Eliminating minimal residual disease as a therapeutic end point: working toward cure for patients with CLL

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Deep remission and prolonged disease-free survival can be achieved with first-line chemoimmunotherapy (CIT), such as combined fludarabine, cyclophosphamide, and rituximab, in the majority of patients with chronic lymphocytic leukemia (CLL). More modest results are reported with less intense regimens like obinutuzumab plus chlorambucil. Clinical assessment has limited sensitivity in detecting residual disease responsible for subsequent relapse, even including morphologic bone marrow (BM) evaluation. Multicolor flow cytometry and polymerase chain reaction (PCR)-based methods can detect minimal residual disease (MRD) to a sensitivity of $\leq 1:10^4$. Achieving BM MRD-negative complete remission (CR) is associated with superior progression-free survival (PFS) and overall survival; MRD status is the single best posttreatment predictor of long-term outcomes after CIT. Newer oral B-cell receptor signaling pathway inhibitors are highly effective at controlling disease, but best monotherapy responses are typically partial remission, and patients must remain on treatment to maintain disease control. Therapeutic progress is still needed for CLL. We propose that targeting MRD provides opportunity to realize this progress. Achieving BM MRD-negative CR is a prerequisite for long-term unmaintained disease-free survival and potential for cure. We review available methodologies for detecting MRD and correlations with posttreatment outcomes. We discuss the potential utility of MRD to direct individualized therapy. Finally, we discuss the importance of MRD-negative status as a surrogate marker for longer PFS in clinical studies to allow more rapid determination of clinical benefit. (Blood. 2016;127(3):279-286)

Introduction

Accurate quantitation of posttreatment residual disease burden in chronic lymphocytic leukemia (CLL) is prognostically relevant. Achievement of complete remission (CR) is associated with superior progression-free (PFS) and overall survival (OS) in first-line and relapsed/refractory CLL. Most patients who achieve CR have persistent, low-level disease, which is not detected with routine imaging and laboratory tests but can be demonstrated in bone marrow (BM) and/or blood with more sensitive flow cytometry (FLC) and molecular methods, so-called minimal residual disease (MRD). These residual cells ultimately are responsible for clinical relapse. The timing of relapse depends on the quantity of residual disease and kinetics of residual leukemia cell division. Sensitive methods to detect and quantify MRD were developed to more accurately predict subsequent clinical outcomes. Efforts to establish standards for MRD detection have implemented multiparameter FLC and polymerase chain reaction (PCR) assays with sensitivity to detect $\leq 1$ CLL cell in 10,000 normal leukocytes (ie, a sensitivity of $\leq 10^{-4}$). Achieving MRD-negative remission is the single most important predictor of PFS and OS in patients treated with chemoimmunotherapy (CIT), independent of clinical remission status and pretreatment patient characteristics.

The ability to achieve deep remission depends on the therapeutic agents and strategy: chlorambucil monotherapy is palliative, with few CRs and limited effect on the natural history of CLL. However, durable, unmaintained CR is achieved in the majority of fit patients treated with fludarabine-based CIT regimens containing CD20 monoclonal antibody (mAb). CR can also be achieved in some elderly patients and those with comorbidities with chlorambucil combined with the CD20 mAbs obinutuzumab and ofatumumab.

The therapeutic paradigm with small molecule inhibitors of the B-cell receptor (BCR) signaling pathway is distinct. The BCR signaling pathway inhibitors ibrutinib and idelalisib achieve rapid and dramatic nodal reduction and improvement in hemoglobin and platelet count. Partial response is achieved by most patients treated with these agents at used for relapsed/refractory CLL, most often due to persistence of bone marrow (BM) disease; MRD-negative CR is rare. Long-term continuous therapy with these agents is required to achieve durable disease control. The CR rate for ibrutinib monotherapy is higher in the first-line setting, but MRD-negative CR remains rare. To date, MRD testing has focused predominantly on its ability to predict clinical outcomes in clinical trials. We speculate that the future will see MRD-directed, individualized therapeutic approaches, for the following reasons: first, widespread availability of highly sensitive MRD testing; second, the association between MRD-negative remission and improved clinical outcomes; finally, the development of therapeutic strategies to achieve MRD-negative remissions in higher numbers of patients with reduced toxicity, such that a higher proportion of patients will be suitable for therapy designed to achieve deep remissions. Such approaches may allow attenuating treatment based on early response and the use of consolidation therapies in patients with persistent MRD positivity and/or high-risk disease biology.

In this review, we discuss available MRD testing methods, existing clinical data using these methods, and how MRD testing may be further developed and applied in the future using existing and novel therapeutic strategies.
Characteristics of an ideal MRD test

In CLL, an ideal MRD assay should have the following characteristics: quantitative, with specific levels predictive of time-to-clinical events; standardized methodology and interpretation of laboratory data, to allow comparison between laboratories and clinical trials; broadly applicable, regardless of pretreatment biological characteristics; high throughput, with rapid turnaround time; and relatively noninvasive, technically simple, with objective and easy to interpret results.

CLL is a multicompartmental disease, nearly always involving BM, blood, lymph nodes, liver, and spleen (macroscopically or microscopically) prior to treatment. Following treatment, 1 or more of these disease sites may act as a “reservoir” for residual disease. As such, MRD testing in CLL is potentially more complex and challenging than for other leukemias, because current methodology focuses on sampling low-level disease from blood or BM, which is likely limiting.

Methodology for detection of MRD in CLL

Table 1 summarizes the methods used for detecting MRD in CLL.22 Standardization currently requires an acceptable MRD assay to have a sensitivity of \( \geq 10^{-4} \) mononuclear cells (0.01% or \( 10^{-5} \)), regardless of site of evaluation.

**Flow cytometry**

Basic 3-color FLC for CLL detects CD5/19 coexpression and k/λ light chain restriction. It is inadequate because sensitivity approximates 1% \( (10^{-3}) \), limited by the presence of normal CD5/19- B cells and difficulty demonstrating monoclonality when B-cell count is low.23-26

Rawstron et al standardized and optimized a 4-color FLC technique with sensitivity of \( \geq 10^{-4} \) (0.01%), consisting of multicolor mAb panels to detect antigens differentially expressed on CLL vs normal B and T cells. It does not require patient-specific reagents and can be performed in most clinical FLC laboratories.2 It is quantitative and can be performed on blood and BM.27 This method has been widely adopted in clinical trials and can be used in routine practice. There were initial concerns that rituximab-induced loss of CD20 expression would impair performance, because dimmer CD20 expression on CLL cells relative to normal B cells is a specific differentiating feature. However, due to normal B-cell depletion by rituximab, this does not affect assay performance in practice.28 Interpretation is, however, somewhat operator dependent, and it is less sensitive than molecular methods; the main determinant of sensitivity is the number of viable leukocytes analyzed.

Sensitivity of \( 10^{-4} \) requires acquisition of \( \geq 500,000 \) events; sensitivity may, therefore, be lower in hypocellular specimens.29 Taking advantage of technological advances, FLC methodology is being further refined; 6-color,2 8-color,29,30 and 10-color methods were developed.31 The latter is a single tube assay and sensitivity approaches \( 10^{-5} \) if \( 1.8 \times 10^6 \) total cells are analyzed. Finally, addition of the tumor-specific antigen CD160 may achieve sensitivity of 0.01% to 0.001% in a 6-color, single-tube assay.32

**PCR-based molecular methodologies**

PCR using consensus primers that bind to conserved regions of the immunoglobulin heavy chain variable (IGHV) gene, adjacent to the complementarity determining region 3 (CDR3), has limited sensitivity in detecting MRD; although as few as 2% CLL cells can be detected in a background of normal B cells, amplification of background polyclonal IGHV limits sensitivity to \( \sim 10^{-2} \).27 The ability to identify the malignant clone in the background of polyclonal B cells depends on the uniqueness of the CDR3 in the CLL clone, with resultant interpatient variability in assay sensitivity.3 Finally, some CLL clones with mutated IGHV will not amplify due to failure of binding of consensus primers.

PCR using patient-specific primers achieves sensitivity of \( 10^{-5} \).9 The IGHV of the malignant clone is sequenced, and allele-specific oligonucleotide (ASO) probes designed for the specific IGHV CDR3 region. The PCR reaction specifically expands the malignant IGHV gene. Using real-time quantitative PCR (RQ-PCR) methodology, the assay is quantitative.33-35 If combined with a consensus PCR step (nested ASO IGHV PCR), sensitivity can be increased \( (\sim 10^{-6}) \), but quantitative ability is lost.36 The quantitative range and sensitivity need to be determined for each individual patient; thus, it is labor intensive, expensive, and time-consuming.9 Advantages compared with flow cytometry include higher sensitivity, requirement for less material and the stability of DNA at room temperature, which allows testing of older samples.9 Standardized procedures have been adopted to improve interlaboratory concordance and reproducibility.35

A novel PCR-based method uses high-throughput sequencing (HTS).37 The patient’s clonotype is sequenced at baseline. In post-treatment samples, consensus (rather than patient-specific) primers universally amplify IGHV gene segments, followed by performance of HTS; the malignant clone is quantified algorithmically within the polyclonal background, which means the assay does not require validation for individual patients.38 This methodology is highly sensitive \( (\sim 10^{-6}) \), quantitative to \( 10^{-5} \). Cells from blood or BM can be evaluated. The assay can also detect cell-free DNA in plasma. In diffuse large B-cell lymphoma, a predominantly nodal disease, HTS for IGHV genes to detect cell-free tumor-associated DNA was both sensitive and specific for the early detection of relapsing disease.39
Plasma evaluation for cell-free DNA was 2 log more sensitive at detecting residual disease than testing the cellular fraction of blood.\textsuperscript{40,41} Cell-free DNA in plasma is likely present due to apoptosis/necrosis of tumor cells in the tissues.\textsuperscript{42} Thus far, HTS MRD testing in CLL has only been performed on the mononuclear cell fraction of blood.\textsuperscript{37} Nonetheless, testing of the plasma component for cell-free DNA may hold promise for assessing the presence of low-level residual disease in the extravascular compartments and its sensitivity is an important future research question.

**Clinical data using MRD analysis**

MRD correlated with PFS and OS in studies using diverse treatments and MRD analysis strategies.

**MRD assessment following combination chemotherapy and CIT in first-line treatment**

Table 2 summarizes studies in treatment-naïve patients treated with combination chemotherapy or CIT, where MRD analysis with a sensitivity of $10^{-5}$ was performed.\textsuperscript{43,45} In the German CLL Study Group (GCLLSG) CLL8 study, the level of MRD in blood and BM at final response assessment correlated with both PFS and OS, independent of pretreatment patient characteristics and treatment group. However, the likelihood of achieving MRD-negative remission was lower in patients with high-risk biological features and those in treated with fludarabine and cyclophosphamide (FC) vs fludarabine, cyclophosphamide, and rituximab (FCR).\textsuperscript{10} Nevertheless, achieving MRD-negative status was an independent predictor of PFS after treatment with combination chemotherapy\textsuperscript{3,30} and both low-intensity\textsuperscript{15} and moderate intensity CIT regimens.\textsuperscript{10,46-48} Level of MRD was significantly associated with OS in all series where it was reported.\textsuperscript{10,46-49}

**MRD assessment post-salvage treatment and in treatment of high-risk populations**

Table 2 summarizes studies reporting clinical outcomes by MRD status in relapsed/refractory or high-risk populations. MRD-negative remission was associated with superior PFS after FCR treatment,\textsuperscript{5} in patients with **TP53** deletion treated with alemtuzumab plus high-dose methylprednisolone,\textsuperscript{50} and with salvage alemtuzumab monotherapy,\textsuperscript{51} independent of clinical response category.

**MRD assessment post-allogenic stem cell transplant**

Studies showed that MRD-negative status at 12 months post–allo-SCT was associated with superior event-free survival (EFS; Table 4), using 4-color FLC (sensitivity $10^{-5}$),\textsuperscript{52} nested ASO IGHV PCR assay (sensitivity $10^{-6}$),\textsuperscript{53} and HTS-based consensus IGHV PCR (sensitivity $10^{-6}$).\textsuperscript{37} Logan et al compared the predictive ability of the HTS PCR assay vs 4-color FLC: EFS at 6-10 years; 53% vs 16%\textsuperscript{37} and not reported Not reported Not reported.

### Table 2. Clinical significance of posttreatment MRD analysis as determined by a method with sensitivity of at least $10^{-4}$, after first-line combination chemotherapy or chemoimmunotherapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>No. of patients with MRD testing, (% MRD negative)</th>
<th>MRD threshold, sample source, method</th>
<th>PFS</th>
<th>$P$ value</th>
<th>Overall survival</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosch et al 2008\textsuperscript{3}</td>
<td>FCM</td>
<td>18 (41%)</td>
<td>$10^{-4}$, FLC BM</td>
<td>MRD-positive CR &lt; MRD-negative CR</td>
<td>.2</td>
<td>Not reported</td>
<td>NA</td>
</tr>
<tr>
<td>Lamanna et al 2009\textsuperscript{39}</td>
<td>F→C→R</td>
<td>23 (52%)</td>
<td>Nested ASO IGHV/PCR $10^{-5}$</td>
<td>35 months vs NR</td>
<td>.007</td>
<td>Not reported</td>
<td>NA</td>
</tr>
<tr>
<td>Maloum et al 2009\textsuperscript{44}</td>
<td>FC</td>
<td>21 (64%)</td>
<td>$10^{-4}$, FLC PB</td>
<td>DFS, median/HR not reported</td>
<td>&lt;.001</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>Bottcher et al 2012\textsuperscript{10}</td>
<td>FC or FCR</td>
<td>290*†</td>
<td>$10^{-2}$ and $10^{-4}$, FLC PB</td>
<td>15, 41, and 69 months for $\geq 10^{-2}$, $\geq 10^{-4}$ to $&lt; 10^{-2}$, and $&lt; 10^{-4}$, respectively</td>
<td>&lt;.001</td>
<td>Significantly inferior for $\geq 10^{-2}$ vs $&lt; 10^{-2}$</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fischer et al 2012\textsuperscript{46}</td>
<td>BR</td>
<td>45 (58%)</td>
<td>$10^{-2}$ and $10^{-4}$, FLC PB</td>
<td>12 months, 32 months, and NR for $\geq 10^{-2}$, $\geq 10^{-4}$ to $&lt; 10^{-2}$, and $&lt; 10^{-4}$, respectively</td>
<td>&lt;.001</td>
<td>23.2 months for $\geq 10^{-2}$ vs NR for $&lt; 10^{-2}$</td>
<td>Not reported</td>
</tr>
<tr>
<td>Abriqueta et al 2013\textsuperscript{37}</td>
<td>R→FCM</td>
<td>63 (56%)</td>
<td>$10^{-4}$, FLC BM</td>
<td>4 years; 86% vs 60%‡</td>
<td>.03</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Strati et al 2014\textsuperscript{48}</td>
<td>FCR</td>
<td>161 (43%)</td>
<td>$10^{-4}$, FLC BM</td>
<td>HR 0.1 (median NR)‡</td>
<td>.03</td>
<td>HR 0.6 (median NR)‡</td>
<td>.02</td>
</tr>
<tr>
<td>Goede et al 2014\textsuperscript{49}</td>
<td>Obinutuzumab + Cib</td>
<td>133 (20%) in BM, 231 (38%) in PB</td>
<td>$10^{-4}$, FLC PB/BM</td>
<td>19.4 months vs NR‡</td>
<td>&lt;.001</td>
<td>Not reported</td>
<td>NA</td>
</tr>
<tr>
<td>Kwok et al 2014\textsuperscript{45}</td>
<td>Predominantly F-based combinations</td>
<td>57 first-line (42%)</td>
<td>$10^{-4}$, FLC BM</td>
<td>5 years; 81% vs 16%‡</td>
<td>&lt;.001</td>
<td>10 years; 53% vs 24%‡</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

BR, bendamustine and rituximab; Cib, chlorambucil; DFS, disease-free survival; F, fludarabine; FCM, fludarabine, cyclophosphamide and mitoxantrone; F→C→R, sequential fludarabine, high-dose cyclophosphamide, and rituximab; PB, peripheral blood.

*Patients with end-of-treatment flow cytometry results only.
†Significance of intermediate MRD ($10^{-2}$-$10^{-4}$) was dependent on the site of assessment; patients with intermediate MRD level in PB had an inferior outcome to those with this level of MRD in marrow only (although numbers were small).
‡MRD showed prognostic significance independent of clinical response and baseline prognostic features.
preemptive intervention, particularly with immunomodulation (withdrawing immunosuppression ± donor lymphocyte infusion), when disease burden is low and the intervention potentially has maximal efficacy. To date, no studies specifically analyzed the efficacy of MRD-directed intervention. In the GCLLSG CLL3X study, 52 patients who were MRD negative had lower relapse risk and superior EFS; MRD-directed preemptive donor lymphocyte infusion achieved MRD-negative CR in 3 of 6 patients. Patient number was small, and interventions were not protocol mandated; the role of MRD-directed, preemptive immunomodulatory therapy requires systematic investigation.

Why should we aim to achieve MRD-negative state and should this be the goal in all patients?

MRD-negative remission after CIT is highly correlated with PFS and OS. In the original phase 2 MD Anderson FCR cohort. 2,14 a ligase-based PCR assay for clonal IGHV was performed at final response assessment. 54 This assay achieved greater discrimination of outcomes than clinical assessment of remission status alone; in patients who achieved CR, median PFS was 13.7 years in MRD-negative patients vs 6.2 years in MRD-positive patients (P < .0001). Patients with mutated IGHV who were MRD negative had a PFS of almost 80% at 13 years. 55 The potential to achieve highly prolonged, disease-free survival after first-line therapy, in a subgroup of patients, places additional importance on MRD as an end point, because achieving MRD-negative CR is a prerequisite for such an outcome.

Potential benefits of achieving MRD-negative status and long-term remission without maintenance therapy include avoidance of potential for cumulative toxicity from maintenance or sequential therapies and reduction in the financial burden on the patient and society through a delay in the use of expensive targeted therapies. Achieving MRD-negative status may reduce the likelihood of developing resistance mutations, although this remains to be proven. Finally, CLL cells induce immune dysfunction, 56 which leads to increased susceptibility to infection, autoreactivity, and potentially other cancers 57; an important future question is whether achieving deep remissions can reverse this immune dysfunction. However, the beneficial effects on immune function from achieving MRD-negative status must be balanced with the toxicity of the therapy required to achieve this. Fludarabine-based CIT, the therapy most able to achieve MRD-negative status, is associated with short and long-term immunosuppression, related to neutropenia and profound lymphopenia. In addition, long-term toxicity must be considered, such as the development of secondary myelodysplasia and acute myeloid leukemia. The only therapy shown to potentially enhance immune reconstitution is lenalidomide, which is associated with increases in immunoglobulin levels with monotherapy 58 and improvements in T-cell function in vitro. 59,60

Recently, several potent novel therapies have shown potential to achieve MRD-negative remission with modest toxicity, bringing this goal potentially within reach of the majority of patients. The type II CD20 mAb obinutuzumab 55 combined with chlorambucil achieved MRD-negative status in 19.5% of patients in BM and 37.5% in blood. Patients were elderly or had comorbidities and the regimen was well tolerated. Long-term follow-up of a phase 2 study of lenalidomide monotherapy as first-line treatment in older patients showed that MRD-negative CR was achieved in 8% of patients. 47 The B-cell CLL/lymphoma 2 (BCL-2) inhibitor venetoclax can also achieve MRD-negative remission in relapsed/refractory patients, as monotherapy; 22% achieved CR/CRi with incomplete marrow recovery (CRi); 5 of 9 CR/CRi patients tested were MRD negative. 61 A phase 1 dose escalation study of venetoclax plus rituximab demonstrated MRD-negative BM in 24 (49%) of 49 patients. 62 As experience is gained withibrutinib

### Table 3. Results of posttreatment MRD assessment and correlation with PFS and survival in patients treated for relapsed/refractory or high-risk CLL

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>No. patients tested for MRD (% MRD negative)</th>
<th>MRD threshold, sample source, method</th>
<th>PFS (months)</th>
<th>P value</th>
<th>Survival (months)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wierda et al 2005 55</td>
<td>FCR</td>
<td>52 with CR (38%) Qualitative PCR, 10⁻⁵, BM</td>
<td>27 vs 44 months (TPP) NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moreton et al 2005 51</td>
<td>Alemtuzumab</td>
<td>34 with CR (53%) 10⁻⁵, FLC BM</td>
<td>20 months vs NR (TFS) &lt;.001</td>
<td>41 months vs NR &lt;.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pettit et al 2012 50</td>
<td>Alemtuzumab + HDMP</td>
<td>25 (36%) 10⁻⁴, FLC BM</td>
<td>Median 24 vs 10 months .009</td>
<td>Not reported NA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HDMP, high-dose methylprednisolone.

### Table 4. Results of landmark analyses of post–Allo-SCT to determine associations between achieving MRD-negative status using highly sensitive testing methodology and subsequent PFS

<table>
<thead>
<tr>
<th>Study</th>
<th>No. patients (% MRD-negative at landmark)</th>
<th>Method</th>
<th>Sensitivity, quantitative?</th>
<th>Time point (months)</th>
<th>PFS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moreno et al 2006 59×</td>
<td>22 (59%) Three- or 4-color FLC</td>
<td>10⁻⁴, yes</td>
<td>3-6</td>
<td>16 vs 75 months</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Farina et al 2009 53</td>
<td>29, all in CR (48%) Nested ASO IGHV PCR from BM</td>
<td>10⁻⁵, 10⁻⁴, no</td>
<td>6</td>
<td>93% vs 46% at 12 months</td>
<td>.012 and .037</td>
<td></td>
</tr>
<tr>
<td>Dreger et al 2010 81</td>
<td>39 (69%) Four-color FLC from BM 10⁻⁴, 12 months</td>
<td>10⁻⁴, yes</td>
<td>12</td>
<td>HR for event-free survival 0.13 if MRD negative</td>
<td>.013</td>
<td></td>
</tr>
<tr>
<td>Logan et al 2013 37</td>
<td>31 (48%) Consensus IGHV PCR using HTS from PB†</td>
<td>10⁻⁶, quantitative from ≥10⁻⁵</td>
<td>12</td>
<td>93 vs 38% at 50 months</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

* Fifteen patients having Allo-SCT had insufficient data to determine effect of MRD on survival. Data presented on patients having autologous stem cell transplant.  
† Cellular fraction of peripheral blood tested.
and ibritinib plus rituximab in the first-line setting, it will be important to note if extended treatment is associated with continued deepening of responses and achievement of MRD-negative state, analogous to that seen in chronic myeloid leukemia treated with imatinib,\textsuperscript{63} and whether this may allow study of treatment cessation in some patients. Combinations of novel therapies are likely to considerably increase the proportion of patients who achieve MRD-negative remission. For example, there is in vitro synergy between ibritinib and venetoclax\textsuperscript{64}, a study is planned at our institution with this combination and a study with a combination of ibritinib, venetoclax and obinutuzumab is ongoing (NCT02427451). Whether achieving MRD-negative remission with such combinations results in durable, unmaintained remission analogous to that seen after CIT will be an important future question, as will the impact of pretreatment disease characteristics on duration of such remissions.

Important unanswered questions in clinical use of MRD analysis

What is the optimal site to test?

Few studies directly compared MRD analysis from blood and BM. Abrisqueta et al showed that 12 of 57 (21\%) patients treated with fludarabine, cytarabine, mitoxantrone, and rituximab were MRD negative in blood but remained MRD positive in BM.\textsuperscript{46} Several studies also reported results from both blood and BM in nonpaired analyses. In the GCLLSG CLL8 study, 63\% of patients were MRD negative in blood at final response and 44\% in BM\textsuperscript{10}; BM assessment was only performed to confirm clinical/radiologic CR, enriching the tested population for patients most likely to have achieved MRD-negative status. In patients treated with bendamustine and rituximab, 57.8\% and 29.2\% were MRD negative after treatment in blood and BM, respectively.\textsuperscript{46} Finally, after low-intensity CIT with obinutuzumab and chlorambucil, 20\% were MRD-negative in BM and 38\% in blood. Again, these were nonpaired specimens, with BM done to confirm CR. Thus, MRD is more likely to be present in BM than blood at completion of CIT.

The site of MRD sampling may significantly affect the predictive ability of the test. Fisher et al showed after treatment with bendamustine and rituximab, the median PFS was significantly shorter for a given level of MRD measured from blood compared with BM (PFS not reached [NR] vs 32.4 vs 11.7 months from blood compared with NR vs NR vs 29.7 months from BM for MRD levels of $<10^{-4}$, $10^{-4}$ to $<10^{-2}$ and $10^{-2}$, respectively). Thus, detection of MRD in blood was associated with short response duration; patients with MRD in blood after treatment may therefore represent an appropriate group in whom to study novel consolidation strategies. For treatment delivered with curative intent (eg, post-allo-SCT), MRD-negative CR is likely essential. Given higher rates of MRD after treatment in BM, assessment of BM MRD would achieve the highest negative predictive value for predicting subsequent relapse risk. In contrast, if the primary purpose of the assay is to identify poor-risk patients who may benefit from consolidation and/or maintenance therapy, then blood assessment may suffice.

While MRD testing can directly assess cells in blood and BM, residual disease in lymph nodes, liver, and spleen cannot be directly or easily assessed by these methods. Computed tomography and positron emission tomography have limited sensitivity and specificity for detecting small volume disease. HTS-based testing of plasma, as discussed earlier, may be evaluated in the future to detect MRD in these sites.

Impact of pretreatment biological characteristics on interpretation of MRD results

Achieving MRD-negative remission is associated with superior PFS and OS, independent of pretreatment prognostic features.\textsuperscript{10} However, the likelihood of achieving MRD-negative remission is lower for patients with high-risk prognostic features and those receiving less effective therapy. Additionally, in multivariable analysis, pretreatment high-risk characteristics, such as unmutated IGHV, ZAP70 positivity, and del(11q), were associated with more rapid reappearance of MRD and shorter PFS.\textsuperscript{3,65}

Disparities between clinical staging and MRD analysis

In the GCLLSG CLL8 study, 39\% and 28\% of patients who remained in partial remission (PR) by clinical criteria were MRD negative in blood and BM, respectively, at final response.\textsuperscript{10} Updated analysis showed no difference in PFS between patients in MRD-negative CR vs MRD-negative PR; patients in MRD-negative PR had superior PFS to those in MRD-positive CR.\textsuperscript{66} Thus, achieving posttreatment MRD-negative remission with CIT may be more important than achieving clinical CR. In first-line treatment with obinutuzumab plus chlorambucil, 38\% of patients in CR were MRD negative in blood compared with 31\% of patients in PR.\textsuperscript{15} Similar results were seen in patients treated with venetoclax plus rituximab, where 50\% of patients in PR and 70\% of patients in CR were MRD negative.\textsuperscript{62} The existence of MRD-negative partial remission appears contradictory. However, there are 2 potential explanations for this phenomenon: first, residual lymph nodes of $\geq1.5$ cm by computed tomography, in the setting of MRD-negative remission, may not contain viable tumor (eg, with residual enlargement instead representing fibrosis). Second, lymph node–resident CLL cells may be more resistant to certain therapies, allowing persistence of viable tumor in lymph nodes, despite clearance of CLL from the blood and marrow. Biopsy of enlarged lymph nodes or novel techniques to detect MRD are needed to determine the clinical relevance of residual nodal enlargement in such patients who are MRD negative by FLC.

Use of MRD results to guide treatment decisions

One proposed goal of MRD assessment is to develop risk-adapted treatment strategies, which requires prospective testing in clinical trial. Patients with MRD at final response assessment could be candidates for treatment intensification, consolidation, or maintenance strategies. Furthermore, patients who achieve early MRD-negative status may be candidates for treatment de-escalation to limit treatment-related toxicity.

Consolidation treatment

Consolidation therapy treats low-level residual disease, expected to otherwise produce clinical relapse, with non–cross-resistant agents, to achieve a greater depth of response. Treatment at a time of lower disease burden could reduce likelihood of developing drug resistance.

Studies evaluated the CD52 mAb alemtuzumab in this setting. Alemtuzumab improved PFS after fludarabine or FC chemotherapy, but was associated with significant infectious toxicity.\textsuperscript{67,68} Alemtuzumab consolidation after fludarabine and rituximab improved response: 61\% of patients in PR achieved CR (50\% MRD negative) and 43\% of patients with MRD-positive CR became MRD negative.
However, infection-related mortality was unacceptably high; consequently, no PFS or OS benefit was seen. Thus, consolidation with alemtuzumab after CIT is not recommended. Lenalidomide consolidation after CIT with pentostatin, fludarabine, and cyclophosphamide improved responses in 24% of patients with measurable disease; 4 became MRD negative, and time to next treatment appeared prolonged relative to historic data. However, many patients required dose reduction, particularly due to hematologic toxicity. The Cancer and Leukemia Group B 10404 study will further clarify the utility of lenalidomide consolidation after fludarabine-based CIT.

Toxicity of any consolidation therapy must be balanced against the benefit of improving depth of response, as the experience with alemtuzumab showed. Availability of potent novel therapies with more favorable toxicity profiles may lead to reexamination of the feasibility of MRD-directed consolidation after CIT. Venetoclax and obinutuzumab can induce MRD-negative CR and would likely be well tolerated as consolidation therapy. In addition, other novel therapies, such as immune checkpoint inhibitor mAbs and cellular therapy, are likely to be tested in this setting in the future.

MRD-guided therapy based on interim-analysis of MRD levels

No published study has prospectively used interim MRD levels to guide therapeutic decisions. In the GCCLLS CL18 study, MRD analysis was performed in blood after 3 cycles of FC/FCR: all patients subsequently received 6 cycles, regardless of interim MRD results; and those achieving MRD-negative status had similar PFS, regardless of whether 3 or 6 cycles were required to achieve MRD-negative remission. Strati et al reported MRD results in treatment-naïve patients treated with FCR: MRD analysis was performed after 3 cycles and at completion of therapy; 17% and 43% of patients achieved MRD-negative status in BM after 3 and 6 cycles, respectively. Those who achieved MRD-negative status after 3 cycles and stopped treatment, most commonly due to therapy-related toxicity, had similar PFS as those who were MRD negative after 3 cycles but received the full 6 planned cycles and also to those who were MRD positive after 3 cycles but achieved MRD-negative status after 6 cycles. Taken together, these data suggest that early treatment cessation in patients who achieve MRD-negative status after 3 cycles of FCR may be feasible without compromising long-term disease control, but this requires prospective study.

Use of MRD analysis as a clinical trial end point

Given the prolonged PFS after CIT, very long follow-up is required to determine whether PFS after receiving novel agents or regimens is superior to existing CIT regimens; this may significantly delay new drug development. Given the strong correlation between achieving MRD-negative status and survival outcomes, MRD status is receiving consideration from regulatory agencies as a potentially meaningful end point for clinical trials. A major caveat to this is that MRD assessment cannot generally be used to assess the efficacy of novel regimens such as ibrutinib as monotherapy or in combination with rituximab, given that these regimens rarely achieve MRD-negative status and are given continuously until disease progression.

Summary

Existing treatment paradigms in CLL are undergoing significant change. Deep remissions are achieved in a large number of fit patients receiving first-line therapy, and those patients with mutated IGHV potentially are treated with curative intent. Many other patients may achieve prolonged treatment-free remissions. Development of rational combinations and sequencing with novel therapies will likely further increase the number of patients achieving such remissions. The availability of sensitive and specific methods to quantify residual disease may allow individualized therapy in the future. Patients who achieve early MRD-negative status may have treatment de-escalated to limit toxicity, whereas those initially failing to achieve MRD-negative remission could receive consolidation or maintenance therapy with non-cross-resistant agents in the setting of low disease bulk, aiming to achieve an MRD-negative state, at which point treatment could be stopped with careful monitoring. For this to be a reality, highly sensitive and specific MRD detection methodology must be available. Whereas multicolor FLC is the current standard, more sensitive methods such as IGHV-HTS may ultimately prove to have superior predictive power; however, this will need to be determined prospectively.

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