The Role of Minimal Residual Disease in Chronic Lymphocytic Leukemia

Othman Al-Sawaf, MD,1,2,3 Michael Hallek, MD,1 and Kirsten Fischer, MD1

1University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine; German CLL Study Group, Center for Integrated Oncology Aachen-Bonn-Cologne-Düsseldorf, Aachen, Germany
2The Francis Crick Institute, London, United Kingdom
3UCL Cancer Institute, University College London, United Kingdom

Abstract: Minimal residual disease (MRD) has evolved as a sensitive and highly prognostic surrogate parameter of response to therapy in chronic lymphocytic leukemia (CLL). Multiple methods have been established to measure and quantify MRD during and after therapy. The improved sensitivity of MRD measurements has made it possible to develop limited-duration therapies, first with chemotherapy and chemoimmunotherapy and now also with combined targeted therapy. Moreover, concepts to integrate MRD information beyond prognostication—to guide duration of treatment and determine sensitivity—are at present being explored in prospective trials. In this review, we summarize currently available methods of MRD detection, provide recent MRD data and outcomes from clinical trials in CLL, and discuss open questions and future approaches for MRD within and outside clinical trials.

Introduction

Assessing and quantifying the response to cancer therapy are a critical part of managing hematologic malignancies. In the context of chemotherapy and chemoimmunotherapy for chronic lymphocytic leukemia (CLL), response assessment at the end of therapy—for instance, after 6 cycles of fludarabine, cyclophosphamide, and rituximab (FCR)—can make it possible to differentiate between a complete response (CR) and a partial response (PR). Response assessment requires a thorough assessment before the start of therapy, including computed tomography (CT) or magnetic resonance imaging (MRI) at baseline, as well as at the end of therapy. The International Workshop on Chronic Lymphocytic Leukemia (iwCLL) guidelines have thresholds that define CR, PR, stable disease, and progressive disease.1 For a CR, a complete recovery of blood cell counts, normal bone marrow histology, and the absence of hepatosplenomegaly or lymphadenopathy of 1.5 cm or greater must be confirmed. Multiple pooled analyses have demonstrated that response to therapy is associated with progression-free survival (PFS), and in some cases
Methods of MRD Detection

Detection of MRD relies on capturing specific features of CLL in a sensitive way to quantify the amount of residual disease. The oldest and most used method of MRD detection is based on flow cytometry. Guidelines on sample preparation, choice of antibodies, and data analyses are regularly provided and updated by the European Research Initiative on CLL (ERIC). The current guidelines recommend an antibody panel of CD5, CD19, CD20, CD43, CD79b, and CD81 at sufficient sensitivity and specificity to detect CLL at a level of 10^4 (ie, 1 CLL cell per 10,000 leukocytes). Higher sensitivity can be achieved with 6-color flow cytometry, with which levels of up to 10^3 can be reached. Generally, it is recommended to use a cutoff of at least 10^4 to define undetectable MRD, given that this level is most established by flow cytometry–based MRD. However, the prognostic ability of very deep MRD responses has become more evident in the context of combined targeted therapy with BCL2 inhibitors.

MRD can be also detected by polymerase chain reaction (PCR) of the immunoglobulin heavy chain variable (IGHV) gene. This method was initially established as a semiquantitative tool to detect MRD with sensitivity of 10^4, on the basis of a set of consensus primers annealing to CLL clone-specific complementary determining region 3 (CDR3). Higher sensitivity can be achieved with primers targeting patient-specific CDR3 to amplify a CLL clone specifically. This so-called “nested” approach allows a high level of sensitivity, up to 10^6, but does not provide quantitative results. Owing to its standardized methodology, many recent clinical trials have used real-time quantitative PCR of CDR3 with allele-specific oligonucleotides, which provides quantitative results with a sensitivity of up to 10^5. This method also requires patient-specific primers and is therefore rarely used in routine clinical care.

The most recent advancement in MRD detection is that based on next-generation sequencing (NGS) of rearranged immunoglobulin heavy (IgH) VDJ or DJ, IgK, and IgL receptor gene sequences or translocated BCL1/IgH(J) and BCL2/IgH(J). This approach does not require patient-specific primers; however, a disease-specific IgH sequence has to be identified before therapy to track the variant during or after therapy. Although NGS requires more sophisticated data analysis than flow cytometry or PCR, it can achieve the highest levels of sensitivity in CLL, of up to 10^-3. The long-term implications of very deep MRD levels of 10^-6 or less have been demonstrated in the context of chemoimmunotherapy as well as targeted combination therapy. Commercial NGS assays have received approval from the US Food and Drug Administration (FDA), although reimbursement and wide clinical use outside trials are still not uniform.

MRD and Chemotherapy/Chemoimmunotherapy

The feasibility of measuring MRD in CLL was first demonstrated in the context of fludarabine plus prednisolone almost 30 years ago. Using low-sensitivity 2-color flow cytometry, Robertson and colleagues reported MRD levels below 10^3 in 89% of patients with a CR, 51% of patients with nodal CRs (nCRs), and 19% of patients with a PR. Despite the rather limited sensitivity, the authors demonstrated significantly better long-term outcomes in patients with undetectable minimal residual disease (uMRD) below 10^-3 than in patients with detectable MRD, even when a formal CR was achieved.

The introduction of CD20 antibodies to chemotherapy was a major milestone in the treatment landscape for CLL that improved both PFS and OS in multiple settings. Along with the improved outcomes, the depth of remission as reflected by uMRD was also improved substantially by the addition of CD20 antibodies. In elderly and/or unfit patients with previously untreated CLL, the CLL11 study showed that the addition of rituximab or obinutuzumab (Gazyva, Genentech) to chlorambucil over 6 cycles improved the depth of remissions; the rate of uMRD was 0% with chlorambucil monotherapy vs 2% with chlorambucil/rituximab and 38% with chlorambucil/obinutuzumab. CLL11 demonstrated that end-of-treatment MRD status is strongly associated with PFS, as indicated by a median PFS of 19.4 months in the patients with detectable MRD vs not reached in those with uMRD at the last study follow-up. Ultimately, longer PFS translated into significantly longer OS in the patients who received chlorambucil/obinutuzumab, supporting the importance of MRD as a prognostic parameter.
Similarly, in fit patients with previously untreated CLL, the FCR combination improved PFS and OS in several studies. The regimen was first tested in a phase 2 study in which uMRD at the end of therapy was reported in 70 of 237 patients (30%). Similar results were seen in the randomized CLL8 study, in which uMRD rates were significantly higher in the FCR arm than in the FC arm (22% vs 12%). In CLL10, a comparison of FCR with bendamustine (Treanda/Bendeka, Teva)/rituximab showed uMRD rates of 49% vs 38%, which translated into a significantly longer PFS after FCR. A pooled analysis of patients treated in CLL8 and CLL10 confirmed that those with uMRD at the end of treatment have the best outcomes in terms of PFS and OS, regardless of whether a PR or CR is achieved. The independent prognostic value of MRD in the frontline setting as well as in the relapsed/refractory setting was also confirmed within other clinical studies.

**MRD and Targeted Therapy**

The advanced understanding of the biology and pathologic mechanism of CLL has allowed targeted therapies to be designed that specifically exploit the vulnerabilities of cancer cells. A major step forward has been the development of Bruton tyrosine kinase (BTK) inhibitors, which inhibit disrupted B-cell antigen receptor (BCR) signaling. Whereas previous treatment regimens based on chemotherapy were designed as limited-duration regimens (eg, administered over 6 cycles of treatment), targeted monotherapies were developed initially as continuous regimens. One of the reasons was the lack of MRD responses; in the case of ibrutinib (Imbruvica, Pharmacycels), fewer than 10% of patients achieved uMRD status at any stage during continuous ibrutinib therapy. Although the CR rate increased over time, the uMRD rate in peripheral blood remained low, at approximately 6%, even after 4 years of therapy. Interestingly, the outcomes of patients taking ibrutinib who have uMRD are not better than the outcomes of those with detectable MRD, highlighting the fundamentally different principle that underlies single-agent continuous therapy of CLL. This finding was demonstrated in a randomized setting in the ECOG1912 study, in which fit, treatment-naive patients with CLL were treated either with 6 cycles of FCR or with continuous ibrutinib. The patients who received FCR had an uMRD rate of 57%, compared with a rate of only 5% in those who received 1 year of ibrutinib. Despite the higher uMRD rate in the chemoimmunotherapy arm, the patients who received ibrutinib therapy had a significantly longer PFS and OS, demonstrating that BTK inhibitors can effectively modulate disease without reducing MRD. Studies of other BTK inhibitors confirmed this observation, such as the ELEVATE TN study of acalabrutinib (Calquence, AstraZeneca) with or without obinutuzumab vs chlorambucil/obinutuzumab. In the intention-to-treat population, the uMRD rate was 12% in the acalabrutinib/obinutuzumab arm, 8% in the chlorambucil/obinutuzumab arm, and 5.5% in the acalabrutinib monotherapy arm. PFS was longer in both acalabrutinib-containing arms than in the chemoimmunotherapy arm, confirming that attainment of uMRD with continuous BTK inhibitor therapy is not required to achieve long-term disease control.

Patients with mutated *IGHV* status have a particularly good prognosis when treated with FCR chemoimmunotherapy, as observed in several prospective studies. In fact, continuous BTK inhibitor therapy has so far not improved outcomes more effectively than FCR in terms of PFS or OS. Approximately half of the patients with mutated *IGHV* status treated with FCR can expect a long-term remission beyond 10 years. It is possible that the combination of a BTK inhibitor with FCR could further increase the rate of long-term remissions. The combinations of ibrutinib plus FCR and FC plus obinutuzumab (iFCR/iFCG) are currently being explored in two phase 2 studies that have so far reported uMRD rates of up to 88%, although longer follow-up must confirm effectiveness in terms of long-term remission.

The BCL2 inhibitor venetoclax (Venclexta, AbbVie) was first introduced as a continuous monotherapy for relapsed/refractory CLL. Compared with other monotherapies, venetoclax showed considerable uMRD rates of between 26% and 36% after approximately 1 year of therapy. Rates of uMRD are considerably higher when venetoclax is combined with other targeted agents; a phase 1b study first demonstrated uMRD rates in peripheral blood of 61% in relapsed/refractory CLL. This finding was confirmed in the randomized MURANO study, which compared venetoclax/rituximab vs bendamustine/rituximab and reported uMRD rates of 62% vs 13% in peripheral blood at the end of combination therapy. A similar pattern was seen with venetoclax plus obinutuzumab in patients with previously untreated CLL in the CLL14 study, in which uMRD rates at the end of treatment were 76% with venetoclax/obinutuzumab vs 35% with chlorambucil/obinutuzumab. In both CLL14 and MURANO, patients with uMRD had the longest PFS regardless of whether they achieved a CR or PR, which indicates that uMRD is one of the most sensitive surrogates for treatment efficacy.

The combination of BCL2 inhibition and BTK inhibition has also been considered in several studies. In theory, the mobilization of CLL cells from lymph node compartments by BTK inhibitors could be complemented by the apoptotic effect of venetoclax, thereby reducing MRD.
CAR T-cell therapy. was highly prognostic for the long-term outcomes after CLL, in which a CR with uMRD was shown in 4 of the 14 efficacy. The first reported successful clinical use of CAR anti-CD19 CAR T-cell therapy has shown promising on both BTK inhibitor and BCL2 inhibitor therapy), larly in patients with double-refractory disease (ie, relapses improve long-term outcomes.

MRD and Cellular Therapy

Allogeneic stem cell transplant has been a critical part of the treatment algorithm for high-risk CLL, but the number of transplants has been declining wherever targeted agents are available.41,42 Notably, allogeneic stem cell transplant can induce sustainable uMRD remissions even in patients with high-risk disease and after multiple relapses. The CLL3X study reported a 10-year OS rate of 51% in 90 patients with high-risk CLL.54,55 As with limited-duration chemoimmunotherapy or targeted treatment, the MRD status close to the time of transplant was prognostic for the long-term outcome. At 1 year after transplant, the uMRD rate was 28%, which was maintained particularly in the patients with the longest PFS. In several cases with initially detectable MRD, uMRD was achieved by immunomodulation—for example, by tapering immunosuppression or by administering donor leukocytes. In this particular setting, MRD can actually be used to guide specific clinical interventions to deepen remissions and potentially improve long-term outcomes.

Most cellular therapy in CLL is currently focused on chimeric antigen receptor (CAR) T-cell therapy. Particularly in patients with double-refractory disease (ie, relapses on both BTK inhibitor and BCL2 inhibitor therapy), anti-CD19 CAR T-cell therapy has shown promising efficacy. The first reported successful clinical use of CAR T-cell therapy was in 14 patients with relapsed/refractory CLL, in which a CR with uMRD was shown in 4 of the 14 patients within 3 months after infusion. The MRD status was highly prognostic for the long-term outcomes after CAR T-cell therapy.54,55 Since then, several phase 1 and 2 studies—but so far, no phase 3 study—have explored the activity of CAR T cells in treating relapsed/refractory CLL. The latest data show uMRD rates in peripheral blood of 75% to 90% after CAR T-cell therapy; some studies are also exploring combinations with ibrutinib to facilitate T-cell recovery and subsequently a better expansion of CD19 CAR T cells.56,57 The follow-up from these studies is still limited, so the long-term implications of the post-infusion MRD status are not yet completely clear.

The Current Role of MRD in Clinical Care

The prognostic value of end-of-treatment MRD has been confirmed in multiple prospective studies covering the frontline and relapsed settings, as well as chemotherapy/ chemoimmunotherapy and targeted combination therapy. Measuring MRD at the end of therapy to assess its success can be therefore recommended whenever reliable MRD measurements are accessible and affordable and whenever the patient and the physician want prognostic information. Tools such as the continuous individualized risk index (CIRI) could be helpful to integrate MRD results into the prognostication of PFS and OS.58,59 Beyond this, currently no evidence supports the extension of MRD-guided treatment outside clinical studies.

In a retrospective analysis of patients treated with FCR, PFS in those with uMRD after 3 cycles of therapy was similar to that of patients who received the full 6 cycles.60 On the basis of such observations, knowledge of MRD could be helpful to support decisions regarding therapy discontinuation in patients who cannot tolerate a certain regimen. Given the lack of prospective evaluations, treatment discontinuation guided by MRD assessment should be reserved for individual cases according to the patient’s characteristics, preferences, and risk status.

The Future Role of MRD in Clinical Care

The next critical step in the research of MRD in CLL is the transition from a prognostic to a predictive tool that guides therapy decisions—that is, treatment initiation and modulation. However, certain caveats must be addressed.

First, the effect of high-risk disease features, such as TP53 aberration and unmutated IGHV as well as complex karyotype, on MRD responses is not yet completely clear. In the frontline setting, the uMRD rates of patients with TP53 aberrations or unmutated IGHV status are fairly similar at end of treatment to the rates of patients with low-risk disease, but PFS is shorter. In contrast, in the relapsed/refractory setting, high-risk disease is associated with lower MRD responses.62,63 However, the uMRD rates in patients with complex karyotype or genomic
complexity are similar in the frontline and relapsed settings, and PFS is not substantially lower than in patients without complex karyotype or genomic complexity. Thus, the varying MRD growth dynamics of patients with high-risk features warrant further investigation.

Second, sufficiently reliable stopping criteria need to be established. Several studies have tested different strategies to guide treatment duration. In a series of phase 2 studies, the German CLL Study Group evaluated a concept by which patients with uMRD in peripheral blood and a CR at 2 subsequent visits within 3 months during maintenance therapy discontinued therapy.52-64 Because high uMRD rates of 87% were observed, particularly with venetoclax/obinutuzumab, the majority of patients were able to discontinue therapy at some point.65 Notably, discontinuation of therapy was also possible in patients with high-risk disease, in whom long-term remissions were observed with this approach.66 Another approach is to consider the individual patient’s time to uMRD attainment to define the specific duration of limited-duration therapy. A group of researchers in the United Kingdom first tested this approach in the CLARITY study with venetoclax/ibrutinib in relapsed/refractory CLL.67 CLARITY allowed treatment duration to be individualized, and the majority of patients were able to discontinue therapy within 2 years. The FLAIR study from the same group of researchers is further exploring this concept, among other questions, in a phase 3 setting.68 Whether any of these approaches will be feasible in routine clinical care or whether limited-duration and continuous approaches will ultimately yield comparable long-term results is still unclear.

Third, the method of measuring MRD and calling uMRD needs to be standardized. Measurements with flow cytometry are standardized and widely used in hematology; however, the sensitivity level of 10−6, although it does have prognostic power, can be improved with NGS-based approaches. In future, a threshold of 10−5 or 10−6 might be more appropriate for defining truly undetectable MRD.

Fourth, no definitive data are available on which compartment is best to capture the MRD status. From the patient’s perspective, peripheral blood is naturally more convenient than bone marrow aspirate for assessment, but in some cases, discordant results have been observed. Hence, some studies propose using “confirmed uMRD”—that is, uMRD confirmed in both peripheral blood and bone marrow—to guide treatment.49 In addition, the prognostic significance of residual lymphadenopathy when no MRD can be detected in peripheral blood is not completely clear.44 Using cell-free DNA from plasma might be a way to obtain insights into MRD across multiple compartments, but the data so far are limited.48

Finally, the alternative treatment paradigm of continuous single-agent therapy without uMRD remissions must be considered. Multiple randomized studies have demonstrated that although fixed-duration chemoimmunotherapy achieves higher uMRD rates than continuous BTK inhibitor treatment, the PFS or OS can be longer with BTK inhibitors despite detectable MRD.46,51,69 In fact, a recent analysis demonstrated that uMRD is not prognostic in the context of continuous ibrutinib therapy.52 The ongoing phase 3 CLL17 study will likely reveal the strengths and weaknesses of MRD-reducing fixed-duration combination therapy (venetoclax/obinutuzumab, venetoclax/ibrutinib) vs those of indefinite monotherapy with ibrutinib (NCT04608318).

**Conclusion**

This review highlights the considerable advancements made in the field of MRD in the management of CLL over the last few years. A large set of robust prospective data confirms the independent prognostic value of MRD for PFS, and in some settings, for OS as well. The next step will be to integrate MRD into clinical decision making—that is, shortening, extending, or even intensifying therapy on the basis of MRD levels at certain points. It is hoped that within the next years, the positive momentum in the CLL community and the large number of ongoing prospective studies will bring us closer to this goal.

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imab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukemia.


