Regence

Medical Policy Manual   Genetic Testing, Policy No. 88

ClonoSEQ® Testing for the Assessment of Measurable Residual Disease (MRD)

Effective: June 1, 2025

Next Review: March 2026
Last Review: April 2025

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

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 such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM), diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma (MCL). MRD is typically assessed by flow cytometry or polymerase chain reaction but can also be assessed using the clonoSEQ® test, which uses next-generation sequencing (NGS).

MEDICAL POLICY CRITERIA

Notes: ClonoSEQ® testing generally includes two components: an initial clonoSEQ® ID test followed by clonoSEQ® MRD testing. These are reviewed together as clonoSEQ® testing.

I. ClonoSEQ® B-cell testing to detect measurable residual disease (MRD) may be considered **medically necessary** for individuals with any of the following:

    A.   B-cell acute lymphoblastic leukemia (B-ALL)

    B.   Chronic lymphocytic leukemia (CLL)

C. Multiple myeloma

II. ClonoSEQ® T-cell testing and ClonoSEQ® testing for all other indications, including but not limited to diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma, is considered **investigational**.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

B-ALL and B-cell lymphoblastic lymphoma are generally considered clinically indistinct, and B-ALL is intended to encompass both entities in this policy.

LIST OF INFORMATION NEEDED FOR REVIEW

REQUIRED DOCUMENTATION:

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

- Name of the test and performing laboratory
- Relevant billing codes
- Brief description of how the test results will guide clinical decisions that would not otherwise be made in the absence of testing
- Medical records related to this test:
  - Diagnosis
  - History and physical exam
  - Date of blood draw for test
  - Conventional testing and outcomes

CROSS REFERENCES

1. [Genetic Testing for Myeloid Neoplasms and Leukemia](#), Genetic Testing, Policy No. 59

BACKGROUND

HEMATOLOGIC DISEASE

There are three main types of hematologic malignancies: lymphomas, leukemias, and myelomas. Lymphoma begins in lymph cells of the immune system, which originate in the 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 the 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. The present evidence review will address B-cell ALL (B-ALL), CLL, MM, diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma (MCL). As B-ALL and B-cell lymphoblastic lymphoma are generally considered clinically indistinct, reference to B-cell ALL is intended to encompass both entities.

B-cell Acute Lymphoblastic Leukemia

B-ALL is the most common cancer diagnosed in children; it represents nearly 25% of cancers in children younger than 15 years and 20% of acute leukemias in adults. Remission of disease is now typically achieved with pediatric chemotherapy regimens in 98% of children with ALL, with up to 85% long-term survival rates. The prognosis after the first relapse is related to the length of the original remission. For example, the leukemia-free survival rate is 40% to 50% for children whose first remission was longer than three years compared with 10% to 15% for those who relapse less than three years after treatment. Between 60% and 80% of adults with ALL can be expected to achieve a complete response after induction chemotherapy; however, only 35% to 40% can be expected to survive two years. "Poor prognosis" genetic abnormalities such as the Philadelphia chromosome (translocation of chromosomes 9 and 22) are seen in 25% to 30% of adult ALL but infrequently in childhood ALL. Other adverse prognostic factors in adult ALL include age greater than 35 years, poor performance status, male sex, and leukocytosis count of greater than 30,000/μL (B-cell lineage) or greater than 100,000/μL (T-cell lineage) at presentation.

Induction therapy aims to reduce the leukemic cell population below the cytological 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 this 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 response 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% of adults will have a resistant disease, and a majority of adults and some children will eventually relapse and die of leukemia.

Chronic Lymphocytic Leukemia

CLL is the most common leukemia in Western countries, representing approximately 25% to 30% of all leukemias. CLL is characterized by progressive accumulation of functionally incompetent monoclonal B lymphocytes. It occurs primarily in older adults, but occurrence in younger adults is not unusual. The incidence of CLL increases with age with a median age at diagnosis of 70 years. Malignant cells in CLL and the non-Hodgkin lymphoma small lymphocytic lymphoma have identical pathologic and immunophenotypic features. The term CLL is used when the disease manifests primarily in the blood, whereas the term small lymphocytic lymphoma is used for primarily nodal manifestation.

Not all patients with CLL will require treatment at the time of diagnosis. Median survival for patients with asymptomatic CLL is 10 years, and some patients with early stage CLL may be asymptomatic without treatment for decades. Importantly, randomized trials evaluating immediate versus delayed treatment strategies have found no improvement in long-term survival with early treatment, survival in some patients will not be different from the normal population, and with the exception of HCT, there is currently no cure for CLL. Therefore, the standard of care for patients with early stage asymptomatic CLL is observation rather than immediate treatment.

Treatment is indicated for patients with disease-related complications, termed "active disease"

by the International Workshop on Chronic Lymphocytic Leukemia.[1] Criteria for active disease include one or more of the following: progressive marrow failure, splenomegaly, lymphadenopathy, progressive lymphocytosis, autoimmune anemia and/or thrombocytopenia, extranodal involvement (e.g., skin, kidney, lung, spine), and constitutional symptoms such as weight loss, fatigue, fever, and night sweats. The goal of therapy is to ameliorate symptoms and improve progression-free survival (PFS) and overall survival (OS). The choice of therapy is based on patient and tumor characteristics and goals of therapy. Most patients will have an initial complete or partial response to treatment but will eventually relapse. Relapse may be asymptomatic but is monitored closely for progression to active disease.

Multiple Myeloma

MM represents approximately 17% of all hematologic cancers, largely occurring in patients over 60 years of age. It is characterized by the proliferation of plasma cells in the bone marrow producing a monoclonal immunoglobulin. The clonal plasma cells frequently result in extensive skeletal destruction with osteolytic lesions, osteopenia, and/or pathologic fractures; additional complications can include hypercalcemia, renal insufficiency, anemia, and infections.

MM is treatable but is typically incurable, with treatment reserved for patients with symptomatic disease (usually progressive). Without effective therapy, symptomatic patients die 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 but more advanced premalignant stage 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 five years, approximately 3% per year for the next five years, and 1% for the next 10 years.

Prognosis and treatment for MM depend on risk stratification based on underlying genetic variants, age, performance status, comorbidities, stage, and response to therapy. Patients are assessed to determine eligibility for HCT because HCT has been shown to prolong both event-free and 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 the 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 respond to initial treatment (refractory disease).

Response to treatment is categorized into clinical response, MRD response, and imaging response. A complete (clinical) response is defined by the International Myeloma Working Group and the National Comprehensive Cancer Network.[2 3] MRD response is defined as a complete response plus the absence of clonal plasma cells by next-generation flow cytometry (NGF) or next-generation sequencing (NGS) at a minimum sensitivity of 1 in 10^{-5} nucleated cells in bone marrow, and there is a category of "imaging plus MRD-negative" in which patients are determined to have a complete response, be MRD negative in the bone marrow, and have also achieved positron emission tomography (PET)/computed tomography (CT)-negativity. "Sustained MRD negativity" is achieved when both imaging and MRD are negative in assessments that are a minimum of one year apart. It is not known whether patients with sustained MRD negative status can be considered cured. MRD measured by NGS is currently used as a surrogate outcome measure in clinical trials, and there are ongoing trials to test the

effectiveness of using NGS-MRD to guide therapy.[4]

Large Diffuse B-Cell Lymphoma

Lymphoma refers to any cancer that starts in the lymph system and includes 2 broad categories of disease, Hodgkin lymphoma and non-Hodgkin lymphoma.[5] There are multiple forms of non-Hodgkin lymphoma with B-cell malignancies comprising 85% of cases.[6] Of the B-cell lymphomas, DLBCL accounts for approximately one-third of cases. DLBCL occurs most commonly in older patients with the mean age of diagnosis of approximately 60 years of age. Although aggressive, DLBCL generally responds well to treatment, and 75% of patients have no signs of disease after initial treatment. Historically, PET and CT imaging have been used to assess lymphoma tumor burden and disease response; however, techniques such as flow cytometry, polymerase chain reaction (PCR)-based methods, and NGS-based techniques are being increasingly used.[7]

Mantle Cell Lymphoma

A small percentage of B-cell lymphomas (about 5%) are categorized as MCL.[6] Similar to DLBCL, it occurs most commonly in patients over 60 years of age and tends to be an aggressive lymphoma; however, the response to treatment has traditionally been poor. Most patients present with advanced stage disease, and treatment is dependent on stage and eligibility for HSCT. Historically, PET and CT imaging have been used to assess lymphoma tumor burden and disease response; however, techniques such as flow cytometry, PCR-based methods, and NGS-based techniques are being increasingly used.[7]

MEASURABLE RESIDUAL DISEASE

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 the bone marrow or blood are referred to as measurable residual disease (MRD), also known as minimal residual disease. MRD assessment is typically performed by flow cytometry or PCR with primers for common variants. Flow cytometry or next generation flow cytometry evaluates blasts based on the expression of characteristic antigens, while PCR assesses specific chimeric fusion gene transcripts, gene variants, and overexpressed genes. PCR is sensitive for specific targets, but clonal evolution may occur between diagnosis, treatment, remission, and relapse that can affect the detection of MRD. NGS has 10- to 100-fold greater sensitivity for detecting clonal cells, depending on the amount of DNA in the sample (see Table 1) 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 available test, clonoSEQ®, uses both PCR and NGS to detect clonal DNA in blood and bone marrow. ClonoSEQ® Clonality (ID) PCR assessment is performed when there is a high disease load (e.g., initial diagnosis or relapse) to identify dominant or "trackable" sequences associated with

the malignant clone. NGS is then used to monitor the presence and level of the associated sequences in follow-up samples. As shown in Table 1, 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 1 in 10^{-5} cells, which is equal to PCR and approaches the limit of detection of NGS (see Table 1).

Table 1. Sensitivity of Methods for Detecting Measurable 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

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® B-cell testing received marketing clearance from the U.S. Food and Drug Administration (FDA) through the de novo classification process to detect MRD in patients with B-ALL or MM. In 2020, clonoSEQ® B-cell testing received marketing clearance from the FDA to detect MRD in patients with CLL.

EVIDENCE SUMMARY

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). Validation of the clinical use of any genetic test focuses on three main principles:

1. Analytic validity, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. Clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. Clinical utility, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

Review of the literature focused on identifying evidence related to clinical validity and clinical utility, particularly whether the tests can be used to improve treatment planning compared with the standard of care, and whether their use results in improved health outcomes. For the evaluation of the clinical validity of the clonoSEQ® test, studies that met the following eligibility criteria were considered:

- Included a suitable reference standard (PFS or OS)

- Evaluated outcomes at different levels of MRD

- Comparative trials that evaluated health outcomes when therapy was guided by NGS

assessment of MRD.

CLONOSEQ® TO DETECT MEASURABLE RESIDUAL DISEASE IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Clinical Validity

Table 2 describes studies that have evaluated prognosis based on MRD levels detected by FC and clonoSEQ®. Overall, higher levels of MRD are associated with a worse prognosis. In an analysis of samples from two multicenter studies, Pulsipher (2022) compared FC at a threshold of 10^{-4} with clonoSEQ® at thresholds of 10^{-4}, 10^{-5}, 10^{-6}, and any detectable level (approximately 10^{-7}) in pediatric and young adult patients with B-ALL who received tisagenlecleucel.[8] In 95 patients with both NGS and FC results, 18% of samples were MRD-positive with FC compared with 22%, 29%, 33%, and 41% with NGS at cutoff values of 10^{-4}, 10^{-5}, 10^{-6}, and any detectable level, respectively. No samples were positive by FC and negative by NGS. Relapse before 12 months occurred without MRD detection in 50% of patients by FC, 31% by clonoSEQ® at 10^{-6}, and 0% of those with clonoSEQ® below the 10^{-6} level. Limitations of the study included limited follow-up and inclusion of only patients treated with tisagenlecleucel. Additional limitations are noted in Table 3.

Liang (2023) reported results of a study of the prognostic performance of the clonoSEQ® assay in 111 adult participants with B-ALL or T-cell ALL (T-ALL) who underwent allogeneic HCT at Stanford University or Oregon Health & Science University between 2014 and 2021.[9] Participants were followed for leukemia relapse and/or death for up to two years after HCT. Relapse was defined as morphologic or clinical. The MRD samples came from either peripheral blood or bone marrow. The median age of the patients was 44 years (range, 19 to 70 years), 62 (56%) were male, and 95 (86%) had B-ALL. Pre-HCT MRD was significantly associated with relapse in multivariable analysis, however detectable post-HCT MRD was the strongest predictor (HR 4.60, 95% CI 3.01 to 7.02).

Table 2. Characteristics of Prognostic Studies Assessing ClonoSEQ® for MRD in B-ALL

Study	Study Population	Design[a]	Reference Standard	Threshold for PIT	Follow-up
Liang (2023)[9]	Blood and bone marrow samples from adults with B-ALL (86%) or T-cell ALL undergoing HCT	Retrospective from banked samples; assessed by NGS	Relapse	B-ALL: a detectable IgH clonotype T-ALL: a detectable TCRβ or TCRγ clonotype Stratified as undetectable (0), low (<10–4), high (≥10–4 to ≤10–3), or very high (>10–3)	Up to two years
Pulsipher (2022)[8]	Blood and bone marrow samples from 143 patients in tisagenlecleucel trials	Retrospective from banked samples with comparison of FC and clonoSEQ®	Relapse	FC at 10^{-4}; NGS at 10^{-4} or less	38.4 months

B-ALL: B-cell acute lymphoblastic leukemia; FC: flow cytometry; MRD: measurable residual disease; PIT: positive index test, T-ALL: B-cell acute lymphoblastic leukemia.

Table 3. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Complete-ness[e]	Statistical[f]
Liang (2023)[9]	2. Selection based on availability of samples from prior studies	1. Blinding was not described				
Pulsipher (2022)[8]	2. Selection based on availability of samples from prior studies	1. Blinding was not described	2. FC analysis was part of the original trials; NGS was performed on frozen samples post hoc			

NGS: next-generation sequencing.

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).

[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.

[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.

[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinical Utility

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. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RCTs). No trials were identified that compared outcomes when treatment was guided by clonoSEQ®.

Section Summary: ClonoSEQ® to Detect Measurable Residual Disease in B-Cell Acute Lymphoblastic Leukemia

Evidence on the clinical validity of clonoSEQ® to risk-stratify patients include two retrospective studies in adults. Comparison with FC showed comparable results when the same threshold (10^{-4}) was used for both NGS and FC, and 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 was found to have lower specificity.

CLONOSEQ® TO DETECT MEASURABLE RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA

Clinical Validity

Study characteristics and results are described in Tables 4 and 5. Study limitations are described in Tables 6 and 7.

Material submitted for U.S. Food and Drug Administration (FDA) approval included data analyzed from two studies that assessed MRD with clonoSEQ® using available blood samples from two clinical trials (NCT02242942 and NCT00759798).[10] The primary endpoint of the first study was to evaluate whether MRD at a threshold of 10^{-5} at three months after treatment could predict PFS. Secondary objectives were to assess different cutoff values and repeated measurements. Patients with MRD greater than 10^{-5} had a 6.64-fold higher event risk compared to MRD negative patients (95% confidence interval [CI] 3.65 to 12.1). The primary distinction was at a cutoff of 10^{-4}, where only 16.5% of patients with MRD in blood greater than 10^{-4} were progression free at four years follow-up, compared to 44%, 49%, and 47% with MRD less than 10^{-6}, 10^{-5}, and 10^{-4}, respectively.

The second study was published by Thompson (2019), who analyzed MRD with clonoSEQ® in stored samples of bone marrow (n=57), blood (n=29) and plasma (n=32) from 62 patients who had previously tested negative for MRD by FC (n=63) in a phase 2 clinical trial.[11] MRD rates by NGS varied according to sample type with fewer patients with undetectable MRD in bone marrow (25%) than blood (55%) or plasma (75%). MRD at the end of treatment was predictive of PFS. Patients with undetectable MRD did not progress by the end of the study (mean 82 months, range 28 to 112 months) compared with PFS of 67 months (bone marrow) or 74 months (blood). The percent of patients who were progression free with MRD less than 10^{-6}, 10^{-5}, and 10^{-4} was 85%, 75%, and 67.5%, respectively. The authors note that "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."

Munir (2023) reported results of the prognostic performance of clonoSEQ® in participants from the GLOW study.[12] GLOW (n=211) was a phase 3 trial comparing fixed-duration ibrutinib+venetoclax to chlorambucil+obinutuzumab in participants with previously untreated CLL who were older and/or had comorbidities. MRD was assessed by clonoSEQ® from samples collected every three to four months from peripheral blood and at 9 and 18 months from bone marrow. Detectable MRD defined as having ≥1 CLL cell per 10,000 leukocytes. Median follow-up was 34 months. PFS at 12 months after the end of treatment with ibrutinib+venetoclax was high regardless of MRD status at the end of treatment: 96% versus 93% in patients with undetectable MRD versus detectable MRD.

Table 4. Characteristics of Prognostic Studies Assessing ClonoSEQ® for MRD in CLL

Study	Study Population	Design[a]	Reference Standard	Threshold for PIT	Follow-up (range)
clonoSEQ® Technical Summary	Patients treated for CLL with blood samples at 3 months after treatment (n=337)	Analysis of prospectively collected blood samples from a phase 3 trial (NCT02242942)	PFS	NGS at 10^{-6}, 10^{-5}, 10^{-4} in blood	4 years
Thompson (2019)[11]	Patients with CLL treated with up to 6 courses of FCR and MRD negative by FC (n=62)	Analysis of prospectively collected samples from a phase 2 trial (NCT00759798)	PFS	NGS at 10^{-6}, 10^{-5}, 10^{-4} in blood, plasma, or bone marrow	82 months (28-112)

Study	Study Population	Design[a]	Reference Standard	Threshold for PIT	Follow-up (range)
Munir (2023)[12]	Patients with CLL treated with ibrutinib+venetoclax (I+V)	Analysis of prospectively collected samples from the phase 3 GLOW trial (NCT03462719)	PFS	NGS at 10^{-4}	1 year

CLL: chronic lymphocytic leukemia; FC: flow cytometry; FCR: fludarabine, cyclophosphamide, and rituximab; MRD: measurable residual disease; PIT: positive index test; PFS: progression-free survival.

Table 5. Results of Prognostic Studies Assessing ClonoSEQ® for MRD in CLL

Study	N	Tissue Source	Progression Free at End of Study n/N (%)					
			EOT MRD $<10^{-6}$	EOT MRD $>10^{-6}$	EOT MRD $<10^{-5}$	EOT MRD $>10^{-5}$	EOT MRD $<10^{-4}$	EOT MRD $>10^{-4}$
clonoSEQ® Technical Summary			33/75 (44.0%)		50/106 (47.2%)		24/49 (49.0%)	17/103 (16.5%)
Thompson (2019)[11]	53	Bone marrow	11/13 (84.6%)	21/40 (52.5%)	18/24 (75.0%)	14/29 (48.3%)	27/40 (67.5%)	5/13 (38.4%)
	29	Blood	7/8 (87.5%)	8/13 (61.5%)				
Munir (2023)[12]	211 (106 I+V)	Bone marrow					I+V: 96% C+O: 83%	I+V: 93% C+O: 59%

C+O: chlorambucil+obinutuzumab; EOT: end of treatment; I+V: ibrutinib+venetoclax; MRD: measurable residual disease; NGS: next-generation sequencing; PFS: progression free survival

Limitations in study relevance, and study design and conduct are shown in Tables 6 and 7.

Table 6. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
clonoSEQ® Technical Summary			3. Did not compare results to FC		
Thompson (2019)[11]					1. Mean follow-up was 82 months (range of 28 to 118 months) which is insufficient to determine PFS in CLL
Munir (2023)[12]			3. Did not compare results to FC		1. Follow-up of 1 year

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

CLL: chronic lymphocytic leukemia; FC. flow cytometry; PFS: progression-free survival.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 7. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
clonoSEQ® Technical Summary		1. Blinding was not described		2. Details from the technical summary are limited and did not discuss the minimal difference of the different thresholds.		
Thompson (2019)[11]	2. Selection based on availability of tissue samples from prior studies	1. Blinding was not described				
Munir[12] (2023)		1. Blinding was not described				

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinical Utility

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. Because these are intervention studies, the preferred evidence would be from RCTs.

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

Section Summary: ClonoSEQ® to Detect Measurable Residual Disease in Chronic Lymphocytic Leukemia

The evidence on clonoSEQ® for detection of MRD includes two studies that were submitted to the FDA. These studies evaluated the association between the level of MRD detected by NGS in the bone marrow or blood and PFS in samples of blood or bone marrow from completed phase 2 and 3 trials. Both studies submitted to the FDA 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. Evidence is sufficient to support the clinical utility of using clonoSEQ® to measure MRD for prognosis based on test results at a sensitivity of 10^{-4}. Analysis of samples from the GLOW study suggests that for participants treated with ibrutinib+venetoclax, PFS was high regardless of MRD status using threshold of 10^{-4} at the end of treatment.

CLONOSEQ® TO DETECT MEASURABLE RESIDUAL DISEASE IN MULTIPLE MYELOMA

Table 8. Definitions of Complete Response and MRD from the International Myeloma Working Group[2]

Standard Response criteria	
Complete response	"Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates"
MRD Response Criteria (requires a complete response)	
Sequencing MRD-negative	Absence of clonal plasma cells with a minimum sensitivity of 1 in 10^{-5} nucleated cells
Imaging plus MRD-negative	MRD negativity by NGF or NGS plus imaging criteria

MRD: minimal residual disease; NGF: next-generation flow cytometry; NGS: next-generation sequencing

Clinical Validity

Three published retrospective studies were identified that evaluated the association between MRD by clonoSEQ® and disease progression in patients with MM (see Tables 9 and 10). Two of the studies assessed MRD levels from patients who had participated in earlier MM treatment trials.

In a study by Perrot (2018), a threshold of 10^{-6} was used to evaluate the association between MRD and PFS, finding that the dichotomous division into MRD positive and MRD negative (no detectable MRD at the limit of detection) was highly predictive of PFS with an HR for MRD negative/MRD positive of 0.19 (p<.001).[13] The median PFS was 29 months in patients who were positive for MRD and was not reached among patients with no detectable MRD.

Martinez-Lopez (2020) 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 clonoSEQ®.[14] MRD assessment by clonoSEQ® was performed after a complete response, but there was no consistent time after treatment; most were performed within one 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 less than 10^{-6} and 129 (55%) had MRD less than 10^{-5}. For both newly diagnosed MM and relapsed MM patients, MRD less than 10^{-5} or less than 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}.

Cavo (2022) analyzed pooled data from four phase 3 studies in patients with relapsed or refractory MM who were ineligible for transplant.[15] MRD was assessed at a sensitivity of 10^{-5}. Patients who achieved a complete response or better and were MRD negative had improved PFS and an 80% reduction in the risk of disease progression or death compared with those who failed to reach complete response or were MRD positive (HR 0.20, p<0.0001).

Oliva (2023) reported results of analyses of MRD status from samples available from the FORTE trial.[16] The FORTE trial was a phase 2, multicenter RCT including participants with newly diagnosed, transplant-eligible multiple myeloma randomized between 2015 and 2021 to one of three induction-intensification-consolidation strategies. Multiparameter FC status was assessed in patients with at least a very good partial response first at premaintenance and then every six months during maintenance treatment until progressive disease. The cut-off for FC MRD positivity was set at ≥20 clonal plasma cells out of the total of nucleated cells, with a sensitivity of ≥10^{-5}. NGS was performed in a subset of participants with at least a suspected complete response at pre-maintenance and monitored every 6 months during maintenance treatment until progressive disease using the clonoSEQ® assay with sensitivities at 10^{-5} and 10^{-6}. There were 2,020 samples available for analysis of FC MRD status and 728 samples available for the analysis of the correlation between FC and NGS in the "suspected complete response population". Median follow-up was 62 months. The hazard ratios for PFS in FC-MRD and NGS-MRD-negative vs. -positive patients were 0.29 (95% CI 0.20 to 0.40) and 0.27 (95% CI 0.18 to 0.39), respectively.

The major limitations of these studies are described in Tables 11 and 12.

Table 9. Characteristics of Studies Assessing ClonoSEQ® for MRD in MM

Study	Study Population	Design	Reference Standard	Threshold
Perrot (2018)[13]	Patients with myeloma enrolled in the IFM 2009 clinical trial[a]	Retrospective	PFS and OS	MRD at 10^{-6}
Martinez-Lopez (2020)[14]	Patients with MM who had been treated at their clinic between 2005 and 2018 (n=234)	Retrospective	PFS	MRD at 10^{-5}
Cavo (2022)[15]	Patients with bone marrow samples from POLLUX, CASTOR, ALCYONE, and MAIA trials[b]	Retrospective	PFS	MRD at 10^{-5}
Oliva (2023)	Patients with bone marrow samples from FORTE trial	Retrospective	PFS	MRD at 10^{-5} and 10^{-6}

MRD: measurable residual disease; NGS: next-generation sequencing; OS: overall survival; PFS: progression-free survival; TTP: time to progression.
[a] IFM 2009 was phase 3 trial from the Intergroupe Francophone du Myelome, conducted between 2010 and 2012, which evaluated the role of autologous cell transplantation in patients with newly diagnosed myeloma.
[b] POLLUX, CASTOR, ALCYONE, and MAIA were daratumumab-based studies in patients with newly diagnosed MM.

Table 10. Results of Prognostic Studies Assessing ClonoSEQ® for MRD in MM

Study	N	MRD Threshold	TTP, months (95% CI)	
Perrot (2018)[13]	509	10^{-6}	MRD negative/MRD positive	
Hazard Ratio for Progression Free Survival (95% CI)			0.19 (0.13 to 0.26)	
p-Value			<0.001	
Martinez-Lopez (2020)[14]			**PFS, months (95% CI)**	**3-year survival (95% CI)**
Newly Diagnosed		$<10^{-6}$		90% (81% to 98.78%)
		$<10^{-5}$	87	85.9% (78.2% to 94.5%)
		$>10^{-5}$	32	46.8% (33.9% to 64.7%)
HR (95% CI)			3.54 (1.94 to 6.45)	
p-Value			<0.001	
Relapsed	27/75 (36%)	$<10^{-6}$	not reached	
	35/75 (47%)	$<10^{-5}$	42	
		$>10^{-5}$	17	
HR (95% CI)			2.45 (1.25 to 4.82)	
p-Value			.01	
Cavo (2022)[15]			**48-month PFS, %**	
	2,510	10^{-5}		
Complete response or better and MRD negative			70.4	
Less than very good partial response or MRD positive			23.9	
Oliva (2023)[16]			**48-month PFS, %**	**48-month OS, %**
MRD positive			46%	78%
MRD negative			83%	94%
HR (95% CI)			0.27 (0.18 to 0.39)	0.31 (0.17 to 0.54)
p-value			<0.01	<0.01

CI: confidence interval; HR: hazard ratio; MRD: measurable residual disease; PFS; progression free survival; OS: overall survival; TTP: time to progression.

Table 11. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Perrot (2018)[13]	4. The study included patients from the IFM 2009 trial who had at least a very good partial response but did not report separately				

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
	on patients with a complete response				
Martinez-Lopez (2020)[14]			3. No comparison to other tests for MRD		
Cavo (2022)[15]			3. No comparison to other tests for MRD		
Oliva (2023)[16]	4. MFC status was assessed in patients with at least a very good partial response and NGS was assessed in patients with at least a suspected complete response				

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 12. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Perrot (2018)[13]	2. Selection based on availability of tissue samples in the original study	1. Blinding not described				1. Post-hoc exploratory analysis, not adjusted for multiple comparisons
Martinez-Lopez (2020)[14]	2. Retrospective assessment of clinical data	1. Blinding not described	2. There was no uniform timing of the test.			
Cavo (2022)[15]			2. MRD assessed at different time points			

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
			in individual studies.			
Oliva (2023)[16]	2. Retrospective assessment of clinical data	1. Blinding not described				

MRD: measurable residual disease

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).

[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.

[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.

[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Kriegsmann (2020) found moderate concordance between NGS and NGF in a study of 113 patients with MM (Table 13).[17] 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 two methods was 0.536 (n=113, p<0.001). 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).

Table 13. Concordance Between NGS and NGF in Study by Kriegsmann (2020)

		Flow Cytometry		
		+	**−**	*Total*
NGS	**+**	42	14	56
	−	14	43	57
	Total	56	57	

NGF: next generation flow cytometry; NGS: next-generation sequencing.

Clinical Utility

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

Costa (2023) reported results of the MASTER multicenter (five centers), single-arm, phase 2 study conducted in the US between 2018 and 2020.[18] MASTER was the first study to use prospective adaptation of treatment duration based on MRD status but MRD status was used to guide therapy in all participants with sufficient unique clonogenic sequences. There is no comparison to management without MRD status. Instead, MASTER demonstrates the feasibility of using MRD to guide therapy. MASTER included 123 adults with newly diagnosed multiple myeloma, life expectancy >12 months, Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and no previous treatment except up to one cycle of therapy containing bortezomib, cyclophosphamide, and dexamethasone. 70 (57%) of the participants were men; 94 (76%) of participants were non-Hispanic White, 25 (20%) were non-Hispanic

Black. The median age was 61 years (IQR, 55 to 68). 53 (43%) had no high-risk chromosome abnormalities (HRCA), 46 (37%) had one HRCA, and 24 (20%) had two or more HRCAs. The median follow-up duration was 42 months (IQR, 35 to 46). MRD status was assessed by clonoSEQ® using a detection threshold of 10^{-5} to adjudicate response-adapted therapy. Five participants had an absence of sufficiently unique clonogenic sequences to enable tracking by the clonoSEQ® assay. There were 84 participants who reached MRD negativity after or during two consecutive treatment phases, who then stopped treatment and began observation with MRD surveillance. Twenty participants who did not reach two consecutive MRD-negative results received maintenance lenalidomide. Ten participants discontinued treatment early: three died, five had disease progression, and two chose to discontinue. Of the 84 participants who transitioned to MRD surveillance, 36-month PFS was 88% (95% CI 77 to 96) for those with no HRCAs, 85% (95% CI 73 to 96) for those with one HRCA, and 60% (95% CI 35 to 82) for those with two or more HRCAs. Of the 84 participants, 23 (27%) resumed therapy due to MRD resurgence or disease progression not preceded by MRD resurgence.

Section Summary: ClonoSEQ® to Detect Measurable Residual Disease in Multiple Myeloma

The evidence on clonoSEQ® for detection of MRD includes three published retrospective studies in patients with MM. These studies evaluated the association between the level of MRD detected by NGS in the bone marrow and the TTP or PFS 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 two 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 clonoSEQ® 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 clonoSEQ® assessment of residual disease. Trials that test the effectiveness of MRD to guide therapy in MM are ongoing.

CLONOSEQ® TO DETECT MEASURABLE RESIDUAL DISEASE IN DIFFUSE LARGE B-CELL LYMPHOMA

Clinical Validity

There are two studies assessing the prognostic value of clonoSEQ® for MRD specifically in patients with DLBCL. One prospective, single-center, observational study by Chase (2021) attempted to correlate MRD with prognosis in patients with newly diagnosed DLBCL receiving conventional treatment; however, attrition limited outcome assessment.[19] Only three patients had early clinical relapse, and no conclusions can be drawn.

In a phase 2, single-center, prospective trial in patients with DLBCL undergoing HSCT, Kambhampati (2021) assessed 15 patients for MRD with NGS.[20] Of the 14 patients with available MRD samples after salvage therapy, 11 were MRD negative and three were MRD positive. MRD tests were predictive of survival in these patients (see Tables 14 and 15). Limitations of the study included the lack of comparator MRD test and the MRD testing threshold was not described.

Table 14. Characteristics of Studies Assessing ClonoSEQ® for MRD in DLBCL

Study	Study Population	Design	Reference Standard	Threshold
Kambhampati (2021)[20]	Patients with relapsed/refractory DLBCL undergoing HSCT enrolled in a phase 2 trial	Single-center, prospective	PFS/OS	NR

DLBCL: diffuse large B-cell lymphoma; HSCT: hematopoietic stem cell transplant; NR: not reported: OS: overall survival; PFS: progression-free survival.

Table 15. Results of Prognostic Studies Assessing ClonoSEQ® for MRD in DLBCL

Study	N	Median OS, mo	Median PFS, mo
Kambhampati (2021)[20]	27 (14 with MRD samples after salvage therapy)		
MRD negative		Not reached	Not reached
MRD positive		3.5	1.3

MRD: measurable residual disease; OS: overall survival; PFS: progression-free survival.

Clinical Utility

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 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. Further studies will be needed to determine whether treatment can be guided by this test.

Section Summary: ClonoSEQ® to Detect Measurable Residual Disease in Diffuse Large B-Cell Lymphoma

The evidence on NGS for detection of MRD in DLBCL includes an analysis from a single-center, prospective trial that did not include comparison to another MRD measure. Although both PFS and OS correlated with MRD positivity, the trial is limited by its small sample-size and inclusion of only patients eligible for HSCT from a single center.

CLONOSEQ® TO DETECT MEASURABLE RESIDUAL DISEASE IN MANTLE CELL LYMPHOMA

Clinical Validity

Characteristics and results of trials evaluating NGS for MRD in MCL are summarized in Tables 16 and 17, and limitations of these trials are summarized in Tables 18 and 19. Smith (2019) conducted a retrospective review of samples from patients enrolled in the ECOG1411 trial which evaluated MCL patients treated with bendamustine-rituximab induction followed by

rituximab (with or without lenalidomide) consolidation and evaluated MRD by both FC and NGS.[21] Concordance between tests was high both after cycle 3 and end of induction. MRD status correlated with PFS. For patients who were MRD negative after cycle 3 by either method, PFS was 58.9 months. For those who were MRD positive by NGS, PFS was 26.9 months and PFS was 29.9 months for those who were positive by FC. The authors concluded both NGS and FC were feasible to assess MRD.

Lakhotia (2022) conducted an exploratory review of circulating tumor DNA analyzed by NGS from a trial of bortezomib induction in 53 MCL patients found patients who had undetectable MRD after two induction cycles had longer PRS and OS than those with MRD.[22] As this was an exploratory analysis, key details are not included, and no firm conclusions can be drawn.

Table 16. Characteristics of Studies Assessing ClonoSEQ® for MRD in patients with MCL

Study	Study Population	Design	Reference Standard	Threshold	Test Version
Smith (2019)[21]	Patients with MCL enrolled in ECOG1411	Retrospective	PFS	MRD at 10^{-4}	"Research version" of clonoSEQ®
Lakhotia (2022)[22]	Patients with MCL enrolled in a trial of bortezomib induction treatment	Retrospective	PFS	NR	Not specified; however, test supplied by Adaptive Biotechnologies

ECOG: Eastern Cooperative Oncology Group; MCL: mantle cell lymphoma; MRD: measurable residual disease; NR: not reported; PFS: progression free survival.

Table 17. Results of Prognostic Studies Assessing ClonoSEQ® for MRD in patients with MCL

Study	N	MRD Threshold	MRD Negative, (%)[a]	PFS	OS
Smith (2019)[21]	214	MRD at 10^{-4}			
FC			95 (peripheral blood)		
NGS			91 (peripheral blood)/90 (bone marrow)		
MRD negative (by NGS)				58.9 mo	
MRD positive (by NGS)				26.9 mo	
Lakhotia (2022)[22]	53				
MRD negative[b]				2.7 yr	13.8 yrs
MRD positive[b]				1.8 yr	7.4 yrs

FC: flow cytometry; MRD: measurable residual disease; NGS: next-generation sequencing; OS: overall survival; PFS: progression free survival.

[a] Results reported at end of induction.

[b] After 2 cycles of induction.

Table 18. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Smith (2019)[21]		2. Unclear if "research version" of clonoSEQ® used in study is same as commercially available test.			1. Data reported from mid-induction or end of induction
Lakhotia (2022)[22]		1,2			

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 19. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Smith (2019)[21]		1. Blinding not described				
Lakhotia (2022)[22]	2. Selection based on availability of tissue samples in the original study	1. Blinding not described				1. Post-hoc exploratory analysis

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).

[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.

[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.

[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinical Utility

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 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. High concordance has been shown between NGS and FC at a threshold of 10^{-4}, indicating that NGS may be considered an alternative to FC at this threshold. Further studies are needed to determine whether treatment can be guided by this test.

Section Summary: ClonoSEQ® to Detect Measurable Residual Disease in Mantle Cell Lymphoma

The evidence on clonoSEQ® for detection of MRD in patients with MCL includes a retrospective study and an exploratory analysis of patients enrolled in treatment clinical trials. When compared with FC, NGS had strong correlation, and MRD positivity with either method was associated with worse PFS. However, the relevance of these findings to the commercial version of clonoSEQ® is unclear as a "research version" was used in the study. An exploratory analysis in patients with MCL enrolled in a treatment trial found improved survival in patients who were MRD negative after two cycles of induction. However, interpretation was limited by imprecision and unspecified NGS testing level.

PRACTICE GUIDELINE SUMMARY

INTERNATIONAL MYELOMA WORKING GROUP

The International Myeloma Working Group developed consensus criteria for response and minimal residual disease (MRD) assessment in multiple myeloma (Table 20).[2]

Table 20. IMWG Criteria

Standard Response Criteria	
Complete response	"Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates"
Stringent complete response	"Complete response as defined below plus normal FLC ratio and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)"
MRD Response Criteria (requires a complete response)	
Sequencing MRD-negative	Absence of clonal plasma cells by NGS using the LymphoSIGHT platform (or validated equivalent) with a minimum sensitivity of 1 in 10^5 nucleated cells
Imaging plus MRD-negative	MRD negativity by NGF or NGS plus imaging criteria
MRD Response Criteria (requires a complete response)	
Sustained MRD-negative	MRD negativity by NGF or NGS, and by imaging, at a minimum of 1 year apart.

FLC: free light chain; IMWG: International Myeloma Working Group; MRD: minimal residual disease; NGF: next-generation flow cytometry; NGS: next-generation sequencing.

INTERNATIONAL WORKSHOP ON CHRONIC LYMPHOCYTIC LEUKEMIA

The 2018 guidelines from the International Workshop on Chronic Lymphocytic Leukemia (CLL) have the following recommendations regarding the assessment of MRD:[1]

"The complete eradication of the leukemia is a desired end point. Use of sensitive multicolor flow cytometry, PCR [polymerase chain reaction], or next generation sequencing can detect MRD in many patients who 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. Refinement and harmonization of these technologies has established that a typical flow cytometry–based assay comprises a core panel of 6 markers (ie, CD19, CD20, CD5, CD43, CD79b, and CD81). As such, patients will be defined as having undetectable MRD (MRD-neg) remission if they have blood or marrow with,1 CLL cell per 10,000 leukocytes."

THE NATIONAL COMPREHENSIVE CANCER NETWORK

The National Comprehensive Cancer Network has published guidelines of relevance to this review (see Table 21).

Table 21. Recommendations on Assessing Measurable Residual Disease

Guideline	Version	Recommendation
Acute lymphoblastic leukemia[23]	3.2024	MRD refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods or standard immunophenotyping. The most frequently used methods for MRD quantification include an FDA-approved NGS-based assay to detect fusion genes or clonal rearrangements in Ig and T-cell receptor (TCR) loci (does not require patient-specific primers) (preferred), flow cytometry assays specifically designed to detect abnormal MRD immunophenotypes at low frequency, real-time quantitative PCR (RQ-PCR) assays (eg, clonally rearranged Ig, TCR genes), and RT-qPCR assays (eg, BCR::ABL1). High-sensitivity flow cytometry with validated analysis algorithms or PCR methods can quantify leukemic cells at a sensitivity threshold of 1×10^{-4} (0.01%) bone marrow mononuclear cells (MNCs). NGS and some PCR methods can detect leukemic cells at a sensitivity threshold of 1×10^{-6} (0.0001%) MNCs.
Chronic lymphocytic leukemia/small lymphocytic lymphoma[24]	3.2025	Evidence from clinical trials suggests that undetectable MRD in the peripheral blood after the end of time-limited treatment is an important predictor of efficacy. MRD evaluation should be performed using an assay with a sensitivity of 10^{-4} according to the standardized European Research Initiative on CLL (ERIC) method or standardized NGS method.
Multiple myeloma[3]	1.2025	Consider baseline clone identification and storage of aspirate sample for future MRD testing by NGS. Bone marrow aspirate and biopsy with FISH, SNP array, NGS, or multi-parameter flow cytometry as clinically indicated. Consider MRD testing as indicated for prognostication after shared decision with the patient.

Guideline	Version	Recommendation
B-cell lymphomas[25]	2.2025	If end-of treatment PET is positive, consider repeat biopsy or if biopsy not feasible, consider circulating tumor DNA (ctDNA) for MRD (ctDNA-MRD) assessment (category 2B), using a test with a detection limit of <1 part per million, prior to additional therapy.

ALL: acute lymphoblastic leukemia, FC: flow cytometry; FISH: fluorescence in situ hybridization; MRD: measurable residual disease; NGS: next-generation sequencing; PCR: polymerase chain reaction; SNP: single nucleotide polymorphism.

SUMMARY

There is enough evidence to show that clonoSEQ® B-cell testing for measurable residual disease (MRD) can improve health outcomes for individuals with B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM) who are being monitored following treatment. Clinical practice guidelines recommend MRD assessment, including the use of NGS testing for these indications. Therefore, clonoSEQ® B-cell testing for measurable residual disease (MRD) may be considered medically necessary for individuals with B-ALL, CLL, or MM.

There is not enough research to show that clonoSEQ® B-cell testing can improve health outcomes for individuals with diffuse large B-cell lymphoma (DLBCL) who are being monitored for residual disease following treatment. In addition, the test has not been approved by the FDA for this condition. Therefore, clonoSEQ® testing is considered investigational for patients with DLBCL.

There is not enough research to show that clonoSEQ® B-cell testing can improve health outcomes for individuals with mantle cell lymphoma (MCL) who are being monitored for residual disease following treatment. Overall, the literature is limited, and guidelines for testing to detect MRD in patients with MCL are lacking. In addition, the test has not been approved by the FDA for this condition. Therefore, clonoSEQ® testing is considered investigational for patients with MCL.

There is not enough research to show that clonoSEQ® T-cell testing or testing for individuals with hematologic malignancies other than B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), or multiple myeloma can improve health outcomes. Only the clonoSEQ® B-cell testing has been approved by the FDA, and only for B-ALL, CLL and multiple myeloma. Therefore, clonoSEQ® T-cell testing and clonoSEQ® for all other indications is considered investigational.

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CODES

Codes	Number	Description
CPT	0364U	Oncology (hematolymphoid neoplasm), genomic sequence analysis using multiplex (PCR) and next-generation sequencing with algorithm, quantification of dominant clonal sequence(s), reported as presence or absence of minimal residual disease (MRD) with quantitation of disease burden, when appropriate
HCPCS	None	

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