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Minimal Residual Disease (MRD)

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I. Policy Description

Minimal residual disease, also called measurable residual disease or MRD, refers to the subclinical levels of residual diseases, such as acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and multiple myeloma (MM).[1-4] MRD is a postdiagnosis, prognostic indicator that can be used for risk stratification and to guide therapeutic options when used alongside other clinical and molecular data.[5] Many different techniques have been developed to detect residual disease. However, PCR-based techniques, multicolor flow cytometry, and deep sequencing-based MRD generally provide the best sensitivity, specificity, reproducibility, and applicability compared to other techniques such as fluorescence in situ hybridization (FISH), Southern blotting, or cell culture.[3]

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with a diagnosis of multiple myeloma (MM), chronic lymphocytic leukemia (CLL), or small lymphocytic lymphoma (SLL), minimal residual disease (MRD) testing by multiparameter flow cytometry **or** next-generation sequencing (NGS) **MEETS COVERAGE CRITERIA**.

2) For individuals with a diagnosis of acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), MRD testing by multiparameter flow cytometry, PCR-based techniques, **or** NGS **MEETS COVERAGE CRITERIA**.

3) For individuals with a diagnosis of human papilloma virus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) who are being monitored for disease recurrence (at least three months after completion of initial definitive therapy), blood-based detection of tumor tissue modified viral (TTMV)-HPV DNA fragments (i.e., NavDx®) **MEETS COVERAGE CRITERIA** once every three months.

4) For individuals with a diagnosis of diffuse large B-cell lymphoma, when end of treatment positron emission tomography is positive **and** biopsy is not feasible, testing for circulating tumor DNA (ctDNA) using a test with a detection limit of <1 part per million prior to additional therapy **MEETS COVERAGE CRITERIA.**

5) For individuals with a diagnosis of Merkel cell carcinoma (MCC), testing for ctDNA **MEETS COVERAGE CRITERIA** once every three months.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) For all situations not addressed above (e.g., MRD testing for all other solid tumors), MRD testing by multiparameter flow cytometry, PCR, or NGS **DOES NOT MEET COVERAGE CRITERIA**.

7) MRD testing by methods other than those discussed above (e.g., mass spectrometry) **DOES NOT MEET COVERAGE CRITERIA.**

III. Table of Terminology

Term	Definition
ABL	*Abelson tyrosine kinase gene*
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
ALL	Pediatric acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AML1	*Acute myeloid leukemia 1 protein gene*
ASCO	American Society of Clinical Oncology
ASCT	Autologous Stem Cell Transplantation
ASH	American Society of Hematology
B-ALL	B-cell acute lymphoblastic leukemia
BCL1	*B-cell lymphoma 1 gene*
BCL2	*B-cell lymphoma 2 gene*
BCP	B-cell precursor
BCR	Break point cluster gene
BM	Bone marrow
BMA	Bone marrow aspirate
BMR	Bone marrow relapse
BRAF	Proto-oncogene B-Raf/v-Raf murine sarcoma viral oncogene homolog B
CA	Cytogenetic abnormalities
CAP	College of American Pathologists
CAR	Post-chimeric antigen receptor
CBF	Core binding factor
CBFB-MYH11	*Cord binding factor subunit beta-myosin 11 fusion gene*
CCO	Cancer Care Ontario
CD19	Cluster of differentiation 19

CD20	Cluster of differentiation 20
CD43	Cluster of differentiation 43
CD49b	Cluster of differentiation 49b
CD5	Cluster of differentiation 5
CD81	Cluster of differentiation 81
CGC	Canadian Consensus Guideline Consortium
2-CldA	Cladribine
CLIA	Clinical Laboratory Improvement Amendments
CLL	Chronic lymphocytic leukemia
CMS	Centers for Medicare and Medicaid Services
CR	Complete response/complete remission
CT	Computed Tomography
ctDNA	Circulating tumor deoxyribonucleic acid
CV	Coefficients of variance
DCF	Pentostatin
DFCI	Dana Farber Cancer Institute
DFS	Disease Free Survival
DJ	Diversity and joining segment rearrangement
DNA	Deoxyribonucleic acid
E2A	Transcription factor E2-alpha gene
EFS	Event-free survival
ELN	European LeukemiaNet
EMN	European Myeloma Network
EOCT	End of combination therapy
ERIC	European Research Initiative on chronic lymphocytic leukemia
ESMO	European Society for Medical Oncology
EWS	Ewing sarcoma
FC	Flow cytometry
FCR	Fludarabine cyclophosphamide and rituximab
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
FUM3	Follow-up month 3
gDNA	Genomic deoxyribonucleic acid
HPV	Human papillomavirus
HR-NB	High-risk neuroblastoma
HSCT	Haematopoietic stem cell transplantation
HTS	High-throughput sequencing
IgH	Immunoglobulin heavy chain
IGH	*Immunoglobulin heavy chain gene*
IgK	Immunoglobulin K

IgL	Immunoglobulin L
IHC	Immunohistochemistry
IMWG	International Myeloma Working Group
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
J	Joining
KIT	KIT proto-oncogene receptor tyrosine kinase
LDT	Laboratory developed test
MCC	Merkel Cell Carcinoma
MFC/mFC	Multicolor flow cytometry
MFC	Mutliparameter flow cytometry
MM	Multiple myeloma
mpFC	Multi parametic flow cytometry
MRD	Minimal residual disease
MRD	Measurable residual disease
NCCN	National Comprehensive Cancer Network
NDMM	Newly diagnosed multiple myeloma
NGF	Next-generation flow cytometry
NGS	Next-generation sequencing
NGS-MRD	Next-generation sequencing-minimal residual disease
non-TBI	Non-total body irradiation
NPV	Negative Predictive Value
NPM1mut	*Nucleophosmin 1 gene mutant allele*
OS	Overall survival
OPSCC	Oropharyngeal squamous cell carcinoma
PBX1	*Pre-B-cell leukemia transcription factor 1 gene*
PCR	Polymerase chain reaction
PET-CT	Positron emission tomography -computed tomography
PFS	Progression-free survival
PML-RARA	*Promyelocytic leukemia-retinoic acid receptor alpha fusion gene*
PPV	Positive predictive value
QALY	Quality adjusted life year
RQ-PCR/RT-qPCR	Real-time quantitative polymerase chain reaction
RRMM	Relapsed refractory multiple myeloma
RT-PCR	Reverse transcriptase polymerase chain reaction
RUNX1-RUNX1T1	*Runt-related transcription factor 1- RUNX1 partner transcriptional co-repressor 1 gene*
sCR	Stringent complete remission
SR	Standard risk
T-ALL	T-cell acute lymphocytic leukemia
TBI	Total body irradiation

TCR	T cell receptor
TEL	*Translocation-Ets-leukemia virus gene*
TRG	*T cell receptor gamma locus gene*
TTMV	tumor tissue modified viral
uMRD	Undetectable minimal residual disease
VDJ	Variable-diversity-joining
VGPR	Very good partial response
WGS	Whole genome sequencing

IV. Scientific Background

The goal of treating cancer has traditionally been "complete remission" (or response), defined as "absence of visible tumor" based on techniques, such as imaging and histological examination of tissue.[6] However, some cancer cells may remain undetected due to a lack of sensitivity of conventional methods, leading to relapse. This subclinical amount of cancer cells is referred to as minimal residual disease or MRD.[7] While many techniques have been developed to determine MRD, multicolor flow cytometry and PCR-based, including next-generation sequencing (NGS), MRD techniques are the most commonly used.[3,8]

Multicolor flow cytometry (MFC), also known as multiparameter flow cytometry, can be used to identify MRD by measuring for the aberrant expression of antigens on cancer cells. MFC uses lasers of different colors to simultaneously determine specific immunophenotypic features of the cells within a sample. "Classic flow cytometry techniques using four to six colors have limited sensitivity and specificity for MRD detection. Current flow cytometry techniques use six to eight colors to assess MRD with a sensitivity which is approximately 10^{-4}, or about 0.5 to 1 log lower than that of polymerase chain reaction" (PCR).[3]

Polymerase chain reaction-based MRD techniques, including NGS, amplify sequences of DNA unique to the cancerous cell. These techniques have amazing sensitivity. In fact, real-time quantitative PCR can be used to detect a single cancerous cell from 10^4 – 10^5 cells.[3,9] The targets of amplification can include T cell receptor (TCR) gene rearrangements, the immunoglobulin heavy chain (IgH), or even fusion gene transcripts.[3,10,11] Reverse transcriptase PCR-based MRD can also be used to detect cancer-related transcripts, including *E2A/PBX1, TEL/AML1,* and *BCR/AB.*[3,12,13]

Proprietary Testing

ClonoSEQ (Adaptive Biotechnologies, Seattle, WA) is a commercially available NGS-based assay intended to assess MRD in certain types of cancer, such as MM and ALL. This test identifies rearrangements in certain receptor gene sequences, representing the level of MRD in a patient. This test typically uses genomic DNA extracted from bone marrow but may use circulating tumor DNA (ctDNA).[14] After testing, a report is provided which includes each nucleotide sequence identified for tracking residual disease, the amount of each identified marker (per million cells), and whether MRD is determined to be present in the sample.[15]

Signatera™ is an MRD assay that uses ctDNA to attempt to inform the likelihood of cancer relapse earlier than standard of care tools. Whole-exome sequencing of an individual's tumor tissue is first performed, allowing the identification of clonal somatic mutations which are expected to be present in all cells for the individual's specific tumor. The test is customized to an individual through selection of 16 clonal, single nucleotide variants (SNVs). After customization, a blood sample may be obtained from the individual, from which the 16 SNVs may be amplified and detected. An individual is considered MRD-positive when at least two SNVs from the set of 16 are detected. According to the test makers, "a positive Signatera™ result predicts relapse with overall positive predictive value more than 98%."[16]

NavDx is a blood test developed by Naveris that detects trace amounts of HPV-associated tumor DNA in the bloodstream, specifically targeting residual disease or early recurrence of HPV-driven oropharyngeal cancers. It detects and analyzes the tumor tissue modified viral (TTMV)-HPV DNA fragments released by dying cancer cells to provide a TTMV-Score. The test indicates cancer activity before it is visible through imaging or other diagnostics.[17]

M-inSight® is a blood test that uses a mass spectrometry assay to monitor MRD in patients with MM. The test measures M-protein, which is secreted by tumor cells. The M-inSight® test first sequences M-protein from serum or bone marrow and selects the best clonotypic peptides for monitoring in an individual patient. The selected clonotypic peptides are then measured over time to determine M-protein levels. A total of "95% of patients MRD positive by NGS in [bone marrow] was detected by M-inSight® in blood." The test offers a personalized and minimally invasive method of monitoring MRD in patients with MM.[18]

Analytical Validity

The EuroFlow Consortium has reported on the analytical validity of the use of an 8-color mFC for MRD. Theunissen, et al. (2017) reported on the use of this methodology for B-cell precursor (BCP) ALL in a multicenter study (319 patients). Using samples containing more than four million cells, they note concordant results in 93% of samples, and "[m]ost discordances were clarified upon high-throughput sequencing of antigen-receptor rearrangements and blind multicenter reanalysis of flow cytometric data, resulting in an unprecedented concordance of 98% (97% for samples with MRD < 0.01%). In conclusion, the fully standardized EuroFlow BCP-ALL MRD strategy is applicable in >98% of patients with sensitivities at least similar to RQ-PCR ($\leq 10^{-5}$), if sufficient cells (>4 × 10^6, preferably more) are evaluated."[19] Another study reports the use of next-generation flow cytometry (NGF) using an "optimized 2-tube 8-color antibody panel" in five cycles to further increase the sensitivity. The authors report "a higher sensitivity for NGF-MRD vs conventional 8-color flow-MRD -MRD-positive rate of 47 vs 34% (P=0.003). Thus, 25% of patients classified as MRD-negative by conventional 8-color flow were MRD-positive by NGF, translating into a significantly longer progression-free survival for MRD-negative vs MRD-positive CR [complete response] patients by NGF (75% progression-free survival not reached vs 7 months; P=0.02)." Another study using a single-tube 10-fluorochrome analysis NGF method of MRD in myeloma reports a five-fold increase over the target minimum of 5 X 10^6 white blood cells per acquisition.[20]

The FDA included an assessment of the analytical validity of ClonoSEQ in their approval summary of a *de novo* request evaluation. A total of 23 patients with MM, 21 patients with ALL, and 22 patients with

other lymphoid malignancies were included. The study tested three different volumes of DNA: 500ng, 2µg, and 20 µg. Six MRD levels were tested for each sample, corresponding to the following amounts of malignant cells: 2.14, 6.13, 21.44, 61.26, 214.40, and 612.56. The authors found the coefficients of variance (%CV) to range from 72% at 2.14 cells to 21% at 612.56 cells. The authors noted that this precision trend was predictable, as ClonoSEQ is dependent on the number of cells evaluated instead of the actual MRD frequency. Regarding DNA extraction reproducibility, all samples were found to pass the "pre-established acceptance criteria of ± 30% MRD frequency." Regarding precision of the nucleotide/base cells, the authors created a set of "baseline calibrating clonotype nucleotide sequences." From this set, replicates of each sample used to create the calibration sequence were created and the disagreement rate was identified. Out of 442.5 million nucleotides, ClonoSEQ was found to have a disagreement rate of 3.5 parts per million. The FDA notes a Phred Score of >30 is considered a "high-quality base call for NGS applications"; ClonoSEQ had a Phred Score of 44.5. When compared to multiparametic flow cytometry (mpFC), both ClonoSEQ and mpFC were tested at five dilutions (from $5x10^{-7}$ to $1x10^{-2}$)s and both techniques were found to be of similar accuracy at frequencies above $1x10^{-4}$.[21]

In 2020, the FDA made a substantially equivalent decision on ClonoSEQ, providing in their summary an assessment with further analytical validity. Here, they looked at patients with CLL, measuring MRD levels from gDNA samples extracted from either bone marrow (22 patients) or blood samples (15 patients). The study tested six MRD levels in three different volumes of DNA: 500ng, 2µg, and 20 µg. In bone marrow samples, precision ranged from 59% CV at 2.14 cells to 20% CV at 612.56 cells. In blood samples, precision ranged from 53% CV at 3.10 cells to 19% CV at 765.70 cells. The authors note that "like BMA [bone marrow aspirate], the precision of the clonoSEQ assay in CLL blood is largely dependent on the number of malignant cells that are being evaluated by the assay." From these data and those presented in the 2018 *de novo* approval document, the indications for use were developed. The authors report that "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 clinicopathological features."[22]

Al-Sawaf, et al. (2020) analyzed the clonal growth patterns of patients treated with venetoclax-obinutuzumab therapy within the CLL14 trial. In this case, MRD was analyzed using NGS via the adaptive clonoSEQ assay with cutoffs of 10^{-4}, 10^{-5}, and 10^{-6}; "the limit-of-quantification of the clonoSEQ assay is less than 10^{-6}." A total of 432 patients with untreated CLL were either treated with chlorambucil or venetoclax, in combination with 7binutuzumab for the first six cycles. Using samples from peripheral blood (PB) collected every three to six months until nine years from last patient enrollment, the researchers found that two months post-treatment completion, among patients treated with venetoclax and obinutuzmab, 40% had uMRD levels $<10^{-6}$, 26% had uMRD levels that were $\geq 10^{-6}$ and $<10^{-5}$, 8% had uMRD levels $\geq 10^{-5}$ and $<10^{-4}$, 5% had uMRD levels $\geq 10^{-4}$ and $<10^{-2}$, and 3% had uMRD levels $\geq 10^{-2}$. In comparison, among patients treated with chlorambucil and obinutuzmab, 7% had uMRD levels $<10^{-6}$, 13% had uMRD levels $\geq 10^{-6}$ and $<10^{-5}$, 14% had uMRD levels $\geq 10^{-5}$ and $<10^{-4}$, 21% had uMRD levels $\geq 10^{-4}$ and $<10^{-2}$, and 26% had uMRD levels $\geq 10^{-2}$. Furthermore, "In a PFS landmark analysis after [end of treatment], patients in the [venetoclax-obinutuzumab] arm with MRD levels $\leq 10^{-5}$ had a 2-year PFS after [end of treatment] of approximately 93%, while patients with detectable MRD $>10^{-2}$ had a 2-year PFS of [approximately] 37%." The average growth rate among patients treated with venetoclax-obinutuzumab

was lower compared to the contrasting arm, and thus had a larger MRD doubling time. Because the clonal growth rate was lower, the study, using NGS, indicated "more effective MRD eradication and clonal growth modulation."[23,24]

Nguyen Hoang, et al. (2024) studied the analytical validity and clinical utilization of K-4CARE™, a comprehensive genomic profiling assay that integrates ctDNA tracking for residual cancer surveillance. K-4CARE™ is proposed to be a more time-efficient and tissue-efficient method than single or small gene panel testing. The assay tests for 473 cancer-relevant genes with a total length of 1.7 Mb. The authors rested the assay with 155 samples from 10 cancer types. "For detection of somatic SNVs and Indels, gene fusion and amplification, the assay had sensitivity of >99%, 94% and >99% respectively, and specificity of >99%." Furthermore, "when CGP-informed mutations were used to personalize ctDNA tracking, the detection rate of ctDNA in liquid biopsy was 79%, and clinical utility in cancer surveillance was demonstrated in 2 case studies." The authors concluded that the K-4CARE™ assay is "comprehensive and reliable" as a biomarker test for targeted therapy and immunotherapy. The authors also state that "integration of ctDNA tracking helps clinicians to further monitor treatment response and ultimately provide well-rounded care to cancer patients."[25]

Gunning, et al. (2023) investigated the DNA stability and the ability to detect TTMV-HPV DNA analytes from patient samples stored in Streck collection tubes over a seven day interval. The ESR1 gene was chosen as the analyte for DNA stability analysis; DNA recovery was reported as the average, minimum, and maximum detected ESR1 per mL of plasma isolated. TTMV-HPV analyte detection was accomplished by measuring the TTMV-HPV DNA percent positivity rate, tracked daily. Percent positivity for TTMV-HPV DNA remained consistently between 26.2% - 32.5% from days one to seven. The average ESR1 values trended upward and more than doubled between days 1-6, which may be due to an increase in cellular lysis and DNA release within the sample over time. The LODs (limit of detection) for TTMV-HPV16, 18, 31, 33 and 35 DNA were reported to be: 0.56, 1.31, 0.63, 1.10 and 0.57 copies per microliter (copies/µL); LOQs (limit of quantitation) were reported as <1.20, 3.56, 4.11, 4.00, and 3.50 copies/ µL. The analytical specificity of this study was reported to be between zero and 0.32 copies/µL for the five high-risk HPV subtypes. The authors investigated "the ability of the NavDx test to obtain a response that is directly proportional to the concentration of analyte standard." It was determined that all five HPV serotypes demonstrated linearity down to the limit of quantitation. The coefficients of determination (R2) equaled one for all serotypes at all timepoints. Intra-assay and inter-assay precision were investigated through determination of % CVs (coefficients of variation) of the measured concentrations of each HPV serotype:

Dilution *	%CVs of Mean Effective Concentration (Copies/µL)									
	Intra-Assay					Inter-Assay				
	TTMV-HPV16	TTMV-HPV18	TTMV-HPV31	TTMV-HPV33	TTMV-HPV35	TTMV-HPV16	TTMV-HPV18	TTMV-HPV31	TTMV-HPV33	TTMV-HPV35
1:1	2.1	2.7	2.2	3.0	1.4	2.9	2.4	1.9	2.5	1.9
1:4	2.1	2.1	1.0	1.5	3.5	3.3	3.6	2.6	4.3	3.8
1:20	3.3	2.7	3.0	5.4	1.2	4.7	2.9	6.3	4.9	3.0
1:40	2.0	6.2	8.0	11.5	1.1	4.6	5.4	7.5	8.4	6.4
1:100	12.2	10.1	9.0	5.3	7.1	7.5	11.9	10.8	10.8	5.8
1:200	13.3	13.8	7.2	13.9	4.7	9.0	20.0	16.5	13.1	11.2

Table 4. Intra-assay precision (repeatability) and inter-assay precision (within-lab on differing days), reflected by percent relative standard deviations of effective concentrations of the five TTMV-HPV DNA types measured at six concentrations in triplicate.

Table borrowed from Gunning, et al. (2023).

Clinical Utility and Validity

The FDA *de novo* approval document for ClonoSEQ contains two clinical validation studies. The first study for MM in ClonoSEQ's *de novo* approval document (DFCI 10-106) included 323 patients. The authors intended "to assess the ability of clonoSEQ to predict progression-free survival (PFS) and disease-free survival (DFS)." At the time of first MRD measurement, ClonoSEQ was found to be predictive of PFS at the MRD threshold of 10^{-5}. Each 10-fold increase in MRD level was associated with a 70% increase in "event" rate across all MRD values.[21]

A second clinical validation study described in the FDA's *de novo* approval document was for ALL (AALL0232, AALL0331). A total of 273 samples were included (210 MRD $\leq 10^{-4}$, [negative], 63 MRD > 10^{-4} [positive]). The authors report that clonoSEQ MRD negativity status was found to predict event-free survival (EFS) at all ages. MRD positivity status was also associated with a 2.74-fold higher event risk compared to MRD negativity status. Across all MRD values, a 10-fold increase in clonoSEQ MRD measurement was associated with a 50% increase in event rate and MRD-negative patients were found to have longer EFS compared to patients with higher frequencies of malignancies.[21]

The FDA's 2020 substantially equivalent approval document for ClonoSEQ analyzed two separate studies to "support that MRD as estimated with the clonoSEQ Assay is prognostic of patient outcomes in CLL…" The first study was for CLL (NCT02242942) and included 337 patients to evaluate the ability of ClonoSEQ to predict PFS. Samples were collected three months or later following treatment (FUM3) and MRD positivity was defined as >1 x 10^{-5} [malignant cells]. Patients found to be MRD-positive had an "event risk" 6.64 times higher than the MRD-negative cohort. A 10-fold increase in MRD was also associated with a 2.35-fold increase in event risk. The authors also analyzed the results for other confounding factors and found "that the MRD level at FUM3 is a stronger predictor of PFS than age, sex, geographic region, Binet stage, or treatment arm of the clinical trial. Together, these results demonstrate the clinical validity of MRD measurement in CLL." This study also found that "patients with clonoSEQ MRD ≤ 10^{-6} or between 10^{-6} and 10^{-5} had longer PFS, followed by patients with MRD between 10^{-5} and 10^{-4} and patients with MRD ≥ 10^{-4} (log-rank P = 4.902 x 10-31, Figure 11). These data demonstrate that patients with MRD ≤ 10^{-5} have better outcomes than patients with MRD > 10^{-5}, and that increasing MRD levels above 10^{-5} are associated with an increased risk of progression within the follow-up time of this study."[22]

The second clinical validation described in FDA's 2020 substantially equivalent approval document was also for CLL (NCT00759798). This study was a "phase 2 clinical trial that evaluated six cycles of fludarabine, cyclophosphamide, and rituximab (FCR) in 111 front-line chronic lymphocytic leukemia (CLL) patients with clonoSEQ ID samples and a corresponding 137 clonoSEQ MRD samples also evaluated by 4-color flow cytometry at an MRD threshold of 10-4 (NCT00759798) and with pertinent co-variate data. Within this cohort of 111 patients with flow MRD results, bone marrow was available for 75 patients and blood was available for 62 patients, of which 26 patients provided both blood and bone marrow. Due to some missing clinical covariates, three patients that provided bone marrow only, were excluded from analyses requiring these covariates. There was an association between PFS and continuous clonoSEQ MRD measurement in both blood and bone marrow, after end of treatment, where PFS is defined as the time from start of treatment until death, disease progression, or last time of disease

assessment ($p = 9.66 \times 10^{-4}$ for blood, $p = 2.13 \times 10^{-4}$ for bone marrow). Additionally, patients who were MRD negative at a threshold $\leq 10^{-5}$ had superior progression-free survival compared to patients with MRD $> 10^{-5}$ ($p = .02$ for blood and $p = 8.17 \times 10^{-5}$ for bone marrow… Taken together these results support the use of the clonoSEQ assay in CLL patients."[22]

Hay, et al. (2019) evaluated the impact of MRD negativity status on relapse rates of ALL patients that post-chimeric antigen receptor [CAR] T cell therapy. Per flow cytometry, 45 of 53 patients achieved an MRD-negative status. At a median follow-up of 30.9 months, the authors found that EFS and overall survival (OS) were significantly better in patients achieving MRD negativity than patients that did not (median EFS: 7.6 months vs 0.8 months; median OS: 20 months vs five months). The authors also identified that the cytometric absence of the *IGH* index malignant clone was associated with better EFS.[27]

Herrera, et al. (2016) evaluated "whether the presence of ctDNA [circulating tumor DNA, measured with next-generation sequencing was associated with outcome after allogeneic haematopoietic stem cell transplantation (HSCT) in lymphoma patients." A total of 88 patients were included from a "phase 3 clinical trial of reduced-intensity conditioning HSCT in lymphoma." Patients with detectable ctDNA three months after HSCT were found to have inferior progression-free survival compared to patients without detectable ctDNA (58% vs 84%, two-year PFS rate). Detectable ctDNA was confered a 10.8-times higher risk of relapse/progression and a 3.9-times higher risk of progression/death compared to the non-detectable ctDNA group. The authors concluded that "detectable ctDNA is associated with an increased risk of relapse/progression, but further validation studies are necessary to confirm these findings and determine the clinical utility of NGS-based minimal residual disease monitoring in lymphoma patients after HSCT."[14]

Perrot, et al. (2018) examined the prognostic value of MRD (measured with NGS) in MM cases. A total of 127 patients achieved MRD negativity (defined as "the absence of tumor plasma cell within 1 000 000 bone marrow cells ($<10^{-6}$) at least once during maintenance therapy. At the start of therapy, MRD was found to be a strong prognostic factor for both progression-free survival as well as overall survival (hazard ratio = .22 and .24 respectively). From a previous cohort, the authors identified 233 patients labeled as MRD-negative, of which 120 were confirmed as MRD-negative with NGS (52%)."[28]

Friend, et al. (2020) investigated the impact of NGS-MRD in predicting relapse in ALL patients. Total body irradiation (TBI)-based regimens were the standard of care for ALL patients requiring allogeneic HSCT, but this procedure has numerous harmful side effects. The authors hypothesized that identifying MRD-negative patients may help some individuals avoid exposure to this radiation. The authors examined outcomes of 57 patients that received TBI and non-TBI regimens and found that relapse rates were similar for both methods of treatment. However, NGS-MRD positivity prior to treatment was "highly" predictive of relapse (for up to three years post-transplant). Based on their data, the authors suggested "that the decision to use either a TBI or non-TBI regimens in ALL should depend on NGS-MRD status, with conditioning regimens based on TBI reserved for patients that cannot achieve NGS-MRD negativity prior to allogeneic HSCT."[29]

Thörn, et al. (2011) performed a comparative analysis of MFC and RT-qPCR based MRD in pediatric ALL. The study, consisting of 726 follow-up samples from 228 children, used an MRD threshold of 0.1% and reported at day 29 a 84% concordance between the two different methods. For B-cell precursor ALL, the

authors note that MFC was better at discriminating higher risk of bone marrow relapse (BMR), whereas RT-qPCR performed better for T-ALL. Regardless, the authors state, "MRD levels of ≥0.1%, detected by either method at day 29, could not predict isolated extramedullary relapse." They conclude that "both methods are valuable clinical tools for identifying childhood ALL cases with increased risk of BMR."[30]

Wood, et al. (2018) compared high-throughput sequencing (HTS) of *IGH* and *TRG* genes to flow cytometry (FC) to evaluate measurable residual disease detection at the end of induction chemotherapy in pediatric patients with newly diagnosed B-ALL [B-cell acute lymphoblastic leukemia]. A total of 619 paired pre-treatment and end of induction bone marrow samples were included. At an MRD threshold of 0.01%, both HTS and FC showed similar event-free survival (EFS) and OS for both MRD-positive and MRD-negative patients. However, HTS identified 55 more patients as "MRD-positive" compared to FC. These "discrepant" patients were found to have worse outcomes than FC MRD-negative patients. HTS was also found to identify 19.9% of "standard risk" (SR) without MRD at any detectable level with excellent EFS and OS (98.1% and 100% respectively). The authors suggested that "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."[31]

Rawstron, et al. (2016) conducted a parallel analysis of MRD using both ClonoSEQ and multiparameter flow cytometry in 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 10^{-5} and 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 one in a million (10^{-6})." The authors also note, "a parallel analysis of high-throughput sequencing using the ClonoSEQ assay showed good concordance with flow cytometry results at the 0.010% (10^{-4}) 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."[32]

Thompson, et al. (2019) evaluated 62 patients with CLL that were considered negative for MRD by flow cytometry (sensitivity of 10^{-4}). Using ClonoSEQ, the authors found that 72.6% of these MRD-negative patients were MRD-positive by ClonoSEQ (a discordant result). Only 27.4% of patients were found to be negative by both methods. The authors also found that patients that were negative by both methods were found to have superior PFS compared to patients that were only negative by flow cytometry, thereby suggesting that ClonoSEQ was a superior prognostic discriminator.[33]

Wang, et al. (2019) published a study on the applicability of multiparameter (multicolor) flow cytometry (MFC) for detecting MRD to predict relapse in patients with AML after allogeneic transplantation. The researchers determined MFC and MRD status using RT-qPCR from 158 bone marrow samples from 44 different individuals and compared the statuses between the two. They noted that "strong concordance was found between MFC-based and RT-qPCR-based MRD status (κ = 0.868)." Moreover, for individuals in complete remission (CR), "the positive MRD status detected using MFC was correlated with a worse prognosis [HRs (*P* values) for relapse, event-free survival, and overall survival: 4.83 (<0.001), 2.23 (0.003),

and 1.79 (0.049), respectively]; the prognosis was similar to patients with an active disease before HSCT [hematopoietic stem cell transplantation]."[34]

Carlson, et al. (2019) published a cost-effectiveness study of NGS-based MRD testing during maintenance treatment for MM. The authors compared use of MRD testing to no MRD testing. A Markov model with six health states was developed; "MRD positive or MRD negative, on or off treatment, relapsed, or dead." From there, the authors compared yearly NGS-MRD to no MRD testing over a lifetime horizon. Overall, the authors found that "MRD testing saved $1,156,600 over patients remaining lifetime." Health outcomes were found to slightly favor MRD testing (0.01 quality adjusted life years [QALYs]) compared to no testing. The authors concluded that "NGS MRD testing is cost saving, with potential QALY gains due to avoidance of [treatment-related adverse events] compared with no testing for MM patients on maintenance therapy."[35]

Medina, et al. (2020) evaluated MRD three months after transplantation in 106 myeloma patients, noting that "detecting persistent minimal residual disease (MRD) allows the identification of patients with an increased risk of relapse and death." In this study, they compared the results of NGS with NGF, where they noted that "correlation between NGS and NGF was high (R2 = 0.905). The three-year PFS rates by NGS and NGF were longer for undetectable vs. positive patients (NGS: 88.7% vs. 56.6%; NGF: 91.4% vs. 50%; $p < 0.001$ for both comparisons), which resulted in a three-year overall survival (OS) advantage (NGS: 96.2% vs. 77.3%; NGF: 96.6% vs. 74.9%, $p < 0.01$ for both comparisons). In the Cox regression model, NGS and NGF negativity had similar results but favoring the latter in PFS (HR: 0.20, 95% CI: 0.09-0.45, $p < 0.001$) and OS (HR: 0.21, 95% CI: 0.06-0.75, $p = 0.02$). All these results reinforce the role of MRD detection by different strategies in patient prognosis and highlight the use of MRD as an endpoint for multiple myeloma treatment."[36]

Goicoechea, et al. (2021) examined MRD as a possible endpoint marker in MM. They note that while patients with MM that carry standard- or high-risk cytogenetic abnormalities (CA) are achieving similar CR rates, high-risk patients have an inferior PFS. They note that this "questions the legitimacy of CR as a treatment endpoint..." Using NGF cytometry to evaluate MRD in MM patients, they compared standard- vs high-risk CAs (n=300 and 90, respectively) and identified mechanisms that determine MRD resistance in both patient subgroups (n=40). In patients achieving undetectable MRD with either standard- or high-risk CAs, the 36-month PFS rates were higher than 90%. In comparison, patients with persistent MRD had a median PFS of about three (standard-risk CA) and two (high-risk CA) years. They found that "further use of NGF to isolate MRD, followed by whole-exome sequencing of paired diagnostic and MRD tumor cells, revealed greater clonal selection in patients with standard-risk CAs, higher genomic instability with acquisition of new mutations in high-risk MM, and no unifying genetic event driving MRD resistance." Ultimately, their results support "undetectable MRD as a treatment endpoint for patients with MM who have high-risk CAs and proposes characterizing MRD clones to understand and overcome MRD resistance."[37]

Subhash, et al. (2022) investigated the "feasibility of using whole-genome sequencing (WGS) data to design tumour-specific polymerase chain reaction (PCR)-based MRD tests (WGS-MRD)" for children with cancers with high-risk of relapse, like ALL, high-risk neuroblastoma (HR-NB), and Ewing sarcoma (EWS). The significance of this study was that pediatric solid tumor MRD DNA-based assays currently remains experimental, and remains focused on mRNA, methylated DNA, or microRNA. Through their

experimentation, the researchers found that "sensitive WGS-MRD assays were generated for each patient and allowed quantification of 1 tumour cell per 10^{-4} (0.01%)-10^{-5} (0.001%) mononuclear cells." They also found that WGS-MRD and Ig/TCR-MRD assays were concordant in ALL, and the WGS-MRD assays "showed good concordance between quantitative PCR and droplet digital PCR formats." The WGS-MRD assay clinical samples also correlated with disease course and was found to be more sensitive than RNA-based MRD assays. This demonstrated how WGS could aid the development of MRD assays for pediatric cancers as it has been done in adults.[38]

Kater, et al. (2019) utilized the MURANO study to investigate the effects of a fixed duration treatment with venetoclax and ritixumab on MRD. The study itself demonstrated "significant progression-free survival (PFS) benefit for fixed-duration venetoclax-rituximab compared with bendamustine-rituximab in relapsed/refractory chronic lymphocytic leukemia." However, among patients who received venetoclax-rituximab, there was a "higher rate of PB [peripheral blood] undetectable MRD (uMRD; less than 10^{-4}) at EOCT [end of combination therapy] (62% v 13%) with superiority sustained through month 24 (end of therapy)," which predicted longer PFS. At the end of the therapy treatment period, 70% of patients remained in uMRD and 98% without disease progression. This demonstrated the correlation between uMRD and PFS using PB MRD in the setting of venetoclax-combination treatment.[39]

Kim, et al. (2022) investigated the clinical utility of NGF-based MRD assessment in a heterogeneous population of patients with MM at the Samsung Medical Center in Korea and found that "sCR samples showed a lower MRD-positive rate (25%) than CR (43%) and VGPR (53%) samples, although the difference was not significant (P=0.051)." When evaluating survival analysis based on clinical response and MRD, "PFS in VGPR patients was lower than that in sCR/CR patients (P<0.001) …whereas PFS in VGPR patients was lower than that in sCR/CR patients (P<0.001)." Cytogenetic risk survival analysis yielded similar results: "There was no significant difference in PFS between patients with high-risk and standard-risk cytogenetics (P=0.222)" and "Further analysis according to MRD status also revealed no significant difference in PFS in patients with standard-risk cytogenetics (P=0.246)," though it is worth noting that "among patients with high-risk cytogenetics, MRD-positive patients showed lower PFS than MRD-negative patients (P=0.016)." Overall, the authors concluded that "Sustained MRD negativity was only observed in patients with sustained sCR, and their PFS was superior to that of patients who were not MRD-negative (P=0.035)," though by their own admission the study was limited by the sample size (n=12 over 18 months).[40]

There is a growing pool of literature investigating the use of ctDNA-based MRD detection in the clinical context of solid tumors. Some studies suggest that inclusion of MRD assessment may offer some improvement over standard of care; this has been reported for gastrointestinal malignancies,[41] skin cancer,[42,43] lung cancer,[44] and especially colorectal cancer, where several reports demonstrate potential benefits of ctDNA measurement, which include response monitoring, prognosis, post-surgical surveillance, chemotherapeutic management, and informing recurrence risk.[45-49]

Signatera™ has been clinically validated across several cancer types for recurrence monitoring. In the context of colorectal cancer, Loupakis, et al. (2021) demonstrated that a two-timepoint analysis of ctDNA (a baseline measure plus last follow-up time point–either the time of radiologic progression or last evidence of radiologic disease's absence) improved the sensitivity of the assay. For patients who were ctDNA positive at both timepoints, the authors reported that the assay demonstrated a sensitivity

of 91.4%, specificity of 93.3%, and positive predictive value (PPV) of 96.7%. Reinert, et al. (2019) also investigated the validity of ctDNA for MRD detection for individuals with stages I-III colorectal cancer and reported promising metrics; the test demonstrated a sensitivity of 88%, specificity of 98%, PPV of 93.3%, and negative predictive value (NPV) of 96.7%. However, Fakih, et al. (2022) recently determined that Signatera™ may not "provide advantages as a surveillance strategy compared with standard imaging combined with CEA levels when performed per National Comprehensive Cancer Network guidelines," after a cohort study failed to demonstrate a sufficient ability (sensitivity) of the test to detect disease recurrence in individuals with resected colorectal cancer.

Coombes, et al. (2019) described the ability of ctDNA to detect disease recurrence ahead of clinical or radiological approaches for individuals with breast cancer with a sensitivity of 89%, specificity of 100%, PPV of 100%, and NPV of 94%. Christensen, et al. (2019) found that ctDNA accurately identified individuals diagnosed with advanced bladder cancer who eventually relapsed (sensitivity of 100% and specificity of 98%). For individuals with non-small cell lung cancer, Abbosh, et al. (2017) reported that Signatera™ detected relapse with 93% sensitivity and 90% specificity.

While evidence supporting MRD analysis for solid tumors continues to emerge, a consensus on the clinical validity and utility of this approach has not yet been reached. Sullivan, et al. (2023) cite several limitations of ctDNA-based approaches to the management of gastrointestinal cancer; O'Sullivan, et al. (2023) argue that the utility of this technology in the context of non-small cell lung cancer is still investigational; Moding, et al. (2021) cite a lack of prospective clinical trials confirming clinical utility of ctDNA-MRD assessment in general; and, Jacome and Johnson (2023) argue that there is not yet consensus on how to apply the results of ctDNA-based testing in the management of colorectal cancer.

Faulkner, et al. (2023) conducted a systematic review and meta-analysis to study the utility of ctDNA for detecting MRD in colorectal cancer. The review included 37 studies involving 3002 patients. The authors calculated hazard ratios and PFS based on ctDNA detection. Overall, there was a "poorer" PFS associated with ctDNA detection at the first liquid biopsy post-surgery, with a hazard ratio of 6.92. Further, "this effect was also seen in subgroup analysis by disease extent, adjuvant chemotherapy and assay type." The authors conclude that "ctDNA detection post-surgery is associated with a greater propensity to disease relapse and is an independent indicator of poor prognosis," but note that "prior to incorporation into clinical practice, consensus around timing of measurements and assay methodology are critical."[60]

Berger, et al. (2022) evaluated the use of circulating tumor tissue modified viral (TTMV)-HPV DNA as a biomarker for detecting recurrence in patients with HPV-driven oropharyngeal squamous cell carcinoma (OPSCC). The authors conducted a retrospective analysis of 1,076 patients from 108 U.S sites who were at least three months post-treatment and had undergone TTMV-HPV DNA testing (NavDx) during routine surveillance between February 2020 and June 2021. The results determined that 80 out of 1,076 (7.4%) tested positive for TTMV-HPV DNA. Among these, 21 patients had known recurrences at the time of the first positive test, while 59 did not, and 93% of the latter group subsequently developed confirmed recurrences. The overall PPV for the TTMV-HPV DNA test was 95%, and the NPV was also 95%. These findings suggest that TTMV-HPV DNA testing can serve as an effective surveillance tool, often identifying recurrences earlier than traditional clinical evaluations and imaging techniques, and may influence future surveillance strategies for HPV-related cancers.[61]

Landgren, et al. (2024) completed a meta-analysis on the use of MRD assessment in patients with MM. The meta-analysis included eight clinical trials with a total of 4907 patients. Overall, the individual-level association between 12-month MRD negativity and progression-free survival had a global odds ratio of 4.02. For specifically relapse or refractory MM, the individual-level association between 12-month MRD negativity and progression-free survival had a global odds ratio of 7.67. The authors concluded that "MRD may be an early clinical end point reasonably likely to predict clinical benefit in MM, that may be used to support accelerated approval and thereby, expedite the availability of new drugs to patients with MM."[62]

Guerrero, et al. (2024) studied the use of MRD negativity as a biomarker to stop treatment in transplant eligible MM patients. The authors were looking for risk factors that indicate MRD resurgence or progressive disease in patients with undetectable MRD. The study included 267 patients, all of whom were newly diagnosed with MM, transplant eligible, and MRD-negative. After 73 months, 42% of patients showed MRD resurgence or progressive disease. "The only prognostic factors at diagnosis that predicted MRD resurgence and/or PD were an International Staging System (ISS) 3 and the presence of ≥0.01% circulating tumor cells (CTCs)." Other predictors were failure to achieve MRD negativity after induction and having two or more risk factors. The authors concluded that "easily measurable risk factors could help refine the selection of patients for whom treatment cessation after MRD negativity is being investigated in clinical trials."[63]

Epstein-Peterson, et al. (2024) studied the use of MRD evaluation for high-risk mantle cell lymphoma patients being treated with immunochemotherapy and lenalidomide. The study included 49 patients. The three-year progression-free survival rate was 63%. At a sensitivity of 1E-5, MRD did not significantly predict prognosis. At a sensitivity of 1E-6, MRD did significantly discriminate progression-free survival. MRD also predicted long-term progression-free survival six months after treatment ended. "MRD status was prognostic and predicted long-term outcomes following [rituximab and high-dose cytarabine] and at 6 months following end-of-treatment." The authors concluded that "sequential MRD assessment is a powerful prognostic tool in patients with MCL."[64]

V. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN has published several relevant guidelines on management of MRD in hematologic malignancies.

Multiple Myeloma (MM)

For MM, MRD is considered an "important" prognostic factor. The NCCN recommends measuring MRD during follow-up/surveillance for symptomatic MM patients after response to primary therapy "as indicated for prognostication." Next-generation flow and next-generation sequencing (or both) are recommended for methodology and a sensitivity of 1 in 10^5 (or better) is recommended for accuracy. The NCCN recommends to "consider baseline clone identification and storage of aspirate sample for future minimal residual disease (MRD) testing by NGS." MRD is a required criterion listed within the IMWG MM response criteria. The NCCN notes that "for MRD there is no need for two consecutive assessments, but information on MRD after each treatment state is recommended (eg, after induction, high-dose therapy/Autologous stem cell transplant (ASCT), consolidation, maintenance). MRD tests

should be initiated only at the time of suspected complete response." Sustained MRD-negative status is only confirmed when taken a minimum of one year apart, but "subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)." The NCCN also notes that MRD is being used in post-hematopoietic stem cell transplantation treatment assessments.[65]

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

The NCCN remarks that "undetectable MRD in the peripheral blood at the end of time-limited treatment is an important predictor of efficacy." The NCCN recommends performing MRD assessment with an assay at a sensitivity of 10^{-4} "according to the standardized European Research Initiative on CLL (ERIC) method or standardized NGS method." NCCN also stated that "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 sequencing (NGS) based assays have been shown to be more sensitive, thus allowing for the detection of MRD at the level of 10^{-6}."[66]

Acute Myeloid Leukemia (AML)

The NCCN recommends measuring MRD "upon achievement of morphologic remission" and "at the time of and following allogeneic HCT." The NCCN also states, "additional time points should be guided by the regimen used." NCCN notes that for NPM1-mutated AML, MRD should be assessed "after two cycles of intensive chemotherapy (eg, one cycle of induction and one cycle of consolidation) and for serial monitoring." For PML:RAR alpha, MRD should be assessed "every 3 months for at least 24 months for patients with AP." NGS-based assays to detect mutated genes are not routinely used in AML, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by NGS. The NCCN states that "if using flow cytometry to assess MRD, it is recommended that a specific MRD assay that incorporates leukemia-associated immunophenotypes (LAIPs) assessment is utilized, but, most importantly, that it is interpreted by an experienced hematopathologist, preferably at the same laboratory as diagnostic flow cytometry for consistency" since there are differences between "diagnostic threshold assessments and MRD assessments." The NCCN further states that "the optimal sample for MRD assessment is a first dedicated pull of the BM. Once MRD-negative remission by BM is achieved, peripheral blood can be utilized for surveillance of MRD for PML::RAR alpha, NPM1, CBFB::MYH11, and RUNX1::RUNX1T1."[67]

Acute Lymphoblastic Leukemia (ALL)/Pediatric Acute Lymphoblastic Leukemia

The NCCN states that "MRD quantification is an essential component of patient evaluation over the course of sequential ALL therapy," noting the prognostic significance of MRD. Techniques used to assess for MRD 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 quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) assays (eg, BCR::ABL1)." NGS is recognized as one of the most sensitive methods at detection levels of 10^{-6}, as can some PCRs. An entire section within the ALL guidelines is devoted to MRD assessment. They note the timing of MRD assessment to be as follows:

- Upon completion of initial induction.
- End of consolidation.
- Additional time points should be guided by the regimen used and risk features.
- 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.

The guidelines also recommend "for patients with negative MRD by flow cytometry but positive MRD by an FDA-approved NGS assay, consider repeat testing before consolidation is started to confirm MRD status."[68] Overall, MRD has a strong correlation with risks for relapse and is considered to have a high prognostic value. MRD has a role in identifying optimal treatments for patients, both adult and pediatric, with ALL.[68,69]

Hairy Cell Leukemia

The NCCN writes that, for a complete response (CR) with or without MRD, "if CR is achieved, an IHC [immunohistochemistry] assessment of the percentage of MRD will be useful to stratify patients based on level of CR (with or without evidence of MRD)." The NCCN notes that "it has been suggested that MRD monitoring as a component of response assessment should be incorporated in all clinical trials for relapsed HCL" but clarifies that "MRD assessment is not recommended (outside of clinical trials) as part of response evaluation."[70]

Merkel Cell Carcinoma (MCC)

The NCCN writes that "circulating tumor DNA (ctDNA) can assess disease burden in both virus-positive and virus-negative MCC and typically becomes positive prior to or at the time of a clinically evident recurrence. For surveillance, this test is often obtained every 3 months." The NCCN also states that "for patients with biopsy-confirmed MCC, additional workup may include complete skin and lymph node examination, imaging studies, and laboratory studies as clinically indicated including MCPyV and MCC circulating tumor (ctDNA) testing."[71]

B-Cell Lymphomas

The NCCN states that, for diffuse large B-cell lymphoma, "if end of treatment [positron emission tomography] is positive, consider repeat biopsy or if biopsy not feasible, consider circulating tumor DNA (ctDNA) for minimal residual disease (MRD) (ctDNA-MRD) assessment."[72]

International Myeloma Working Group (IMWG)

The IMWG recommends assessing MRD response at a sensitivity of $1/10^5$ nucleated cells or better.[73]

European Myeloma Network (EMN)

Regarding NGS in assessment of MRD in MM, the EMN writes that "results from next-generation sequencing are highly concordant with flow-based MRD detection, highly reproducible and reach a

sensitivity of 10^{-6}" and that the primary restraints for NGS are "a lack of standardization and limited commercial availability."[74]

American Society of Clinical Oncology (ASCO) and Cancer Care Ontario (CCO)

These joint guidelines focus on treatment of MM. Their MRD-related recommendations are listed below:

- "There is insufficient evidence to make modifications to maintenance therapy based on depth of response, including MRD status"
- "MRD-negative status has been associated with improved outcomes, but it should not be used to guide treatment goals outside the context of a clinical trial"
- "There is insufficient evidence to support change in type and length of therapy based on depth of response as measured by conventional IMWG approaches or MRD"
- "There are not enough data to recommend risk-based versus response-based duration of treatment (such as MRD)."[75]

The ASCO also fully endorsed the "Initial Diagnostic Work-Up of Acute Leukemia" released jointly by the College of American Pathologists and the American Society of Hematology in 2018.[76]

European LeukemiaNet (ELN) MRD Working Party

The ELN states, "measurable residual disease (MRD; previously termed minimal residual disease) is an independent, postdiagnosis, prognostic indicator in acute myeloid leukemia (AML) that is important for risk stratification and treatment planning, in conjunction with other well-established clinical, cytogenetic, and molecular data assessed at diagnosis." The ELN remarks that quantitative PCR is applicable to approximately 40% of AML patients with "1 or more suitable abnormalities." However, NGS for MRD assessment may provide assessment to an additional 40%-50% of AML patients, as NGS can "theoretically, be applied to all leukemia-specific genetic aberrations." The ELN recommends a sensitivity of at least 1 / 103 cells, and states that NGS platforms will be used after careful validation.[5]

International Workshop on Chronic Lymphocytic Leukemia (iwCLL)

The iwCLL published guidelines on CLL in 2018. In it, they consider MRD assessment to be a necessary component in identifying complete remission of CLL. The iwCLL also writes that eradication of leukemia is a "desired end point." They go on to state: "Use of sensitive multicolor flow cytometry, PCR, or next-generation sequencing can detect MRD in many patients who achieved a complete clinical response… 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."[77]

College of American Pathologists (CAP) and the American Society of Hematology (ASH)

This CAP/ASH joint guideline was published in 2017 and focuses on Initial Diagnostic Workup of Acute Leukemia (AL). The guideline strongly recommends that "For patients with suspected or confirmed AL, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of MRD." The guideline also notes that MRD is a "powerful" predictor of adverse outcome in patients with AL.[78]

This guideline was endorsed by the American Society of Clinical Oncology in 2018.[78]

European Society for Medical Oncology (ESMO)

Chronic Lymphocytic Leukaemia

The ESMO notes that "detection of MRD by multicolour flow cytometry or RT-PCR has a strong prognostic impact following CIT73,74 as well as venetoclax plus CD20-antibody combinations. A total of 75 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… 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."[79] The guidelines are also endorsed by the European Hematology Association.[80]

Acute Lymphoblastic Leukaemia

The ESMO writes that "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…If MRD is measured by flow cytometry, a good MRD response is often defined as less than 10^{-3}, although MRD levels less than 10^{-4} can be achieved with the 8–12 colour flow cytometers."[81]

Multiple Myeloma

The ESMO states, "one of the most significant improvements in the response criteria is the introduction of minimal residual disease (MRD) both in the bone marrow (BM) [using either next-generation sequencing or next-generation flow cytometry (NGF)] and outside the BM [using positron emission tomography-computed tomography (PET-CT); imaging MRD]. MRD negativity in the BM in patients who have achieved conventional complete response (CR) consistently correlates with prolonged progression-free survival (PFS) and overall survival (OS) in both newly diagnosed MM (NDMM) and relapsed/refractory MM (RRMM) patients." ESMO also notes that "MRD has been found to be a surrogate endpoint for PFS in patients receiving first-line treatment. Therefore, MRD may be used as an endpoint to accelerate drug development. The use of MRD to drive treatment decisions is under investigation."[82]

Acute Myeloid Leukaemia

The ESMO includes MRD status as part of the treatment algorithm for AML. They state, "morphological enumeration of the blast percentage should be refined by immunophenotypic or molecular MRD assessment in patients with <10% blasts. ELN recommendations on MRD assessment in AML specify its clinical use and technical requirements. It is recommended to assess MRD by reverse transcriptase polymerase chain reaction (RT-PCR) for patients positive for *NPM1*^{mut}*, RUNX1-RUNX1T1, CBFB-MYH11* or *PML-RARA* fusion genes; ~40% of all AML patients. In the remaining patients, MRD should be assessed by MFC, which relies on antigens aberrantly expressed by leukaemic cells that can be found in >90% of AML patients. Many clinical studies have shown the strong prognostic impact of MRD, as measured by MFC, with levels 0.1% defined as positive."[83]

Hairy Cell Leukaemia

Concerning hairy cell leukemia, ESMO notes, "recently, monoclonal antibodies that detect the mutated BRAF protein have been developed and shown to be useful for the diagnosis and detection of minimal residual disease (MRD)." Within the section on response evaluation, ESMO states, "immunophenotypic analysis of peripheral blood or bone marrow is not required but is useful to detect MRD… The eradication of MRD is generally not recommended in routine clinical practice. Assessment of response should be performed 4 – 6 months after treatment with 2-CldA and after 8 – 9 courses of DCF. Relapse is defined as any deterioration in blood counts related to the detection of hairy cells in peripheral blood and/or bone marrow."[84]

Canadian Consensus Guideline Consortium (CGC)

The CGC released recommendations on "first line treatment of newly diagnosed transplant eligible multiple myeloma." For the assessment of treatment response and the evaluation of relapse, the CGC recommends, "MRD evaluation should not yet be used in clinical practice to guide treatment outside of the research setting, but could be used for its prognostic value."[85]

VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On August 5, 2020, the FDA approved ClonoSEQ, marketed by Adaptive Biotechnologies, as a substantially equivalent DNA-based test for minimal residual disease. It is "an in vitro diagnostic device that identifies and quantifies specific nucleic acid sequences isolated from human specimens to estimate the percentage of cells that harbor the specific sequence pathology test." In its Decision Summary, the FDA states that "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), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). The clonoSEQ Assay measures 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 clinicopathological-p features."[22]

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). LDTs are

not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status.

HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

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IX. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)
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 Proprietary test: MyMRD® NGS Panel Lab/Manufacturer: Laboratory for Personalized Molecular Medicine
0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient-specific panel for future comparisons to evaluate for MRD Proprietary test: Invitae PCM Tissue Profiling and MRD Baseline Assay Lab/Manufacturer: Invitae Corporation
0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD Proprietary test: Invitae PCM MRD Monitoring Lab/Manufacturer: Invitae Corporation
0340U	Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate Proprietary test: Signatera™ Lab/Manufacturer: Natera, Inc

Avalon Healthcare Solutions is an independent company providing laboratory benefits management on behalf of HMSA.

CPT	Code Description
0356U	Oncology (oropharyngeal or anal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence Proprietary test: NavDx® Lab/Manufacturer: Naveris, Inc
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 Proprietary test: clonoSEQ® Assay Lab/Manufacturer: Adaptive Biotechnologies
0422U	Oncology (pan-solid tumor), analysis of DNA biomarker response to anti-cancer therapy using cell-free circulating DNA, biomarker comparison to a previous baseline pre-treatment cell-free circulating DNA analysis using next-generation sequencing, algorithm reported as a quantitative change from baseline, including specific alterations, if appropriate. Proprietary test: Guardant360 Response™ Lab/Manufacturer: Guardant Health, Inc,
0467U	Oncology (bladder), DNA, next-generation sequencing (NGS) of 60 genes and whole genome aneuploidy, urine, algorithms reported as minimal residual disease (MRD) status positive or negative and quantitative disease burden Proprietary test: UroAmp MRD Lab/Manufacturer: Convergent Genomics, Inc
0470U	Oncology (oropharyngeal), detection of minimal residual disease by next-generation sequencing (NGS) based quantitative evaluation of 8 DNA targets, cell-free HPV 16 and 18 DNA from plasma Proprietary test: HPV-SEQ Test Lab/Manufacturer: Sysmex Inostics, Inc
0560U	Oncology (minimal residual disease [MRD]), genomic sequence analysis, cell-free DNA, whole blood and tumor tissue, baseline assessment for design and construction of a personalized variant panel to evaluate current MRD and for comparison to subsequent MRD assessments Proprietary test: Haystack MRD™ Baseline Lab/Manufacturer: Quest Diagnostics ®
0561U	Oncology (minimal residual disease [MRD]), genomic sequence analysis, cell-free DNA, whole blood, subsequent assessment with comparison to initial assessment to evaluate for MRD Proprietary test: Haystack MRD™ Monitoring Lab/Manufacturer: Quest Diagnostics ®
0569U	Oncology (solid tumor), next-generation sequencing analysis of tumor methylation markers (>20,000 differentially methylated regions) present in cell-free circulating tumor DNA (ctDNA), whole blood, algorithm reported as presence or absence of ctDNA with tumor fraction, if appropriate

CPT	Code Description
	Proprietary test: Guardant Reveal™ Lab/Manufacturer: Guardant Health, Inc

X. Policy History

Action Date	Action
June 01, 2023	Policy created
March 17, 2026	Policy approved by Medical Directors
March 27, 2026	Policy approved at UMC
August 01, 2026	Policy effective date following notification period