been treated for hematologic malignancies.

There are 3 main types of hematologic malignancies: lymphomas, leukemias, and myelomas. Lymphoma begins in lymph cells of the immune system, which originate in

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bone marrow and collect in lymph nodes and other tissues. Leukemia is caused by the overproduction of abnormal white blood cells in the bone marrow, which leads to a decrease in production of red blood cells and plasma cells. The most common forms of leukemia are acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML). Multiple myeloma (MM), also called plasma myeloma, is a malignancy of plasma cells in the bone marrow.

Treatment depends on the type of malignancy and may include surgery, radiotherapy, chemotherapy, targeted therapy, plasmapheresis, biologic therapy, or hematopoietic cell transplant. Treatment of the acute leukemias can lead to complete remission. MM and chronic leukemias are treatable but generally incurable. Patients are typically followed by complete blood count and morphologic assessment of bone marrow. Complete hematologic response is defined as a bone marrow blast (immature cells) composition of less than 5% and hematologic recovery (normal neutrophil and platelet count) without the need for red blood cell transfusions.

Measurable Residual Disease (MRD)

Relapse is believed to be due to residual clonal cells that remain following "complete response" after induction therapy but are below the limits of detection using conventional morphologic assessment. Residual clonal cells that can be detected in bone marrow are referred as measurable residual disease (MRD), also known as minimal residual disease. MRD assessment is typically performed by flow cytometry or polymerase chain reaction (PCR) with primers for common variants. Flow cytometry evaluates blasts based on the expression of characteristic antigens, while PCR assesses specific chimeric fusion gene transcripts, gene variants, and overexpression genes. PCR is sensitive for specific targets, but clonal evolution may occur between diagnosis, treatment, remission and relapse can affect the detection of MRD. Next-generation sequencing (NGS) has 10 to 100- fold greater sensitivity for detecting clonal cells, depending on the amount of DNA in the same and does not require patient-specific primers. For both PCR and NGS a baseline sample at the time of high disease load is needed to identify tumor-specific sequences. MRD with NGS is frequently used as a surrogate measure of treatment efficacy in drug development.

It is proposed that by using a highly sensitive and sequential MRD surveillance strategy, one could expect better outcomes when therapy is guided by molecular markers rather than hematologic relapse. However, some patients may have hematologic relapse despite no MRD, while others do not relapse despite residual mutation-bearing cells. Age-related clonal hematopoiesis, characterized by somatic variants in leukemia-associated genes with no associated hematologic disease, further complicates the assessment of MRD. One commercially available test is clonoSEQ (Adaptive Biotechnologies, Seattle WA) which uses both PCR and NGS to detect receptor gene sequences (IgH [VDJ], IgH [DJ], IgK and IgL) as well as translocated sequences (BCL1/IgH [J] and BCL2/IgH [J]) in DNA extracted from blood and bone marrow in patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM). ClonoSEQ PCR assessment is performed

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when there is a high disease load (e.g., initial diagnosis or relapse) to identify dominant or “trackable” sequences associated with malignant clone. NGS is then used to monitor the presence and level of the associated sequences in follow-up samples. NGS can detect clonal cells with greater sensitivity than either flow cytometry or PCR, although next-generation flow techniques have reached a detection limit of one in 10^{-5} cells which is equal to PCR and approaches the limit of detection of NGS (see below table). The clonoSEQ assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment.

Sensitivity of Methods for Detecting Minimal Residual Disease

Technique	Sensitivity	Detection Limit of Blasts per 100,000 Nucleated Cells
Microscopy (complete response)		50,000
Multiparameter flow cytometry	10^4	10
Next-generation flow cytometry	10^5	1.0
Polymerase chain reaction	10^5	1.0
Quantitative next-generation sequencing	10^5	1.0
Next – generation sequencing	10^6	0.1

Next-Generation Sequencing to Detect Measurable Residual Disease in B-Cell Acute Lymphoblastic Leukemia

Clinical Context and Test Purpose
Acute lymphoblastic leukemia (ALL) is a heterogenous hematologic disease characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs. The age-adjusted incidence rate of ALL in the United States is 1.8 per 100,000 individuals per year, with approximately 5,690 new cases and 1,580 deaths estimated in 2021. The median age at diagnosis for ALL is 17 years with 53.5% of patients diagnosed at younger than 20 years of age. In contrast, 29.6% of cases are diagnosed at 45 years or older and only approximately 13.7% of patients are diagnosed at 65 years or older. ALL represents 75% to 80% of acute leukemia among children, making it the most common form of childhood leukemia; by contrast, ALL represents approximately 20% of all leukemias among adults.

The cure rates and survival outcomes for patients with ALL have improved dramatically over the past several decades, primarily young children. Improvements are largely owed to advances in the understanding of the molecular genetics and pathogenesis of the disease, the incorporation of minimal residual disease (MRD) testing, the refinement of

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risk-adapted treatment algorithms, the advent of new targeted agents, and the use of allogeneic hematopoietic cell transplantation (HCT).

Analyses from the SEER (Surveillance, Epidemiology, and End Results Program) database have shown improvements in survival for children and AYA patients with 5-year overall survival (OS) rates of 89% and 61% respectively. However, survival for adult patients remain low at approximately 20% to 40%. Survival rates are especially poor in older adult patients at approximately 20%. Although exact OS percentage can vary based on how the age range is defined for pediatric, AYA, and adult patients, the trend is nonetheless clear that OS decreases substantially with increased age.

The intent of induction therapy is to reduce tumor burden by clearing as many leukemic cells as possible from the bone marrow below the cytologic detection limit (about 10^{10} cells or one malignant cell for every 20 to 100 normal cells), but it is believed that remaining leukemic cells that are below the level of clinical and conventional morphologic detection lead to relapse if no further treatment were given. Consolidation and intensification therapy is intended to eradicate the residual disease. The type of post-remission therapy (chemotherapy or autologous or allogeneic hematopoietic cell transplantation, HCT) depends on the expected rate of relapse and patient characteristics such as age and comorbidities. Bone marrow is examined every three to six months for a minimum of two years to determine clinical relapse. If a patient is in complete remission (CR) for seven to eight-years, they are considered cured. Most children and up to one-half of adults will have prolonged disease-free survival, but up to 20 percent of adults will have resistant disease, and a majority of adults and some children will eventually relapse and die of leukemia.

Measurable, or minimal, residual disease (MRD) is used to assess subclinical residual disease. Patients with detectable MRD have an increased risk of relapse, but the absolute risk varies depending on the timing of MRD evaluation, the sensitivity of the method used, and baseline characteristics of the patient and tumor. In addition, not all patients with MRD positivity will relapse clinically because some cells with abnormal markers may lack the ability to create disease. Other patients will relapse despite no detectable disease as a result of malignant progenitor cells that lack the initially identified markers. MRD is most commonly measured with polymerase chain reaction (PCR) and flow cytometry (FC).

MRD assays are routinely used in the clinical care of children and increasingly in adults with ALL, although the choice of tests may depend on how the results will impact patient care. FC may be preferred if there are plans to escalate care, because results are rapidly available and the likelihood of relapse with this less sensitive test is high. PCR may be preferred to identify patients with a low risk of relapse when a reduction in treatment intensity is being considered. Some clinicians use more than one technique to minimize false-negative results, or at multiple time points to assess disease trajectory, and ongoing trials are evaluating whether children who demonstrate a rapid clearance of tumor cells during induction therapy may be candidates for less intensive therapy. In adults who have

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a high rate of relapse, MRD is being studied to identify patients who require intensified treatment. Next generation sequencing (NGS) is a newer technique which is commercially available (e.g., clonoSEQ). NGS is more sensitive than other methods and can detect up to 1 leukemic cell in 1,000,000 cells if there is sufficient DNA in the sample.

Test Purpose
The main use of measurement of MRD with next generation sequencing (NGS) is to risk stratify and inform treatment management.

Measures of MRD can be used to assess whether a patient has failed to fully response to treatment or is progressing after responding to treatment. If a patient meets criteria for nonresponse or for relapse, the clinical decision generally would be to provide additional therapy prior to transplant. The analytic framework for the use of MRD for ALL, based on current guideline from the National Comprehensive Cancer Network (NCCN) are below:

Minimal/Measurable Residual Disease Assessment

- The preferred sample for MRD assessment is the first small volume (of up to 3 mL) pull of the bone marrow aspirate if feasible.
- MRD in ALL refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who achieved a CR by morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow
- MRD is an essential component of patient evaluation over the course of sequential therapy. If validated MRD assessment technology with appropriate sensitivity (at least 10^{-4}) is not available locally, there are commercially available tests.
- Studies in both children and adults with ALL have demonstrated the strong correlation between MRD and risks for relapse, as well as the prognostic significance of MRD measurements during and after initial induction therapy.
- The most frequently employed methods of MRD assessment include at least 6-color flow cytometry assays specifically designed to detect abnormal MRD immunophenotypes, real-time quantitative polymerase chain reaction (RQ-PCR) assays to detect fusion genes (e.g., BCR-ABL1) and NGS- based assays to detect clonal rearrangements in immunoglobulin (Ig) heavy chain genes and/or T-cell receptor (TCR) genes.
- Current 6-color flow cytometry can detect leukemic cells at a sensitivity threshold of $<1 \times 10^{-4}$ (<0.01%) bone marrow mononuclear cells (MNCs). PCR/NGS methods can detect leukemic cells at sensitivity threshold $<1 \times 10^{-6}$ (<0.0001%) bone MNCs. The concordance rate for detecting MRD between these methods is generally high. Methods not achieving these sensitivity levels are not suitable.
    - For flow cytometric analysis of MRD, notify lab performing the MRD assessment if immunotherapy (such as rituximab, blinatumomab, inotuzumab ozogamicin, or tisagenlecleucel) has been used.

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- Timing of MRD assessment:
  - Upon completion of initial induction
  - Additional time points should be guided by the regimen used
  - Serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden
  - For some techniques a baseline sample may be needed or helpful for the MRD assessment to be valid

Populations
The relevant population of interest is patients who have received induction therapy acute lymphoblastic leukemia. Patients who receive a clinical complete response (CR) following induction therapy would be assessed for MRD to determine whether additional therapy might be recommended prior to HCT. Patients who have relapsed or refractory disease would assessed for Philadelphia chromosome and if negative may undergo assessment for MRD.

Interventions
The test being considered is MRD assessment by next generation sequencing (NGS) (e.g., clonoSEQ). This test is proposed as an adjunct to clinical assessment and an alternative to flow cytometry (FC) and polymerase chain reaction (PCR). NGS detects receptor gene sequences (IgH [VDJ], IgH [DJ], IgK and IgL) as well as translocated sequences (BCL1/IgH [J] and BCL2/IgH [J]) in DNA extracted from blood and bone marrow in patients with B-cell acute lymphoblastic leukemia (ALL). This technique does not require the use of patient-specific primers, but baseline bone marrow samples are required in order to identify the dominant clonotype. MRD positivity or negativity is reported at all thresholds (e.g., positive at 10^{-4} but negative at 10^{-5}. The sensitivity of this technique can reach up to 10^{-6} depending on the quantity of DNA available from the bone marrow sample.

Comparators
The following tests are currently being used to inform treatment decisions for those acute lymphoblastic leukemia (ALL): Flow cytometry (FC) (sensitivity of 10^{-4}) and PCR (sensitivity of 10^{-5}).

Outcomes
The general outcomes of interest are remission and relapse in the short term and survival at longer follow-up.

Beneficial outcomes of a true-positive test result (presence of clinically significant residual disease) would be the administration of an effective treatment leading to a reduction in relapse and improvements in overall survival (OS). The beneficial outcome of a true-negative test (absence of clinically significant disease) is the avoidance of unnecessary treatment and reduction of adverse events.

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Harmful outcomes of a false-positive tests are unnecessary treatment for ALL resulting in treatment-related harms. Harmful outcomes of false-negative tests are a reduction in necessary treatment that would delay treatment, with potential impact on PFS and OS.

Direct harms of the test are repeated bone marrow biopsy, although bone marrow samples are needed for flow cytometry (FC). Harms of repeated bone marrow biopsy may include tenderness or pain, bleeding, or bruising, and swelling.

Relapse of ALL may be measured in 2 years. Change in survival from ALL would be observable at a minimum of 5 years.

Clinically Valid
A test must detect the presence of absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In 2018, Wood et. al. compared high-throughput sequencing (HTS) of IGH and TRG genes versus flow cytometry (FC) for measurable residual disease (MRD) detection at the end of induction chemotherapy in pediatric patients with newly diagnosed B-ALL. Six hundred nineteen paired pretreatment and end-of-induction bone marrow samples from Children's Oncology Group studies AALL0331 (clinicaltrials.gov #NCT00103285) (standard risk [SR]; with MRD by FC at any level) and AALL0232 (clinicaltrials.gov #NCT00075725) (high risk; with day 29 MRD <0.1% by FC) were evaluated by HTS and FC for event-free (EFS) and overall survival (OS). HTS and FC showed similar 5-year EFS and OS for MRD-positive and -negative patients using an MRD threshold of 0.01%. However, there was a high discordant rate with HTS identifying 55 (38.7%) more patients MRD positive at this threshold. These discrepant patients have worse outcomes than FC MRD-negative patients. In addition, the increased analytic sensitivity of HTS permitted identification of 19.9% of SR patients without MRD at any detectable level who had excellent 5-year EFS (98.1%) and OS (100%). The higher analytic sensitivity and lower false-negative rate of HTS improves upon FC for MRD detection in pediatric B-ALL by identifying a novel subset of patients at end of induction who are essentially cured using current chemotherapy and identifying MRD at 0.01% in up to one-third of patients who are missed at the same threshold by FC. The authors concluded given that HTS has increased analytic sensitivity and specificity compared with FC and is more objective, requiring less interpretative judgment, HTS is a robust clinical platform that can drive assay standardization for MRD determination.

In 2015, Pulsipher et. al. reported on whether the increased sensitivity of next-generation sequencing (NGS)– minimal residual disease (MRD) better identifies pre- and post-HCT (hematopoietic cell transplantation) relapse risk, they performed immunoglobulin heavy chain (IgH) variable, diversity, and joining (V[D]J) DNA sequences J NGS-MRD on 56 patients with B-cell ALL (acute lymphoblastic leukemia) enrolled in Children's Oncology Group trial ASCT0431. NGS-MRD predicted relapse and survival more accurately than MFC-MRD (*P* < .0001), especially in the MRD negative cohort (relapse, 0% vs 16%; *P* = .02; 2-year overall survival, 96% vs 77%; *P* = .003). Post-HCT NGS-

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MRD detection was better at predicting relapse than multichannel flow cytometry (MFC)-MRD ($P < .0001$), especially early after HCT (day 30 MFC-MRD positive relapse rate, 35%; NGS-MRD positive relapse rate, 67%; $P = .004$). Any post-HCT NGS positivity resulted in an increase in relapse risk by multivariate analysis (hazard ratio, 7.7; $P = .05$). Absence of detectable IgH-V(D)J NGS-MRD pre-HCT defines good-risk patients potentially eligible for less intense treatment approaches. Post-HCT NGS-MRD is highly predictive of relapse and survival, suggesting a role for this technique in defining patients early who would be eligible for post-HCT interventions. The trial was registered at www.clinicaltrials.gov as #NCT00382109. Th authors concluded our study suggests a role for IgH-V(D)J NGS-MRD detection in clinical management of patients with ALL undergoing allogeneic HCT. Additional studies will be needed to appropriately characterize deep sequencing MRD approaches for patients with T-cell ALL and non–IgH-V(D)J recombinations of B-cell ALL. In addition, our trial included CR1 and CR2 patients, but no patients in CR3 and no patients treated with non-TBI myeloablative or reduced intensity regimens. Further study of different populations of patients with ALL undergoing HCT with differing approaches will assist in more precisely defining the role of this technique for MRD detection compared with other methods in determining risk and guiding therapy.

Section Summary

Evidence on the clinical validity of next generation sequencing (NGS) to risk stratify patients includes two retrospective studies in pediatric patients with ALL who had participated in earlier trials by the Children's Oncology Group. The largest study was conducted in stored samples from before and after induction therapy, and MRD negativity as one of several factors that were used to risk stratify patients. Comparison with flow cytometry (FC) showed comparable results when the same threshold (10^{-4}) was used for both NGS and FC, and overall survival (OS) in pediatric patients with MRD positivity was significantly lower than in pediatric patients who were MRD negative. However, NGS at the limit of detection (10^{-6} 1 leukemic cell in 1,000,000 normal cells) was found to have lower specificity. In the study of over 600 pediatric patients with B-ALL undergoing induction, risk stratification based on NGS, and FC were comparable at a threshold of 10^{-4} but NGS had more false positives at lower thresholds.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patents receive correct therapy, or more effective therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. No trials were identified that compared outcomes when treatment was guided by NGS.

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Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

There is sufficient evidence on test performance when results of the NGS are reported at 10^4, which is comparable to other established methods of measuring MRD such as flow cytometry (FC). However, performance characteristics at lower thresholds are uncertain, and there is some evidence that false positives may be increased with more sensitive test. Therefore, a chain of evidence cannot be constructed regarding the clinical utility of measurement of MRD at less than 10^4 in patients with ALL.

Section Summary
Evidence is sufficient to support the clinical utility of using NGS to measure MRD when patient management is based on test results at a sensitivity of 10^4. Evidence is insufficient to evaluate benefits and harms when treatment decisions are made based on NGS results at threshold lower than 10^4. Few studies have been performed to assess whether identification of 1 in 1,000,000 cells identifies clinically significant residual disease, false positives may be increased resulting in harms from over treatment. Further study is needed to clarify which threshold of NGS should be considered when risk stratifying patients, and whether treatment decision based on the more sensitive assays improves the net health outcome.

Next Generation Sequencing to Detect Measurable Residual Disease in Multiple Myeloma

Clinical Context and Test Purpose
Multiple myeloma (MM) is a malignant neoplasm of plasma cells that accumulate in bone marrow, leading to bone destruction and marrow failure. MM accounts for about 1.8% of all cancers and 18% of hematologic malignancies in the United States. MM is more frequently diagnosed among people aged 65 to 74 years, with median age being 69 years. The American Cancer Society has estimated 34, 470 new MM cases in the United States in 2022, with an estimated 12,640 deaths.

MM is treatable but is typically incurable, with treatment reserved for patients with symptomatic disease (usually progressive). Without effective therapy, symptomatic patients within a median of six months. Asymptomatic patients are observed because there is little evidence that early treatment of asymptomatic MM prolongs survival compared with therapy delivered at the time of symptoms or end-organ damage. In some patients, an asymptomatic or more advanced premalignant state is referred to as smoldering MM. Patients with smoldering MM may remain stable for prolonged periods, with an overall risk of disease progression from smoldering to symptomatic MM of 10% per year for the first 5 years approximately 3% per year for the next 5 years, and 1% for the next 10 years.

Prognosis and treatment for MM depends on the risk stratification based on underlying genetic variants, age, performance status, comorbidities, stage and response to therapy.

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Patients are assessed to determine eligibility for hematopoietic cell transplantation (HCT), because HCT has been shown to prolong both event-free and overall survival (OS) compared with chemotherapy alone. The response to treatment is usually determined by a morphologic evaluation and visual quantitation of the percentage of plasma cells in bone marrow. Most patients with MM will have an initial response to treatment, but will ultimately progress with serial relapse, and will be treated with most available agents at some point during their disease course. Other patients will not response to initial treatment (refractory disease).

Response to treatment is categorized into clinical response, MRD response, and imaging response. Response criteria for MM is defined by the International Myeloma Working Group (IMWG) and included in the current National Comprehensive Cancer Network (NCCN) guideline for multiple myeloma as shown in the below tables. MRD response requires a complete response plus the absence of clonal plasma cells by next generation flow (NGF) or next generation sequencing (NGS) at a minimum sensitivity of 1 in 10^5 nucleated cells or higher in the bone marrow, and also there is a category of "imaging plus MRD-negative" in which patients are determined to have a complete response defined by MRD negativity in the bone marrow by NGF or NGS plus disappearance of every area of increased uptake found at baseline or a preceding FDG PET/CT or decrease to less mediastinal blood pool standardized uptake value (SUV) or decrease to less than that or surrounding normal tissue. Sustained MRD-negativity is achieved when imaging plus MRD in the marrow by NGF or NGS (or by both NGF and NGS) are negative in assessments that are a minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity. MRD measured by NGS is currently used as surrogate outcome measure in clinical trials, and there are ongoing trials to test the effectiveness of using NGS-MRD to guide therapy.

IMWG Criteria for Response Assessment Including Criteria for Minimal Residual Disease (MRD)

Note: IMWG MRD criteria requires a complete response as defined below

Response Category	Response Criteria
Sustained MRD-negative	MRD negativity in the marrow (next-generation flow [NGF], next generation sequencing [NGS], or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e. g., MRD-negative at 5 years)
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in

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	multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using a validated equivalent method with a minimum sensitivity of 1 in 10^5
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding FDG PET/CT or decrease to less mediastinal blood pool standardized uptake value (SUV) or decrease to less than that of surrounding normal tissue.

Standard IMWG Response Criteria

Response Category	Response Criteria
Stringent complete response	Complete response as defined below plus normal FLC ratio and absence of clonal cells in bone marrow biopsy by immunohistochemistry.
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates.
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h.
Partial response	≥50% reduction in serum M-protein plus reduction in 24-h urinary M-protein by ≥90% or to <200 mg per 24 h. If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria.

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	If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (sum of products of the maximal perpendicular diameters [SPD] of measured lesions) of soft tissue plasmacytomas is also required.
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50%-89%. In addition to the above listed criteria, if present at baseline, a 25%-49% reduction in SPD of soft tissue plasmacytomas is also required.
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease.

Progressive disease	Any one or more of the following criteria
	• Increase of 25% from lowest confirmed response value in one or more of the following criteria:
	    ▪ Serum M-protein (absolute increase must be ≥0.5 g/dL);
	    ▪ Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL;
	    ▪ Urine M-protein (absolute increase must be ≥200 mg/24 h);
	• In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL);

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	• In patients without measurable serum and urine M-protein levels and without measurable FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥ 10%); • Appearance of new lesion(s), ≥ 50% increase in the longest diameter of a previous lesion >1 cm in short axis; • ≥ 50% increase in circulating plasma cells (minimum of 200 cells per uL) if this is the only measure of disease.
Clinical relapse	Clinical relapse requires one or more of the following criteria: • Direct indicators of increasing disease and/or end organ dysfunction (calcium elevation, renal failure, anemia, lytic bone lesions [CRAB features] related to the underlying clonal plasma cells proliferative disorder. It is not used in calculation of time to progression or progressive-free survival but is listed as something that can be reported optionally for use in clinical practice; • Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fracture do not constitute progression; • Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially be the SPD of the measurable lesion; • Hypercalcemia (>11 mg/dL); • Decrease in hemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; • Rise in serum creating by 2 mg/dL or more from the start of the

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	therapy and attributable to myeloma; • Hyperviscosity related to serum paraprotein.
Relapse from complete response (to be used only if the endpoint is disease-free-survival)	Any one or more of the following criteria: • Reappearance of serum or urine M-protein by immunofixation or electrophoresis; • Development of ≥5% plasma cells in the bone marrow; • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion or hypercalcemia).
Relapse from MRD negative (to be used only if the endpoint is disease-free survival)	Any one or more of the following criteria: • Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study of recurrent of myeloma) • Reappearance of serum or urine M-protein by immunofixation or electrophoresis; • Development of ≥5% plasma cells in the bone marrow; • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion or hypercalcemia)

Test Purpose

The main use of measurement of measurable residual disease (MRD) is to inform treatment management.

Measure of MRD can be used to assess whether a patient has responded to treatment, has not fully responded to treatment or has progressed. The analytic framework for the use of MRD for MM, based on guidelines from the current NCCN guideline is outlined below:

Multiple myeloma (symptomatic) → induction therapy → consider minimal residual disease (MRD) as indicated for prognostication

- Response after primary therapy → stem cell transplant or continue myeloma therapy or maintenance therapy → consider MRD as indicated for prognostication
- Relapse or progressive disease after primary therapy → therapy for previously treated myeloma or assess for autologous hematopoietic cell transplant candidacy.

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Populations
The relevant population of interest is patients who are undergoing or have undergone treatment for multiple myeloma (MM).

Interventions
The test being considered is measurable residual disease (MRD) assessment by next generation sequencing (NGS) e.g., ClonoSEQ. NGS utilizes locus-specific primers for immunoglobulin gene rearrangements, which are rearranged in myeloma patients. Baseline bone marrow samples at the time of high disease load are required to identify the dominate clonotype. With the ClonSEQ test, dominant ("clonogenic") sequences can be identified in 92% of MM patients, while dominant sequences cannot be identified in the other 8% of patients.

Comparators
Evaluation of disease progression in multiple myeloma (MM) typically includes serum protein electrophoresis (SPEP), serum immunofixation, 24-hour urine protein electrophoresis (UPEP), urine immunofixation, and serum-free light chain (FLC), hemoglobin, serum calcium, and creatinine. A bone marrow aspirate and biopsy are not always needed but can clarify disease status and determine if a change in the cytogenetic characteristics has occurred. MRD detection by NGS would be an adjunct to clinical measures of progression and an alternative to flow cytometry (FC), which has sensitivity of 10^{-5}.

Outcomes
The general outcomes of interest are clinical progression in the short term and survival at longer follow-up.

Beneficial outcomes of a true-positive test result (detection of clinically significant disease) would be intensification or continuation of an effective treatment leading to longer PFS. The beneficial outcome of a true-negative test (absence of clinically significant residual disease) is the avoidance of unnecessary treatment and reduction of adverse events.

Harmful outcomes of a false-positive test include an increase or continuation of unnecessary treatment resulting in treatment-related harms. Harmful outcomes of a false-negative test include a reduction in necessary treatment that would delay treatment, with a potential impact in disease progression.

Direct harms of the test are repeated bone marrow biopsy. Harms of repeated bone marrow biopsy may include tenderness or pain, bleeding or bruising and swelling.

Utility of MRD to guide treatment of MM may be measured in months for progression of disease, with survival measured in years.

Clinically Valid

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A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In 2020, Martinez-Lopez et. al. reported a retrospective analysis of patients (N=234) treated at their center for newly diagnosed or relapsed MM who had been evaluated for MRD by NGS. MRD assessment by clonoSEQ was performed after a CR, but there was no consistent time after treatment; most were performed within 1 year. Successful identification of at least one trackable sequence in the pretreatment sample was obtained in 234 out of 251 (93%) patients. Sensitivity was assessed at 10^{-4}, 10^{-5}, and 10^{-6}. Out of all patients, 91 (39%) had MRD $<10^{-6}$ and 129 (55%) had MRD $<10^{-5}$. For both newly diagnosed MM and relapsed MM patients, MRD $<10^{-5}$ or $<10^{-6}$ was associated with prolonged survival. In patients who had repeat testing, rising MRD levels preceded clinical relapse by a median of 13 months (range 1 to 28 months). Patients who reached a molecular response at 10^{-5} had similar outcomes to those who achieved MRD negativity at 10^{-6}.

Kriegsmann et. al., (2020) found moderate concordance between next generation sequencing (NGS) and next generation flow cytometry (NGF) in a study of 113 patients with MM. Concordance between methods was obtained in 68% of patients while discordant results were found in 28 patients (11.2% in each direction). Cohen's kappa coefficient for interrater agreement between the MRD status of the 2 methods was 0.536 (n = 113, p <.001). A threshold of 10^{-5} was chosen as the best fit MRD cut-off for evaluation as it met the international guidelines and resulted in a tolerable proportion of nonassessable cases in both methods (1.6%, n = 2 in NGS and 8.0%, n = 10 in NGF).

In 2018 Perrot et. al. performed a retrospective review to assess the prognostic value of minimal residual disease (MRD) measured during maintenance therapy by next-generation sequencing (NGS). MRD negativity was defined as the absence of tumor plasma cell within 1 000 000 bone marrow cells ($<10^{6}$). Data was analyzed from a phase 3 clinical trial (IMF 2009 trial) that evaluated the role of transplantation in newly diagnosed myeloma patients treated with lenalidomide, bortezomib, and dexamethasone (RVD). MRD negativity was achieved at least once during maintenance in 127 patients (25%). At the start of maintenance therapy, MRD was a strong prognostic factor for both progression-free survival (adjusted hazard ratio, 0.22; 95% confidence interval, 0.15-0.34; $P < .001$) and overall survival (adjusted hazard ratio, 0.24; 95% confidence interval, 0.11-0.54; $P = .001$). Patients who were MRD negative had a higher probability of prolonged progression-free survival than patients with detectable residual disease, regardless of treatment group (RVD vs transplant), cytogenetic risk profile, or International Staging System disease stage at diagnosis. These results were similar after completion of maintenance therapy. The authors concluded our findings confirm the value of MRD status, as determined by NGS, as a prognostic biomarker in multiple myeloma, and suggest that this approach could be used to adapt treatment strategies in future clinical trials.

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Clinical validation studies of the clonoSEQ assay for multiple myeloma (MM) include two separate studies to support that MRD as estimated with clonoSEQ assay is associated with patient outcomes in MM.

Samples for the analysis of the clonoSEQ assay performance in MM were obtained from an ongoing randomized, open label, phase III study of lenalidomide and bortezomib in combination therapy regimen (DFCI Study 10-106). Multiple timepoints were assessed in this two-arm analysis and not all patients have the same number of MRD assessments. Patients on Arm A (blinded to Adaptive Biotechnologies) had assessments after eight cycles of RVD (lenalidomide, bortezomib, and dexamethasone), and then after lenalidomide maintenance. Patients on Arm B (blinded to Adaptive Biotechnologies) were assessed following 3 cycles of RVD, following auto transplant, and again after two more cycles of RVD consolidation, and then following lenalidomide maintenance. A subset of 365 of the 720 patients originally enrolled in DFCI Study 10-106 had leftover samples of sufficient amount to be tested with the clonoSEQ assay. The populations characteristics between these 365 patients were compared against the remaining 355 patients that were not tested and there were no significant differences in any characteristic that was evaluated, including age, gender, ISS staging, cytogenetic status and progression free survival. Samples from 365 patients were tested and results from 323 patients were evaluable and passed QC. Seventy-five (75) of these samples were from patients in complete response (CR) at the time of first MRD assessment. This study aimed to demonstrate the association of the first MRD measurement with PFS in patients who achieved CR and with PFS in all evaluable patients. Samples from 75 patients who had achieved CR were evaluable for analysis. Continuous clonoSEQ MRD levels were modestly associated with DFS in patients who have achieved CR (P=0.064), such that patient with lower MRD levels were less likely to progress. The ability of the clonoSEQ Assay MRD measurements to predict PFS in all 323 evaluable patients was also assessed. clonoSEQ measurements demonstrated that MRD status at a threshold of $1x10^5$ at the time of first MRD measurement significantly predicts PFS in all patients (P= 0.027). Cox regression analysis using a continuous measure of MRD was also associated with disease progression ($P=1.9x10^7$). For every 10-fold increase in continuous clonoSEQ MRD measurement, the likelihood of an event is 1.69 times higher (95% CI:1.071-2.67).

The ALCYONE Trial was a multicenter, randomized, open-label, active-controlled phase 3 trial that evaluated daratumumab plus bortezomib, melphalan and prednisone (D-VMP) versus bortezomib, melphalan and prednisone (VMP) in 706 patients with newly diagnosed multiple myeloma who were ineligible for stem-cell transplantation. The result of this study was reported in Mateos et. al. 2018. Within this trial, minimal residual disease was assessed by means of the clonoSEQ assay using bone marrow aspirate collected at screening, at the time of confirmation of complete response or stringent complete response, and at 12, 18, 24, and 30 months after the first dose in patients having a complete response or stringent complete response. Patients who did not achieve a CR were considered to be MRD positive. An MRD threshold of 10^5 was used for analysis. Regardless of treatment group, patients who were MRD negative by the clonoSEQ assay at $\leq 10^5$ had longer progression-free survival (PFS) compared to MRD positive patients.

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The FDA summary of assessment of benefit included the following: “The Adaptive Biotechnologies clonoSEQ Assay is a next generation sequencing-based in vitro diagnostic to evaluate minimal residual disease, evaluating rearranged IgH (VDJ), IgH(DJ), IgK, and IgL receptor gene sequences, as well as translocated BCL1/IgH(J) and BCL2/IgH(J) sequences. This assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment. There is significant potential benefit associated with the use of this device in the clinical setting to assist clinicians manage patients with ALL or MM in accordance with professional guidelines and in conjunction with clinicopathological features.”

In 2014, Martinize-Lopez et. al. performed a retrospective study to assess the prognostic value of minimal residual disease (MRD) detection in multiple myeloma (MM) patients using a sequencing-based platform in bone marrow samples from 133 MM patients in at least very good partial response (VGPR) after front-line therapy. Deep sequencing was carried out in patients in whom a high-frequency myeloma clone was identified and MRD was assessed using the IGH-VDJ_H, IGH-DJ_H, and IGK assays. The results were contrasted with those of multiparametric flow cytometry (MFC) and allele-specific oligonucleotide polymerase chain reaction (ASO-PCR). The applicability of deep sequencing was 91%. Concordance between sequencing and MFC and ASO-PCR was 83% and 85%, respectively. Patients who were MRD negative by sequencing had a significantly longer time to tumor progression (TTP) (median 80 vs 31 months; $P < .0001$) and overall survival (median not reached vs 81 months; $P = .02$), compared with patients who were MRD positive. When stratifying patients by different levels of MRD, the respective TTP medians were: MRD $\geq 10^3$ 27 months, MRD 10^3 to 10^5 48 months, and MRD $<10^5$ 80 months ($P = .003$ to .0001). Ninety-two percent of VGPR patients were MRD positive. In complete response patients, the TTP remained significantly longer for MRD negative compared with MRD positive patients (131 vs 35 months; $P = .0009$). The authors concluded this study suggests that MRD assessment by sequencing is a useful method for patient risk stratification, and the definition of molecular CR in clinical trials can be extended to include the sequencing method.

Section Summary

The evidence on next generation sequencing (NGS) for detection of measurable residual disease (MRD) include two retrospective studies and additional retrospective studies from the FDA Summary of Safety and Effectiveness of the de novo application for clonoSEQ assay in patients with multiple myeloma (MM). These studies evaluated the association between the level of MRD detected by NGS in bone marrow and the TTP from the completed phase 3 trials. All of the studies demonstrated an association between the level of MRD and PFS with longer TTP in patients who exhibit MRD negativity below 10^5 or 10^6 compared to patients who have detectable residual disease. There was also high concordance between NGS and flow cytometry (FC).

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if

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patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. The preferred evidence would be from randomized controlled trials (RCTs).

No RCTs assessing the clinical utility of MRD by NGS to guide therapy were identified.

Indirect evidence on clinical utility rests on clinical validity. There was high concordance between NGS and flow cytometry (FC) at a threshold of 10^4 indicating that NGS may be considered an alternative to FC at this threshold.

Section Summary

The evidence on NGS for detection of MRD includes 3 published retrospective studies and additional retrospective studies from the Summary of Safety and Effectiveness of the de novo application for ClonoSEQ in patients with MM. These studies evaluated the association between the level of MRD detected by NGS in the bone marrow and the TTP from the completed phase 3 trials or from a clinical population. All of the studies demonstrated an association between the level of MRD and PFS with longer TTP in patients who exhibit MRD negativity below 10^{-5} or 10^{-6} compared to patients who have detectable residual disease. There was also high concordance between NGS and FC. Patients who were discordant for the 2 tests had outcomes that were intermediate between patients who were positive for both tests and those who were negative for both tests.

In exploratory analysis of the largest study, the median PFS was 29 months in patients who were positive for MRD and was not reached among patients with no detectable clones, suggesting that assessment of MRD might have utility in guiding therapy. About one-quarter of MRD negative patients progressed within 36 months in these trials, raising questions about whether NGS could be used to guide therapy. It is unknown whether progression is due to very low levels of residual disease or to new clonal rearrangements in MM. Direct evidence from RCTs is needed to evaluate whether patient outcomes are improved by changes in postinduction care (e.g., continuing or discontinuing therapy, avoiding unnecessary adverse events) following NGS assessment of residual disease. Trials that test the effectiveness of MRD to guide therapy in MM are ongoing.

Next Generation Sequencing to Detect Measurable Residual Disease in Chronic Lymphocytic Leukemia (CLL)

Clinical Context and Test Purpose

Chronic lymphocytic leukemia (CLL) are characterized by a progressive accumulation of leukemic cells in the peripheral blood, bone marrow, and lymphoid tissues. Morphologically these leukemic cells appear as small, mature lymphocytes that may be found admixed with occasional larger or atypical cells, or prolymphocytes. CLL remains the most prevalent adult leukemia. In 2022, an estimated 20,160 people will be diagnosed with CLL in the United States, and an estimated 4,410 people will die from the disease.

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CLL and SLL are different manifestations of the same disease are managed in much the same way. The major difference is that in CLL, a significant number of the abnormal lymphocytes are found in the peripheral blood in addition to bone marrow and lymphoid tissue, while in SLL, the bulk of disease is in lymph nodes, bone marrow, and other lymphoid tissues and there are few (if any) abnormal lymphocytes circulating in the peripheral blood.

The choice of first-line treatment for CLL should be based on the disease stage, presence or absence of del(17p) or TP53 mutation, IGHV mutation status (if considering chemoimmunotherapy), patient's age, performance status, comorbid conditions, and the agent's toxicity profile. Ibrutinib and acalabrutinib ± obinutuzumab are preferred first-line therapy options for all patients including in high-risk subgroups such as those with del(11q) or del(17p)/TP53 mutation and unmutated IGHV. Venetoclax + obinutuzumab is an effective fixed-duration chemotherapy-free first-line treatment option for all patients including those with del(17p)/TP53 mutation. Idelalisib is not indicated in first-line treatment. FCR is preferred for patients <65 years with untreated IGHV-mutated CLL as it offers a defined treatment course and the majority of patients with IGHV-mutated CLL who receive first-line FCR are expected to have more than 10 years of PFS and may potentially be cured of their disease. Ibrutinib, idelalisib (± rituximab), acalabrutinib, develisib, and venetoclax ± rituximab are effective treatment options for relapse/refractory CLL/SLL.

Response to treatment is categorized into clinical response (tumor load and hematopoietic system [or marrow]) and MRD response. Response to treatment was developed by the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) and National Comprehensive Cancer Network (NCCN) Response Definition after Treatment for CLL as outlined below:

Response Definition after Treatment for CLL

Parameter	**Complete Revision (CR)** **Note:** All criteria have to be met	**Partial Remission (PR)** **Note:** At least 2 of the parameters of Group A and 1 parameter of Group B need to improve if previously abnormal; if only 1 parameter of	**Progressive Disease (PD)** **Note:** At least 1 of the criteria of Group A or Group B has to be met	**Stable Disease (SD)** **Note:** All of the criteria have to be met; constitutional symptoms alone do not define PD

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		both Groups A and B is abnormal before therapy, only 1 needs to improve		
Group A – Criteria define the tumor load				
Lymph nodes	None ≥ 1.5 cm	Decrease ≥50% (from baseline)	Increase ≥ 50% from baseline or from response	Change of -49% to +49%
Liver and/or spleen size	Spleen size <13 cm; liver size normal	Decrease ≥50% (from baseline)	Increase ≥ 50% from baseline or from response	Change of -49% to +49%
Constitutional symptoms	None	Any	Any	Any
Circulating lymphocyte count	Normal	Decrease ≥50% (from baseline)	Increase ≥ 50% from baseline	Change of -49% to +49%
Group B – criteria define the function of the hematopoietic system (or marrow)				
Platelet count	≥100,000/uL	≥100,000/uL or increase ≥50% over baseline	Decrease ≥50% over baseline secondary to CLL	Change of -49% to +49%
Hemoglobin	≥11 g/dL (untransfused and without erythropoietin)	≥11 g/dL or increase ≥50% over baseline	Decrease of ≥2 g/dL from baseline secondary to CLL	Increase <11.0 g/dL or <50% over baseline, or decrease <2 g/dL

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Marrow	Normocellular, no CLL cells, no B-lymphoid nodules	Presence of CLL cells, or of B-lymphoid nodules, or not done	Increase of CLL cells by $\geq$50% on successive biopsies	No change in marrow infiltrate
Neutrophils without growth factors	$\geq$1500/uL	$\geq$1500/uL or >50% improvement over baseline		

Minimal Residual Disease MRD Assessment

- Evidence from clinical trials suggest that undetectable MRD in the peripheral blood after the end of treatment is an important predictor of treatment efficacy.
- Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}. Next Generation DNA sequencing (NGS) based assays have been shown to be more sensitive, thus allowing for the detection of MRD at the level of 10^{-6}.
- MRD evaluation should be performed using an assay with a sensitivity of 10^{-4} according to the standardized ERIC method or standardized NGS method.

Undetectable minimal residual disease (MRD; $<10^{-4}$ detectable leukemic cells in peripheral blood or marrow) after the end of treatment (EOT) is associated with long-term survival.

Test Purpose

The main use of measurement of minimal residual disease (MRD) with next generation sequencing (NGS) in patients with chronic lymphocytic leukemia (CLL) is to predict treatment efficacy.

Measures of MRD can be used to assess whether a patient has responded to treatment, has not fully responded to treatment, or has progressed.

Populations

The relevant population of interest is patients who are undergoing or have undergone treatment for chronic lymphocytic leukemia (CLL).

Interventions

The test being considered is minimal residual disease (MRD) assessment by next-generation sequencing (NGS) (e.g., clonSEQ). NGS utilizes locus-specific primers for immunoglobulin gene rearrangements, which are rearranged in CLL patients. Baseline blood or bone marrow samples at the time of high disease load are required in order to identify the dominant clonotype.

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Comparators
MRD detection by NGS would be an adjunct to clinical measures of progression and an alternative to FC or next generation flow cytometry (NGF), which has a sensitivity of 10^{-5}.

Outcomes
The general outcomes of interest are clinical progression in short term and survival at longer follow-up.

Beneficial outcomes of a true-positive test result (detection of clinically significant disease) would be intensification or continuation of an effective treatment leading to longer PFS. The beneficial outcome of a true-negative test (absence of clinically significant residual disease) is the avoidance of unnecessary treatment and reduction of adverse events.

Harmful outcomes of a false-positive test include an increase or continuation of unnecessary treatment resulting in treatment-related harms. Harmful outcomes of a false-negative test include a reduction in necessary treatment that would delay treatment, with a potential impact in disease progression.

Direct harms of the test are repeated bone marrow biopsy. Harms of repeated bone marrow biopsy may include tenderness or pain, bleeding or bruising and swelling.
MRD detection by NGS would be an adjunct to clinical measures of progression and an alternative to PCR and flow cytometry. Undetectable MRD in the peripheral blood after the end of treatment is an important predictor of treatment efficacy, supporting the integration of MRD assessment as part of response evaluation. Achieving undetectable MRD (U-MRD) status after chemoimmunotherapy predicts longer progression-free and overall survival.

Systematic Review and Meta-Analysis
In 2019, Molica et.al. conducted a systematic review and meta-analysis to assess minimal residual disease (MRD) and survival outcomes in patients with chronic lymphocytic leukemia (CLL). Patients with chronic lymphocytic leukemia (CLL) who achieve undetectable minimal residual disease (U-MRD) (i.e., $< 10^4$ detectable leukemic cells in peripheral blood or bone marrow) have better outcomes than those with detectable MRD. Eleven studies comprising 2457 patients with CLL treated in upfront with chemotherapy (CT) or chemo-immunotherapy (CIT) were considered suitable for inclusion in the quantitative meta-analysis. Nine studies (n = 2088) provided data on the impact of MRD on PFS and 6 (n = 1234) on overall survival (OS). MRD was the main endpoint in only 2 of these studies (n = 213). Tests of heterogeneity revealed significant differences among studies for progression free survival (PFS) and OS, which highlights differences across studies. U-MRD status was associated with significantly better PFS overall ($P < .001$) and in patients who achieved conventional complete remission ($P = .01$). Regarding OS, U-MRD predicted longer OS globally ($P < .001$) but not in patients having achieved complete remission ($P = .82$). The authors concluded U-MRD status after treatment with

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CT or CIT in newly diagnosed CLL is associated with long-term survival. These findings provide quantitative evidence to support the integration of MRD assessment as an end point in clinical trials of CLL.

Clinically Valid
The test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In 2019, Thompson et. al. conducted a prospective, phase 2 clinical trial assessing minimal residual disease (MRD) in patients with chronic lymphocytic leukemia (CLL) using next-generation sequencing (NGS) after chemoimmunotherapy. Patients with chronic lymphocytic leukemia (CLL) who achieve blood or bone marrow (BM) undetectable minimal residual disease (U-MRD) status after first-line fludarabine, cyclophosphamide, and rituximab (FCR) have prolonged progression-free survival (PFS), when assessed by an assay with sensitivity 10^4 (MRD4). Despite reaching U-MRD4, many patients, especially those with unmutated *IGHV*, subsequently relapse, suggesting residual disease $<10^4$ threshold and the need for more sensitive MRD evaluation. MRD evaluation by next-generation sequencing (NGS) has a sensitivity of 10^6 (MRD6). To better assess the depth of remission following first line FCR treatment, next generation sequencing (NGS) assay clonoSEQ (Adaptive Biotechnologies Corporation) was used to assess MRD in 62 patients, all of whom had BM U-MRD by multicolor flow cytometry (sensitivity 10^4) at end-of-FCR treatment. Samples from these patients included 57 BM samples, 29 peripheral blood mononuclear cell (PBMC) samples, and 32 plasma samples. Only 27.4% of the 62 patients had U-MRD by NGS. Rate of U-MRD by NGS was lowest in BM (25%), compared with PBMC (55%) or plasma (75%). No patient with U-MRD by NGS in BM or PBMC was MRD positive in plasma. Patients with mutated *IGHV* were more likely to have U-MRD by NGS at the end of treatment (EOT; 41% vs 13%, $P = .02$) than those with unmutated *IGHV*. Median follow-up was 81.6 months. Patients with U-MRD at EOT had superior PFS versus MRD positive patients, regardless of sample type assessed (BM, $P = .02$, median not reached [NR] versus 67 months; PBMC, $P = .02$, median NR versus 74 months). More sensitive MRD6 testing increases prognostic discrimination over MRD4 testing. The authors concluded the optimal sample type to use in CLL for MRD testing by NGS remains to be determined. In part, this will be dependent upon the specific clinical scenario. At this time, no additional treatment is offered to eradicate low-level MRD ($<10^4$) after first-line treatment of CLL, given the generally favorable prognosis for such patients. Thus, if NGS-MRD is used for purely prognostic purposes, analysis of PBMC after first-line chemoimmunotherapy may be adequate. In the future, however, we anticipate that treatment decisions may be made on the basis of highly sensitive MRD results: first, in patients where first-line treatment is given with curative intent, consolidation treatment may be offered to patients with high-risk biological features; second, in patients receiving venetoclax-based combinations, U-MRD4 or U-MRD6 may be used as a trigger for treatment discontinuation. In these scenarios, the most sensitive sample type (BM) may be preferred. Additional studies of follow-up samples from this cohort are planned to further elucidate the temporal dynamics of low-level residual disease.

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In 2016, Rawstron et. al. conducted a parallel analysis of minimal residual disease (MRD) using both clonoSEQ and multiparameter flow cytometry in chronic lymphocytic leukemia (CLL) as part of the European Research Initiative on CLL (ERIC) study. The MFC approach used within the ERIC study is validated to the level of 105, and it consists of six different markers CD5, CD19, CD20, CD43, CD49b, and CD81. The ERIC study reports that the clonoSEQ method provides good linearity to a detection limit of 1 in a million (106). The authors also note, A parallel analysis of high-throughput sequencing (HTS) using the clonoSEQ assay showed good concordance with flow cytometry results at the 0.010% (104) level, the MRD threshold defined in the 2008 International Workshop on CLL guidelines. The combination of both technologies would permit a highly sensitive approach to MRD detection while providing a reproducible and broadly accessible method to quantify residual disease and optimize treatment in CLL.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. The preferred evidence would be from randomized controlled trials (RCTs).

No RCTs assessing the clinical utility of MRD by NGS to guide therapy were identified.

Indirect evidence on clinical utility rests on clinical validity.

Section Summary

The International Workshop on Chronic Lymphocytic Leukemia (iwCLL) updated their guidelines for diagnosis, indications for treatment, response assessment and supportive management of CLL in 2018, which states the following regarding minimal residual disease (MRD): The complete eradication of leukemia is a desired end point. Use of sensitive multicolor flow cytometry, PCR or next generation sequencing (NGS) can detect MRD in many patients who have achieved a complete clinical response. Prospective clinical trials have provided substantial evidence that therapies that are able to eradicate MRD usually result in an improved clinical outcome. The techniques for assessing MRD have undergone a critical evaluation and have become well standardized. Six-color flow cytometry (MRD flow), allele-specific oligonucleotide PCR or high-throughput sequencing using the ClonoSEQ assay are reliably sensitive down to a level of < 1 CLL cell in 10,000 leukocytes.

The current NCCN guideline for Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia Version 1.2022 states the following:

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Minimal Residual Disease (MRD) Assessment

- Evidence from clinical trials suggests that undetectable MRD in the peripheral blood after the end of treatment is an important predictor of treatment efficacy.
- Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD-flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}. Next generation DNA sequencing (NGS) based assays have been shown to be more sensitive thus allowing for the detection of MRD at the level of 10^{-6}
- MRD evaluation should be performed using an assay with a sensitivity of 10^{-4} according to the standardized ERIC method or standardized NGS method

In the combined analysis of two randomized phase III studies conducted by the German CLL Study Group (GCLLSG) (CLL8 and CLL10), MRD status at the end of chemoimmunotherapy correlated with better survival in a multivariate analysis.

The findings support the integration of MRD assessment as part of response evaluation. Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}. Next generation DNA sequencing (NGS) based assays have been reported to be more sensitive allowing for the detection of MRD at the level of 10^{-6}.

Evidence is sufficient to support the clinical utility of using NGS to measure MRD for prognosis based on test results at a sensitivity of 10^{-4}.

Next Generation Sequencing to Detect Measurable Residual Disease in Acute Myeloid Leukemia (AML)

Clinical Context and Test Purpose

Acute myeloid leukemia (AML) is a heterogenous hematologic malignancy characterized by the clonal expansion of myeloid blasts in the peripheral blood, bone marrow and/or other tissues. It is the most common form of acute leukemia among adults and accounts for the largest number of annual deaths from leukemias in the United States. An estimated 20,050 people will be diagnosed with AML in 2022, and 11,540 patients will die of the disease. According to the SEER Cancer Statistics Review, the median age at diagnosis is 68 years, other registries report 71 years with approximately 54% of patients diagnosed at 65 years or older (and approximately a third diagnosed at $\geq$ 75 years of age).

Treatment of AML has been divided into induction chemotherapy and post-remission (e.g., consolidation) therapy. Although obtaining a remission is the first step in controlling the disease, it is also important for patients to emerge from the induction phase in a condition to tolerate subsequent, more intensive treatments during consolidation to achieve durable disease control. In some cases, patients who either received post-remission therapy or those who do not experience relapse usually within 6 to 9 months. Post-remission therapy is recommended for patients younger than 60 years and/or who are fit for intensive therapy. The induction strategy is influenced by

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individual patient characteristics such as age, presence of comorbid conditions affecting performance status, and preexisting myelodysplasia. Patients whose performance status would make them poor candidates for the standard antineoplastic regimens may still be able to participate in clinical trials or low intensity therapy plus oral agents designed to target this underserved patient population. Supportive care may also be an appropriate choice. In younger patients, strategies for consolidation are based on the potential risk of relapse, with higher-risk patients receiving more aggressive therapy. Cytogenetic and molecular abnormalities are the most significant prognostic indicators; however, failure to achieve remission after 1 cycle of induction therapy or high tumor burden, defined as WBC count ≥40,000/mcL, are included as poor-risk factors for long-term remission. Therefore, response is based on bone marrow morphology and cytogenetic and molecular responses taken at several points during the course of treatment. The use of flow cytometry and/or molecular methods to assess minimal residual disease (MRD) is emerging as a novel determinant to assess the depth of therapeutic response at the time of morphologic remission in AML patients.

Response Criteria for AML

- Morphologic leukemia-free state
    - BM <5% blasts in an aspirate with spicules; at least 200 cells must be enumerated
    - No blasts with Auer rods or persistence of extramedullary disease
    - If there is a question of residual leukemia, a BM aspirate/biopsy should be repeated in one week
    - A BM biopsy should be performed if spicules are absent from the aspirate sample
- Complete response (CR)
    - Morphologic CR – patient independent of transfusions
        - Absolute neutrophil count >1000/mcL (blasts <5%)
        - Platelets ≥ 100,000/mcL (blasts <5%)
    - CR without MRD (CR_{MRD-})
        - If studied pretreatment, CR with negativity for a genetic marker by RQ-PCR or CR with negativity by MFC
        - Sensitivity varies by marker and method used; analyses should be done in experienced laboratories Molecular CR – molecular studies negative
    - CRh – partial hematologic recovery defined as <5% blasts in the BM, no evidence of disease and partial recovery of peripheral blood counts (platelets > 50 x 10^9/L and ANC > 0.5 x 10^9/L)
    - CR with complete hematologic recovery (CRi) – All CR criteria and transfusion independence but with persistence of neutropenia (<1,000/mcL) and thrombocytopenia (<100,000/mcL)
    - Responses less than CR may still be meaningful depending on the therapy
- Partial remission (PR)
    - Decrease of at least 50% in the percentage of blasts to 5% to 25% in the BM aspirate and the normalization of blood counts as noted above

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- Relapse following CR
    - Is defined as reappearance of leukemic blasts in the peripheral blood or the finding of more than 5% blasts in the BM, not attributable to another cause (e.g., BM regeneration after consolidation therapy) or extramedullary relapse
- Induction failure
    - Failure to attain CR or CR following exposure to at least 2 courses of intensive induction therapy

Minimal residual disease (MRD) in AML refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who have received a complete remission (CR) by morphologic assessment alone can still harbor a large number of leukemia cells in the bone marrow. Due to the rapidly evolving nature of this filed and the undeniable need for monitoring, MRD is still under investigation. While morphologic assessment is the first step in a cure for AML, there remains a level of MRD that currently lacks any standardized method of monitoring. Two of the most commonly used techniques are real-time quantitative PCR (RQ-PCR) and flow cytometry. RQ-PCR amplifies leukemia associated genetic abnormalities, while flow cytometric profiling detects leukemia-associated immunophenotypes (LAIPs). Both methods have higher sensitivity than conventional morphology. RQ-PCR has a detection range of 1 in 1000 to 1 in 100,000, while flow cytometry has sensitivity between 10^4 to 10^5. The challenge of incorporating these techniques into routine practice is a lack of standardization and established cutoff values, through ongoing research is focused on addressing these limitations. Most of what is known about MRD monitoring has been done in the APL population, however, these techniques are expanding to include other AML subtypes. Emerging technologies include digital PCR and NGs. NGS-based assays can be used to detect mutated genes through targeted sequencing gene panels, though higher sensitivities are observed in PCR and flow cytometry- based methods compared to conventional NGS.

The current NCCN guideline Acute Myeloid Leukemia version 1.2022includes the following regarding measurable (minimal) residual disease assessment:

- The role of MRD in prognosis and treatment is evolving. Participation in clinical trials is encouraged.
- MRD in AML refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. MRD is a component of patient evaluation over the course of sequential therapy. If the patient is not treated in an academic center, there are commercially available tests available that can be used for MRD assessment.
- The most frequently employed methods for MRD assessment include real-time quantitative polymerase chain reaction (RQ-PCR) assays (i.e. NPM1, CBFB-MYH11, RUNX1-RUNX1T1) and multicolor flow cytometry (MFC) assays specifically designed to detect abnormal MRD immunophenotypes. The threshold to defined MRD positive and MRD negative samples depends on the technique and subgroup of AML. Next-generation sequencing (NGS) based assays to detect mutated genes (targeted sequencing 20-50 per gene panel) is not routinely used as

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the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. Mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) and aging (i.e., DNMT3A, TET2, potentially ASKL1) are also not considered reliable markers for MRD.

- Based on the techniques, the optimal sample for MRD assessment is either peripheral blood (NPM1 PCR-based techniques) or an early, dedicated pull of the BM aspirate (other PCR or flow cytometry).
- Timing of MRD assessment:
    - Upon completion of initial induction
    - Before allogeneic transplantation
    - Additional time points should be guided by the regimen used.

Test Purpose

The main use of measurement of measurable residual disease (MRD) with next generation sequencing (NGS) is to inform treatment management.

Measures of MRD can be used to assess whether a patient has responded to treatment, has not fully responded to treatment, or has progressed.

Populations

The relevant population of interest is patients who are undergoing or have undergone treatment for acute myeloid leukemia (AML).

Interventions

The test being considered is MRD assessment by next generation sequencing (NGS) (e.g., clonoSEQ). This test is proposed as an adjunct to clinical assessment and an alternative to FC and PCR.

Comparators

Evaluation for disease progression in AML typically includes CBC, platelets every 1-3 months for 2 years, then every 3-6 months up to 5 years. Bone marrow (BM) aspirate and biopsy only if peripheral smear is abnormal or cytopenia develop.

Outcomes

The general outcome of interest is clinical progression in the short term and survival at longer follow-up.

Beneficial outcomes of a true-positive test result (detection of clinically significant disease) would be intensification or continuation of an effective treatment leading to longer PFS. The beneficial outcome of a true-negative test (absence of clinically significant residual disease) is the avoidance of unnecessary treatment and reduction of adverse events.

Harmful outcomes of a false-positive test include an increase or continuation of unnecessary treatment resulting in treatment-related harms. Harmful outcomes of a false-

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negative test include a reduction in necessary treatment that would delay treatment, with a potential impact in disease progression.

Direct harms of the test are repeated bone marrow biopsy. Harms of repeated bone marrow biopsy may include tenderness or pain, bleeding or bruising and swelling.

The utility of MRD in prognosis and treatment of AML is evolving. Participation in clinical trials is encouraged. MRD in AML refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. MRD is a component of patient evaluation over the course of sequential therapy.

Clinically Valid
The test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In 2018, Thol et. al. in a pilot study tested next generation sequencing (NGS) for minimal residual disease (MRD) considering mutations in NPM1 and FLTR3-ITD. Patients were included if they were age $\geq$ 18 years, had a diagnosis of AML excluding acute promyelocytic leukemia (APL), underwent allogeneic HCT in complete morphologic remission (CR) and had DNA available at diagnosis and in CR just before allogeneic HCT (median time from diagnosis to CR 91 days; median time from sample to transplantation 24 days; range 5-71 days). A total of 116 patients were identified and underwent myeloid panel sequencing to identify a suitable molecular MRD marker, which could be a mutation in any gene except DNMT3A and NPM1, which we excluded from MRD markers because of the association of DNMT3A with clonal hematopoiesis and the established methodology to measure NPM1 MRD. DNA from the relapse sample was available for 20 patients and was analyzed by panel sequencing. MRD was measured in CR samples from peripheral blood or bone marrow before allogeneic HCT and identified 12 patients with persistence of an ancestral clone (variant allele frequency [VAF] >5%). The remaining 96 patients formed the final cohort of which 45% were MRD positive (median VAF, 0.33%; range, 0.016%-4.91%). In competing risk analysis, cumulative incidence of relapse (CIR) was higher in MRD positive than in MRD negative patients (hazard ratio [HR], 5.58; $P < .001$; 5-year CIR, 66% vs 17%), whereas non-relapse mortality was not significantly different (HR, 0.60; $P = .47$). In multivariate analysis, MRD positivity was an independent negative predictor of CIR (HR, 5.68; $P < .001$), in addition to *FLT3-ITD* and *NPM1* mutation status at the time of diagnosis, and of overall survival (HR, 3.0; $P = .004$), in addition to conditioning regimen and *TP53* and *KRAS* mutation status. A high sensitivity of error-corrected NGS-MRD assessment was confirmed by comparing NGS-MRD with the current gold standard of qRT-PCR in NPM1-mutated patients. Additional studies are needed to guide how to interpret persistence of an ancestral clone with leukemic potential for MRD detection. The study is limited by the use of only 1 to 3 MRD markers per patient rather than the whole gene panel to monitor clonal evolution. Generally, NGS-MRD is advantageous because it is flexible, applicable to many patients, and easy to standardize. Limitations of this technique are currently clonal evolution of the cells and the need to further increase the

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sensitivity of the assay. The authors concluded NGS-MRD is widely applicable to AML patients, is highly predictive of relapse and survival when measured in CR before allogeneic HCT and may help refine transplantation and post-transplantation management in AML patients.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. The preferred evidence would be from randomized controlled trials (RCTs).

No RCTs assessing the clinical utility of MRD by NGS to guide therapy were identified.

Indirect evidence on clinical utility rests on clinical validity.

Section Summary
Methods of evaluating measurable residual disease (MRD) in acute myeloid leukemia are evolving and awaiting standardization. The most used methods for monitoring MRD in AML include quantitative polymerase chain reaction (Q-PCR) and multiparameter flow cytometry (MFC). Some studies have used next generation sequencing (NGS) to detect gene mutations for MRD analysis, however, NGS is not routinely used as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. While MRD assessment appears to have prognostic value, it is unknown whether MRD assessment will have therapeutic consequences that will improve long-term outcomes. These and other logistical aspects to MRD monitoring must be clarified and validated in clinical trials before MRD monitoring can be become part of the routine follow-up of all patients with AML.

A consensus document from the European LeukemiaNet MRD Working Party regarding minimal/measurable residual disease in AML updated in 2021 includes the following:

- MRD should be assessed to refine relapse risk in patients who achieve morphology remission, with full or partial hematologic recovery.
- For patients with mutant NPM1, CBF AML (RUNX-1-RUNX1T1 or CBFB-MYH1 1) or APL (PML-RARA) we recommend molecular MRD assessment by qPCR or dPCR.
- AML patients who are not included in the molecularly defined subgroups should be monitored by MRD by MFC.
- NGS-MRD monitoring is useful to refine prognosis to MFC but, to date, there are insufficient data to recommend NGS-MRD as a stand-alone technique.

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Molecular MRD testing
For qPCR-MRD, the prognostic value of log reduction of transcript levels between diagnosis and postinduction time points is under evaluation in clinical trials. For NGS-MRD, the prognostic and predictive relevance of different time points, tissues, and target genes are all under investigation. Bioinformatics approaches also need standardization and quality control rounds. Further studies are needed on how to interpret NGS results when monitoring several gene mutations in a single patient, and whether there are prognostic differences if one, some, or all genes remain detectable. Finally, it is important to identify the benefits and limitations of targeted vs panel approaches for NGS-MRD assessment.

The current National Comprehensive Cancer Network (NCCN) guideline for Acute Myeloid Leukemia Version 1.2022 states the following: "The most frequently employed methods for MRD assessment include real-time quantitative polymerase chain reaction (RQ-PCR) assays (i.e., NPM1, CBFB-MYH11m RUNX1-RUNX1T1) and multicolor flow cytometry (MFC) assays specifically designed to detect abnormal MRD immunophenotypes. The threshold to define MRD positive and MRD negative samples depends on the technique and subgroup of AML. Next-generation sequencing (NGS) based assays to detect mutated genes (targeted sequencing 20-50 genes per panel) is not routinely used, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. Mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) and aging (i.e., DNMT3A, TET2, potentially ASXL1) are also not considered reliable markers for MRD.

Next Generation Sequencing to Detect Measurable Residual Disease in Lymphoid Cancer (Lymphoma)

Clinical Context and Test Purpose
Lymphoma (Hodgkin lymphoma and non-Hodgkin lymphoma) is the most common type of blood cancer. Lymphoma develops when lymphocytes multiply and grow uncontrollably. The two principal types of cells that develop into lymphomas are B-lymphocytes (B-cells) and T-lymphocytes (T-cells). There are other types of B-cell NHL, including but not limited to follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), DLBCL, MM, and MCL. Lymphoblastic or lymphocytic leukemia is a related cancer and is considered either a lymphoma or leukemia, depending on how much of the bone marrow is involved.

Treatment options for lymphoma vary depending upon the disease subtype and may include surgery, radiation therapy, chemotherapy, targeted therapy, plasmapheresis, biologic therapy, stem cell transplant and watchful waiting. In certain cancers, an important consideration in determining success of treatment and monitoring disease progression and prognosis is the monitoring of minimal residual disease (MRD). Research is being done to determine if MRD detection and quantification using next generation sequencing (NGS) can be used in managing individuals with lymphoid cancer.

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While some individuals who undergo treatment for lymphoma will achieve a complete remission (CR) and experience prolonged disease-free survival, others may experience a recurrence or die from the disease. Relapse is thought to be the result of residual cancer cells that remain following a complete remission (CR) but are below the limits of detection using conventional morphologic assessment. These subclinical levels of residual cancer cells, referred to as measurable residual disease (MRD, previously termed minimal residual disease [MRD]), are an important consideration in determining the success of the treatment, forming a prognosis, monitoring disease progression and choosing therapies.

Current generally accepted methods for MRD assessment include allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and flow cytometry (FC). Each technique has its advantages and disadvantages. ASO-PCR provides high sensitivity (generally 1 leukemic cell in 100,000; 0.001%) and has wide applicability given that many malignant cells in many individuals with leukemia, lymphoma, or another malignant hematologic disease have acquired clonal chromosomal abnormalities. But ASO-PCR is time-intensive, typically requires bone marrow and the development of unique patient-specific primers and probes for quantitative PCR. FC also has wide applicability, can be accomplished within 1 day from bone marrow or whole blood and can provide information on both benign and malignant cells, but has lower sensitivity (generally 1 leukemic cell in 10,000; 0.01%). Neither ASO-PCR nor FC is capable of capturing changes associated with immunophenotypic drift during disease progression.

Research is ongoing to determine if high-throughput (deep) sequencing to detect or quantify MRD can be used to manage individuals with lymphoid cancer. Unlike PCR and FC based assays, deep sequencing allows for both the monitoring of MRD of original clones and of clonal evolution during therapy. In initial tests, deep sequencing detected one leukemic cell among greater than 1 million leukocytes. The ability to make an early diagnosis of relapse may allow effective treatment strategies to be implemented at a time when disease burden is still minimal. Additionally, researchers are exploring the role of high-throughput (deep) sequencing in stratifying individuals at highest risk of relapse to guide the selection of the most appropriate adjuvant therapy.

Test Purpose
The main use of measurement of measurable residual disease (MRD) with next generation sequencing (NGS) is to inform treatment management.

Measures of MRD can be used to assess whether a patient has responded to treatment, has not fully responded to treatment, or has progressed.

Populations
The relevant population of interest is patients who are undergoing or have undergone treatment for lymphoid cancers.

Interventions

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The test being considered is MRD assessment by next generation sequencing (NGS) (e.g., clonoSEQ)..

Comparators
Current generally accepted methods for MRD assessment include allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and flow cytometry (FC).

MRD detection by NGS would be an adjunct to clinical measures of progression and an alternative to flow cytometry.

Outcomes
The general outcome of interest is clinical progression in the short term and survival at longer follow-up.

Beneficial outcomes of a true-positive test result (detection of clinically significant disease) would be intensification or continuation of an effective treatment leading to longer PFS. The beneficial outcome of a true-negative test (absence of clinically significant residual disease) is the avoidance of unnecessary treatment and reduction of adverse events.

Harmful outcomes of a false-positive test include an increase or continuation of unnecessary treatment resulting in treatment-related harms. Harmful outcomes of a false-negative test include a reduction in necessary treatment that would delay treatment, with a potential impact in disease progression.

Direct harms of the test are repeated bone marrow biopsy. Harms of repeated bone marrow biopsy may include tenderness or pain, bleeding or bruising and swelling.

Research is ongoing to determine if high-throughput (deep) sequencing to detect or quantify MRD can be used to manage individuals with lymphoid cancer. The ability to make an early diagnosis of relapse may allow effective treatment strategies to be implemented at a time when disease burden is still minimal. Additionally, researchers are exploring the role of high-throughput (deep) sequencing in stratifying individuals at highest risk of relapse to guide the selection of the most appropriate adjuvant therapy.

Clinically Valid
The test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In 2019, Galimberti et. al. completed a review focused on the history of the minimal residual disease (MRD) in NHLs, focusing on the new frontiers of the molecular MRD detection, advantages and disadvantages of the different techniques and how MRD could lead in a next future to drive the therapeutic strategies, which includes the following: "Minimal residual disease (MRD) in non-Hodgkin's lymphomas (NHLs) still represents matter of interest and debate." The authors concluded in the last 20 years many studies

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demonstrated that MRD play an evident prognostic role, especially in terms of progression-free survival (PFS), in NHLs; nevertheless, few studies included MRD as endpoint in their initial design, and also the guidelines edited by the European Society for Medical Oncology (ESMO), even if recognizing the role of MRD in NHLs, do not yet include the molecular tests in the necessary and routine work-up of NHL patients, sustaining the need of a definitive standardization of the molecular tools. Clinical trials are in progress and these results may convince the scientific community about the real possibility of exporting MRD in the clinical practice.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. The preferred evidence would be from randomized controlled trials (RCTs).

No RCTs assessing the clinical utility of MRD by NGS to guide therapy were identified.

Indirect evidence on clinical utility rests on clinical validity.

Section Summary
Current generally accepted methods for MRD assessment include allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and flow cytometry (FC). Research is ongoing to determine if high-throughput (deep) sequencing (next generation sequencing [NGS]) to detect or quantify MRD can be used to manage individuals with lymphoid cancer (non-Hodgkin and Hodgkin). The ability to make an early diagnosis of relapse may allow effective treatment strategies to be implemented at a time when disease burden is still minimal. Additionally, researchers are exploring the role of high-throughput (deep) sequencing (next generation sequencing [NGS]) in stratifying individuals at highest risk of relapse to guide the selection of the most appropriate adjuvant therapy.

Summary of Evidence
For individuals with B-cell acute lymphoblastic leukemia (ALL) who are being monitored for residual disease following treatment who receive next generation sequencing (NGS) for measurable residual disease (MRD) at threshold of 10^{-4}, the evidence includes retrospective comparison of data from two earlier trials by the Children's Oncology Group. Comparison of NGS and the established standard of flow cytometry (FC) showed good concordance when the same threshold (10^{-4}) as used for both NGS and FC. Overall survival (OS) in pediatric patients with MRD positivity was significantly lower than in pediatric patients who were MRD negative at this threshold. The relatively small subset of patients who were discordant for FC and NGS results had

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outcomes that were midway between patients who were concordant as MRD positive or MRD negative for both tests. As most patients had concordant results for NGS and FC at a threshold of 10^{-4}, NGS can be considered an alternative to FC for monitoring MRD in patients with B-ALL. The evidence is sufficient to determine that the technology results in a meaningful improvement in net health outcome.

For individuals with B-cell acute lymphoblastic leukemia (ALL) who are being monitored for measurable residual disease (MRD) following treatment who receive next generation sequencing (NGS) for MRD at a threshold less than 10^{-4}, NGS can be more sensitive than flow cytometry (FC) to detect the presence of residual leukemia cells, but specificity may be decreased at the more sensitive thresholds resulting in potential harms from over treatment. Further studies are needed to clarify whether MRD at lower levels than 1 in 10,000 cells represents clinically significant disease and if the more sensitive test can be used to risk stratify patients with B-ALL. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with multiple myeloma (MM) who have achieved a compete response (CR) following treatment who receive next generation sequencing (NGS) for measurable residual disease (MRD) at threshold of 10-5, the evidence includes retrospective comparison of NGS with flow cytometry (FC) data from multiple myeloma treatment trials and from clinical series. Concordance has been demonstrated between NGS and the established standard of FC at 10^{-4} as well as with NGF at a threshold of 10^{-5}. PFS in patients with MRD positivity is significantly shorter than in patients who are MRD negative at these thresholds. The relatively small subset of patients who were discordant for FC and NGS results had outcomes that were, on average, midway between patients who were concordant as MRD positive or MRD negative for both tests. Retrospective studies also indicate improved PFS when MRD is less than 10^{-5} compared to patients who have MRD greater than 10^{-5}. This threshold is consistent with current guideline-based care for prognostication using either NGF or NGS. The evidence is sufficient to determine that the technology results in meaningful improvement in net health outcome.

For individuals with multiple myeloma (MM) who have achieved a complete response (CR) following treatment who receive next generation sequencing (NGS) for measurable residual disease (MRD) at a threshold of less than 10^{-5}, the evidence includes retrospective studies on prognosis. There is some evidence that MRD may be a prognostic marker, but there is insufficient evidence on the number of false positives in patients with complete response (CR) at the more sensitive threshold provided by NGS to guide therapy. A chain of evidence regarding management changes based on the assessment of MRD with NGS to detect 1 malignant clonal sequence out of 1,000,000 cells cannot be completed. Direct evidence from randomized controlled trials (RCTs) is needed to evaluate whether patient outcomes are improved by measurable residual disease (MRD) at threshold lower than 10^{-5}. Several trials that will test the effectiveness of NGS to guide therapy in MM are ongoing.

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For individuals with chronic lymphocytic leukemia (CLL) who are being monitored for residual disease following treatment who receive NGS for MRD at a threshold of 10^{-4}, the evidence includes analysis of samples from 2 clinical trials. These studies evaluated the association between the level of MRD detected by NGS in bone marrow or blood and progression-free survival in completed phase 2 and 3 trials. Both studies demonstrated an association between the level of MRD and PFS with lower risk of progression in patients who exhibit MRD negativity below 10^{-4} compared to patients who have detectable residual disease. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with chronic lymphocytic leukemia (CLL) who are being monitored for residual disease following treatment who receive NGS for MRD at a threshold of less than 10^{-4}, the evidence includes analysis of samples from 2 clinical trials. NGS can be more sensitive than FC to detect the presence of residual leukemic cells, but it is not clear if prognosis is improved at the lower thresholds. Currently, no additional treatment is offered to eradicate low-level MRD ($<10^{-4}$) after first-line treatment of CLL. Further study is needed to clarify whether MRD at levels lower than 1 in 10000 cells represents clinically significant disease and if the more sensitive test can be used for prognosis in patients with CLL.

For individuals with acute myeloid leukemia (AML) the methods of evaluating measurable residual disease (MRD) are evolving and awaiting standardization. The most used methods for monitoring MRD in AML include quantitative polymerase chain reaction (Q-PCR) and multiparameter flow cytometry (MFC). Some studies have used next generation sequencing (NGS) to detect gene mutations for MRD analysis, however, NGS is not routinely used as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. While MRD assessment appears to have prognostic value, it is unknown whether MRD assessment will have therapeutic consequences that will improve long-term outcomes. These and other logistical aspects to MRD monitoring must be clarified and validated in clinical trials before MRD monitoring can be become part of the routine follow-up of all patients with AML. The current National Comprehensive Cancer Network (NCCN) guideline for Acute Myeloid Leukemia Version 1.2022 states the following: "The most frequently employed methods for MRD assessment include real-time quantitative polymerase chain reaction (RQ-PCR) assays (i.e., NPM1, CBFB-MYH11m RUNX1-RUNX1T1) and multicolor flow cytometry (MFC) assays specifically designed to detect abnormal MRD immunophenotypes. The threshold to define MRD positive and MRD negative samples depends on the technique and subgroup of AML. Next-generation sequencing (NGS) based assays to detect mutated genes (targeted sequencing 20-50 genes per panel) is not routinely used, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. This technology is not as beneficial as established alternatives MRD assessment utilizing flow cytometry (FC) and quantitative polymerase chain reaction (Q-PCR) testing.

For individuals with non-Hodgkin and Hodgkin lymphomas the current generally accepted methods for MRD assessment include allele-specific oligonucleotide

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polymerase chain reaction (ASO-PCR) and flow cytometry (FC). Research is ongoing to determine if high-throughput (deep) sequencing (next generation sequencing [NGS]) to detect or quantify MRD can be used to manage individuals with lymphoid cancer (non-Hodgkin and Hodgkin). The ability to make an early diagnosis of relapse may allow effective treatment strategies to be implemented at a time when disease burden is still minimal. Additionally, researchers are exploring the role of high-throughput (deep) sequencing (next generation sequencing [NGS]) in stratifying individuals at highest risk of relapse to guide the selection of the most appropriate adjuvant therapy. This technology is not as beneficial as established alternatives MRD assessment utilizing flow cytometry (FC) and) allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) testing.

Practice Guidelines and Position Statements

National Comprehensive Cancer Network (NCCN)
Acute Lymphoblastic Leukemia Version 4.2021 (Adult and AYA)

Minimal/Measurable Residual Disease Assessment

- The preferred sample of MRD assessment is the first small volume (of up to 3 mL) pull of the bone marrow aspirate, if feasible.
- MRD in ALL refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who achieved a CR by morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow.
- MRD is an essential component of patient evaluation over the course of sequential therapy. If validated MRD assessment technology with appropriate sensitivity (at least 10^4) is not available locally, there are commercially available tests.
- Studies in both children and adults with ALL have demonstrated the strong correlation between MRD and risk for relapse, as well as the prognostic significance of MRD measurements during and after initial induction therapy.
- The most frequently employed methods for MRD assessment include at least 6-color flow cytometry assays specifically designed to detect abnormal MRD immunophenotypes, real-time quantitative polymerase chain reaction (RQ-PCR) assays to detect fusion genes (e.g., BCR-ABL1) and NGS based assays to detect clonal rearrangements in immunoglobulin (Ig) heavy chain genes and/or T-cell receptor (TCR) genes.
- Current 6-color flow cytometry can detect leukemic cells at a sensitivity threshold $<1 \times 10^4$ (<0.01%) bone marrow mononuclear cells (MNCs). PCR/NGS methods can detect leukemic cells at a sensitivity threshold of $<1 \times 10^6$ (<0.0001%) bone MNCs. The concordance rate for detecting MRD between these methods is generally high. Methods not achieving these sensitivity levels are not suitable.
    - For flow cytometric analysis of MRD, notify lab performing the MRD assessment if immunotherapy (such as rituximab, blinatumomab, inotuzumab, ozogamicin, or tisagenlecleucel) has been used.
    - Timing of MRD assessment:

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    - Upon completion of initial induction
    - Additional time points should be guided by the regimen used
    - Serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden
    - For some techniques a baseline sample may be needed or helpful for the MRD assessment to be valid.

Pediatric Acute Lymphoblastic Leukemia Version 1.2022

Minimal Residual Disease

- MRD in ALL refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who achieved a CR by morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow.
- MRD is an essential component of patient evaluation over the course of sequential therapy. If a validated MRD assessment technology with appropriate sensitivity is not available locally there are commercially available tests.
- Studies in both children and adults with ALL have demonstrated the strong correlation between MRD and risk for relapse, as well as the prognostic significance of MRD measurements during and after initial therapy.
- There are data to support the importance of MRD testing in T-cell ALL (all immunophenotypes), de novo and relapsed B-ALL, and infant ALL.
- The most frequently employed methods for MRD assessment include 6-color flow cytometry assays, specifically designed to detect abnormal MRD immunophenotypes, real-time quantitative polymerase chain reaction (RQ-PCR) assays (e.g., clonally arranged immunoglobulin (Ig), T-cell receptor (TCR) genes), reverse transcriptase quantitative PCR (RT-qPCR) assays (e.g., BCR/ABL1), and NGS based assays to detect fusion genes or clonal rearrangements in Ig an TCR loci (does not require patients specific primers).
  - Prior treatment with immunotherapy of HCT can affect interpretation of flow cytometry based MRD results. MRD should be performed in a laboratory with experience performing MRD in this setting.
- The optimal sample for MRD assessment is the first pull or early pull of the bone marrow aspirate.
- Current flow cytometry or PCR methods can detect leukemic cells at a sensitivity threshold of $<1 \times 10^{-4}$ (<01.01%) bone marrow mononuclear cells (MNCs). PCR/NGS methods can detect leukemic cells at a sensitivity threshold of $<1 \times 10^{-6}$ (<0.0001%) bone MNCs. The concordance rate for detecting MRD between these methods is generally high. Methods not achieving these sensitivity levels are not recommended.
  - Timing of MRD assessment:
    - Upon completion of induction (de novo or relapse)
    - End of consolidation
    - Additional time points should be guided by the regimen used

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    - Serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden.
    - For some techniques, a baseline sample (i.e., prior to treatment) is needed to characterize the leukemic clone for subsequent MRD assessment.
- MRD quantification can be affected by bone marrow aplasia and some protocols require count recovery prior to sending MRD. MRD sent during aplasia may need to be repeated after count recovery.

Acute Myeloid Leukemia Version 1.2022

Measurable (Minimal) Residual Disease Assessment

- The role of MRD in prognosis and treatment is evolving. Participation in clinical trials is encouraged.
- MRD in AML refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. MRD is a component of patient evaluation over the course of sequential therapy. If the patient is not treated in an academic center, there are commercially available tests available that can be used for MRD assessment. Patients who achieved a CR by morphologic assessment alone can still harbor a large number of leukemic cells in the BM. The points discussed below are relevant to intensive approaches (induction chemotherapy) but have not been validate for the modalities of treatment.
- The most frequently employed methods for MRD assessment include real-time quantitative polymerase chain reaction (RQ-PCR) assays (i.e., NPM1, CBFB-MYH11m RUNX1-RUNX1T1) and multicolor flow cytometry (MFC) assays specifically designed to detect abnormal MRD immunophenotypes. The threshold to define MRD+ and MRD- samples depends on the technique and subgroup of AML. NGS based assays to detect mutated genes (targeted sequencing 20-50 genes per panel) is not routinely used, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. Mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) and aging (i.e., DNMT3A, TET2, potentially ASXL1) are also not considered reliable markers for MRD.
    - There are distinct differences between diagnostic threshold assessments. If using flow cytometry to assess MRD, it is recommended that a specific MRD assay is utilized but most importantly that it is interpreted by an experienced hematopathologist.
- Based on the techniques the optimal sample for MRD assessment is either peripheral blood (NPM1 PCR-based techniques) or an early dedicated pull of the BM aspirate (i.e., other PCR, flow cytometry, NGS). The quality of the sample is of paramount importance to have a reliable evaluation.
- Studies in both children and adults with AML have demonstrated the correlation between MRD and risks for relapse as well as the prognostic significance of MRD measurements after initial induction therapy.

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- MRD positivity is not proof of relapse. However, a persistently positive MRD result after induction which depends on the technique used and the study, is associated with an increased risk of relapse.
    - For favorable-risk patients if MRD is persistently positive after induction and/or consolidation consider a clinical trial or alternative therapies (e.g., including allogeneic transplantation).
    - Some evidence suggest MRD testing may be more prognostic than KIT mutation status in CBF AML, but this determination depends on the method used to assess MRD and treated of detectable MRD.
    - After completed of therapy "molecular relapses" can predict hematologic relapses within a 3-to-6-month timeframe.
- Timing for MRD assessment:
    - Upon completion of initial induction
    - Before allogeneic transplantation
    - Additional time points should be guided by the regimen used.

Role of MRD Monitoring
MRD in AML refers to the presence of leukemic cells below the threshold detection by conventional morphologic methods. Patients have achieved a CR by morphologic assessment alone can still harbor a large number of leukemic cells in the bone marrow. Due to the rapidly evolving nature of this field and the undeniable need for monitoring, MRD is still under investigation, with NCCN recommendations as discussed below.

While morphologic assessment is the first step in the cure for AML, there remains a level of MRD that currently lacks any standardized method of monitoring. Two of the most commonly used techniques are real-time quantitative PCR (RQ-PCR) and flow cytometry. RQ-PCR amplifies leukemia-associated genetic abnormalities, while flow cytometric profiling detects leukemia-associated immunophenotypes (LAIPs). Both methods have a higher sensitivity than conventional morphology. RQ-PCR has a detection range of 1 in 1000 to 1 in 100,000 while flow cytometry has sensitivity between 10^4 to 10^5. The challenge of incorporating these techniques into routine practice is a lack of standardization and established cutoff values, though ongoing research is focused on addressing these limitations. Most of what is known about MRD monitoring has been done in the APL population, however, these techniques are expanding to include other AML subtypes. Emerging technologies include digital PCR and NGS. NGS based assays can be used to detect mutated genes through targeted gene sequencing panels though higher sensitivities are observed in PCR and flow cytometry-based methods compared to conventional NGS. The data from these methods have been correlated with AML treatment outcome and the primary results are promising, Refinement of these methods that take into account variables including the intrinsic nature of the transcript as well as factors of the patient population, including age, disease severity, and treatment, will make MRD monitoring in patients with AML are more reliable tool.

Because a high-quality sample is essential for reliable treatment evaluation, the NCCN AML Panel recommends that the optimal sample for MRD assessment is either

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peripheral blood for NPM1 PCR-based techniques or the first pull/early pull of the bone marrow aspirate for other PCR, flow cytometry and NGS based assays. The timing of MRD assessments will vary and depend on the regimen used but may occur after completion of initial induction and before allogeneic transplantation.

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Version 1.2022

Response Definition After Treatment for CLL/SLL
Minimal Residual Disease (MRD) Assessment

- Evidence from clinical trials suggests that undetectable MRD in the peripheral blood after the end of treatment is an important predictor of treatment efficacy.
- Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD-flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}. Next generation DNA sequencing (NGS) based assays have been shown to be more sensitive thus allowing for the detection of MRD at the level of 10^{-6}
- MRD evaluation should be performed using an assay with a sensitivity of 10^{-4} according to the standardized ERIC method or standardized NGS method

In the combined analysis of two randomized phase III studies conducted by the German CLL Study Group (GCLLSG) (CLL8 and CLL10), MRD status at the end of chemoimmunotherapy correlated with better survival in a multivariate analysis.

The findings support the integration of MRD assessment as part of response evaluation. Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}. Next generation DNA sequencing (NGS) based assays have been reported to be more sensitive allowing for the detection of MRD at the level of 10^{-6}.

Multiple Myeloma Version 4.2022
Initial Diagnostic Work-up

- **Useful in Certain Circumstances**
  - Consider baseline clone identification and storage of aspirate sample for future minimal residual disease (MRD) testing by NGS

Multiple Myeloma (symptomatic)

- **Follow-up/Surveillance**
  - Consider minimal residual disease (MRD) as indicated for prognostication after shared decision with patient

Response after Primary Therapy

- **Follow-up/Surveillance**
  - Consider MRD as indicated for prognostication after shared decision with patient

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Response Criteria for Multiple Myeloma
(Revised based on the new criteria by International Myeloma Working Group [IMWG])

IMWG Criteria for Response Assessment Including Criteria for Minimal Residual Disease

- IMWG MRD criteria (requires a complete response as defined below)

Response Category	Response Criteria
Sustained MRD-negative	MRD negativity in the marrow (next-generation flow [NGF], next generation sequencing [NGS], or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g., MRD-negative at 5 years)
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using a validated equivalent method with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or preceding FDG PET/CT or decrease to less mediastinal blood pool standardized uptake value (SUV) or decrease to less than that of surrounding normal tissue
Complete Response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates

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Relapse from MRD negative (to be used only if the endpoint is disease-free survival)	Any one or more of the following criteria: • Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); • Reappearance of serum or urine M-protein by immunofixation or electrophoresis; • Development of $\geq$5% clonal plasma cells in the bone marrow; • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion or hypercalcemia)

All response categories require two consecutive assessments made any time before starting any new therapy; for MRD there is no need for two consecutive assessments, but information on MRD after each treatment stage is recommended (e.g., after induction, high-dose therapy/ASCT, consolidation, maintenance). MRD tests should be initiated only at the time of suspected completed response. All categories of complete response and MRD require no evidence of progressive or new bone lesions if radiographic studies were performed. However, radiographic studies are not required to satisfy these response requirements except for the requirement for FDG PET if imaging MRD-negative status is reported.

Sustained MRD negativity when reported should also annotate the method used (e.g., sustained flow MRD-negative, sustained sequencing MRD-negative)

Imaging should be performed once MRD negativity is determined by MFC or NGS.

Positive immunofixation alone in a patient previously classified as achieving a complete response will not be considered progression. For purposes of calculating time to progression and progression-free survival, patient who have received complete response are and MRD-negative should be evaluated using criteria listed for progressive disease. Criteria for relapse from a complete response or relapse from MRD should be used only when calculating disease – free survival.

Additional Diagnostic Testing
The Panel also suggests baseline clone identification or storage of bone marrow aspirate sample for clone identification for future minimal residual disease (MRD testing for NGS if required, and assessment for circulating plasma cells in peripheral blood, as clinically indicated).

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Follow-up after Hematopoietic Cell Transplantation
Follow-up tests after HCT are similar to those done after primary myeloma therapy. In addition, MRD assessment is increasingly being incorporated into post-treatment assessments. MRD has been identified as an important prognostic factor. A prospective study of patients with newly diagnosed MM evaluated MRD in bone marrow samples and showed that at median follow-up at 57 months, MRD negativity after autologous HCT transplanted to significantly improved PFS and OS rates. Similarly, in another study MRD negativity after autologous HCT was predictive of favorable PFS and OS. Similar results have also been reported int eh allogeneic HCT setting where the presence of MRD after allogeneic HCT has been associated with a significantly adverse PFS and OS. The NCCN Panel recommends assessing for MRD during follow-up as indicated prognostication after shared decision with patient.

B-Cell Lymphomas Version 5.2021
The current guideline does not mention or specifically address the use of NGS to detect or monitor MRD.

Hodgkin Lymphoma Version 1.2022
The current guideline does not mention or specifically address the use of NGS to detect or monitor MRD.

Pediatric Hodgkin Lymphoma Version 3.2021
The current guideline does not mention or specifically address the use of NGS to detect or monitor MRD.

Myelodysplastic Syndromes Version 3.2022

The current guideline does not mention or specially address the use of NGS to detect or monitor MRD.

Myeloproliferative Neoplasms Version 2.2021
The goal of treatment is to reduce symptom burden and minimize the risk of leukemic transformation. Changes in symptom status could be a sign of disease progression. Therefore, change in symptom status should prompt evaluation of treatment efficacy and/or disease status. Evaluation of treatment efficacy should include CBC to assess normalization of blood counts, monitoring symptom status using MPN-SAF TSS, and monitoring spleen size either by palpation or imaging.

The guidelines recommend monitoring response (anemia response, spleen response and symptom response), signs and symptoms of disease progression every 3 to 6 months during the course of treatment. Bone marrow aspirate and biopsy should be performed as clinically indicated.

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European Society for Medical Oncology (ESMO)

Chronic Lymphocytic Leukemia (CLL)
In 2020, the European Society for Medical Oncology (ESMO) issued clinical practice guidelines for diagnosis, treatment and follow up for chronic lymphocytic leukemia (CLL) which included the following:

Response evaluation. Response evaluation includes a careful physical examination and a blood cell count. A bone marrow biopsy and MRD assessment should be carried out to define complete remission and MRD status within clinical trials as well as CT scans. For evaluation of response outside clinical trials, bone marrow biopsy and CT scan may be helpful but are not mandatory. For evaluation of efficacy of novel treatments with continuous administration within clinical trials, more than one CT scan might be necessary.

Detection of MRD by multicolor flow cytometry or RT-PCR has a strong prognostic impact following CIT as well as venetoclax plus CD20-antibody combinations. Patients with undetectable MRD after therapy show a longer response duration and survival. Additional clinical consequences of MRD positivity after therapy with respect to treatment escalation remain unclear, except for patients who underwent allogeneic SCT, where a positive MRD signal may trigger the reduction of immunosuppressive therapies, the administration of donor lymphocyte infusions or the start of anti-leukemic maintenance therapy. Therefore, MRD assessment is not generally recommended for monitoring after therapy outside clinical studies. This may change soon, as increasing efforts are made to determine whether therapy with targeted agents could be discontinued on the basis of MRD status.

Recommendations
- Except after allogeneic SCT MRD measurement is not yet recommended as a clinical routine test.

Acute Myeloid Leukemia (AML)

In 2020, the European Society for Medical Oncology (ESMO) issued clinical practice guidelines for issued clinical practice guidelines for diagnosis, treatment and follow-up of acute myeloid leukemia (AML) which included the following:
- Flow cytometric MRD should be assessed from BM, while molecular MRD should be assessed from both blood and BM.
- NGS-MRD needs thorough standardization and validation before recommendation for clinical use.

Acute Lymphoblastic Leukemia (ALL)
In 2016, the European Society for Medical Oncology (ESMO) issued clinical practice guidelines for diagnosis, treatment and follow-up of acute lymphoblastic leukemia (ALL) which included the following:

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- Quantification of MRD is a major and well-established risk factor and should be obtained whenever possible for all patients also outside of clinical trials.

Multiple Myeloma (MM)
In 2017, the European Society for Medical Oncology (ESMO) issued clinical practice guidelines for diagnosis, treatment and follow-up of multiple myeloma (MM) which included the following:

- Sustained MRD-negative MRD-negative in the marrow (next-generation flow and/or NGS) and by imaging as defined below, confirmed one year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g., MRD-negative at 5 years)
- Flow MRD-negative absence of phenotypically aberrant clonal plasma cells by next-generation flow cytometry on BM aspirates using the EuroFlow standard operation procedure for MRD detection in MM (or validated equivalent method) with a minimum sensitivity of 1 in 105 nucleated cells or higher
- Sequencing MRD-negative Absence of clonal plasma cells by NGS on BM aspirates in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of BM aspirates using the LymphosightVR platform (or validated equivalent method) with a minimum sensitivity of 1 in 105 nucleated cells or higher
- Imaging MRD-negative as defined by next-generation flow cytometry or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET-CT or decrease to < mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue

International Workshop on Chronic Lymphocytic Leukemia (iwCLL)
In 2021, the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) updated their guideline for the diagnosis, indications for treatment, response assessment and supportive management of CLL which includes the following regarding the use of MRD status for clinical evaluation:

Minimal Residual Disease
The complete eradication of the leukemia is a desired end point. Use of sensitive multicolor flow cytometry, PCR or next-generation sequencing can detect MRD in many patients who have achieved a complete clinical response. Prospective clinical trials have provided substantial evidence that therapies that are able to eradicate MRD usually result in an improved clinical outcome. The techniques for assessing MR have undergone a critical evaluation and have become well standardized. Six-color flow cytometry (MRD flow), allele specific oligonucleotide PCR, or high-throughput sequencing using the ClonoSEQ assay are reliable sensitive down to a level of <1 CLL cell in 10,0000 leukocytes.

European LeukemiaNet MRD Working Party
In 2021, the European LeukemiaNet (ELN) MRD Working Party evaluated standardization and harmonization of MRD in an ongoing manner and has updated the

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2018 ELN MRD recommendations on MRD in acute myeloid leukemia based on significant developments in the field. The recommendations state the following:

- MRD should be assessed to refine relapse risk in patients who achieve morphology remission, with full or partial hematologic recovery.
- For patients with mutant NPM1, CBF AML (RUNX-1-RUNX1T1 or CBFB-MYH1 1) or APL (PML-RARA) we recommend molecular MRD assessment by qPCR or dPCR.
- AML patients who are not included in the molecularly defined subgroups should be monitored by MRD by MFC.
- NGS-MRD monitoring is useful to refine prognosis to MFC but, to date, there are insufficient data to recommend NGS-MRD as a stand-alone technique.

Molecular MRD testing
For qPCR-MRD, the prognostic value of log reduction of transcript levels between diagnosis and postinduction time points is under evaluation in clinical trials. For NGS-MRD, the prognostic and predictive relevance of different time points, tissues, and target genes are all under investigation. Bioinformatics approaches also need standardization and quality control rounds. Further studies are needed on how to interpret NGS results when monitoring several gene mutations in a single patient, and whether there are prognostic differences if one, some, or all genes remain detectable. Finally, it is important to identify the benefits and limitations of targeted vs panel approaches for NGS-MRD assessment.

Regulatory Status
The clonoSEQ Minimal Residual Disease Test is offered by Adaptive Biotechnologies. ClonoSEQ was previously marketed as clonoSIGHT (Sequenta), which was acquired by Adaptive Biotechnologies in 2015. ClonoSIGHT was a commercialized version of the LymphoSIGHT platform by Sequenta for clinical use in MRD detection in lymphoid cancers. In September 2018, clonoSEQ received marketing clearance from the Food and Drug Administration through the de novo classification process to detect MRD in patients with ALL or MM.

The clonoSEQ Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK, and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM).

The clonoSEQ Assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment. The test is indicated for use by qualified healthcare professionals in accordance with professional guidelines for clinical decision-making and in conjunction with other clinicopathologic features.

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The clonoSEQ Assay is a single-site assay performed at Adaptive Biotechnologies Corporation.

A search of the FDA device database on of “minimal residual disease” and “MRD” resulted in no additional pertinent results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

- MyMRD NGS Panel (Invivoscribe)

PRIOR APPROVAL

Not required

POLICY

Next generation sequencing (NGS) using the clonoSEQ assay to detect or quantify measurable (minimal) residual disease (MRD) in individuals diagnosed with and receiving treatment for acute lymphoblastic leukemia (ALL), is considered **medically necessary** when one of the following indications is met:

- Upon completion of induction therapy; **or**
- Serial monitoring in patients with molecular relapse or persistent low-level disease burden.

Next generation sequencing (NGS) using the clonoSEQ assay to detect or quantify measurable (minimal) residual disease (MRD) in individuals diagnosed with and receiving treatment for chronic lymphocytic leukemia (CLL), is considered **medically necessary** when the following indication is met:

- At the end of treatment

Next generation sequencing (NGS) using the clonoSEQ assay to detect or quantify measurable (minimal) residual disease (MRD) in individuals diagnosed with and receiving treatment for multiple myeloma (MM), is considered **medically necessary** when one of the following indications is met:

- During follow-up/surveillance after response to primary therapy; **or**
- After each treatment state (e.g., following induction therapy, high dose therapy/stem cell transplant, and consolidation).

Next generation sequencing (NGS) to detect or quantify measurable (minimal) residual disease (MRD) for indications not meeting the above criteria are considered **not medically necessary.**

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Next generation sequencing (NGS) to detect or quantify measurable (minimal) residual disease (MRD) (including use of clonSEQ assay or MyMRD NGS Panel) for all other indications including but not limited to the following are considered **not medically necessary:**

- Acute myeloid leukemia (AML)
- Hodgkin lymphoma
- Non-Hodgkin lymphomas

Based on the peer reviewed medical literature the current generally accepted methods for measurable (minimal) residual disease (MRD) assessment for the above indications are polymerase chain reaction (PCR)-based assays and/or flow cytometry (FC) as the sensitivity of polymerase chain reaction (PCR)-based assays and flow cytometry (FC) was found to be more superior (e.g., more clinically effective) to what is achieved by next generation sequencing (NGS) in measurable (minimal) residual disease (MRD) assessment. Therefore, the technology of next generation sequencing (NGS) is not as beneficial as established alternatives in MRD assessment utilizing flow cytometry (FC) and quantitative polymerase chain reaction (Q-PCR) testing.

Policy Guidelines

Definitions

Allele-specific oligonucleotide PCR (ASO-PCR): A two-step nested polymerase chain reaction (PCR) technique that allows the direct detection of any point mutation in human DNA.

Flow cytometry: A diagnostic test which identifies the arrangement and amount of DNA in a cell.

Measurable (minimal) residual disease (MRD): The cancer cells that may remain in the body during or following treatment. These cells are present at levels undetectable by traditional microscopic (morphologic) examination of blood, bone marrow or a lymph node biopsy.

Next-generation sequencing: Any of the technologies that allow rapid sequencing of large numbers of segments of DNA, up to and including entire genomes. This technology includes but is not limited to high-throughput (deep) sequencing.

- **Deep Sequencing:** A testing strategy in which sequencing a genomic region is done multiple times, sometimes hundreds or even thousands of times, allowing the detection of rare clonal types, cells, or microbes. Deep sequencing increases the yield with low purity tumors, highly polyclonal tumors, and applications that require high sensitivity (identifying low frequency clones).

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PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81479 Unlisted molecular pathology procedures (when specified as next generation sequencing [NGS] for measurable residual disease (MRD) or minimal residual disease (MRD), to include but not limited to clonoSEQ assay)
- 81599 Unlisted multianalyte assay with algorithmic analysis ((when specified as next generation sequencing [NGS] for measurable residual disease (MRD) or minimal residual disease (MRD), to include but not limited to clonoSEQ assay)
- 0171U Targeted genomic sequence analysis panel, acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms, DNA analysis, 23 genes, interrogation for sequence variants, rearrangements, and minimal residual disease, reported as presence/absence (MyMRD NGS Panel)

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POLICY HISTORY

Date	Reason	Action
January 2022	Annual Review	Policy Revised
January 2021		New medical policy created

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

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Medical Policy Analyst
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