The Role of Minimal Residual Disease in Chronic Lymphocytic Leukemia

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

[1]University 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

[2]The Francis Crick Institute, London, United Kingdom

[3]UCL Cancer Institute, University College London, United Kingdom

Corresponding author:
Othman Al-Sawaf, MD
University Hospital of Cologne
Kerpener Str 62
50937 Cologne
Germany
Tel: +49 221 478 88220
Email: othman.al-sawaf@uk-koeln.de

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.

Keywords
Chemoimmunotherapy, chronic lymphocytic leukemia (CLL), fixed-duration therapy, minimal residual disease (MRD), targeted agents

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

with overall survival (OS) as well.[2,3] Notably, patients with MRD levels below a sensitivity threshold of 10^{-4} at the end of therapy can expect a similarly good outcome regardless of whether they have a CR or a PR.[2] Mild residual lymphadenopathy or splenomegaly often reflects not residual disease activity but rather residual tissue scars; MRD measurement can overcome these uncertainties.[2,3] These observations confirm the role of end-of-treatment MRD as an independent prognostic factor.

This review provides an overview of the methods of MRD detection in CLL, and their advantages and disadvantages. Moreover, we summarize data from pivotal clinical trials, with emphasis on MRD outcomes. Finally, we provide an outlook on how MRD can be further explored and used within and outside clinical trials.

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).[4,5] 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).[6] Higher sensitivity can be achieved with 6-color flow cytometry, with which levels of up to 10^{-5} can be reached.[7] 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.[1] However, the prognostic ability of very deep MRD responses has become more evident in the context of combined targeted therapy with BCL2 inhibitors.[8]

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).[9,10] 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.[11] 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}.[12,13] This method also requires patient-specific primers and is therefore rarely used in routine clinical care.[14]

The most recent advancement in MRD detection of CLL is 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).[15] 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^{-6}.[16] 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.[17,18] 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.[19] Using low-sensitivity 2-color flow cytometry, Robertson and colleagues reported MRD levels below 10^{-1} 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^{-1} 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.[20,21] 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.[22]

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%).[23] 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%).[24,25] 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.[26] 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.[2] 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.[27]

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.[28] 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, Pharmacyclics), fewer than 10% of patients achieved uMRD status at any stage during continuous ibrutinib therapy.[29,30] Although the CR rate increased over time, the uMRD rate in peripheral blood remained low, at approximately 6%, even after 4 years of therapy.[31] 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.[32] 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.[30] 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, Astra Zeneca) with or without obinutuzumab vs chlorambucil/obinutuzumab.[33] In the intention-to-treat population, the uMRD rate was 12% in the acalabrutinib/obinutuzumab arm, 8% in the chlorambucil/obinutuzumab arm, and 0.5% 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.[24,34] In fact, continuous BTK inhibitor therapy has so far not improved outcomes more effectively than FCR in terms of PFS or OS.[30] 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.[35,36]

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.[37-39] 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.[40,41] 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.[42] 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.[43] 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.[44,45]

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

more effectively.[46] This hypothesis was first explored in patients with relapsed/refractory CLL in the phase 2 CLARITY study, which reported a uMRD rate of 58% after 1 year of combination therapy.[47] Similar findings were reported in patients with previously untreated CLL, in whom 1 year of combination therapy achieved uMRD rates of approximately 75% in 2 phase 2 studies.[48,49] In the phase 3 GLOW study, in which treatment-naive patients with CLL were assigned either to fixed-duration venetoclax/ibrutinib (3 cycles of ibrutinib monotherapy, 12 cycles of combination therapy) or to chlorambucil/obinutuzumab (6 cycles of combination therapy), the uMRD rates in peripheral blood were 55% after the end of venetoclax/ibrutinib treatment and 39% in the chlorambucil/obinutuzumab arm.[50] The difference was more pronounced in the bone marrow (52% vs 17%); however, further analyses are warranted to understand the effect of factors such as patient selection and characteristics on the lower MRD responses in the phase 3 studies than in the phase 2 studies.

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.[51] 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.[52,53] 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.[42,45,61] 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.[62-64] 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.[47] 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.[67] 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^{-4}, 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.[68]

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.[30,33,69] In fact, a recent analysis demonstrated that uMRD is not prognostic in the context of continuous ibrutinib therapy.[32] 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.

Disclosures

Dr Al-Sawaf has received research funding from AbbVie, Roche, Janssen, and BeiGene; and honoraria and personal fees from AbbVie, Adaptive Biotechnologies, AstraZeneca, BeiGene, Gilead, Janssen, and Roche. Dr Hallek has received grants, nonfinancial support, and personal fees from Roche, Gilead, Mundipharma, Janssen, Celgene, Pharmacyclics, and AbbVie. Dr Fischer has received research funding from Roche, and honoraria and personal fees from Roche and AbbVie.

References

1. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745-2760.
2. Kovacs G, Robrecht S, Fink AM, et al. Minimal residual disease assessment improves prediction of outcome in patients with chronic lymphocytic leukemia (CLL) who achieve partial response: comprehensive analysis of two phase III studies of the German CLL Study Group. *J Clin Oncol*. 2016;34(31):3758-3765.
3. 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.
4. Rawstron AC, Böttcher S, Letestu R, et al; European Research Initiative in CLL. Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL. *Leukemia*. 2013;27(1):142-149.
5. Rawstron AC, Fazi C, Agathangelidis A, et al. A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual

disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. *Leukemia*. 2016;30(4):929-936.
6. 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.
7. Rawstron AC, Kreuzer KA, Soosapilla A, et al. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: an European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. *Cytometry B Clin Cytom*. 2018;94(1):121-128.
8. Al-Sawaf O, Zhang C, Lu T, et al. Minimal residual disease dynamics after venetoclax-obinutuzumab treatment: extended off-treatment follow-up from the randomized CLL14 Study. *J Clin Oncol*. 2021;39(36):4049-4060.
9. Böttcher S, Stilgenbauer S, Busch R, et al. Standardized MRD flow and ASO IGH RQ-PCR for MRD quantification in CLL patients after rituximab-containing immunochemotherapy: a comparative analysis. *Leukemia*. 2009;23(11):2007-2017.
10. Böttcher S, Ritgen M, Pott C, et al. Comparative analysis of minimal residual disease detection using four-color flow cytometry, consensus IgH-PCR, and quantitative IgH PCR in CLL after allogeneic and autologous stem cell transplantation. *Leukemia*. 2004;18(10):1637-1645.
11. Böttcher S, Ritgen M, Dreger P. Allogeneic stem cell transplantation for chronic lymphocytic leukemia: lessons to be learned from minimal residual disease studies. *Blood Rev*. 2011;25(2):91-96.
12. Raponi S, Della Starza I, De Propris MS, et al. Minimal residual disease monitoring in chronic lymphocytic leukaemia patients. A comparative analysis of flow cytometry and ASO IgH RQ-PCR. *Br J Haematol*. 2014;166(3):360-368.
13. van der Velden VH, Cazzaniga G, Schrauder A, et al; European Study Group on MRD detection in ALL (ESG-MRD-ALL). Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21(4):604-611.
14. Thompson PA, Wierda WG. Eliminating minimal residual disease as a therapeutic end point: working toward cure for patients with CLL. *Blood*. 2016;127(3):279-286.
15. Logan AC, Gao H, Wang C, et al. High-throughput VDJ sequencing for quantification of minimal residual disease in chronic lymphocytic leukemia and immune reconstitution assessment. *Proc Natl Acad Sci USA*. 2011;108(52):21194-21199.
16. Ching T, Duncan ME, Newman-Eerkes T, et al. Analytical evaluation of the clonoSEQ Assay for establishing measurable (minimal) residual disease in acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma. *BMC Cancer*. 2020;20(1):612.
17. Al-Sawaf O, Zhang C, Robrecht S, et al. Venetoclax-obinutuzumab for previously untreated chronic lymphocytic leukemia: 4-year follow-up analysis of the randomized CLL14 study [ICML abstract 137]. *Hematol Oncol*. 2021;39(suppl 2).
18. 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.
19. 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.
20. 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.
21. Langerak AW, Ritgen M, Goede V, et al. Prognostic value of MRD in CLL patients with comorbidities receiving chlorambucil plus obinutuzumab or rituximab. *Blood*. 2019;133(5):494-497.
22. Goede V, Fischer K, Dyer MJ, et al. Overall survival benefit of obinutuzumab over rituximab when combined with chlorambucil in patients with chronic lymphocytic leukemia and comorbidities: final survival analysis of the CLL11 study. Presented at: 23rd Congress of the European Hematology Association; June 14-17, 2018; Stockholm, Sweden. Abstract S151.
23. 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.
24. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*. 2016;127(2):208-215.
25. 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.
26. 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.
27. 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.
28. Woyach JA, Johnson AJ, Byrd JC. The B-cell receptor signaling pathway as a therapeutic target in CLL. *Blood*. 2012;120(6):1175-1184.
29. Burger JA, Sivina M, Jain N, et al. Randomized trial of ibrutinib vs ibrutinib plus rituximab in patients with chronic lymphocytic leukemia. *Blood*. 2019;133(10):1011-1019.
30. Shanafelt TD, Wang XV, Kay NE, et al. Ibrutinib-rituximab or chemoimmunotherapy for chronic lymphocytic leukemia. *N Engl J Med*. 2019;381(5):432-443.
31. 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.
32. Wang XV, Hanson CA, Tschumper RC, et al. Measurable residual disease does not preclude prolonged progression-free survival in CLL treated with ibrutinib. *Blood*. 2021;138(26):2810-2827.
33. Sharman JP, Egyed M, Jurczak W, et al. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzmab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. *Lancet*. 2020;395(10232):1278-1291.
34. 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.
35. Davids MS, Brander DM, Kim HT, et al; Blood Cancer Research Partnership of the Leukemia & Lymphoma Society. Ibrutinib plus fludarabine, cyclophosphamide, and rituximab as initial treatment for younger patients with chronic lymphocytic leukaemia: a single-arm, multicentre, phase 2 trial. *Lancet Haematol*. 2019;6(8):e419-e428.
36. Jain N, Thompson PA, Burger JA, et al. Ibrutinib, fludarabine, cyclophosphamide, and obinutuzumab (GA101) (iFCG) for previously untreated patients with chronic lymphocytic leukemia (CLL) with mutated IGHV and non-del (17p) [ASCO abstract 7522]. *J Clin Oncol*. 2017;35(15)(suppl).
37. Roberts AW, Ma S, Kipps TJ, et al. Efficacy of venetoclax in relapsed chronic lymphocytic leukemia is influenced by disease and response variables. *Blood*. 2019;134(2):111-122.
38. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol*. 2016;17(6):768-778.
39. Kater AP, Arslan O, Demikran F, et al. Efficacy of venetoclax in patients with relapsed/refractory chronic lymphocytic leukemia: primary endpoint analysis of the international phase 3b trial (VENICE I). Presented at: 25th Congress of the European Hematology Association; June 11-21, 2020; Virtual. Abstract S156.
40. Brander DM, Seymour JF, Kipps TJ, et al. Durability of responses on continuous therapy and following drug cessation with venetoclax and rituximab: long-term follow-up analysis of a phase 1b study in patients with relapsed CLL [ASH abstract 3036]. *Blood*. 2019;134(1)(suppl).
41. Seymour JF, Ma S, Brander DM, et al. Venetoclax plus rituximab in relapsed or refractory chronic lymphocytic leukaemia: a phase 1b study. *Lancet Oncol*. 2017;18(2):230-240.
42. 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.
43. 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.
44. Al-Sawaf O, Zhang C, Tandon M, et al. Venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab for previously untreated chronic lymphocytic leukaemia (CLL14): follow-up results from a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2020;21(9):1188-1200.
45. 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.
46. Haselager MV, Kielbassa K, Ter Burg J, et al. Changes in Bcl-2 members after

ibrutinib or venetoclax uncover functional hierarchy in determining resistance to venetoclax in CLL. *Blood*. 2020;136(25):2918-2926.
47. 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.
48. Jain N, Keating M, Thompson P, et al. Ibrutinib and venetoclax for first-line treatment of CLL. *N Engl J Med*. 2019;380(22):2095-2103.
49. Wierda WG, Allan JN, Siddiqi T, et al. Ibrutinib plus venetoclax for first-line treatment of chronic lymphocytic leukemia: primary analysis results from the minimal residual disease cohort of the randomized phase II CAPTIVATE study. *J Clin Oncol*. 2021;39(34):3853-3865.
50. Kater A, Owen C, Moreno C, et al. Fixed-duration ibrutinib and venetoclax (I+V) versus chlorambucil plus obinutuzumab (CLB+O) for first-line (1L) chronic lymphocytic leukemia (CLL): primary analysis of the phase 3 GLOW study. Presented at: 26th Congress of the European Hematology Association; June 9-17, 2021; Virtual. Abstract LB1902.
51. Gribben JG. How and when I do allogeneic transplant in CLL. *Blood*. 2018;132(1):31-39.
52. Dreger P, Döhner H, Ritgen M, et al; German CLL Study Group. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. *Blood*. 2010;116(14):2438-2447.
53. Krämer I, Stilgenbauer S, Dietrich S, et al. Allogeneic hematopoietic cell transplantation for high-risk CLL: 10-year follow-up of the GCLLSG CLL3X trial. *Blood*. 2017;130(12):1477-1480.
54. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365(8):725-733.
55. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015;7(303):303ra139.
56. Gauthier J, Hirayama AV, Purushe J, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. *Blood*. 2020;135(19):1650-1660.
57. Siddiqi T, Soumerai JD, Dorritie KA, et al. Phase 1 TRANSCEND CLL 004 study of lisocabtagene maraleucel in patients with relapsed/refractory CLL or SLL [published online October 26, 2021]. *Blood*. doi:10.1182/blood.2021011895.
58. Al-Sawaf O, Esfahani MS, Zhang C, et al. A continuous individualized risk index for refined outcome prediction after targeted therapy for patients with chronic lymphocytic leukemia (CIRI-CLL). *Hematol Oncol*. 2021;39(suppl 2):205-207.
59. Kurtz DM, Esfahani MS, Scherer F, et al. Dynamic risk profiling using serial tumor biomarkers for personalized outcome prediction. *Cell*. 2019;178(3):699-713.e19.
60. Strati P, Keating MJ, O'Brien SM, et al. Eradication of bone marrow minimal residual disease may prompt early treatment discontinuation in CLL. *Blood*. 2014;123(24):3727-3732.
61. Kater AP, Wu JQ, Kipps T, et al. Venetoclax plus rituximab in relapsed chronic lymphocytic leukemia: 4-year results and evaluation of impact of genomic complexity and gene mutations from the MURANO phase III study. *J Clin Oncol*. 2020;38(34):4042-4054.
62. Cramer P, Tresckow JV, Robrecht S, et al. Bendamustine, followed by ofatumumab and ibrutinib in chronic lymphocytic leukemia (CLL2-BIO): primary endpoint analysis of a multicenter, open-label phase-II trial. *Haematologica*. 2021;106(2):543-554.
63. Cramer P, von Tresckow J, Bahlo J, et al. Bendamustine followed by obinutuzumab and venetoclax in chronic lymphocytic leukaemia (CLL2-BAG): primary endpoint analysis of a multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2018;19(9):1215-1228.
64. von Tresckow J, Cramer P, Bahlo J, et al. CLL2-BIG: sequential treatment with bendamustine, ibrutinib and obinutuzumab (GA101) in chronic lymphocytic leukemia. *Leukemia*. 2019;33(5):1161-1172.
65. Cramer P, von Tresckow J, Bahlo J, et al. Durable remissions after discontinuation of combined targeted treatment in patients with chronic lymphocytic leukemia (CLL) harbouring a high-risk genetic lesion (del(17p)/TP53 mutation) [ASH abstract 694]. *Blood*. 2018;132(1)(suppl).
66. Cramer P, Tausch E, von Tresckow J, et al. Durable remissions following combined targeted therapy in patients with CLL harboring TP53 deletions and/or mutations. *Blood*. 2021;138(19):1805-1816.
67. Collett L, Howard DR, Munir T, et al. Assessment of ibrutinib plus rituximab in front-line CLL (FLAIR trial): study protocol for a phase III randomised controlled trial. *Trials*. 2017;18(1):387.
68. Yeh P, Hunter T, Sinha D, et al. Circulating tumour DNA reflects treatment response and clonal evolution in chronic lymphocytic leukaemia. *Nat Commun*. 2017;8(1):14756.
69. Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. *N Engl J Med*. 2018;379(26):2517-2528.