

# Clinical Significance and Management of MRD in Adults With Acute Lymphoblastic Leukemia

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**Abstract:** Measurable residual disease (MRD) quantification is an essential component of caring for patients with acute lymphoblastic leukemia (ALL). Many studies in pediatric and adult populations have validated the prognostic significance of MRD early in and throughout the course of treatment for ALL, and it is generally accepted that achievement of MRD less than  $10^{-4}$  (0.01%) is a critical milestone. ALL is uniquely amenable to quantification of MRD by multiple techniques, including multiparameter flow cytometry, various allele-specific and mutation-specific quantitative polymerase chain reaction methods, and more recently amplicon-based next-generation sequencing. Quantification of MRD with these high-sensitivity methods not only facilitates risk stratification, but also is used to determine appropriateness of intensified therapy, such as allogeneic hematopoietic cell transplant, as well as MRD-targeted therapy with blinatumomab. We review the data supporting the use of MRD quantification in ALL to guide clinical decision-making.

## Introduction

Measurable residual disease (MRD, previously known as minimal residual disease) in acute lymphoblastic leukemia (ALL) can be defined as the amount of residual leukemic cells that remain in the bone marrow and/or circulating in the peripheral blood after treatment. Although the goal of cytoreductive therapy is to eradicate all malignant cells, a substantial portion of patients have MRD after therapy that ultimately leads to relapse (Figure, A). With the development of high-sensitivity quantification, such as multiparameter flow cytometry (MFC), reverse transcription quantitative polymerase chain reaction (RQ-PCR), quantitative PCR (qPCR), and amplicon-based next-generation sequencing (NGS), clinicians are now able to more directly and deeply assess how well patients respond to therapy. MRD quantification using these high-sensitivity techniques facilitates assessment of patient-specific responses far beyond the narrow dynamic range within which clinical remission

## Keywords

Acute lymphoblastic leukemia, measurable residual disease, minimal residual disease

is classically defined (ie, achievement of <5% blasts). The level of MRD at any time reflects the response achieved to primary induction and consolidation therapies, as well as immunologic effects that have the potential to counteract relapse. Response to treatment is antagonized by high-risk disease features, niche protective effects, and immunologic evasion (Figure, B). An MRD threshold of  $10^{-4}$  (0.01%) leukemia cells has been recognized in several studies as a cut-off that can differentiate between patients at higher risk of relapse and those with a lower, but non-negligible, risk of relapse. Achieving MRD negativity at this threshold is the single most important prognostic factor in treating patients with ALL.

In a recent meta-analysis evaluating 39 studies with 13,637 patients in both pediatric and adult populations with ALL, MRD negativity uniformly correlated with significantly better outcomes in all studies.<sup>1</sup> Ten-year event-free survival within the first 3 cycles of chemotherapy was 64% for adults achieving MRD-negative status vs 21% in those with MRD-positive disease. It is important to recognize that this meta-analysis incorporated results of trials across different types of chemotherapy and protocols around the globe, and all demonstrated the value of assessing MRD status early in the course of therapy. Based on the wealth of data supporting MRD quantification as a prognostic indicator and clinical decision-making tool, the National Comprehensive Cancer Network and the European Society for Medical Oncology clinical practice guidelines both advocate for MRD quantification to be a standard component of caring for pediatric and adult patients with ALL.<sup>2,3</sup> Further recommendations for algorithmic application of MRD testing in B- and T-cell ALL have also been proposed.<sup>4</sup>

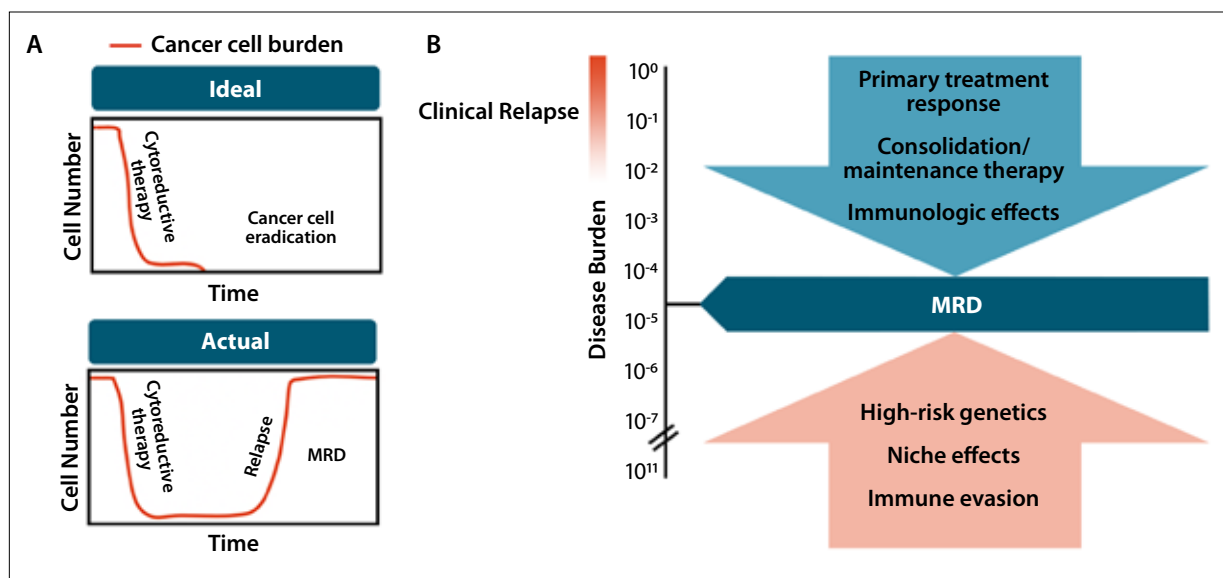
## Methods of MRD Monitoring

Because no gold standard method remains for quantifying MRD, clinical practice around the world varies. Most clinical trials and retrospective studies to date have used PCR-based techniques or MFC.

For patients with Philadelphia chromosome-positive (Ph+) ALL, the most common method of disease monitoring is *BCR-ABL* fusion gene quantification by RQ-PCR. This fusion gene is present in approximately 25% of B-cell ALL in adults. RQ-PCR measures RNA expression and is used in this setting to detect the number of *BCR-ABL* transcripts in the cells that remain in a bone marrow or peripheral blood specimen during or after treatment. The *BCR-ABL* subtype p190 is most commonly identified in B-cell ALL, but the p210 subtype may also be seen in de novo B-cell ALL and in cases arising from chronic myeloid leukemia (CML) in lymphoid blast crisis; both subtypes are readily quantified by RQ-PCR.<sup>5,6</sup> Several studies have

shown that MRD in patients treated with chemotherapy and tyrosine kinase inhibitors can predict outcomes in patients with Ph+ ALL early in the course of treatment.<sup>7-9</sup> Ravandi and colleagues, for instance, published a series of 122 patients with newly diagnosed Ph+ ALL and demonstrated that MRD monitoring by RQ-PCR after induction predicts outcomes. In this study, patients who achieved a major molecular response, defined as *BCR-ABL/ABL* (ie, *BCR-ABL* transcript count divided by *ABL* transcript count) less than 0.1% at 3, 6, 9, and 12 months, had significantly better overall survival (OS; 73% vs 33% at 3 months and 85% vs 25% at 12 months) than those who did not achieve a major molecular response.<sup>7</sup> The study further demonstrated that assessment for major molecular response was more specific as a predictor of outcome than MFC or immunoglobulin (Ig) heavy chain PCR using consensus PCR primers (which are less sensitive and specific than the allele-specific oligonucleotide PCR [ASO-PCR] or NGS methods for quantifying IG heavy chain rearrangements, discussed below). Short and colleagues analyzed data for a population of patients treated with conventional chemotherapy and imatinib, dasatinib (Sprycel, Bristol-Myers Squibb), or ponatinib (Iclusig, Ariad), and found that achievement of complete molecular remission (CMR) vs molecular response not achieved at 3 months of therapy strongly correlated with both median OS (126.5 vs 20.4 months, respectively;  $P=.005$ ) and median relapse-free survival (RFS; 125.7 vs 12.1 months, respectively;  $P=.002$ ).<sup>8</sup> This study defined CMR as the absence of a *BCR-ABL* transcript with a sensitivity less than 0.01%.

Most of the remaining patients with Ph-negative (Ph-) ALL do not have a unique translocation, such as *BCR-ABL*, that is suitable for mutation-associated molecular MRD quantification. Without a specific mutation for RNA amplification, MRD can be evaluated by other methods, such as MFC or immunoreceptor rearrangement quantification using qPCR or NGS. MFC uses a panel of fluorochrome-conjugate antibodies that can identify leukemic cells with a specific aberrant leukemia-associated phenotype. Quantification based on leukemia-associated phenotype can be achieved in more than 90% of cases and has a reliable sensitivity of detection of  $10^{-4}$  cells, provided the assay is specifically validated for MRD quantification.<sup>10</sup> Rarely, specialized labs may be capable of reporting deeper levels of sensitivity with adequately cellular specimens. The EuroFlow Consortium has demonstrated sensitivity in ALL to  $10^{-5}$  using a 2-tube 8-color antibody panel with at least  $4 \times 10^6$  cells analyzed.<sup>11</sup> For practical reasons, it is important to recognize that MFC performed for standard clinical immunophenotyping (which generally will have a detection limit of only ~0.1%) usually does not achieve adequate sensitivity for quantifying MRD.



**Figure.** Measurable residual disease (MRD) represents the remaining malignant cells present after cytoreductive therapy that ultimately lead to relapse (A). MRD quantification with highly sensitive techniques facilitates assessment of patient-specific responses to treatment (B).

MRD, measurable residual disease.

Another technique that has been widely used in clinical trials is qPCR, also known as ASO-PCR. This method relies on quantification of Ig and/or T-cell receptor (TCR) rearrangements using specific primers and a qPCR probe designed for each patient. With this method, junctional variable, diversity, and joining (VDJ)-specific sequences must be identified in leukemic cells at the time of diagnosis. Leukemia-specific Ig/TCR rearrangements may be identified from the initial diagnostic sample through NGS or a panel of screening PCRs and conventional Sanger sequencing.<sup>12-17</sup> Subsequently, patient-specific primer/probe sets must be generated for use in ASO-PCR-based quantification of the precise number of residual leukemic cells in a post-treatment specimen. The sensitivity of this method is at least  $10^{-4}$  in most cases and is technically feasible in roughly 90% of patients; however, the development of patient-specific assays is laborious, costly, and not amenable to approval by regulatory agencies in the United States, which has severely limited the applicability of this method. This approach is nevertheless used extensively by members of the European Study Group on MRD Detection in ALL (EuroMRD) throughout Europe.<sup>18,19</sup>

Amplicon-based NGS has more recently emerged as a powerful method for quantifying MRD that overcomes many of the logistical and regulatory barriers encountered with ASO-PCR. As with ASO-PCR, the NGS technique requires a diagnostic sample to identify leukemia-specific immunoreceptor gene rearrangements. Identification of Ig/TCR sequences uses multiplexed PCR with consensus primers to amplify the entire repertoire of 1 or more

specific Ig/TCR immunoreceptor genes within a sample, followed by NGS to quantify specific sequences. The sample can be derived from bone marrow aspirate, peripheral blood, or biopsy of tissues such as lymph nodes. Leukemia-specific rearrangements are then monitored through the course of therapy via bioinformatic interrogation of the repertoire derived from NGS analysis of serial samples.<sup>20-22</sup> Importantly, unlike ASO-PCR, the NGS technique accomplishes highly sensitive and specific repertoire analysis without the need to generate patient-specific oligonucleotides for qPCR. This method is applicable to approximately 90% of ALL cases and can readily achieve  $10^{-6}$  (0.0001%) sensitivity with adequately cellular samples.<sup>21-23</sup> Furthermore, it is the only MRD quantification method that has been cleared by the US Food and Drug Administration (FDA).

The NGS approach has been demonstrated to be more sensitive than FCM in both B- and T-cell ALL.<sup>24,25</sup> High degrees of correlation between NGS and both FCM and ASO-PCR have been observed when MRD burden is greater than  $10^{-4}$ .<sup>22,23,26</sup> Evidence exists to favor NGS over FCM for clinically relevant predictive value. In a study quantifying MRD in children who underwent hematopoietic cell transplant (HCT), NGS was found to be more specific than RQ-PCR. These findings were likely due to the massive B-lymphocyte regeneration that occurs in the post-transplant setting.<sup>27</sup> Another study also affirmed an advantage to using NGS over FCM because it was predictive of outcome at much earlier times (day +30 and day +100) after allogeneic HCT (alloHCT).<sup>28</sup>

## Clinical Significance of MRD Monitoring

In both pediatric and adult patients, MRD has been identified as the single most robust prognosticator of OS and leukemia-free survival. Studies contributing to the evidence base supporting use of MRD are plentiful and have been reviewed in 2 meta-analyses.<sup>1,29</sup> Therefore, this review provides a few instructive examples that demonstrate the utility of MRD quantification early in the course of treatment; the remainder of the review focuses on MRD quantification in specific settings.

The German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) evaluated the significance of MRD in adults with standard-risk leukemia.<sup>30</sup> In this study, the method of MRD quantification was Ig/TCR ASO-PCR, with a sensitivity threshold of  $10^{-4}$ . The trial evaluated 196 standard-risk patients who received induction with asparaginase-based BFM-like chemotherapy. MRD quantification was performed on days 11 (middle of induction phase 1), 24 (end of induction phase 1), and week 16 (end of first consolidation). Using the MRD status on days 11 and 24 and week 16, patients were stratified into low-, intermediate-, and high-risk categories. Low-risk patients had negative MRD on days 11 and 24. Ten percent of patients were in this category, and for this group the 3-year cumulative incidence of relapse was 0. For patients who were MRD-positive at both day 24 and week 16, 94% had relapsed within 3 years. Patients who did not meet criteria for low- or high-risk were considered in the intermediate-risk group, and had a relapse rate of 47%.<sup>30</sup>

In another study, Vidriales and colleagues demonstrated the importance of MRD as a prognostic factor by using MFC to evaluate MRD in adolescents and young adults.<sup>31</sup> A total of 102 individuals with ALL were evaluated, and only patients who were in complete morphologic remission after induction were included. Mandatory MRD evaluation of bone marrow samples was performed by MFC on day 35 and showed that patients with residual blast cells lower than 0.05% had significantly longer RFS than those with levels of 0.05% or higher (42 vs 16 months;  $P=.001$ ). All patients with MRD greater than 0.1% relapsed within 2 years.<sup>31</sup>

The Polish Adult Leukemia Group ALL 4-2002 MRD study also assessed the clinical significance of MRD status after induction in adults with Ph- ALL.<sup>32</sup> The method of MRD quantification used was MFC, which was assessed at the end of induction and consolidation therapy. The endpoint of the study was the prognostic significance of achieving MRD below 0.1% ( $10^{-3}$ ) after the end of induction and consolidation. The induction treatment consisted of asparaginase-based chemotherapy, and the consolidation included 2 courses of high-dose cytarabine and

cyclophosphamide. After consolidation, patients were separated into standard-risk and high-risk groups and treated accordingly. MRD was evaluated in 115 of 116 patients in complete remission (CR) after induction, and was considered positive ( $>0.1\%$ ), classifying patients as high risk, in 33%. After consolidation, 21.6% were positive and 42% had at least 1 positive MRD result at any point in the study. Achieving MRD less than 0.1% after induction was associated with a significant decrease in relapse (26% vs 81%) and improved leukemia-free survival (61% vs 17%) at 3 years.<sup>32</sup>

## MRD in the Setting of Allogeneic Hematopoietic Cell Transplant

AlloHCT is a potentially curative long-term consolidation therapy for patients with ALL. The therapeutic implications of MRD before and after alloHCT remain under investigation and it is yet unclear which patients with MRD-positive disease will derive the most benefit from this modality of treatment, particularly in the era of immunotherapy alternatives for MRD-directed therapy and treatment of frankly relapsed disease.

GRAALL (Group for Research on Adult Acute Lymphoblastic Leukemia) retrospectively analyzed transplant outcomes in patients who were treated in the GRAALL 2003 (Feasibility of Risk-Adapted Therapy in Young Adult Acute Lymphoblastic Leukemia: a Multicenter Trial) and GRAALL 2005 (Treatment of Acute Lymphoblastic Leukemia in Younger Adults) trials, which assessed the use of pediatric-inspired chemotherapy in adults with ALL.<sup>33</sup> A total of 282 patients who received a transplant in first CR (CR1) were included. All patients were considered high risk by conventional methods and had at least 1 of the following: central nervous system involvement, low hypodiploidy/near triploidy, early resistance to corticosteroids, poor early bone marrow blast clearance, late CR, and Ig/TCR MRD greater than  $10^{-2}$  after induction. Quantitative PCR-based MRD evaluation was performed 6 weeks after induction initiation and after the 3 blocks of consolidation (12 weeks after induction). The 3-year post-transplant nonrelapse mortality was 15.5% and survival was almost 70%. None of the high-risk criteria were associated with evidence of benefit from alloHCT. Good MRD response, defined as MRD less than  $10^{-3}$  after the first induction, was not associated with benefit from transplant. In patients who were MRD-positive, alloHCT was associated with longer RFS (hazard ratio [HR], 0.4). This suggests that MRD status is helpful for guiding the decision of whether chemotherapy consolidation or transplant should be offered to patients in first remission.

The GMALL also retrospectively analyzed data from adults with Ph- ALL who were enrolled in their trials

between April 1999 and July 2009.<sup>34</sup> MRD evaluation was consistently performed at days 11, 26, 46, and 71, and at week 16. The MRD quantification method was RQ-PCR with a minimum sensitivity of  $10^{-4}$ . A total of 1648 patients were studied and data from 580 patients met criteria for inclusion in the analysis. In this population, 76% of patients achieved MRD-negative disease ( $<10^{-4}$ ) by week 16. MRD-negative ALL was associated with higher continuous morphologic CR (69% vs 29%) and better OS (80% vs 42%) at 5 years from diagnosis. Patients with MRD-positive disease who were not undergoing alloHCT relapsed after a median of 7.6 months; continuous CR and survival at 5 years were 12% and 33%, respectively. A total of 120 patients had MRD-positive disease on week 16, and 47% underwent alloHCT in first CR. For patients who were MRD-positive, the probability of continuous CR after 5 years was significantly higher for patients who received alloHCT in CR1 compared with those not receiving alloHCT in CR1 (66% vs 12%;  $P<.0001$ ). These data suggest that patients with MRD-positive ( $>10^{-4}$ ) status after induction benefit from allogeneic (alloHCT), whereas survival is dismal in the absence of transplant.

### Use of MRD to Escalate Therapy

In the modern treatment of ALL, knowledge that MRD-negative status of less than  $10^{-4}$  has been associated with better clinical outcomes has been incorporated into practice patterns. Owing to this fact, escalation of therapy in order to achieve deeper MRD response is often believed to lead to better survival rates. In the face of abundant data demonstrating the relapse risk associated with MRD greater than  $10^{-4}$ , achieving complete hematologic remission alone is no longer the most crucial endpoint, so studies have investigated methods for achieving MRD negativity in those with detectable MRD at the end of induction.

In children, MRD-triggered interventions with therapy escalation were studied in the UKALL 2003 trial (United Kingdom Childhood Acute Lymphoblastic Leukaemia Randomised Trial) that enrolled ALL patients aged 1 to 24 years and classified them as clinical standard-risk, intermediate-risk, or high-risk.<sup>35</sup> A total of 553 MRD high-risk patients (those with MRD  $\geq 10^{-4}$  at day 29 of induction) were randomly assigned to receive standard therapy or augmented post-remission therapy. The augmented treatment regimen included 8 additional doses of pegylated asparaginase, vincristine, and dose-escalated intravenous methotrexate without folinic acid rescue. The 5-year event-free survival was better in the augmented treatment group than in the standard therapy group (89.6% vs 82.8%, respectively; odds ratio, 0.61;  $P=.04$ ).

The use of alloHCT as intensified consolidation based on MRD was evaluated in children at intermediate risk for relapse. The ALL-REZ BFM 2002 study (Multi-Center Study for Children With Relapsed Acute Lymphoblastic Leukemia) from the Berlin-Frankfurt-Münster Group evaluated patients with an MRD level of at least  $10^{-3}$  (by RQ-PCR) at the end of induction.<sup>36</sup> Patients deemed MRD-negative received standard consolidation chemotherapy, and patients with MRD positivity were assigned to alloHCT with related or unrelated donors. In the MRD-positive group, 83% had donors and received alloHCT. The RFS for patients with MRD positivity was 64%. This result was significantly better than that observed in the predecessor ALL-REZ BFM P95/96 study, in which only 18% of patients with these characteristics remained event-free without early assignment to transplant. This was achieved owing to the reduction of relapse with the ALL-REZ BFM 2002 regimen, including MRD intervention (59% vs 27%;  $P<.001$ ).<sup>36</sup>

In adults, initial attempts to optimize treatments of patients with MRD-positive disease investigated the use of consolidation with alloHCT. The Northern Italy Leukemia Group (NILG) studied patients who were MRD-positive ( $>10^{-4}$ ) prior to consolidation, and assigned patients to standard chemotherapy or alloHCT based on MRD status.<sup>37</sup> MRD was evaluated at weeks 10, 16, and 22 using RQ-PCR. Ph+ or t(4;11) patients were automatically assigned to the alloHCT arm. MRD positivity ( $>10^{-4}$ ) after consolidation was more significant than other genetic or clinical features as a predictor of relapse (HR, 5.33;  $P=.001$ ), demonstrating the power of MRD quantification to predict outcomes across a spectrum of genetic risk groups. After a median of 3 years of follow-up, the OS and disease-free survival rates were 0.75 and 0.72, respectively, in the MRD-negative group vs 0.33 and 0.14, respectively, in the MRD-positive group, indicating that this strategy was successful at identifying a population (MRD-negative  $<10^{-4}$ ) in which the majority could do well without transplant.<sup>37</sup> Unfortunately, the study also demonstrated poor outcomes for MRD-positive patients, even with alloHCT employed based on the presence of MRD after consolidation, reaffirming the need for better strategies to help such patients.

The PETHEMA ALL-AR-03 trial (Treatment of High Risk Adult Acute Lymphoblastic Leukemia) also explored the same question in patients with Ph- ALL who were assigned to alloHCT vs chemotherapy depending on their early cytologic response ( $>10\%$  blast in bone marrow at day 14) or an MFC-based MRD level greater than  $5 \times 10^{-4}$  at the end of consolidation.<sup>38</sup> Patients with good early cytologic response and MRD negativity were assigned to delayed consolidation and maintenance, and alloHCT was reserved for patients with MRD positivity

or poor early cytologic response. A total of 179 patients were assigned by intention to treat to receive alloHCT (n=71) or chemotherapy (n=108). The 5-year disease-free survival and OS were 32% and 37%, respectively, for patients assigned to alloHCT, and 55% and 59% for those assigned to chemotherapy, respectively, again demonstrating poor outcomes for patients who remain MRD positive after consolidation and raising questions about whether the benefit of early alloHCT seen in other studies is generally applicable.

### Blinatumomab in Patients With MRD-Positive B-Cell ALL

Blinatumomab (Blinicyto, Amgen) is a CD19/CD3 bispecific T-cell engager (BiTE) antibody construct that induces lysis of CD19-expressing leukemic cells by stimulating intercellular connection between the target cells and cytotoxic T lymphocytes. It was initially approved by the FDA to treat relapsed or refractory precursor B-cell ALL based on results of the multicenter phase 3 TOWER trial (Blinatumomab Versus Standard of Care Chemotherapy in Patients With Relapsed or Refractory Acute Lymphoblastic Leukemia), in which patients were randomly assigned to blinatumomab or standard cytotoxic chemotherapy reinduction.<sup>39</sup> Among 405 patients who were randomly assigned in this study, CR rates within 12 weeks after treatment were higher in the blinatumomab group than in the standard chemotherapy group (34% vs 16%;  $P<.001$ ) and CR with full, partial, or incomplete hematologic recovery was observed in 44% vs 25% ( $P<.001$ ) of the groups, respectively. Patients receiving blinatumomab also had significantly longer OS (7.0 vs 4.0 months;  $P=.01$ ). Improvement in OS was greater when blinatumomab was given in first CR with MRD (median, 36.5 months) vs second or later CR with MRD (median, 19.1 months).<sup>40</sup> Long-term survival data from the initial phase 2 blinatumomab trial also described outcomes of the 36 patients who received blinatumomab and demonstrated a median OS of 13 months, which was better than observed in the phase 3 study.<sup>41</sup> Ten out of the 36 patients were long-term survivors (OS >30 months). All long-term survivors were MRD-negative. Achievement of an MRD-negative response to blinatumomab was associated with significantly longer OS ( $P=.0009$ ), indicating that MRD negativity after salvage immunotherapy is also predictive of OS, as it is following conventional chemotherapy.

Prior to its application to relapsed/refractory B-cell ALL, blinatumomab was originally studied in patients in MRD-positive remission by Topp and colleagues.<sup>42</sup> In an initial study, 20 patients with MRD-positive disease (defined as  $>10^{-3}$ ) in first remission received blinatumomab.

Eighty percent of patients achieved an MRD-negative response, and after 33 months of median follow-up, RFS was 61%.<sup>42</sup> Although all patients were at high risk for relapse based on persistent MRD after 3 cycles of treatment, 6 of 11 long-term survivors did not receive alloHCT as additional consolidation, providing tantalizing data suggesting that blinatumomab treatment for MRD positivity may be associated with long RFS, even in the absence of subsequent alloHCT.

These positive data were subsequently confirmed in the larger phase 2 BLAST trial. In this trial, Gökbuget and colleagues again studied blinatumomab in patients in CR after 3 cycles of chemotherapy who remained MRD-positive ( $>10^{-3}$ ).<sup>40</sup> After 1 cycle of blinatumomab (15  $\mu\text{g}/\text{m}^2$  per day in continuous infusion for 28 days), patients could proceed to alloHCT. The total number of patients enrolled was 116 and 78% achieved complete MRD response ( $<10^{-4}$ ). The RFS at 18 months was 54% and median OS was 36.5 months. Complete MRD responders had longer RFS (23.6 vs 5.7 months;  $P=.002$ ) and OS (38.9 vs 12.5 months;  $P=.002$ ). A further report from the same phase 3 trial described the long-term OS for patients who had a minimum follow-up of 3 years after treatment with blinatumomab.<sup>43</sup> In the subgroup of 110 patients with Ph- ALL in CR, the RFS at 18 months was 54% and the OS was 36.5 months. MRD responders had longer RFS (23.6 vs 5.7 months;  $P=.002$ ) and OS (38.9 vs 12.5 months;  $P=.002$ ) compared with MRD nonresponders. A total of 74% of the patients received alloHCT while they were in remission after treatment with blinatumomab. Nine (25%) of 36 patients without alloHCT or additional chemotherapy remained in continuous CR. Although the number of patients were small, the analysis of the subgroup who received alloHCT vs blinatumomab alone in CR1 did not show significant statistical difference. In contrast, patients in second CR (CR2) appeared to benefit from alloHCT. Overall, relapse was lower in the allografted group, whereas transplant-associated mortality appeared to decrease the benefit of transplant vs blinatumomab alone on OS.<sup>43</sup>

In March 2018, blinatumomab received an additional accelerated approval for the indication of B-cell precursor ALL in CR1 or CR2 with MRD greater than or equal to 0.1% ( $10^{-3}$ ). Approval was based on the results of the BLAST study in comparison with historical controls. Because this approval was based on nonrandomized phase 2 data, the FDA requires confirmation in another trial, which will be addressed by the ECOG-E1910 study (NCT02003222). Blinatumomab is also being studied in combination with tyrosine kinase inhibitors in Ph+ ALL with MRD<sup>44</sup> and as frontline therapy.<sup>45</sup> Early results appear promising, suggesting this option may also be useful for eliminating chemotherapy to achieve MRD-negative remissions for

Ph+ ALL. Additional data are needed to fully clarify which patients with MRD-positive Ph+ or Ph- ALL benefit from alloHCT after blinatumomab.

### Strategies to Maximize MRD Response in the First-Line Setting

The ongoing randomized phase 3 E1910 trial (NCT02003222) is currently investigating the use of blinatumomab in the frontline setting immediately following induction and consolidation chemotherapy. This study design is based on the hypothesis that blinatumomab will deepen the CRs that are typically achieved by the vast majority of patients with ALL following induction chemotherapy. This approach is based on the observation from other studies that blinatumomab is more effective in patients with lower disease burdens. If proven safe and effective, this approach may help more patients achieve MRD-negative CRs early in the course of therapy. In turn, this holds promise for reducing the proportion of ALL patients who may need alloHCT, with its significant inherent risks, to maintain long-term remission.

An alternative to the debulking approach with cytotoxics prior to blinatumomab is represented by the ongoing phase 2 SWOG 1318 study (Blinatumomab and Combination Chemotherapy or Dasatinib, Prednisone, and Blinatumomab in Treating Older Patients With Acute Lymphoblastic Leukemia). This study is using blinatumomab as first therapy, followed by prednisone, vincristine, methotrexate, and 6-mercaptopurine (POMP) maintenance, in newly diagnosed elderly patients with Ph- ALL.<sup>46</sup> In a preliminary report from this study, 29 eligible patients received 1 or 2 cycles of induction with blinatumomab until CR or incomplete CR, then received 3 cycles of blinatumomab after remission followed by 18 months of POMP maintenance. The ORR was 66%, and 12 of 13 (92%) responders evaluable for MRD were negative. Although these data are promising, with higher response rates than seen in the TOWER trial employing blinatumomab in the relapsed/refractory setting, the treatment success was lower than in the BLAST study, in which 80% of patients achieved MRD negativity (although the BLAST study was not restricted to the elderly population). These findings suggest that blinatumomab therapy may benefit from prior cytoreduction with chemotherapy.

### MRD Surveillance After Treatment

Serial testing of MRD is recommended and is performed in many centers, although robust trial evidence to substantiate the optimal timing and frequency remains wanting. The importance of repeating MRD assessment

in patients who achieve MRD-negative disease after treatment is unclear. It is logical to assume, though, that before a leukemic patient relapses, a transition through an MRD-positive state is required, and this may be an opportunity for intervention. The GMALL prospectively evaluated samples from 105 patients who were enrolled in the GMALL 06/99 and 07/03 trials. MRD was performed using RQ-PCR with intervals of 3 months.<sup>47</sup> All patients evaluated were in remission, had completed first-year chemotherapy, and were previously MRD-negative at less than  $10^{-4}$ . From the initial 105 patients, 27% converted to MRD positivity and 61% of those patients relapsed during a median follow-up of 16.1 months. The median time from molecular to clinical relapse was 9.5 months, suggesting an ample window for intervention. Of the 77 patients who were continuously MRD-negative with quarterly monitoring, only 5 (6%) relapsed.<sup>47</sup> On the basis of these data, we suggest suitable times for MRD quantification throughout the course of ALL therapy, with recognition that specific application of MRD quantification depends to some degree on the treatment regimen and patient factors (Table).

As discussed above, achievement of an MRD-negative response after blinatumomab for either relapsed/refractory disease or MRD-positive CR status was associated with improved OS, demonstrating that MRD remains prognostic in the setting of salvage therapy. Saygin and colleagues found that MRD negativity in CR2, regardless of which treatment method achieved it, was associated with improved RFS ( $P=.001$ ), but not OS ( $P=.36$ ).<sup>48</sup> Similarly, Jabbour and colleagues found MRD less than  $10^{-4}$  by MFC to be associated with an improvement in 2-year RFS—46% vs 17% ( $P=.06$ )—in first salvage, but MRD status was not useful in second salvage among a heterogeneous group of patients treated with several different salvage regimens.<sup>49</sup> The same group reported a more homogenous patient population ( $n=48$ ) treated in first salvage with inotuzumab ozogamicin combined with mini-hyperfractionated cyclophosphamide, vincristine, and dexamethasone (mini-HCVD). The median OS was numerically higher in those achieving MRD negativity ( $<10^{-4}$ ) than in those with MRD greater than  $10^{-4}$ , at 47 vs 5 months ( $P=.065$ ), but the difference was not statistically significant.<sup>50</sup>

### Discussion

Achieving an MRD-negative status of less than  $10^{-4}$  within the first 2 to 3 cycles of induction/consolidation therapy is a critical milestone that should be pursued in all patients with ALL. As shown in many trials in the adult and pediatric populations, MRD is the single most important factor that will predict long-term survival and risk of

**Table.** When and How to Assess MRD in ALL

ALL Subtype	Methods	End of Induction	Consolidation/ Intensification	Maintenance	Pre-transplant	Post-transplant
Ph–	NGS (Ig/TCR) or MFC	Essential	Every 2-3 mo	Every 3-6 mo	Essential	Every 2-3 mo
Ph+	BCR-ABL RQ-PCR, NGS (Ig/TCR), or MFC	Essential	Every 2-3 mo	Every 3-6 mo	Essential	Every 2-3 mo

ALL, acute lymphoblastic leukemia; Ig, immunoglobulin; MFC, multiparameter flow cytometry; mo, months; MRD, measurable residual disease; NGS, next-generation sequencing; Ph, Philadelphia chromosome; RQ-PCR, real-time quantitative polymerase chain reaction; TCR, T-cell receptor.

relapse. Quantification of MRD can be performed using different methods as discussed herein, but it is seemingly unlikely that a randomized clinical trial will be performed comparing the different methods of MRD assessment. Therefore, in the context of available data, it is reasonable to advocate that MRD testing be done with whatever method is most readily available. The NGS approach remains the only method with FDA clearance for quantification of MRD in ALL. This approach, which has been commercialized as the clonoSEQ Assay from Adaptive Biotechnologies, is available as a send-out test from any medical facility in the United States. The availability of this test should improve the rates of MRD quantification in ALL patients, regardless of where they are treated. The optimal frequency of MRD evaluation remains to be clarified, but as indicated in many trials, MRD evaluation after induction and consolidation should be considered the minimum standard for assessing the risk of relapse for patients. Serial follow-up is advisable. Additionally, based on the importance of MRD status before and especially after alloHCT, patients should be assessed for MRD at these times whenever feasible, with appropriate interventions made based on the data. There are currently limited data to guide decisions based on MRD detectable below  $10^{-4}$ , but given the availability of NGS with sensitivity to  $10^{-6}$  disease burden, this will become increasingly important. Early data suggest that MRD less than  $10^{-4}$  may be just as predictive of outcome as MRD greater than  $10^{-4}$ .<sup>51</sup>

Patients with MRD-positive status in CR can now receive blinatumomab, and a confirmatory phase 3 trial is ongoing to determine if this approach unequivocally provides benefit. Controversy remains regarding whether achieving MRD-negative status with blinatumomab prior to alloHCT will translate into better clinical outcomes than proceeding directly to transplant, but based on historical experience—with generally poor outcomes for MRD-positive patients even with alloHCT—the early data provided by the BLAST study are compelling and provide reason for optimism. It is also intriguing that some patients treated with blinatumomab for MRD may experience long-term remission without alloHCT. It remains uncertain how such patients may be identified

to help avoid potential treatment-related mortality and morbidity. The deep sensitivity of NGS-based MRD quantification may, however, be a tool to help distinguish patients who do not necessarily need alloHCT in this setting. It is important for future studies to determine whether MRD lower than  $10^{-6}$  in multiple serial evaluations might be sufficient to identify patients who do not need alloHCT after blinatumomab administered for MRD (or potentially as frontline therapy, as is currently being investigated).

Another question remaining to be addressed is how many cycles of blinatumomab patients should receive after achieving an MRD-negative remission. Most patients who achieve MRD negativity with blinatumomab will do so within the initial 2 cycles. The previous and ongoing trials that studied blinatumomab have included 4 to 8 cycles of treatment. It is unclear at this point whether additional therapy produces deeper molecular responses, or if achieving deeper molecular responses are clinically meaningful. An alternative approach to consider would be treatment with blinatumomab to MRD less than  $10^{-6}$ , followed by monitoring and preemptive re-treatment for MRD progression. This approach should be compared with serial consolidation for a specific number of cycles, even if MRD clearance below the  $10^{-6}$  threshold is achieved early in the course of blinatumomab treatment. These questions need to be addressed in the future to minimize exposure to the adverse effects that can be associated with BiTE therapy, including cytokine release syndrome and neurotoxicity, but also for important health economic reasons.

Ultimately, it will be important to determine how best to utilize high-sensitivity MRD quantification in combination with novel therapies including blinatumomab and inotuzumab, and with cellular therapies, such as chimeric antigen receptor (CAR) T cells, to maximize the likelihood of ALL cure from the first line of treatment. MRD quantification may also help guide studies seeking to reduce what are currently very long treatment commitments for patients, with roughly 3 years of therapy for those who complete induction, consolidation, and maintenance regimens without use of alloHCT. It is thus certain that MRD quantification will remain a critical



assessment at multiple times for all patients undergoing treatment for ALL, and it is imperative that all providers caring for ALL patients identify an approach to obtaining MRD quantification routinely.

### Disclosures

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