Clinical Roundtable Monograph

The Evolving Use of Minimal Residual Disease (MRD) Assessment in Chronic Lymphocytic Leukemia

Discussants

Bruce D. Cheson, MD
Scientific Advisor
Lymphoma Research Foundation
New York, New York

Anthony R. Mato, MD, MSCE
Director, CLL Program
Memorial Sloan Kettering Cancer Center
New York, New York

Abstract: The treatment of chronic lymphocytic leukemia (CLL) has changed remarkably throughout the past decade, with patients achieving deeper and more durable responses. Importantly, this clinical activity has been found to translate to prolonged survival. With some treatments, these responses are now allowing patients to stop therapy after 1 or 2 years, a concept referred to as "fixed duration." However, not all patients experience these outcomes. How to determine which patients can safely stop treatment remains unclear. Minimal residual disease (MRD) is emerging as a prognostic biomarker. In CLL, undetectable MRD has been shown to correlate with prolonged progression-free survival and, in some cases, overall survival. The incorporation of MRD status into clinical decision-making is not yet widely done, primarily based on the lack of prospective clinical trial data. As the endpoint of MRD status becomes more common in clinical trials of CLL, the role in the clinical setting will become more clear. Furthermore, prognostic models will help to determine the utility of MRD as a surrogate endpoint in clinical studies. This monograph examines clinical trial data regarding the role of MRD in CLL, and provides recommendations on how to incorporate MRD assessment into clinical management.

Clinical Advances in
HEMATOLOGY&ONCOLOGY ™

A Peer-Reviewed Journal

Table of Contents

A Clinical Perspective on Minimal Residual Disease (MRD) Assessment in Chronic Lymphocytic Leukemia

Bruce D. Cheson, MD

Scientific Advisor
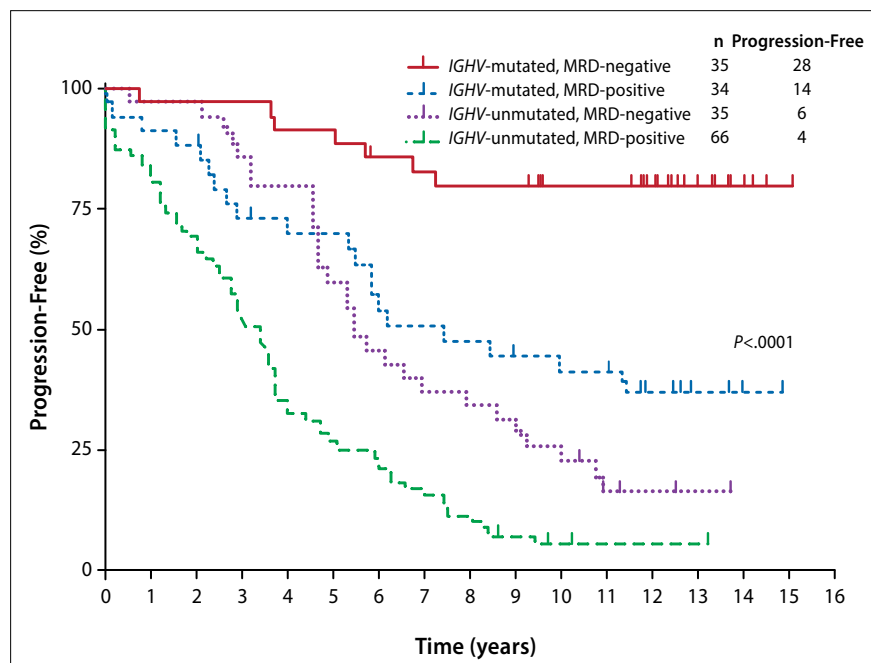Lymphoma Research Foundation
New York, New York

The treatment of chronic lymphocytic leukemia (CLL) has undergone a seismic shift in recent years. This shift was initiated by the finding that rituximab provides a survival advantage when added to chemotherapy, establishing chemoimmunotherapy as the backbone of treatment.[1-3] These results prompted the comparison of bendamustine plus rituximab vs fludarabine, cyclophosphamide, and rituximab (FCR).[4] An advantage in progression-free survival (PFS) was reported for younger patients with FCR vs bendamustine plus rituximab. However, bendamustine plus rituximab was the preferred regimen for older patients, primarily because of its improved tolerability. These studies are now best viewed in the rearview mirror owing to the availability of an increasing number of targeted therapies. The first targeted therapies approved by the US Food and Drug Administration (FDA) for CLL were idelalisib and rituximab, followed by ibrutinib. Although these agents were associated with high response rates, most of the responses were partial, and patients require indefinite treatment that is limited primarily by tolerability.[5-7] Newer strategies with drugs such as venetoclax demonstrated not only higher response rates, but more complete remissions.[8] Many patients achieved undetectable minimal residual disease (uMRD). This finding was important given that venetoclax-based strategies are most often time-limited.

Eradication of MRD was first shown to be possible in 1992[9] with drugs such as alemtuzumab. More recently, better technologies for assessment of MRD have demonstrated a correlation between MRD eradication and PFS (Figure 1),[6,10] and, in some instances, correlation with overall survival has also been shown (Figure 2).[11] Thus,

Figure 1. Six-month landmark progression-free survival according to mutation status and post-treatment MRD status in a study of long-term disease-free survival among patients with chronic lymphocytic leukemia treated with fludarabine, cyclophosphamide, and rituximab. *IGHV*, immunoglobulin heavy chain variable; MRD, minimal residual disease. Adapted from Thompson PA et al. *Blood.* 2016;127(3):303-309.[6]
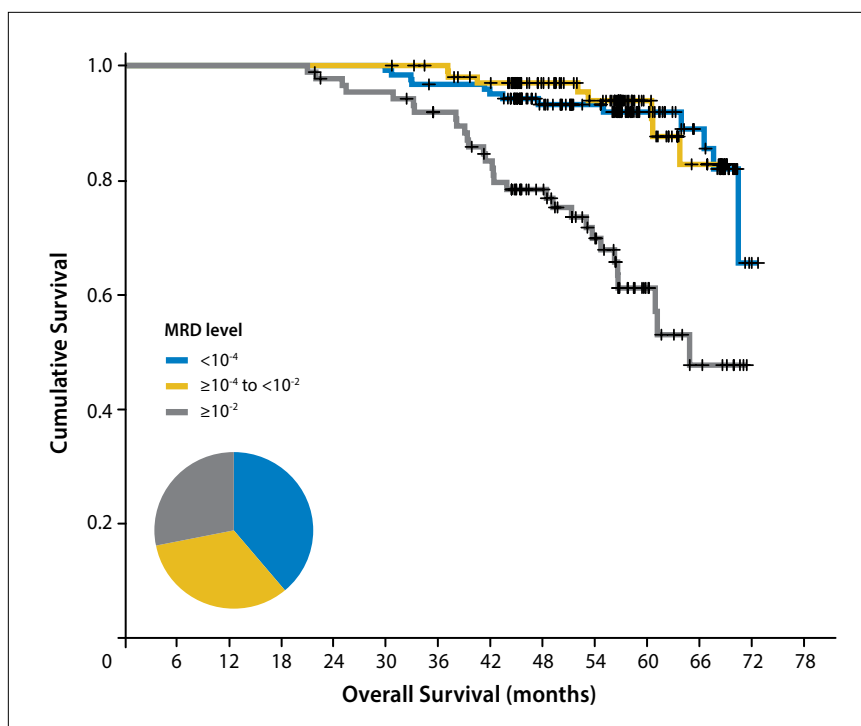

	n	Progression-Free
IGHV-mutated, MRD-negative	35	28
IGHV-mutated, MRD-positive	34	14
IGHV-unmutated, MRD-negative	35	6
IGHV-unmutated, MRD-positive	66	4

$P<.0001$

Figure 2. Overall survival among patients with chronic lymphocytic leukemia grouped according to levels of MRD assessed in the peripheral blood during follow-up of the GCLLSG CLL8 trial. The pie chart illustrates the frequency distributions. *P*<.0001 for the analysis according to a log-rank test. GCLLSG, German CLL Study Group; MRD, minimal residual disease. Adapted from Böttcher S et al. *J Clin Oncol.* 2012;30(9): 980-988.[11]

we are learning the most appropriate clinical settings in which to study MRD and the best ways to apply this information in the management of patients with CLL.

There are several techniques that measure MRD. The most common, 4-color flow cytometry with CD19/CD25 and kappa/lambda, achieves a sensitivity of approximately 10^{-4} (ie, 1 in 10,000 cells).[12] The addition of antibodies to detect antigens such as CD79B, CD43, and CD81 with flow cytometry can potentially improve sensitivity to 10^{-5}, but this option is less readily available.[13] Investigators are evaluating different antigen combinations to better identify the presence or absence of MRD. Flow cytometry requires samples that are less than 48 hours old, but the procedure is widely standardized across institutions. It is directly quantitative, and the results are quickly available. The main limitation of flow cytometry is low sensitivity.

Real-time quantitative polymerase chain reaction (PCR) using patient-specific primers (a technique referred to as allele-specific oligonucleotide [ASO] PCR) achieves better sensitivity, routinely to 10^{-5}.[14] This technique detects the disease-specific immunoglobulin heavy chain variable (*IGHV*) gene. It does not require fresh material, but DNA extraction must be performed within 48 hours. The approach is standardized and highly sensitive. However, there are several limitations. This technique is not directly quantitative, and it requires a baseline sample. It is time- and labor-intensive, as well

as expensive. Studies are currently evaluating high-throughput sequencing, which can achieve a sensitivity of 10^{-6}.

More recently, next-generation sequencing has been shown to be a highly sensitive measure of MRD, reaching a level of 10^{-6}. In September 2018, the FDA cleared the clonoSEQ® Assay (Adaptive Biotechnologies; Seattle, WA) for the detection and monitoring of MRD in bone marrow samples from patients with multiple myeloma or B-cell acute lymphoblastic leukemia.[15,16] The clonoSEQ Assay is a laboratory-developed test also used to assess MRD in other B-cell and T-cell malignancies, such as CLL, as well as with other specimen types, such as peripheral blood. By using multiple primer sets to amplify gene segments, the assay identifies immunoglobulin (Ig) rearrangements within *IgH* (VDJ), *IgH* (DJ), *IgK*, and *IgL* receptor gene sequences, as well as translocated *BCL1/IgH* (J) and *BCL2/IgH* (J) sequences.[17]

Importantly, in CLL, the clonoSEQ Assay can test peripheral blood, in addition to bone marrow. In studies using flow cytometry to measure MRD in patients treated with regimens such as fludarabine plus cyclophosphamide, results obtained from the peripheral blood and bone marrow correlate in approximately 90% to 95% of cases.[10,18] Using ASO-PCR at 10^{-5}, the correlation remains strong. However, the correlation between results obtained from the peripheral blood vs the bone
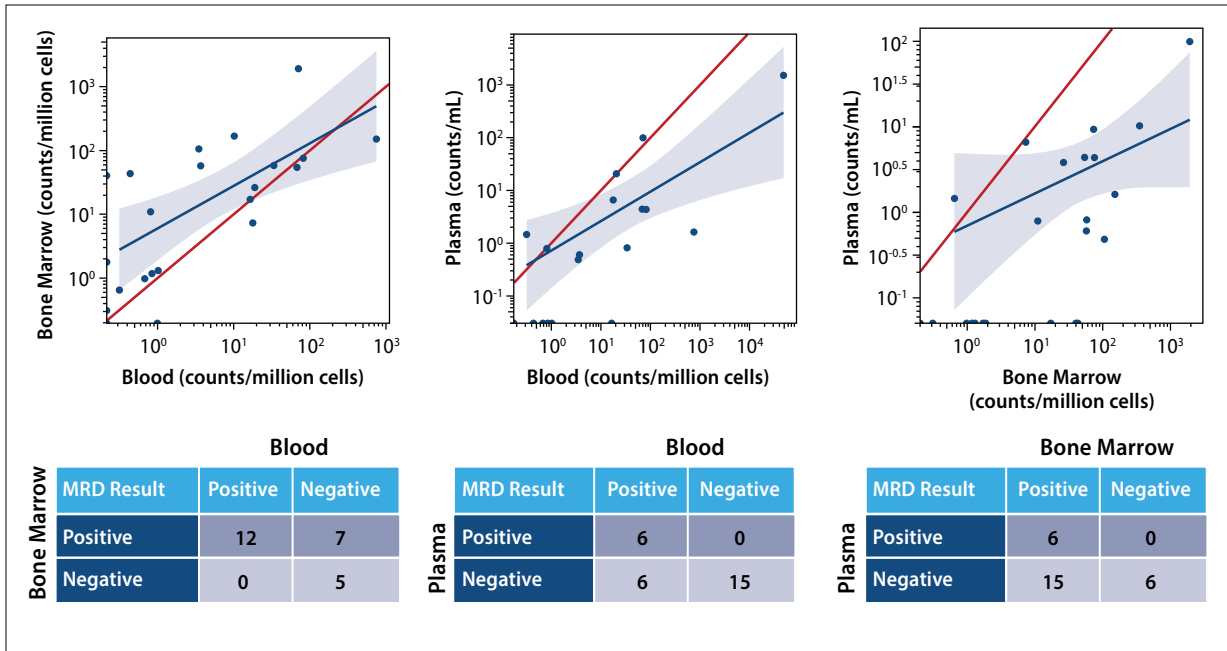
Figure 3. MRD as assessed by next-generation sequencing according to sample type in an analysis of patients with chronic lymphocytic leukemia treated with first-line fludarabine, cyclophosphamide, and rituximab. MRD, minimal residual disease. Adapted from Thompson PA et al. *Blood.* 2019;134(22):1951-1959.[10]

marrow worsens once MRD levels reach 10^{-6}, which is possible with next-generation sequencing. Several studies suggest that some drugs rapidly clear lymphocytes from the peripheral blood, whereas other drugs, such as rituximab, have a slower effect on the bone marrow. In the German CLL8 and CLL10 trials, approximately 65% of patients had uMRD in the peripheral blood.[3,11] With testing of the bone marrow, only 41% of patients had uMRD. In the CLL14 trial, there was also a dramatic difference.[19] The rates of uMRD in the peripheral blood were 76% with venetoclax plus obinutuzumab vs 35% with chlorambucil plus obinutuzumab. In the bone marrow, these rates were 57% vs 17%, respectively. An interesting study from Thompson and colleagues evaluated 62 patients with uMRD according to flow cytometry at the end of FCR therapy.[10] Of the samples that had uMRD by flow cytometry, only 27.4% were undetectable with next-generation sequencing. Among the samples assessed with next-generation sequencing, 25% had uMRD in the bone marrow, 55% had uMRD in the peripheral blood, and 75% had uMRD when assessing plasma (Figure 3).

When evaluating MRD, it is necessary to consider not only the test and its sensitivity, but also the compartment. For example, MRD that is detectable in the peripheral blood will also typically be detectable in the bone marrow. If a patient has uMRD according to peripheral blood assays, it is still necessary to confirm this finding with a bone marrow sample.

The use of MRD is increasing among patients treated with fixed-duration regimens. Many of the venetoclax-based regimens used 1 or 2 years of treatment.[8,19] It is currently unclear how to manage patients with detectable MRD at the conclusion of treatment. The trajectory of MRD may provide some insight. Among patients with continually decreasing levels of MRD, treatment might be continued. If there is no continued improvement in the amounts of detectable MRD, then discontinuing treatment might be the appropriate approach. A randomized trial is needed to provide that information definitively. The MURANO trial (A Study to Evaluate the Benefit of Venetoclax Plus Rituximab Compared With Bendamustine Plus Rituximab in Participants With Relapsed or Refractory Chronic Lymphocytic Leukemia [CLL]) was a time-limited study showing that venetoclax plus rituximab was superior to bendamustine plus rituximab.[8] Among the patients who completed treatment, uMRD was reported in 62% of the venetoclax arm vs 13% of the bendamustine arm. Patients with uMRD had longer PFS than patients who had low levels of detectability, and much longer PFS than patients with higher levels of detectable MRD. Rates of uMRD over time for each treatment group are shown in Figure 4.

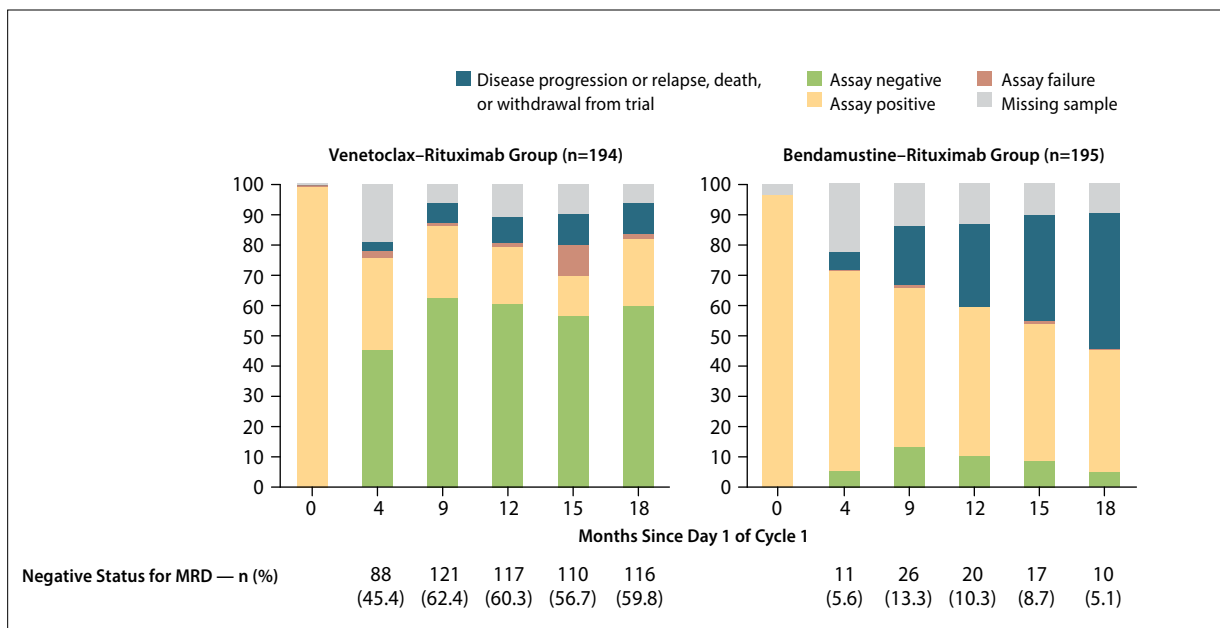There are other situations where MRD is being

Figure 4. Rates of MRD detected in peripheral blood samples over time in the phase 3 MURANO trial. Samples were categorized as missing when patients reached a specific time point but a sample was not obtained from them at that time, or when patients did not yet reach a specific time point owing to reasons other than disease progression, disease relapse, or death. The threshold for MRD was defined as 1 tumor cell per 10^4 white cells. MRD, minimal residual disease; MURANO, A Study to Evaluate the Benefit of Venetoclax Plus Rituximab Compared With Bendamustine Plus Rituximab in Participants With Relapsed or Refractory Chronic Lymphocytic Leukemia (CLL). Adapted from Seymour JF et al. *N Engl J Med*. 2018;378(12):1107-1120.[8]

evaluated in patients with CLL. For example, MRD might be assessed in patients treated with chimeric antigen receptor (CAR) T-cell therapy or allogeneic stem cell transplant. In these cases, it is thought that MRD may provide insight into the potential for cure.

MRD in Clinical Practice
I have used MRD in several settings. Before the MURANO data were available, I had a number of patients with relapsed/refractory disease receiving venetoclax. Some patients would ask if treatment could be stopped. I would assess MRD with peripheral blood flow cytometry. If negative, I would attempt to confirm with a bone marrow aspirate. Among patients with uMRD, I would raise the possibility of discontinuing treatment, and then starting it again if the disease returned. I might also modify treatment with chemoimmunotherapy. Some patients would want to stop chemoimmunotherapy, particularly FCR, after 3 or 4 cycles. We would assess MRD to see if treatment could be discontinued.

Disclosure
Dr Cheson is a consultant for AbbVie, Pharmacyclics/Janssen, TG Therapeutics, BeiGene, Celgene, and AstraZeneca. He has received research support (directed to his prior institu-

tion) from AbbVie, Pharmacyclics/Janssen, AstraZeneca, TG Therapeutics, and Adaptive Biotechnologies. He has consultancies with MorphoSys and Symbio.

References
1. Robak T, Dmoszynska A, Solal-Céligny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. *J Clin Oncol*. 2010;28(10):1756-1765.
2. Byrd JC, Rai K, Peterson BL, et al. Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. *Blood*. 2005;105(1):49-53.
3. Hallek M, Fischer K, Fingerle-Rowson G, et al; International Group of Investigators; German Chronic Lymphocytic Leukaemia Study Group. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet*. 2010;376(9747):1164-1174.
4. Eichhorst B, Fink AM, Bahlo J, et al; international group of investigators; German CLL Study Group (GCLLSG). First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol*. 2016;17(7):928-942.
5. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2014;370(11):997-1007.
6. Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. *Blood*. 2016;127(3):303-309.
7. Burger JA, Tedeschi A, Barr PM, et al; RESONATE-2 Investigators. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. *N Engl J Med*. 2015;373(25):2425-2437.

8. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med.* 2018;378(12):1107-1120.

9. Robertson LE, Huh YO, Butler JJ, et al. Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: clinical, pathologic, immunophenotypic, and molecular analysis. *Blood.* 1992;80(1):29-36.

10. Thompson PA, Srivastava J, Peterson C, et al. Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. *Blood.* 2019;134(22):1951-1959.

11. Böttcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. *J Clin Oncol.* 2012;30(9):980-988.

12. Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. *Leukemia.* 2007;21(5):956-964.

13. Zalcberg I, D'Andrea MG, Monteiro L, Pimenta G, Xisto B. Multidisciplinary diagnostics of chronic lymphocytic leukemia: European Research Initiative on CLL–ERIC recommendations [published online September 11, 2019]. *Hematol*

Transfus Cell Ther. doi:10.1016/j.htct.2019.07.006.

14. Noy A, Verma R, Glenn M, et al. Clonotypic polymerase chain reaction confirms minimal residual disease in CLL nodular PR: results from a sequential treatment CLL protocol. *Blood.* 2001;97(7):1929-1936.

15. US Food and Drug Administration. FDA authorizes first next generation sequencing-based test to detect very low levels of remaining cancer cells in patients with acute lymphoblastic leukemia or multiple myeloma. Posted September 28, 2018. https://www.fda.gov/news-events/press-announcements/fda-authorizes-first-next-generation-sequencing-based-test-detect-very-low-levels-remaining-cancer. Accessed April 22, 2020.

16. Evaluation of atuomatic class II designation for clonoSEQ® Assay. Decision summary. US Food and Drug Administration. https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170080.pdf. Accessed April 22, 2020.

17. clonoSEQ® Assay. Technical information. Adaptive Biotechnologies Corporation; Seattle, WA. PNL-10027-01.

18. Fürstenau M, De Silva N, Eichhorst B, Hallek M. Minimal residual disease assessment in CLL: ready for use in clinical routine? *Hemasphere.* 2019;3(5):e287. doi: 10.1097/HS9.0000000000000287.

19. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. *N Engl J Med.* 2019;380(23):2225-2236.

Assessment of Minimal Residual Disease (MRD) in Chronic Lymphocytic Leukemia: A Review of the Data and Future Directions

Anthony R. Mato, MD, MSCE

Director, CLL Program
Memorial Sloan Kettering Cancer Center
New York, New York

Until recently, MRD had been relegated to the role of a prognostic factor in patients with CLL. However, in both the United States and Europe, there is tremendous interest in using MRD for other purposes. The first is as a potential surrogate endpoint in the context of clinical trials, in order to allow those trials to provide results earlier. That surrogate endpoint would potentially be for PFS, which is a standard endpoint used in key clinical trials in CLL at this time. The second is use of MRD as a measure of depth of response to guide clinical decision-making. At this time, neither of these uses are clinically accepted practices. Instead, most clinical trials in CLL use MRD status as a secondary or exploratory endpoint. Most of the clinical trials that have explored chemotherapy and/or novel agents have utilized methods such as flow cytometry, ASO-PCR, or next-generation sequencing (NGS) for measuring MRD, but only in the context of a prognostic factor. Although it is tempting to alter patient management based on persistent MRD, or even to shorten therapy based on uMRD in the peripheral blood or the bone marrow, there are no validated clinical trial data to fully support these practices today.

MRD as a Surrogate Endpoint: Clinical Trial Data

Kwok and colleagues conducted a retrospective analysis of a historical cohort of patients with CLL who were treated at a single institution in order to evaluate the long-term prognostic value of MRD status in a real-world setting across different treatment modalities.[1] All patients had completed treatment between 1996 and 2007, achieved at least a partial response, and received a bone marrow MRD assessment within 6 months of treatment completion. A total of 133 patients met the inclusion criteria, and were treated with either combination chemotherapy or chemoimmunotherapy (n=67), single-agent chemotherapy (n=31), autologous stem cell transplant (n=7), or chemotherapy-free regimens (n=28; primarily monoclonal antibody therapy). A total of 55 patients (41%) had uMRD after treatment, including 46 patients with a complete response/complete response with incomplete bone marrow recovery and 9 patients with a partial response/nodular partial response.

After a median follow-up of 10.1 years (range, 7.8-18.6) among surviving patients, the median PFS was
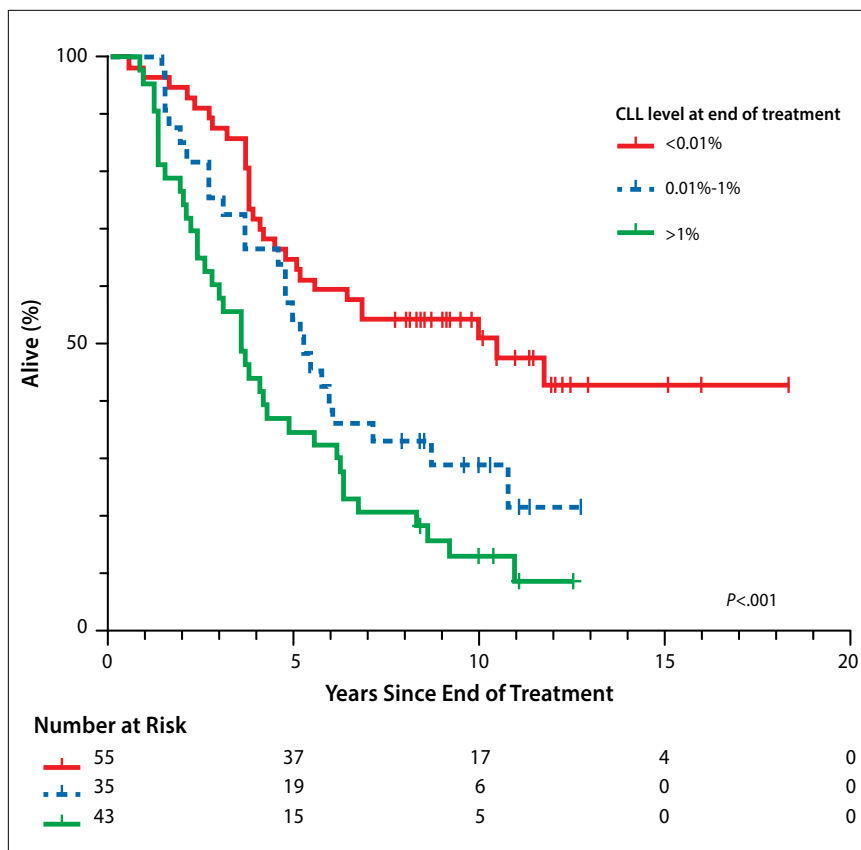
Figure 5. Overall survival according to the level of MRD at the end of treatment in a retrospective analysis of patients with chronic lymphocytic leukemia with follow-up lasting up to 18 years. Post-treatment MRD levels were obtained within 6 months after the end of treatment with multiparameter flow cytometry to a sensitivity of 10^{-4} (0.01%). The log-rank *P* value is shown. CLL, chronic lymphocytic leukemia; MRD, minimal residual disease. Adapted from Kwok M et al. *Blood.* 2016;128(24):2770-2773.[1]

7.6 years in patients with uMRD (<.01%), 3.3 years in patients with low levels of MRD (0.01%-1%), and 2.0 years in patients with high levels of MRD (>1%).[1] The median overall survival was 10.6 years, 5.3 years, and 3.6 years, respectively (Figure 5).

Outcomes were best among patients with uMRD and a complete response, intermediate among those with uMRD and a partial response, and worst among those with detectable MRD and either a complete or partial response.[1] When MRD response was considered together with established prognostic factors (eg, age, Binet stage, cytopenias, prior treatment, and adverse cytogenetics), as well as type of treatment and degree of response, only MRD response status and adverse cytogenetics emerged as significant for PFS. Overall survival according to MRD status and mutational status at the end of treatment is shown in Figure 6. In a multivariate analysis, only MRD response, age, stage, and prior treatment were significant for overall survival.

The benefits associated with achieving uMRD were stronger with upfront therapy vs subsequent therapy.[1] Among patients treated in the upfront setting, the 10-year PFS was 65% in those with uMRD vs 10% in those with detectable MRD. The 10-year overall survival was 70% vs 30%, respectively. In contrast, among patients treated

in the relapsed/refractory setting, the 10-year rate of PFS was 30% in patients with uMRD vs 0% in patients with detectable MRD. The 10-year overall survival was 47% vs 11%, respectively.

Interestingly, among patients with deletion 17p or deletion 11q, uMRD appeared to at least partially overcome the poor prognosis associated with these poor-risk cytogenetic abnormalities, suggesting that targeting uMRD may be of great value in these patients.

The study authors concluded that these results support the long-term benefit of achieving uMRD, regardless of the therapeutic setting and treatment modality.[1] These data also provided further rationale to support the use of MRD as a prognostic marker for long-term PFS, making uMRD a potential therapeutic goal in CLL.

The GCLLSG Trials

The potential to use MRD as a surrogate endpoint for long-term outcomes in CLL clinical trials was demonstrated in three randomized phase 3 studies of frontline chemoimmunotherapy, all conducted by the German CLL Study Group (GCLLSG). The CLL8, CLL10, and CLL11 trials showed that MRD response correlated with PFS and, in some cases, overall survival.[2-4] However, the

Figure 6. Overall survival according to MRD status and mutational status at the end of treatment in a retrospective analysis of patients with chronic lymphocytic leukemia with follow-up lasting up to 18 years. Cytogenetic aberrations were evaluated by fluorescence in situ hybridization. The balance of patients with del(17p) and del(11q), respectively, was comparable between the MRD-negative and MRD-positive groups. In the MRD-negative del(17p/11q) group, 3 of 9 patients (33%) had del(17p), whereas 6 of 9 patients (67%) had del(11q). In the MRD-positive del(17p/11q) group, 6 of 15 patients (40%) had del(17p), whereas 9 of 15 patients (60%) had del(11q). MRD, minimal residual disease; MRD-neg, MRD-negative; MRD-pos, MRD-positive. Adapted from Kwok M et al. *Blood.* 2016;128(24):2770-2773.[1]
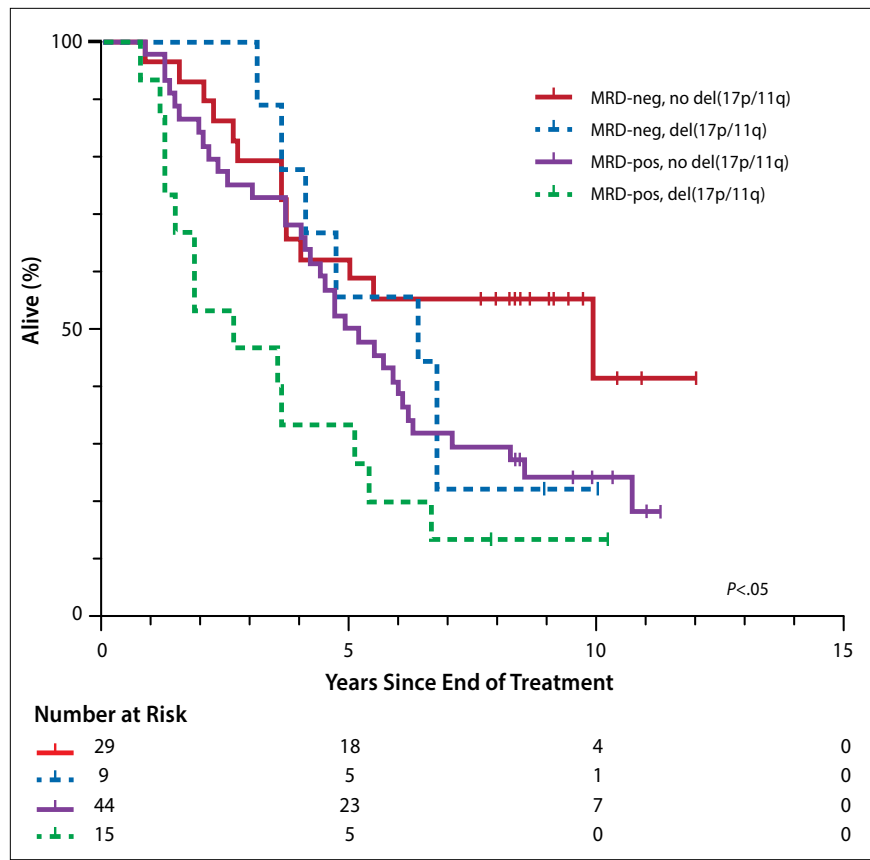
correlation was insufficient to establish MRD as a longer-term endpoint, such as PFS. Instead, the prognostic value of MRD for that endpoint must be established, with evidence to support that uMRD can reliably predict the effect of treatment over the longer term. Thus, Dimier and colleagues evaluated MRD status (negative vs positive) as a surrogate endpoint for PFS in patients with CLL.[5] To do this, a meta-regression model using combined data from CLL8, CLL10, and CLL11 was applied to predict the treatment effect on PFS using MRD as a surrogate endpoint. The study defined uMRD as a sensitivity of less than 10^{-4}.

In these studies, MRD was quantified using an international standardized approach by flow cytometry analysis in CLL8 and CLL10, and by ASO PCR according to the EuroMRD guidelines in CLL11.[2-4] With the exception of increased age and frequency of comorbidities in the CLL11 study, the baseline demographics of the study populations were similar across the 3 trials. The analysis included 2162 randomized patients. Peripheral blood MRD data were available for 393 patients from CLL8, 337 from CLL10, and 474 from CLL11.[5] These patients comprised the MRD evaluable population. Demographic characteristics and efficacy endpoint results were comparable between

the MRD evaluable population and the intention-to-treat population. Across the 3 trials, PFS was longer in the experimental arm vs the control arm. In addition, a larger proportion of patients achieved uMRD in the experimental arm vs the control arm.

A meta-regression model was developed to predict treatment effects on PFS using the treatment effect on peripheral blood MRD.[5] This model demonstrated a statistically significant relationship between the treatment effect on peripheral blood MRD and the treatment effect on PFS (Figure 7). As the difference in rates of MRD increased between the treatment arms, the risk of progression or death decreased. Thus, for each unit increase in the log ratio of MRD rates between arms, the log of the PFS hazard ratio decreased by −0.188 (95% CI, −0.321 to −0.055; P=.008).

After the model was developed, it was externally validated with data from the REACH trial.[6] This trial compared FCR vs fludarabine and cyclophosphamide in patients with previously treated CLL. uMRD was identified in 43% of patients in the FCR arm and 31% of patients in the fludarabine/cyclophosphamide arm (for a relative risk of 1.39). Using these data, the model predicted a hazard ratio for PFS of 0.63, which is consistent with the hazard ratio for
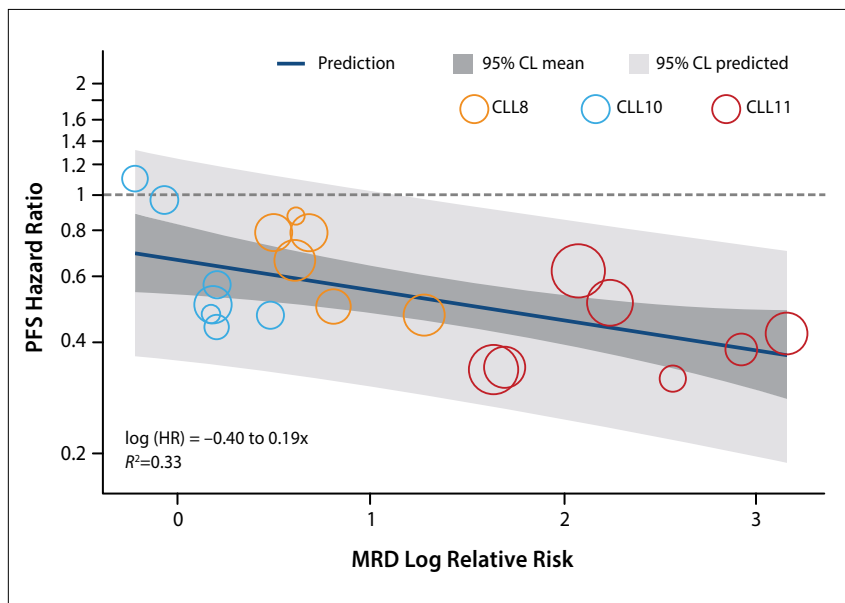
Figure 7. A meta-regression model of data from the CLL8 (orange circles), CLL10 (blue circles), and CLL11 (red circles) trials identified a significant relationship between treatment effects on MRD and PFS. The circle size reflects weighting of each subgroup to the overall model; those with the least variability in PFS HR have the largest circle. The clustering of circles by trial reflects the overall treatment effect for MRD and PFS in the trials. CL, confidence limits; HR, hazard ratio; MRD, minimal residual disease; PFS, progression-free survival; R2, the coefficient of determination. Adapted from Dimier N et al. *Blood.* 2018;131(9):955-962.[5]

PFS of 0.65 that was reported by the REACH trial. The study authors concluded that these data supported the reliability of the model predictions.[5]

The MURANO Trial

The global, open-label, randomized, phase 3 MURANO trial demonstrated a significant benefit with PFS for the 2-year fixed duration combination of venetoclax plus rituximab vs bendamustine plus rituximab in patients with relapsed/refractory CLL.[7,8] At a median follow-up of 36.0 months, the estimated PFS was 71.4% (95% CI, 64.8%-78.1%) with venetoclax plus rituximab vs 15.2% (95% CI, 9.1% to 21.4%) with bendamustine plus rituximab.[8] Three-year overall survival was 87.9% vs 79.5%, respectively (hazard ratio [HR], 0.50; 95% CI, 0.30-0.85; *P*=.0093).

MRD status in the MURANO trial was assessed with both ASO-PCR and flow cytometry. Both modalities demonstrated high correlation and concordance. The study defined uMRD as less than 10^{-4}. There was also high concordance for MRD status between peripheral blood and bone marrow measurements. Among patients treated with venetoclax plus rituximab, 49 had uMRD in the peripheral blood. Testing of pair samples showed that 44 of these patients (90%) also had uMRD in the bone marrow.[8]

Following completion of the combination treatment, uMRD in the peripheral blood was reported among 62.4% of patients treated with venetoclax plus rituximab vs 13.3% of those who received bendamustine plus rituximab (*P*<.001).[8] This significant difference was maintained at all assessments during and after treatment with single-agent venetoclax. Overall, the rate of uMRD as best

MRD response at any time during the study was 82.5% in patients treated with venetoclax plus rituximab vs 23.1% in those treated with bendamustine plus rituximab.

uMRD was also associated with improved PFS.[8] At the end of combination treatment, patients with uMRD showed a longer duration of PFS compared with patients who had detectable MRD, regardless of the treatment arm. Even among patients with detectable MRD, those with lower levels of MRD had a longer duration of PFS compared with those who had higher MRD levels.

In the venetoclax/rituximab arm, rates of PFS were similar among patients with uMRD who achieved a partial response or a complete response (HR, 0.71; 95% CI, 0.24-2.14).[8] In contrast, PFS was inferior among patients with a partial response who continued to have detectable MRD.

The kinetics of uMRD in the MURANO trial were also explored.[8] In the venetoclax/rituximab arm, the high rate of uMRD achieved at the end of treatment persisted during serial assessments. At the end of 2 years of venetoclax treatment (median of 9.9 months off venetoclax), MRD status was negative in 64% of 130 patients and low in 18%. In this follow-up, just 2 patients with uMRD at the completion of treatment had developed progressive disease.

Univariate analysis of the 130 patients who completed treatment showed a strong association between MRD level and disease progression, with uMRD repeatedly associated with better outcomes.[8] Mutation status appeared to affect the rate of uMRD, as more patients without deletion 17p and/or mutated *TP53* achieved uMRD without disease progression at the end

of treatment compared with patients who had one of these mutations. Among patients with uMRD at the end of treatment, most sustained this status (70% of 83 patients). The remaining 30% developed detectable MRD (in most cases, low-level MRD), and all remained progression-free.

The improvement in rates of uMRD seen with venetoclax plus rituximab vs bendamustine plus rituximab persisted after patients completed treatment.[8] Furthermore, among these patients, uMRD was durable and predicted longer PFS. The authors suggested that these data provide evidence supporting the impact of MRD on the PFS benefit associated with fixed-duration venetoclax plus rituximab.

The CLL14 Trial

The CLL14 trial compared the fixed-duration combination of venetoclax plus obinutuzumab vs obinutuzumab plus chlorambucil.[9] The study met the primary endpoint of investigator-assessed PFS. At 24 months, PFS was 88.2% with venetoclax plus obinutuzumab vs 64.1% with obinutuzumab plus chlorambucil (HR, 0.35; 95% CI, 0.23-0.53; P<.001). uMRD ($<10^{-4}$) in peripheral blood and bone marrow was assessed by ASO-PCR, and then confirmed by flow cytometry, as a secondary endpoint. The median time to treatment completion was 17.1 months (range, 0.0-30.4) with venetoclax plus obinutuzumab and 17.9 months (range, 0.0-30.2) with obinutuzumab plus chlorambucil. Three months after treatment completion, uMRD in the peripheral blood was observed in 75.5% of the venetoclax/obinutuzumab arm vs 35.2% of the obinutuzumab/chlorambucil arm (P<.001). uMRD in the bone marrow was seen in 56.9% vs 17.1%, respectively (P<.001). uMRD was achieved more frequently across all patient subgroups in the venetoclax/obinutuzumab arm.

The percentage of patients with both a complete response and uMRD, a key secondary endpoint, was significantly higher with venetoclax plus obinutuzumab vs obinutuzumab plus chlorambucil.[9] uMRD in peripheral blood was reported in 42.1% vs 14.4%, respectively (P<.001), and uMRD in bone marrow was reported in 33.8% vs 10.6% (P<.001).

The CLARITY Trial

The single-arm, phase 2 CLARITY trial (Assessment of Venetoclax [ABT-199] in Combination With Ibrutinib in Relapsed/Refractory Chronic Lymphocytic Leukaemia) evaluated the combination of ibrutinib and venetoclax in patients with relapsed/refractory CLL.[10] The primary endpoint was uMRD in both the peripheral blood and bone marrow after 12 months of combination treatment (month 14 of total therapy). MRD was assessed by flow cytometry, and defined as fewer than 1 CLL cell in 10,000 leukocytes (uMRD4). A secondary endpoint was

the eradication of MRD (below MRD4) in peripheral blood and bone marrow after 6 months of ibrutinib plus venetoclax (at month 8) and 24 months of ibrutinib plus venetoclax (at month 26). Other secondary endpoints were investigator-assessed response according to criteria from the International Workshop on CLL (iwCLL), PFS, overall survival, and safety.

The primary endpoint was reached in 36% of 53 evaluable patients.[10] This rate met the assumption by the investigators that the regimen would be of interest if more than 30% of patients had uMRD. At month 14, 53% of patients had uMRD in the peripheral blood (Figure 8) and 36% had uMRD in the bone marrow. Importantly, uMRD was observed regardless of the prior therapy. Among 20 patients who developed relapsed disease within 36 months of FCR or bendamustine plus rituximab, 70% achieved uMRD in the peripheral blood and 45% achieved it in the bone marrow. Similarly, among 9 patients treated with prior idelalisib, 67% achieved uMRD in the peripheral blood, and 56% achieved uMRD in the bone marrow. Continuous improvement was apparent by the degree of MRD reduction, with 44% of patients achieving an MRD level of 10^{-4} or below according to flow cytometry at month 26. The study investigators concluded that the combination of ibrutinib plus venetoclax was highly active and well-tolerated in patients with relapsed/refractory CLL, and was associated with a high rate of uMRD.[10]

The CAPTIVATE Trial

The multicenter, randomized, phase 2 CAPTIVATE trial (Ibrutinib Plus Venetoclax in Subjects With Treatment-Naive Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma) assessed the safety and efficacy of frontline treatment with ibrutinib plus venetoclax in patients with CLL or SLL.[11,12] Patients initiated treatment with ibrutinib (420 mg once daily for three 28-day cycles), followed by 12 cycles of ibrutinib at the same dose plus venetoclax (ramp up dosing to 400 mg once daily). After patients completed the twelve 28-day cycles, their MRD status was determined. Those with uMRD ($<10^{-4}$ by 8-color flow cytometry) were stratified by *IGHV* mutation status and randomly assigned to double-blind treatment with either single-agent ibrutinib or placebo. Patients with detectable MRD were randomly assigned to open-label treatment with single-agent ibrutinib or ibrutinib plus venetoclax. Data reported at the 2019 American Society of Hematology (ASH) meeting summarized results following the 12 cycles of ibrutinib plus venetoclax, before MRD-guided randomization.[12] Data from treatment under MRD-guided randomization are expected at a later date.

The CAPTIVATE study had several primary endpoints, including the uMRD response rate.[12] Among the evaluable patients, 75% achieved uMRD
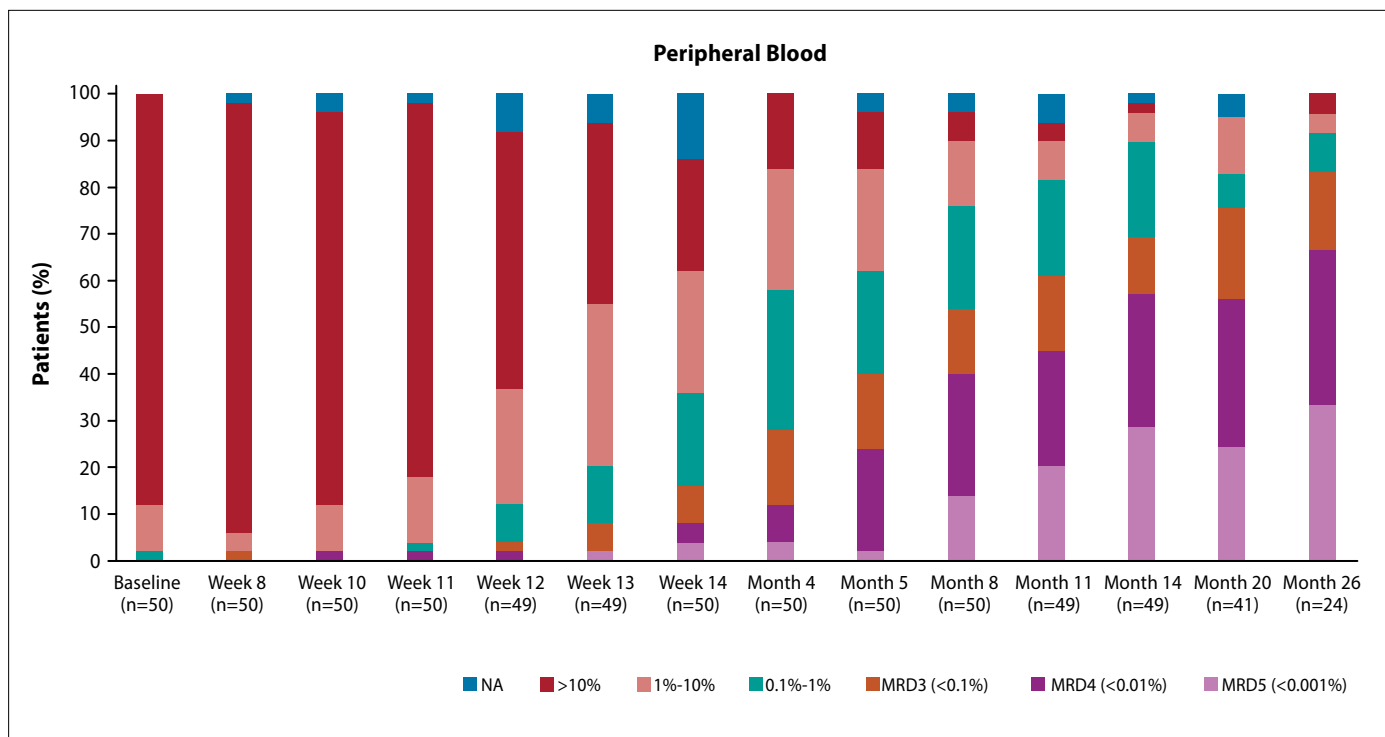
Figure 8. Rates of MRD in the peripheral blood over time in the CLARITY trial. CLARITY, Assessment of Venetoclax (ABT-199) in Combination With Ibrutinib in Relapsed/Refractory Chronic Lymphocytic Leukaemia; MRD3, minimal residual disease to fewer than 1 CLL cell in 1000 leukocytes; MRD4, minimal residual disease to fewer than 1 chronic lymphocytic leukemia cell in 10,000 leukocytes; MRD5, minimal residual disease undetectable below a threshold of 1 CLL cell in 100,000 leukocytes; NA, not available. Adapted from Hillmen P et al. *J Clin Oncol.* 2019;37(30):2722-2729.[10]

in the peripheral blood (n=163) at some point after baseline, while 72% achieved uMRD in the bone marrow (n=155). Among the patients with uMRD in the peripheral blood who had matching bone marrow samples, 93% also had uMRD in the bone marrow. The proportion of patients with uMRD in the peripheral blood increased throughout the 12 cycles of ibrutinib plus venetoclax. uMRD was present in 0% at baseline, 57% after 6 cycles, 68% after 9 cycles, and 73% after 12 cycles. High rates of uMRD in both the peripheral blood and bone marrow were consistently observed across high-risk patient subgroups, including those with deletion 17p/mutated *TP53*, deletion 11q, and unmutated *IGHV*.

Use of Next-Generation Sequencing for Measuring MRD in CLL

In recent years, the use of next-generation sequencing techniques has been explored for its potential to measure MRD in patients with CLL. The quantitative next-generation clonoSEQ Assay can assess MRD in the bone marrow and peripheral blood.[13] The assay is a feasible way to measure sensitivity of 10^{-6} in patients with lymphoid malignancies.[14-17] Thompson and colleagues explored the use of next-generation sequencing in CLL with pro-

spectively banked, post-treatment bone marrow (n=57), peripheral blood (n=29), and plasma samples (n=32) from patients treated with first-line FCR in a prospective, phase 2 clinical trial.[16,17]

All patients had achieved uMRD4 in the bone marrow by flow cytometry.[17] However, when these samples were analyzed by next-generation sequencing, only 27.4% of the 62 patients were deemed to have uMRD. The rate of uMRD by next-generation sequencing was 25% in bone marrow, 55% in peripheral blood, and 75% in plasma.[16] All patients with detectable MRD in the plasma also had detectable MRD in either the bone marrow or the peripheral blood. Sensitivity of at least 10^{-6} was achieved in 74% of bone marrow samples and 62% of peripheral blood samples.

The median follow-up was 81.6 months.[17] The median PFS was not reached among patients with uMRD according to testing in bone marrow and peripheral blood (Figure 9). In contrast, the median PFS was 67 months among patients with MRD in the bone marrow and 74 months among those with MRD in the peripheral blood (*P*=.02 for both comparisons).

The study authors concluded that more sensitive 10^{-6} testing, using next-generation sequencing techniques, has the potential to increase prognostic discrimination
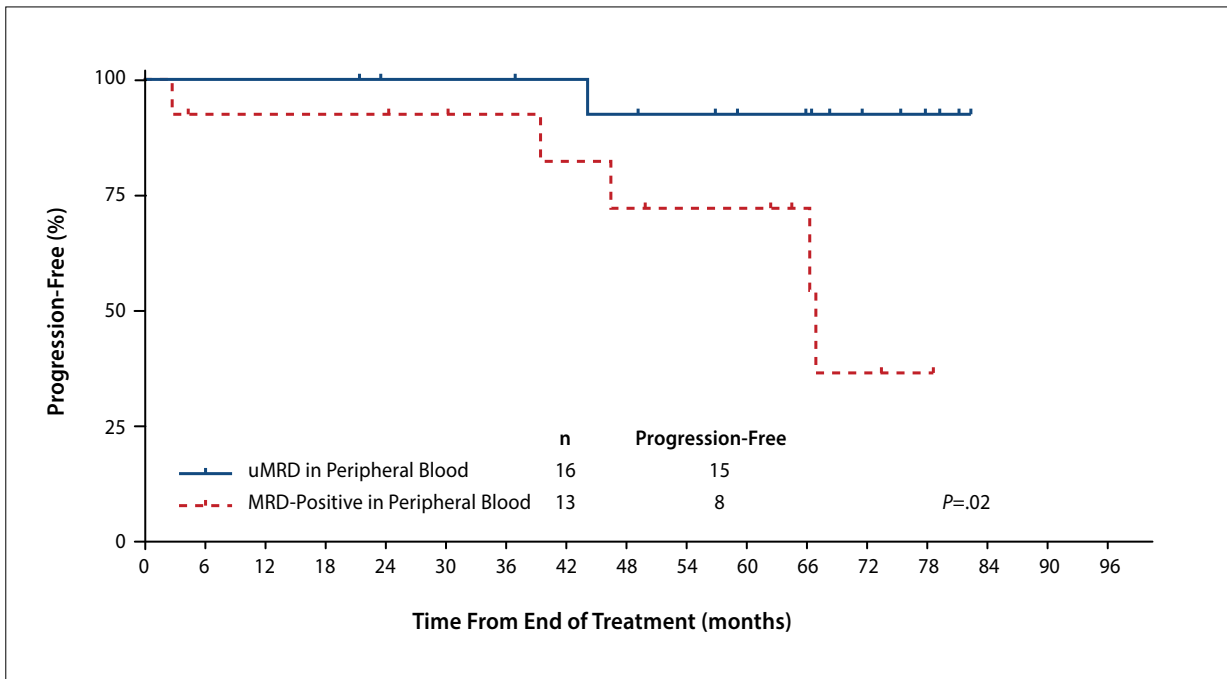
Figure 9. Progression-free survival from the end of treatment according to MRD status (detectable vs undetectable) in PBMC by next-generation sequencing. PBMC specimens were available for 29 patients. Results were categorized as detectable vs undetectable, regardless of sensitivity. MRD, minimal residual disease; PBMC, peripheral blood mononuclear cell; uMRD, undetectable MRD. Adapted from Thompson PA et al. *Blood.* 2019;134(22):1951-1959.[17]
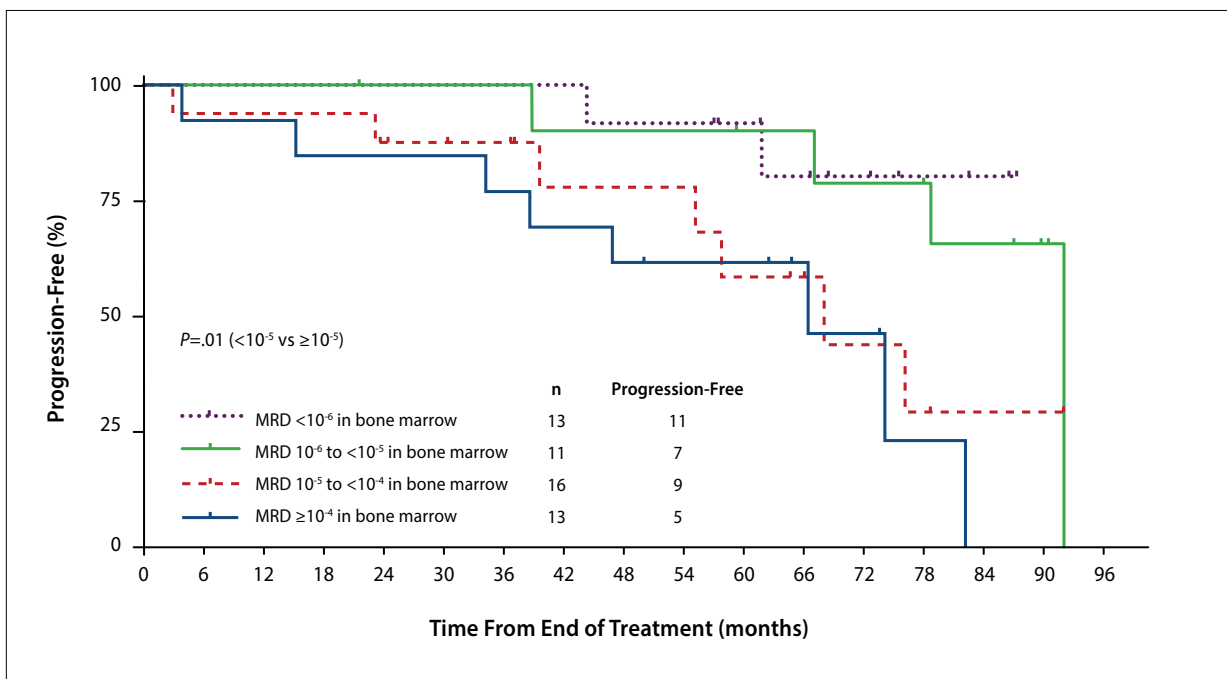

Figure 10. Progression-free survival from the end of treatment according to absolute MRD level, as measured in the bone marrow. Among the 57 bone marrow specimens, 53 were included in this analysis; 4 were not included because MRD was undetectable, but sensitivity did not reach 10^{-6}. Adapted from Thompson PA et al. *Blood.* 2019;134(22):1951-1959.[17]

compared with testing that has a sensitivity of 10^{-4} (Figure 10).[17] The authors also noted that despite the disease burden, the prognosis for patients with uMRD in the peripheral blood by next-generation sequencing was generally excellent. Given that currently no additional therapy is used in these cases following frontline treatment, next-generation sequencing of MRD is used for purely prognostic purposes. After first-line chemoimmunotherapy, analysis of MRD using peripheral blood may be sufficient.

Future Directions of MRD in CLL

Compelling evidence across the spectrum of protocols suggests that 60% to 80% of patients can have uMRD in the frontline and relapsed/refractory settings after treatment with venetoclax in combination with an anti-CD20 antibody.[7-12] However, because these study designs all used fixed-duration strategies, there is no information as to how one could alter therapy at the end of treatment. It is known that MRD status at the end of venetoclax-based therapy is strongly predictive for either superior or poor outcomes, which was made particularly evident with longer-term follow-up data from the MURANO trial presented at the 2019 ASH meeting.[18]

Disclosure

Dr Mato has received ARM grants, personal fees, and other compensation from TG Therapeutics. He has received grants and personal fees from Pharmacyclics, Janssen, Genentech, AbbVie, Adaptive Biotechnologies, and AstraZeneca. He has received grants from Loxo, Sunesis, Regeneron, and DTRM. He has received grants and other compensation from Celgene and personal fees from BeiGene.

References

1. Kwok M, Rawstron AC, Varghese A, et al. Minimal residual disease is an independent predictor for 10-year survival in CLL. *Blood.* 2016;128(24):2770-2773.
2. Böttcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. *J Clin Oncol.* 2012;30(9):980-988.
3. Eichhorst B, Fink AM, Bahlo J, et al; international group of investigators; German CLL Study Group (GCLLSG). First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol.* 2016;17(7):928-942.
4. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N Engl J Med.* 2014;370(12):1101-1110.
5. Dimier N, Delmar P, Ward C, et al. A model for predicting effect of treatment on progression-free survival using MRD as a surrogate end point in CLL. *Blood.* 2018;131(9):955-962.
6. Robak T, Dmoszynska A, Solal-Céligny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. *J Clin Oncol.* 2010;28(10):1756-1765.
7. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med.* 2018;378(12):1107-1120.
8. Kater AP, Seymour JF, Hillmen P, et al. Fixed duration of venetoclax-rituximab in relapsed/refractory chronic lymphocytic leukemia eradicates minimal residual disease and prolongs survival: post-treatment follow-up of the MURANO phase III study. *J Clin Oncol.* 2019;37(4):269-277.
9. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. *N Engl J Med.* 2019;380(23):2225-2236.
10. Hillmen P, Rawstron AC, Brock K, et al. Ibrutinib plus venetoclax in relapsed/refractory chronic lymphocytic leukemia: the CLARITY study. *J Clin Oncol.* 2019;37(30):2722-2729.
11. Wierda WG, Siddiqi T, Flinn I, et al. Phase 2 CAPTIVATE results of ibrutinib (ibr) plus venetoclax (ven) in first-line chronic lymphocytic leukemia (CLL) [ASCO abstract 7502]. *J Clin Oncol.* 2018;36(15 suppl).
12. Tam CS, Siddiqi T, Allan JN, et al. Ibrutinib plus venetoclax for first-line treatment of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL): results from the MRD cohort of the phase 2 CAPTIVATE study [ASH abstract 35]. *Blood.* 2019;134(suppl 1).
13. clonoSEQ® Assay. Technical information. Adaptive Biotechnologies Corporation; Seattle, WA. PNL-10027-01.
14. Perrot A, Lauwers-Cances V, Corre J, et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. *Blood.* 2018;132(23):2456-2464.
15. Wood B, Wu D, Crossley B, et al. Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. *Blood.* 2018;131(12):1350-1359.
16. Thompson PA, Peterson CB, Strati P, et al. Serial minimal residual disease (MRD) monitoring during first-line FCR treatment for CLL may direct individualized therapeutic strategies. *Leukemia.* 2018;32(11):2388-2398.
17. Thompson PA, Srivastava J, Peterson C, et al. Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. *Blood.* 2019;134(22):1951-1959.
18. Seymour JF, Kipps TJ, Eichhorst BF, et al. Four-year analysis of MURANO study confirms sustained benefit of time-limited venetoclax-rituximab (VenR) in relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) [ASH abstract 355]. *Blood.* 2019;134(suppl 1).

The Evolving Use of Minimal Residual Disease (MRD) Assessment in Chronic Lymphocytic Leukemia: Q&A

Bruce D. Cheson, MD, and Anthony R. Mato, MD, MSCE

H&O How are you incorporating MRD assessment into clinical practice?

Anthony R. Mato, MD, MSCE There are 2 situations where I am using MRD to make clinical decisions. The first is in the small minority of patients with CLL who are *IGHV*-mutated without a *TP53* mutation or deletion 17p, who are treated with FCR. In this population, I assess MRD by flow cytometry in the bone marrow after the first 3 cycles of therapy. This strategy is based on a retrospective unplanned analysis of the FCR300 trial that suggested these patients may have similar outcomes regardless of whether they stop FCR early or complete 6 cycles.[1,2] It should be noted, however, that this endpoint was not included in the original design of the trial. There were some patients who underwent MRD assessment after 3 cycles and then had to discontinue therapy. It turns out that these patients did well.

The other way that I am using MRD in practice is in the setting of venetoclax fixed-duration strategies. I check MRD at the end of the CLL14 regimen or the MURANO regimen after 12 or 24 months of therapy, respectively.[3-5] Among patients with persistent disease, I raise the possibility of continuing venetoclax as monotherapy. This approach is based on the fact that these trials have shown a clear difference in PFS based on MRD status at the end of therapy. However, whether continuing therapy for another year will help to overcome this difference remains unknown. In the clinical trials that we are designing at our center, these are the types of questions we hope to address. For patients with persistent disease treated with ibrutinib or venetoclax, we sometimes add other novel agents to deepen the MRD response.

A study presented at the 2019 ASH meeting evaluated the addition of venetoclax to ibrutinib in the setting of persistent disease.[6] This regimen was associated with deep remissions that could potentially lead to discontinuation of targeted therapy. In clinical trials, we are evaluating the possibility of either lengthening therapy or adding other novel agents based on MRD status. However, this strategy is not the standard of care at this time.

Bruce D. Cheson, MD We were using MRD in similar ways at my former institution. In addition, we were assessing MRD as an exploratory parameter in patients receiving CAR T-cell therapy, where it could suggest the potential for cure.

There are several widely used drugs, such as ibrutinib, that do not eradicate MRD but are still associated with good outcomes.[7] It is therefore important to consider that the use of MRD is drug-dependent.

Anthony R. Mato, MD, MSCE Yes, I agree. Assessment of MRD makes sense only for patients treated with agents or combinations that are capable of inducing deep remissions. Therefore, in the context of Bruton tyrosine kinase (BTK) inhibitors or CD20 antibodies, there is no real utility for MRD at this time based on the current studies. Notably, BTK inhibitors were designed and studied to be administered continuously. It would be helpful to have the option of discontinuing a regimen such as acalabrutinib plus obinutuzumab or ibrutinib plus obinutuzumab, which can lead to uMRD in approximately one-third of patients. However, it is not yet possible to do so because this approach has not been tested in clinical trials. Based on the current trials, the pathway forward for use of MRD for clinical decision-making in CLL is largely with venetoclax plus CD20 antibodies or BTK inhibitors.

Bruce D. Cheson, MD Which MRD assay do you typically use?

Anthony R. Mato, MD, MSCE In general, I use 4-color flow cytometry. Most of the data validation has been at a sensitivity of 10^{-4}. Our center now uses up to 10-color flow cytometry, but I am comfortable using 4-color flow cytometry per guidelines from the European Research Initiative on CLL (ERIC). ERIC consists of approximately 1300 members from around the world. ERIC helped to standardize flow cytometry as a method of measuring MRD, and developed guidance regarding the methodology, the markers, the appropriate depth of response, and how and where to measure MRD. These guidelines are

used worldwide, establishing a standard for comparisons across clinical trials and for communication among clinicians.

The clonoSEQ Assay has a sensitivity of 10^{-5} or 10^{-6}, depending on the cell input.[8] It is an exciting technology that we are utilizing in several clinical trials. It is easy to perform and reproducible, thereby reducing some of the subjectivity typically associated with flow cytometry. In our clinical trials, we are increasingly using next-generation sequencing, achieving 10^{-5} to 10^{-6} sensitivity. It is not yet known how this increased sensitivity might impact patient outcomes based on the current regimens. Some of the modeling data suggest that a deeper MRD response corresponds with a more durable remission. However, when thinking about MRD and depth of response, it becomes obvious that regimens consisting of more therapies are associated with increased adverse events. Whether there is a longer-term clinical benefit for patients remains to be seen.

Bruce D. Cheson, MD The correlation between the depth of MRD and outcome was seen in long-term follow-up of the MURANO trial.[5]

Anthony R. Mato, MD, MSCE The MURANO data are interesting because the study utilized the newer concept of low vs high MRD-positive status.[5] Per the ERIC consortium criteria, the concept of low MRD (10^{-2} to 10^{-4} detectability) is not defined.

Bruce D. Cheson, MD A study by Thompson and colleagues suggested that outcomes were better among patients with uMRD at a sensitivity of 10^{-6} vs those with uMRD defined by a lower sensitivity.[9]

Anthony R. Mato, MD, MSCE I agree. However, it is difficult to assess the benefit of the improved PFS in the context of the increased adverse events associated with the number of treatments needed to achieve a deeper MRD, particularly in the setting of novel agents.

Bruce D. Cheson, MD Because of ERIC, the use of MRD has been approved by the European Medicines Agency.

Anthony R. Mato, MD, MSCE The FDA may be amenable to using MRD as a primary endpoint. However, I am not sure if there is an ongoing study in CLL with a registrational pathway based on MRD as a primary endpoint.

H&O How do you explain MRD to patients?

Anthony R. Mato, MD, MSCE I usually start by describing the standard iwCLL response criteria, such

as the use of physical examination, computed tomography, peripheral blood counts, and bone marrow biopsy to assess the number of abnormal cells. I note that often there are relatively few cells for the pathologist to count. Complete remission as defined by the iwCLL may represent the tip of the iceberg. There may be significant disease burden that is not measurable by these criteria. I then explain that a technology exists that can allow us to look for responses beyond the standard response criteria, even beyond complete remission. Methods such as flow cytometry or next-generation sequencing can detect the absence of cells to a level of 1 in 10,000, 1 in 100,000, or even greater. Assessment of MRD incorporates different technologies to look beyond standard response criteria for the presence or absence of CLL cells.

Bruce D. Cheson, MD Although standard methodologies can indicate the absence of any residual CLL and identify clinical complete remission, almost all of these patients will relapse. Therefore, there is remaining disease that is undetectable. MRD employs assays that are much more sensitive and can help assign a level of response beyond the clinical response established by the iwCLL guidelines. It is now possible to eradicate even minute amounts of disease, and correlate this eradication with outcome. It is especially helpful to know whether disease is eradicated in settings where therapy can be stopped. uMRD is in the eye of the beholder. A patient can have uMRD according to flow cytometry, but still relapse. Some techniques are more sensitive than others. However, it is a good thing for a patient to have uMRD. This state seems to translate into a better outcome, although not necessarily a cure.

Anthony R. Mato, MD, MSCE I sometimes tell patients that uMRD is likely a requirement for cure, but it does not necessarily define cure. It may in the future.

Bruce D. Cheson, MD Data from the CLL10 trial and other studies showed that outcomes were similar for patients with uMRD regardless of the treatment arm.[10] Outcomes were also similar for patients with detectable MRD, regardless of the treatment. Thus, the ability of a particular regimen to bring a higher number of patients to an undetectable state shows it is more effective. Would you agree with that?

Anthony R. Mato, MD, MSCE I do agree. Unfortunately, within a regimen, there are still limited data to predict which patients will have uMRD. There is some information regarding achievement of uMRD with older chemotherapy-based regimens, and fewer data for novel agents. There are some emerging data about which

pretreatment markers are associated with a deeper remission. This correlation will be determined in future trials.

Disclosure

Dr Cheson is a consultant for AbbVie, Pharmacyclics/Janssen, TG Therapeutics, BeiGene, Celgene, and AstraZeneca. He has received research support (directed to his prior institution) from AbbVie, Pharmacyclics/Janssen, AstraZeneca, TG Therapeutics, and Adaptive Biotechnologies. He has consultancies with MorphoSys and Symbio. Dr Mato has received ARM grants, personal fees, and other compensation from TG Therapeutics. He has received grants and personal fees from Pharmacyclics, Janssen, Genentech, AbbVie, Adaptive Biotechnologies, and AstraZeneca. He has received grants from Loxo, Sunesis, Regeneron, and DTRM. He has received grants and other compensation from Celgene and personal fees from BeiGene.

References

1. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood.* 2008;112(4):975-980.
2. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol.* 2005;23(18):4079-4088.
3. Fischer K, Al-Sawaf O, Fink AM, et al. Venetoclax and obinutuzumab in chronic lymphocytic leukemia. *Blood.* 2017;129(19):2702-2705.
4. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med.* 2018;378(12):1107-1120.
5. Kater AP, Seymour JF, Hillmen P, et al. Fixed duration of venetoclax-rituximab in relapsed/refractory chronic lymphocytic leukemia eradicates minimal residual disease and prolongs survival: post-treatment follow-up of the MURANO phase III study. *J Clin Oncol.* 2019;37(4):269-277.
6. Thompson PA, Keating MJ, Jain N, et al. Venetoclax added to ibrutinib in high-risk CLL achieves a high rate of undetectable minimal residual disease [ASH abstract 642]. *Blood.* 2019;134(suppl 1).
7. Ahn IE, Farooqui MZH, Tian X, et al. Depth and durability of response to ibrutinib in CLL: 5-year follow-up of a phase 2 study. *Blood.* 2018;131(21):2357-2366.
8. US Food and Drug Administration. FDA authorizes first next generation sequencing-based test to detect very low levels of remaining cancer cells in patients with acute lymphoblastic leukemia or multiple myeloma. Posted September 28, 2018. https://www.fda.gov/news-events/press-announcements/fda-authorizes-first-next-generation-sequencing-based-test-detect-very-low-levels-remaining-cancer. Accessed April 22, 2020.
9. Thompson PA, Srivastava J, Peterson C, et al. Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. *Blood.* 2019;134(22):1951-1959.
10. Eichhorst B, Fink AM, Bahlo J, et al; international group of investigators; German CLL Study Group (GCLLSG). First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol.* 2016;17(7):928-942.

Slide Library

Assessment of Minimal Residual Disease (MRD) in Chronic Lymphocytic Leukemia

- Eradication of MRD was first shown to be possible in 1992[1]
- More recently, better technologies for assessment of MRD have demonstrated a correlation between MRD eradication and:
  - Progression-free survival[2,3]
  - Overall survival[4]
- Clinicians are learning the most appropriate clinical settings in which to study MRD, and the best ways to apply this information in the management of patients with CLL

CLL, chronic lymphocytic leukemia. 1. Robertson LE et al. Blood. 1992;80(1):29-36. 2. Thompson PA et al. Blood. 2016;127(3):303-309. 3. Thompson PA et al. Blood. 2019;134(22):1951-1959. 4. Böttcher S et al. J Clin Oncol. 2012;30(9):980-988.

Four-Color Flow Cytometry

- Four-color flow cytometry with CD19/CD25 and kappa/lambda achieves a sensitivity of approximately 10^{-4} (ie, 1 in 10,000 cells)[1]
- Flow cytometry requires samples that are less than 48 hours old
- The procedure is widely standardized across institutions
- It is directly quantitative, and the results are quickly available
- The main limitation of flow cytometry is low sensitivity

1. Rawstron AC, et al. Leukemia. 2007;21(5):956-964.

Real-Time Quantitative ASO-PCR

- Real-time quantitative ASO-PCR using patient-specific primers routinely reaches 10^{-5} sensitivity[1]
- This technique detects disease-specific *IGHV*
- It does not require fresh material, but DNA extraction must be performed within 48 hours
- The approach is standardized and highly sensitive
- There are several limitations. This technique is not directly quantitative, and it requires a baseline sample. It is time- and labor-intensive, as well as expensive

ASO-PCR, allele-specific oligonucleotide polymerase-chain reaction; IGHV, immunoglobulin heavy chain variable. 1. Noy A et al. Blood. 2001;97(7):1929-1936.

Next-Generation Sequencing in CLL

- In September 2018, the FDA cleared the clonoSEQ Assay® (Adaptive Biotechnologies; Seattle, WA) for the detection and monitoring of MRD in bone marrow samples from patients with multiple myeloma or B-cell acute lymphoblastic leukemia[1,2]
- Next-generation sequencing is a highly sensitive measure of MRD, reaching 10^{-6}
- The clonoSEQ Assay is also used to assess MRD in other B-cell and T-cell malignancies, such as CLL, as well as with other specimen types, such as peripheral blood

1. US Food and Drug Administration. FDA authorizes first next generation sequencing based test to detect very low levels of remaining cancer cells in patients with acute lymphoblastic leukemia or multiple myeloma. https://www.fda.gov/news-events/press-announcements/fda-authorizes-first-next-generation-sequencing-based-test-detect-very-low-levels-remaining-cancer. Posted September 28, 2018. Accessed April 22, 2020. 2. Evaluation of automatic class II designation for clonoSEQ® Assay. Decision summary. US Food and Drug Administration. https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170080.pdf. Accessed April 22, 2020.

Next-Generation Sequencing in CLL: Study Data

- A prospective, phase 2 clinical trial explored the use of next-generation sequencing in CLL[1,2]
- All patients had achieved uMRD4 in the bone marrow by flow cytometry. However, when these samples were analyzed by next-generation sequencing, only 27.4% of the 62 patients were deemed to have uMRD
- The median PFS was not reached among patients with uMRD according to testing of the bone marrow and peripheral blood. In contrast, median PFS was 67 months among patients with MRD in the bone marrow and 74 months among those with MRD in the peripheral blood (*P*=.02 for both comparisons)

PFS, progression-free survival; uMRD, undetectable minimal residual disease. 1. Thompson PA et al. Leukemia. 2018;32(11):2388-2398. 2. Thompson PA et al. Blood. 2019;134(22):1951-1959.

A Retrospective Analysis Evaluating the Long-Term Prognostic Value of MRD

- A retrospective analysis of a historical cohort of patients with CLL treated at a single institution evaluated the long-term prognostic value of MRD status in a real-world setting across different treatment modalities
  - After a median follow-up of 10.1 years among surviving patients, the median PFS was 7.6 years in patients with uMRD (<.01%), 3.3 years in patients with low levels of MRD (0.01%-1%), and 2.0 years in patients with high levels of MRD (>1%)[1]
  - The median overall survival was 10.6 years, 5.3 years, and 3.6 years, respectively

1. Kwok M et al. Blood. 2016;128(24):2770-2773.

MRD as a Surrogate Endpoint for Long-Term Outcomes in Clinical Trials

- A meta-regression model using combined data from the CLL8, CLL10, and CLL11 trials was applied to predict the treatment effect on PFS using MRD as a surrogate endpoint.[1] The study defined uMRD as a sensitivity of less than 10⁻⁴
- The model demonstrated a statistically significant relationship between the treatment effect on peripheral blood MRD and the treatment effect on PFS
- The model was externally validated with data from the REACH trial.[2] The model predicted a hazard ratio for PFS of 0.63, which is consistent with the hazard ratio for PFS of 0.65 that was reported by the REACH trial

1. Dimier N et al. Blood. 2018;131(9):955-962. 2. Robak T et al. J Clin Oncol. 2010;28(10):1756-1765.

MRD in the MURANO Trial

- MRD status in the MURANO trial was assessed with both ASO-PCR and flow cytometry.[1] The study defined uMRD as less than 10⁻⁴
- uMRD was associated with improved PFS.[2] At the end of combination treatment, patients with uMRD showed a longer duration of PFS compared with patients who had detectable MRD, regardless of the treatment arm
- Even among patients with detectable MRD, those with lower levels of MRD had a longer duration of PFS compared with those who had higher MRD levels

1. Seymour JF et al. N Engl J Med. 2018;378(12):1107-1120. 2. Kater AP et al. J Clin Oncol. 2019;37(4):269-277.

The CLARITY Trial

- The single-arm, phase 2 CLARITY trial evaluated the combination of ibrutinib and venetoclax in patients with relapsed/refractory CLL[1]
- The primary endpoint was uMRD in both the peripheral blood and bone marrow after 12 months of combination treatment (month 14 of total therapy)
- At month 14, 53% of patients had uMRD in the peripheral blood and 36% had uMRD in the bone marrow
- uMRD was observed regardless of the prior therapy
- Continuous improvement was apparent by the degree of MRD reduction, with 44% of patients achieving an MRD level of 10⁻⁴ or below according to flow cytometry at month 26

1. Hillmen P et al. J Clin Oncol. 2019;37(30):2722-2729.

The Potential Use of MRD in CLL

- Until recently, MRD had been relegated to the role of a prognostic factor in patients with CLL. However, there is tremendous interest in using MRD for other purposes, such as:
  – A potential surrogate endpoint in the context of clinical trials, in order to allow those trials to provide results earlier. That surrogate endpoint would potentially be for PFS, which is a standard endpoint used in key clinical trials in CLL at this time
  – A measure of depth of response to guide clinical decision-making
- At this time, neither of these uses are clinically accepted practices. Instead, most clinical trials in CLL use MRD status as a secondary or exploratory endpoint

For a free electronic download of these slides, please direct your browser to the following web address:

http://www.hematologyandoncology.net