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LEUKEMIA (A AGUAYO, SECTION EDITOR)

Minimal Residual Disease in Acute Lymphoblastic Leukemia: How to Recognize and Treat It

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Abstract In recent years, the identification of minimal residual disease (MRD) that persists after chemotherapy has emerged as the most powerful tool in determining the prognosis of patients with ALL, often superseding historically relevant prognostic factors. Multiple methods to detect MRD exist, each with their own advantages and disadvantages. Multiparameter flow cytometry and quantitative polymerase chain reaction are the most commonly used methods of MRD detection in clinical practice, although there is promise in the use of more sensitive assays utilizing next-generation sequencing that may be able to further refine MRD-based risk stratification. By accurately identifying patients with persistent MRD who are at highest risk for relapse, we may be able to better design rational post-remission therapies using novel agents, such as inotuzumab ozogamicin, blinatumomab, and CD19-directed chimeric antigen receptor T cells, all of which have been shown to be effective in achieving MRD negativity, even in patients with relapsed or refractory disease. Future studies will be required to determine whether these post-remission strategies can obviate the need for allogeneic stem cell transplantation for patients with ALL in whom MRD can be eradicated.

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Introduction

Achievement of a morphological remission (i.e., bone marrow blasts <5%) is a prerequisite for long-term survival of patients with ALL. However, morphological assessment of the bone marrow is not sufficient to identify patients who are most likely to relapse, as the vast majority of patients with ALL achieve remission with standard chemotherapy regimens, and yet, many of these patients ultimately relapse and die from their disease [1, 2]. Standard morphological assessment of a remission bone marrow cannot distinguish normal hematopoietic recovery of non-malignant blasts from those that represent residual ALL clones. Compared to morphological assessment alone, more sensitive methods of minimal residual disease (MRD) detection are better able to estimate the reduction of disease burden after treatment and also provide information about the inherent leukemia biology in a particular patient. This information can be used to risk stratify patients according to treatment response and has been shown in a number of studies to be the most powerful prognostic marker in ALL, superseding many historically relevant prognostic factors such as age, white blood cell (WBC) count at diagnosis, and cytogenetics [3, 4, 5••, 6•]. While most studies have evaluated the impact of MRD in Philadelphia chromosome (Ph)-negative ALL, more recent studies have also identified the strong impact of MRD status in Ph-positive ALL [7].

Ultimately, the goal of risk stratification is to inform the most appropriate post-remission therapies for patients. In the pediatric population, MRD response at various time points has been well validated to predict outcomes and has been incorporated into consensus guidelines for post-remission

therapies, including intensification of chemotherapy and use of allogeneic stem cell transplantation (allo-SCT) in first remission [8]. There is less standardization of how MRD information should be used for risk stratification and treatment decisions in adults with ALL. In some studies of adults with ALL, allo-SCT in first remission has resulted in improved outcomes of patients with persistently detectable MRD after frontline chemotherapy [4, 9]. However, with the development of novel antibody constructs such as the anti-CD19 bispecific T cell engager blinatumomab and the anti-CD22 drug-antibody conjugate inotuzumab ozogamicin, both of which are effective in eradicating MRD, the question remains whether the use of these agents may allow us to cure patients who remain MRD-positive after standard chemotherapy without the need for allo-SCT.

Methods of MRD Assessment

Several methods of MRD detection are currently available in clinical practice and in research settings. Regardless of the methodology used, for optimal sensitivity, MRD assessment should be performed on a remission bone marrow sample, rather than on the peripheral blood. This is especially true for patients with B cell ALL (B-ALL), in whom levels of MRD may be 1- to 3-log lower in the blood than in the bone marrow [10]. Ultimately, all methods of MRD detection require differentiation of ALL cells from normal leukocytes, and the targets used to distinguish these two entities vary according to the MRD assay. These include detection of aberrant leukemia-associated immunophenotypes (LAIPs), pathogenic translocations, and immunoglobulin or T cell receptor (TCR) gene rearrangements. For patients with Ph-negative B-ALL or T cell ALL (T-ALL), the most commonly used methods are multiparameter flow cytometry (MFC) to identify LAIPs and quantitative polymerase chain reaction (PCR) of the immunoglobulin or TCR genes. Both of these methods can be applied to >90% of patients with ALL. The use of one of these assays over the other is driven largely by institutional resources and familiarity; MFC is commonly used in the USA and many Asian countries, and PCR is more commonly used in European countries. For patients with Ph-positive disease, PCR-based monitoring of *BCR-ABL1* is the preferred method of MRD assessment. The advantages and disadvantages of the various methods of MRD assessment are summarized in Table 1.

Flow Cytometry

MRD by MFC generally uses a six-color flow cytometric assay in order to identify LAIPs in the remission bone marrow that correspond to the immunophenotype of the pretreatment sample. This methodology identifies MRD with a sensitivity of approximately one leukemic cell per 10^4 nucleated cells. Difficulty in distinguishing aberrant ALL clones from normal regenerating blasts, which may have similar immunophenotypes, partly attenuate the sensitivity of this assay. Additionally, there is the potential for phenotypic shifts that may occur over the course of treatment; if the residual ALL that persists during remission or that emerges at the time of relapse differs phenotypically from the dominant clone at diagnosis, then MFC may not detect this clone. Due to the challenges and nuances associated with interpretation of MFC patterns, MFC-based MRD assessment requires significant expertise by the technician and pathologist, leading to potentially inconsistent inter-laboratory interpretation of MRD by this method. However, despite these potential disadvantages, MFC is significantly faster, less expensive, and less labor-intensive than PCR-based methods. Newer techniques using ≥8-color flow cytometry appear to be able to achieve improved sensitivities (as low as 10^{-6}) and thus may further improve on the utility of MFC in the risk assessment of ALL [10].

Polymerase Chain Reaction

Quantitative PCR may be used to identify either (1) clonal immunoglobulin or TCR gene rearrangements or (2) pathogenic translocations, such as *BCR-ABL1* in patients with Ph-positive ALL. In the former scenario, the target of the assay is the immunoglobulin H (IgH) gene rearrangement that occurs through recombination of the V, D, and J gene segments. While this is typically a random event that occurs in normal B cells early in the maturation process, this recombination event results in identical IgH gene rearrangements among malignant B cell clones. To optimize the sensitivity of this assay, the PCR is directed at the junctional regions, which are the most diverse in terms of size and composition. Similar rearrangements in the TCR can be used to identify MRD, particularly among patients with T-ALL. In European countries, much effort has been put into place to ensure standardization of PCR-based MRD assessment, which has led to significantly better reproducibility of MRD results compared to MFC assays [11]. While there is evidence that the sensitivity of PCR may be approximately 1-log higher than that achieved with MFC [12–14], the time-consuming and laborious nature of constructing the allele-specific oligonucleotide primers necessary for this assay limits the universal adoption of PCR-based techniques. Additionally, as with MFC, minor subclones present at diagnosis may not be appreciated when developing patient-specific primers and therefore may be overlooked in remission samples.

PCR can also be used in patients with known translocations, such as *BCR-ABL1*, *MLL-AF4*, or *TCF3-PBX1*, in order to identify MRD with a sensitivity of 10^{-4} to 10^{-5} (similar to that of the other PCR-based assays described above). MRD

Table 1 Methods of minimal residual disease assessment in ALL

Method	Sensitivity	Advantages	Disadvantages
Flow cytometry for LAIPs	10^{-4}	• Fast • Relatively inexpensive	• Difficult to distinguish malignant clones from hematogones • Requires significant technical expertise • Potential for immunophenotypic shift • Limited standardization
RQ-PCR for IgH/TCR gene rearrangements	10^{-4} to 10^{-5}	• Sensitive • Well-standardized with consensus guidelines	• Time-consuming and labor-intensive • Requires significant technical expertise • Expensive
RQ-PCR for gene fusions	10^{-4} to 10^{-5}	• Sensitive • Simple (uses standard primers used for diagnostic purposes)	• Applicable to <50% of ALL cases • Limited standardization
Next-generation sequencing	10^{-6}	• Very sensitive • Relatively fast (uses consensus primers) • Can identify small subclones and clonal evolution	• Not standardized • Requires complex bioinformatics • Minimal clinical validation

LAIPs leukemia-associated immunophenotypes, *RQ-PCR* real-time quantitative polymerase chain reaction, *IgH* immunoglobulin H, *TCR* T cell receptor

assays for gene translocations are performed with the same primer probes used for diagnostic purposes, which makes the process simpler than other methods of MRD detection. The primary limitation of this approach is that it can only be used in the minority of ALL cases with a pathogenic gene fusion. Additionally, unlike quantitative PCR of IgH/TCR genes, PCR-based detection of gene fusions is not standardized. Even in the case of *BCR-ABL1* in which careful standardization of molecular response has been developed for patients with chronic myeloid leukemia [15], these molecular response milestones cannot be extrapolated to patients with the more common p190 transcript observed in the majority of ALL cases.

Next-Generation Sequencing

A shift towards NGS platforms may help to resolve many of the limitations of both MFC and PCR described above. Thus far, efforts employing NGS in the detection of MRD have predominantly focused on targeting the same IgH and TCR gene rearrangements as with PCR-based MRD assays [16, 17]. Because NGS allows for rapid, parallel sequencing using consensus primers, it does not require the laborious construction of patient-specific primers and can be performed in significantly less time than standard PCR assays. Early reports suggest that the sensitivity of NGS may be 1- to 2-log better than that which can currently be achieved with MFC- and PCR-based methods [17]. In addition to the potential for improved sensitivity compared to standard methods of MRD detection, NGS has the advantage of being able to identify and monitor small malignant subclones that may be present at diagnosis but not appreciated by either MFC or PCR. The importance of these subclones leading to ALL relapse is becoming increasingly appreciated [18]. Nevertheless, despite these potential advantages of NGS-based MRD assessment, much work still needs to be done to standardize NGS methodologies. NGS requires complex bioinformatics, and experience with its application to MRD is still limited. Therefore, while it remains a potentially promising tool, NGS for the purpose of MRD detection is still only available in research settings.

Prognostic Impact of MRD in ALL

Accurate detection of MRD is imperative for patients with ALL, primarily because this information identifies patients at highest risk for relapse and death. Whereas risk stratification in ALL historically was determined by age, WBC count at diagnosis, cytogenetics, and other pretreatment host- and disease-related factors, the detection of MRD using sensitive laboratory methods has superseded many of these previously relevant prognostic factors [3, 4, 5••, 6•]. Because measurement of MRD is, by definition, a response-based assessment, MRD serves as an in vivo measure of chemosensitivity and disease biology that may not have been predicted by pretreatment characteristics alone.

T-ALL and Ph-Negative B-ALL

The achievement of MRD negativity in response to frontline chemotherapy is predictive for long-term survival among patients with ALL, both in pediatric and adult populations and regardless of whether MFC and PCR-based assays are used [3, 4, 5••, 6•, 19–21]. When comparing studies, the prognostic impact of MRD varies according to the MRD assay and

chemotherapy regimen used, whether the study was performed in a pediatric or adult population, relative rates of allo-SCT, timing of MRD assessment, and other factors. However, despite these differences, MRD has consistently emerged as one of the most powerful predictors of relapse across multiple studies. In fact, in several reports, MRD status was the only factor identified as independently prognostic for long-term remission and survival, suggesting that risk stratification could potentially be based on MRD assessment alone [3–5••].

More recent reports have suggested that MRD information may be combined with molecular subtyping to further improve risk stratification in both children and adults [6•, 9, 22–25]. In adult patients treated on two GRAALL trials, positive MRD (defined as a level $\geq 10^{-4}$ by PCR for IgH/TCR gene rearrangement) detected after 6 weeks of treatment was associated with a significantly increased risk of relapse for both patients with B-ALL (hazard ratio [HR] 3.45, $P < 0.001$) and T-ALL (HR 2.93, $P < 0.001$) [6•]. By multivariate analysis, MRD status, presence of *IZKF1* gene deletion, and *MLL* gene rearrangement were associated with increased risk of relapse in patients with B-ALL; in patients with T-ALL, MRD status and poor-risk genetic profile (defined as the absence of *NOTCH1/FBXW7* mutation and/or the presence of *NRAS/KRAS* mutation or *PTEN* alteration) were independently associated with worse outcomes. Similarly, in another study, allo-SCT in first remission was associated with improved RFS and OS in patients with B-ALL harboring focal *IZKF1* gene deletion, but not in those with intact *IKZF1* [9]. Future prospective studies are needed to determine how MRD assessment should be integrated with genetic profiling to inform post-remission therapies after frontline chemotherapy.

For patients who receive allo-SCT, levels of MRD before transplant are prognostic for post-transplant relapse [26•, 27–30]. In one study of children with relapsed ALL, patients with MRD $\geq 10^{-4}$ by PCR detected prior to allo-SCT had a significantly worse event-free survival (EFS) and a higher cumulative incidence of subsequent relapse (CIR) compared to those with lower levels of MRD (5-year EFS rate: 27 vs 60%, respectively, $P = 0.004$; 5-year CIR rate: 57 vs 13%, respectively, $P < 0.001$) [26•]. Only pre-transplant MRD status was prognostic for EFS in multivariate analysis. Conversely, higher levels of MRD after allo-SCT have been shown to predict impending relapse, particularly when detected after day +60 after transplantation [31].

Ph-Positive ALL

Compared to Ph-negative ALL, the utility of MRD monitoring in Ph-positive ALL is less defined. In individual studies of chemotherapy plus a tyrosine kinase inhibitor (TKI), achievement of a deeper molecular response (as measured by PCR of *BCR-ABL1* transcripts) has been associated with improved survival [32–34]. Additionally, in analyses of patients who received hyper-CVAD plus a TKI but did not undergo allo-SCT in first complete remission (CR), patients who achieved deep molecular responses were found to have excellent long-term survival [7, 35]. In one study, patients who achieved a complete molecular response (defined as the absence of a detectable *BCR-ABL1* transcript by quantitative PCR) after 3 months of treatment had a 4-year overall survival (OS) rate of 66%, despite not undergoing allo-SCT in first CR; achievement of MRD negativity was the only variable found to be independently predictive for OS [7]. These results suggest that MRD assessment should be considered when deciding whether to pursue allo-SCT for patients with Ph-positive ALL.

Relapsed or Refractory ALL

There are relatively few reports on the prognostic impact of MRD assessment in patients with relapsed/refractory ALL. Most studies examining this question have evaluated children in first relapse receiving cytotoxic chemotherapy. These reports have suggested that lower levels of MRD are associated with improved outcomes [36–39], and in one study, allo-SCT was associated with decreased risk for relapse or death in children who had an unsatisfactory MRD response to salvage therapy [40]. In adults, information about the prognostic impact of MRD in the salvage setting comes predominantly from trials of novel monoclonal antibodies such as inotuzumab and blinatumomab. Patients with relapsed/refractory ALL treated with blinatumomab who achieved MRD negativity using an allele-specific PCR assay had longer survival than patients who remained MRD-positive [41, 42]; overall, MRD response was associated with a 67% reduction in the risk of death [42]. In patients treated with inotuzumab in the salvage setting, achievement of MRD negativity by MFC has been associated with longer remission durations, although survival analyses stratified by MRD response have not been reported for these prospective trials [43, 44].

Interestingly, the impact of achieving MRD negativity by MFC in the salvage setting may differ according to the number of prior lines of therapy received. In one retrospective analysis of adults in first or second salvage who received inotuzumab- or blinatumomab-containing regimens, MRD negativity was associated with improved survival only in patients in first salvage; patients in second salvage had poor outcomes regardless of MRD response [45]. In the relapsed/refractory setting, patients are generally referred to allo-SCT if remission is achieved; so, while MRD assessment may provide prognostic information, it is unlikely to have significant implications for post-remission therapies. However, the poor outcomes of adult patients in second salvage and beyond regardless of MRD response suggest that that most effective salvage regimen should be used at the time of first relapse (and followed by allo-SCT for fit patients with a suitable

donor) in order to maximize the chance for long-term survival in patients with relapsed/refractory disease.

Therapeutic Implications of MRD

The detection of MRD serves not only to predict patient outcomes, but it also can inform risk-adapted strategies for patients with ALL. By tailoring therapies according to MRD response, patients at highest risk of relapse can selectively receive more aggressive therapy, such as allo-SCT in first CR, intensification of chemotherapy, or the introduction of novel agents. Notably, the treatment-related mortality for adults with ALL who undergo allo-SCT has been reported as high as 40% (depending on the intensity of the conditioning regimen), with rates of acute and chronic graft-versus-host disease approaching 50% [46]. Thus, perhaps equally important as identifying high-risk patients, MRD-based prognostication is crucial to the identification of patients at relatively low risk of relapse who may be cured with chemotherapy alone and may be spared the potential morbidity of these more intensive treatment approaches.

In some studies, allo-SCT has been shown to improve outcomes of patients with ALL and suboptimal MRD response to frontline chemotherapy [4, 9]. In one study of adult patients with Ph-negative ALL receiving a pediatric-inspired regimen, allo-SCT was found to benefit patients with poor MRD response (HR for relapse-free survival [RFS] 0.37, $P = 0.001$; HR for OS 0.41, $P = 0.005$), but not those with adequate MRD response [9]. Other studies have also bolstered these findings that allo-SCT may be safely avoided in many patients with good MRD response. The PETHEMA ALL-AR-03 trial evaluated adolescent and adult patients with Ph-negative B-ALL with high-risk pretreatment characteristics (i.e., age 30 to 60 years, WBC >30 × 10^6/L or *MLL* gene rearrangement) [5••]. Patients with poor day 14 bone marrow morphological response and/or suboptimal MRD response after induction and at the end of early consolidation were assigned to allo-SCT, whereas other patients received chemotherapy alone. Patients with good morphological and MRD response assigned to receive chemotherapy alone had 5-year RFS and OS rates of 55 and 59%, respectively, despite the presence of historically adverse pretreatment prognostic characteristics. More recently, a report from the Dutch Childhood Oncology Group has suggested both that de-escalation of chemotherapy in children who achieve MRD negativity is safe and that intensification of chemotherapy with or without allo-SCT can improve outcomes in patients with suboptimal MRD response [47].

In addition to informing decisions whether or not to pursue allo-SCT in first CR, MRD assessment can also identify patients who may benefit from non-transplant-based novel therapies. This is especially important for older adults or those with significant comorbidities who may not be candidates for allo-SCT as well as for those in whom an adequate donor is not identified. Inotuzumab and blinatumomab have shown significant promise in the management of relapsed/refractory ALL [41, 42, 44]. The apparent improved survival observed with these novel monoclonal antibodies may be in part mediated through the higher MRD negativity rates achieved with these agents as compared to standard cytotoxic chemotherapy. The use of CD19-directed chimeric antigen receptor T cells in patients with relapsed/refractory ALL has also resulted high rates of MRD negativity [48, 49], which have in turn resulted in impressive long-term survival in some responders. These findings raise the question as to whether incorporating these agents into frontline regimens can increase rates of MRD negativity compared to chemotherapy alone, possibly with less toxicity. In the most recent update of a study of dose-reduced chemotherapy (mini-hyper-CVD) plus inotuzumab in older patients with newly diagnosed ALL, MRD negativity by MFC was achieved in 80% of patients who achieved CR after 1 cycle of therapy; notably, no patients experienced early death due to toxicity [50]. In contrast, in one large retrospective study of older patients receiving full-intensity hyper-CVAD, the induction mortality rate was 10% and the death in CR rate was 34% [51]. The encouraging results observed with the novel combination of inotuzumab and dose-reduced chemotherapy suggest that such combinations can result in high rates of MRD negativity with significantly less toxicity than full-intensity chemotherapy regimens. Given the known significant impact of MRD response on long-term outcomes, there is promise that regimens able to induce deeper remissions will ultimately translate into improved survival for patients with ALL.

It remains an open question whether patients who have suboptimal MRD response to frontline chemotherapy can be salvaged without allo-SCT. In an ongoing study of blinatumomab for patients with Ph-negative B-ALL and inadequate MRD response after initial treatment, achievement of MRD negativity with blinatumomab was associated with an improvement of RFS compared to those who continued to have detectable MRD (median RFS: 35.2 vs 7.1 months, respectively, $P = 0.002$) [52••]. Interestingly, while allo-SCT was associated with a longer duration of response, it did not significantly improve either RFS or OS in patients who were in first CR. While these data are still preliminary, they suggest that after initial suboptimal MRD response, subsequent MRD eradication with novel agents can be associated with improved outcomes and may ultimately alter our risk assessment of these patients.

In patients with Ph-positive ALL, failure to achieve MRD negativity is associated with relatively poor outcomes, and therefore, allo-SCT should be strongly considered for these patients [7]. Conversely, achievement of MRD negativity may obviate the need for allo-SCT for many patients, although

randomized trials to validate this approach are needed. Given the association between MRD response and outcomes in Ph-positive ALL, it is reasonable to incorporate TKIs with the highest rates of complete molecular response into frontline regimens; these strategies may decrease the number of patients with Ph-positive disease for whom allo-SCT is ultimately necessitated. Rates of MRD negativity vary according to the TKI added to chemotherapy, ranging from 28 to 50% with imatinib [32, 53, 54], 45–65% with dasatinib [34, 55], and 78% with ponatinib [56]. Comparing across studies, survival rates appear to improve with each successive generation of TKI. Although no randomized trial has been performed to confirm this observation, a propensity score analysis of patients who received frontline hyper-CVAD plus either dasatinib or ponatinib found that the ponatinib-based regimen was associated with significantly longer EFS ($P = 0.003$) and OS ($P = 0.001$) compared to hyper-CVAD plus dasatinib [57]. Moreover, in the phase 2 trial of frontline hyper-CVAD plus ponatinib for adults with Ph-positive ALL, a 3-year OS rate of 80% was reported; this was likely mediated through the relatively high MRD negativity rate achieved with this combination [56].

Conclusions

MRD has emerged as the most influential factor in determining risk of relapse in patients with ALL, surpassing the prognostic information obtained from analysis of pretreatment characteristics alone. However, while the prognostic impact of MRD assessment is clear across ALL subtypes, many questions remain about how to best incorporate this information into risk-adapted strategies. Ultimately, to improve the outcomes of patients with ALL, two parallel strategies are required. First, MRD-based risk assessment should be used to identify both patients at high risk of relapse who are candidates for treatment intensification as well as patients with lower risk of relapse in whom the potential treatment-related morbidity and mortality associated with intensive chemotherapy or allo-SCT outweigh the potential clinical benefits. Second, we must continue to develop novel treatment strategies designed to induce the deepest remission possible. This requires incorporating our most effective therapies, whether monoclonal antibodies or TKIs, in the frontline setting in order to induce MRD negativity and offer patients the best chance for cure.

Compliance with Ethical Standards

Conflict of Interest Nicholas J. Short and Elias Jabbour declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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