



# Minimal Residual Disease in Acute Lymphoblastic Leukemia

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Acute lymphoblastic leukemia (ALL) is the most common malignancy in children accounting for 25 and 75% of childhood cancers and leukemia, respectively, cited as the major success stories in the world of oncology where the cure rates have gone up to 80% (event-free survival [EFS]) from literally zero in the 1950s.<sup>1–3</sup> Prognostic factors play an important role in the strategic standard management of ALL wherein minimal residual disease (MRD) is now widely regarded as a clinically significant tool. A meta-analysis has proven that MRD negativity is directly proportional to the powerful predictors of disease-free survival (DFS) (hazard ratio [HR]: 0.23, [95% Bayesian credible interval [BCI]: 0.18–0.28] for pediatric patients and 0.28 [95% BCI: 0.24–0.33] for adults) and overall survival (OS) (HR: 0.28, [95% BCI: 0.19–0.41] and 0.28 [95% BCI: 0.20–0.39] for children and adults with ALL, respectively).<sup>4</sup>

It now provides information depending on when the MRD assessment was performed: after induction therapy, after consolidation therapy (CT), or before and after stem cell transplant (SCT) and genomic information for targetable therapies available today, as shown in ►Table 1. As of today, for the management of ALL, induction therapy to aim complete hematological recovery and complete remission (CR), followed by CT after attainment of CR, with standard central nervous system (CNS) prophylaxis, is imperative. It is followed by SCT in few subsets. Mostly all pediatric and adult ALL guidelines have introduced informative checkpoints during the management of ALL. For pediatric subgroup, MRD negativity on day 15 of induction chemotherapy defines excellent outcomes, wherein in adults, MRD is taken later in the course at 4 weeks of starting induction chemotherapy and defines better survival rates.<sup>5,6</sup>

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## Molecular Detection Methods for Minimal Residual Disease

Molecular detection methods for MRD identify cells either through patterns of phenotypic markers or differential gene expression through analysis by flow cytometry (FCM), polymerase chain reaction (PCR), or next-generation sequencing (NGS) (►Fig. 1).

An extensive marker screening panel of multiplex PCR assays targeting immunoglobulin/T-cell receptor (Ig/TR) gene rearrangements of a primary diagnosis sample is used to identify tumor-specific Ig/TR rearrangements. To discriminate malignant clonal rearrangements against a polyclonal background, PCR fragments from Ig/TR PCR assays are analyzed. The most frequently used methods for this fragment analysis are GeneScan or denaturing high pressure liquid chromatography, followed by heteroduplex analysis, which is comparable to multiplex PCR.<sup>7</sup>

## Sample Prerequisites

Many large-scale studies have confirmed that the bone marrow sample is more informative than peripheral blood for the detection of MRD.<sup>8–10</sup> There has been a difference of 1–3 log of MRD being lower in a paired peripheral blood than a bone marrow sample.<sup>11</sup> Therefore, bone marrow assessments might be replaced by analysis of blood samples in T-ALL but not in BCP-ALL. The difference of residual tumor load is more apparent in B-ALL as compared with that of T-ALL. Bone marrow aspirate, however, remains the sample of choice for MRD detection. It is advisable that the first sample of aspirate should be used for MRD studies. Care should be taken not to dilute it with peripheral blood, and, usually, a 2 mL,

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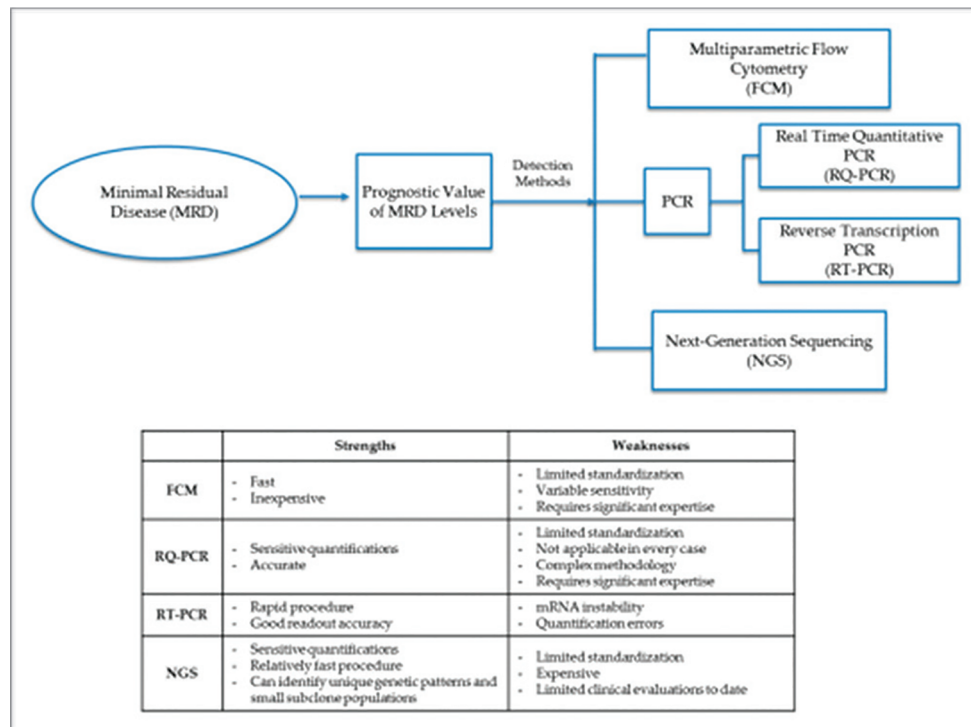
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**Table 1** Genetic classification by prognosis of B-cell acute lymphoblastic leukemia

Good prognosis	Intermediate prognosis	Poor prognosis	Undetermined prognosis
Hyperdiploid karyotypes	t(1; 19); TCF3-PBX1	Hypodiploid karyotypes	t(5; 14); IL3-IGH <sup>a</sup>
t(12; 21); ETV6-RUNX1 (TEL-AML1)		t(9; 22); BCR-ABL	
		Philadelphia-like ALL	
		11q23 MLL arrangements	

Abbreviations: ALL, acute lymphoblastic leukemia; MLL, mixed-lineage leukemia.

<sup>a</sup>t(5; 14); IL3-IGH is a World Health Organization-classified acute leukemia and prognosis data has not been determined.



**Fig.1** Detection methods for minimal residual disease. Methods to diagnose minimal residual disease either through phenotypic marker patterns or differential gene patterns through analysis by flow cytometry, polymerase chain reaction, real-time quantitative-polymerase chain reaction, reverse transcription-polymerase chain reaction, or next-generation sequencing.

but <5 mL, sample is sufficient to recover cells that give a sensitivity of B 10–4.<sup>12</sup> Ethylenediaminetetraacetic acid or heparin-anticoagulated samples are good and preferably to be assayed for MRD detection within 24 to 48 hours.

### Minimal Residual Disease in Ph-Negative Acute Lymphoblastic Leukemia and Time Points

MRD is a time point-dependent variable. MRD levels at different time points have different prognostic values for relapse: early MRD assessment at the end of induction or early consolidation identifies patients with a rapid tumor clearance and a very low risk of relapse representing a good prognosis, whereas any persisting MRD at the end of CT is associated with a particularly poor prognosis. The Programa para el Tratamiento de Hemopatías Malignas (PETHEMA) group evaluated the role of MRD (by FCM, cutoff:  $5 \times 10^{-4}$ ) in 326 adult high-risk Philadelphia (Ph)-negative ALL patients and confirmed that the

only prognostic factor was represented by MRD persistence after induction and early consolidation.<sup>13</sup> Similarly, Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) group in 955 patients assessing the role of SCT post-MRD after induction showed that the persistence is not abrogated by transplant procedures and that MRD-negative patients could be spared this approach.<sup>14</sup> The Northern Italian Study Group with MRD post end of induction at week 4 and afterward 10, 16, and 22 weeks to assess liposome-encapsulated cytarabine for CNS prophylaxis proved profound prognostic effect. The relapse risk (RR) was very low (17% at 5 years) in the group of week 4 MRD responders and significantly lower (28%) than that in nonresponders (57%) when week 10 MRD results were examined.<sup>15</sup> The German Multicenter Study Group for Adult ALL for Ph negative patients with SR/HR features (580 patients) in CR showed MRD after standard induction and consolidation treatment was the only significant prognostic factor for remission duration and survival in both risk groups,<sup>16</sup> which has been confirmed later by many trials.<sup>13-15</sup>

## Minimal Residual Disease in Ph-Positive Acute Lymphoblastic Leukemia

This subset of ALL, defined as a more high-risk group with Ph chromosome-forming breakpoint cluster region-Abelson gene (BCR/ABL) rearrangement seen in 20 to 25% of ALL patients, is more common in adults than the pediatric population and increases in incidence with increasing age group. This subgroup was always defined as the highest risk group till tyrosine kinase inhibitors (TKIs) were brought into practice that revolutionized treatment till the present date where this Ph-positive ALL subset can attain CR in almost all cases with the TKIs such as imatinib, dasatinib, and ponatinib with or without chemotherapy as a therapeutic strategy, further improving EFS and also the OS with the number of Ph-positive patients who could further receive SCT.<sup>17-21</sup>

Like in the Ph- subset where MRD reduction serves as a prognostic tool for improved EFS and OS, the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) trial proves again in the Ph-positive subset that MRD reduction correlates with the EFS and OS irrespective of the inhibitor used<sup>22</sup> and that a very early clearance has a better prognosis.<sup>23</sup> Initially, all Ph-positive patients would be taken for SCT irrespective of the treatment used, but now, cases that are persistently MRD negative could avoid this and the debate continues.<sup>24,25</sup> For therapeutic purposes too, MRD persistence or positivity or its reversal can signify the presence of a clone of mutation resistance, like T315-I that warrants novel TKIs (ponatinib) or combinations with TKI and monoclonal antibodies (blinatumomab).

Today, clinicians can vouch for more from MRD testing where more than one marker could be identified, like in pediatric ALL pH-positive subsets, 20% or more children could have significantly higher levels of BCR/ABL, which is evaluated by estimating both DNA and RNA fusion levels shown by Hovorkova et al<sup>26</sup> than Ig/TR/TKZF1 deletions, proving that BCR/ABL1 signals could arise from different hematopoietic progenitors. Similarly, a trial by Cazzaniga et al proved that positive MRD reports by assessing Ig/TR deletion levels at after induction and consolidation are strongly prognostic of relapse,<sup>27</sup> wherein a formal correlation between Ig/TR and BCR/ABL1 proves again a similar conclusion for relapse with similarity of the technique used (69%). In adult patients, a trial by Clappier et al<sup>28</sup> found discordance in genomic and RNA-BCR/ABL1 levels because of p210 isoforms, Ikaros family zinc finger protein 1 (IKZF1) deletions, again proving that signals arise from different hematopoietic cells than lymphoblasts and represents an altogether different subset of "CML-like subtype"; however, there is no consensus as to which is suitable for treatment decisions.

## Ph-Like Acute Lymphoblastic Leukemia

The rate of CR/CR with incomplete platelet recovery (CRp) was similar in the three disease subgroups (Ph-like ALL, 89%; Ph-positive ALL, 93%; and B-other, 94%;  $p = 0.57$ ).<sup>29</sup> However, patients with Ph-like ALL were statistically significantly less

likely to achieve MRD-remission as assessed by FCM (30% for Ph-like ALL vs. 56% for Ph-positive ALL vs. 87% for B-other;  $p < 0.001$ ), with achievement of MRD negativity at the time of remission having no impact on inferior long-term outcomes. In addition, there was no difference in the CR/CRp rate and MRD remission rate between the Ph-like CRLF2-positive and Ph-like non-CRLF2 groups. Because the majority of patients with Ph-like ALL receive hyper-cyclophosphamide, vincristine, Adriamycin, and dexamethasone-based treatment, intensification of chemotherapy treatment is unlikely to benefit adult patients with Ph-like ALL. It remains to be determined whether addition of novel monoclonal antibodies (such as inotuzumab ozogamicin) or bispecific antibodies (such as blinatumomab) could improve the outcome of this group of patients.<sup>30</sup>

## Minimal Residual Disease and Stem Cell Transplantation

SCT is a procedure still regarded as the one with high mortality and toxicity, which is performed for ALLs with MRD persistence/positivity and remains a major tool for decision-making, which could be seen in 20% of patients.<sup>31</sup> Trials have proven the prognostic impact of MRD positive on SCT from time to time<sup>32,33</sup> and also the relevance of performing MRD for pretransplant assessment.<sup>34-36</sup> MRD levels of  $>10^3$  at week 16/22 post consolidation had worst prognosis and posttransplant mortality with 6-year RR of 64 vs. 23% for MRD  $<10^3$  shown by Bassan et al.<sup>37</sup> A meta-analysis on 21 reports including  $>20,000$  patients has proven the same MRD positivity results in posttransplant mortality and reduced relapse-free survival, EFS, and OS.<sup>38</sup> MRD positivity pretransplant could therapeutically benefit from immunotherapeutic compounds such as blinatumomab and inotuzumab and possibly chimeric antigen receptor (CAR)-T-cells in future too, aiming to obtain a MRD-negative status, and also help in identifying early molecular relapses when done at day 30 as it will help taper immunosuppression early or preemptively start TKIs in a Ph positive B-ALL.

MRD positivity posttransplant accounts for significantly worse outcomes as compared with their MRD-negative counterparts<sup>39</sup> but is less commonly practiced as donor chimerism provides risk for early relapse.<sup>40</sup>

## Minimal Residual Disease and Novel Markers

Extensive genetics and molecular markers of ALL mandate combining MRD with other markers, for example, in a 400 young adult cohort of Ph-negative ALL, the GRAAL group identified a high-risk relapsed population by MRD-del IKZF1 positive, absent NOTCH1/FBXW7 mutation, N/K-RAS mutation, and/or PTEN gene alteration in T-cell ALL positive.<sup>41</sup> Similarly, in pediatric ALL, presence of IKZF1 intragenic deletion and P2RY8-CRLF2 provides additional prognostic information over MRD alone.<sup>42</sup>

## Minimal Residual Disease and Novel Agents

Blinatumomab is a bispecific anti-CD19 and anti-CD3 construct, recruiting cytotoxic T-cells against CD19 positive blast T-cells, bridging malignant B-cells directly to CD3 positive T-cells, bypassing T-cell receptor specificity and major histocompatibility complex class 1 molecules,<sup>43,44</sup> and inducing T-cell activation and release of inflammatory cytokines.<sup>45</sup> It has been approved for refractory ALL and more recently for MRD positive patients (response rates of 43–69%),<sup>46,47</sup> Patients with MRD-status have demonstrated a longer median OS and median DFS compared to MRD-positive counterparts.<sup>48,49</sup> In MRD-positive patients, blinatumomab induced a complete MRD response in 78% of cases and as expected, MRD responders had a longer RFS than nonresponders. A small fraction of complete MRD responders did not undergo transplant and is still in continuous CR.<sup>50,51</sup>

Inotuzumab ozogamicin—an antibody drug conjugate of monoclonal antibody directed to CD22 and a cytotoxic agent—is approved as monotherapy of relapsed/refractory adult CD22-positive ALL patients. Patients treated with inotuzumab ozogamicin reached response rates ranging from 58 to 81%, with 72 to 78% of these having MRD results below 0.01%<sup>52,53</sup> by FCM assessment. While this compound appears to be extremely effective in reinducing responses, it must be underlined that CR duration is usually short, and therefore SCT must be performed as soon as possible.

CAR-T-cells are patient-derived or, less frequently, donor-derived normal T-cells molecularly engineered to express a T-cell receptor-mediating cytotoxicity toward anti-CD19 (in most cases). After CAR-T-cells are infused into a patient, they act as a “living drug” against cancer cells: they bind to the target, become activated, proliferate, and exert their cytotoxic activity. Several groups have shown that most of the responding patients (both children and adults) become MRD negative (at least by FCM)<sup>54,55</sup> and maintain this status for several months or years.<sup>56,57</sup> Data on the prognostic value of MRD in this setting are still preliminary. However, differently from first-line chemotherapeutic approaches, relapse is observed also in patients reaching an MRD negativity, mostly because of the loss of CD19. Therefore, MRD response in this setting seems to be an essential but not sufficient criterion for the definition of long-term remissions. Higher sensitivities or earlier MRD assessments might be necessary to identify a subgroup of patients with a particularly rapid and deep MRD response and a better prognosis.

## Conclusions

MRD stands as an independent predictor of DFS and OS for both pediatric and adult patients of ALL during treatment, and also for pre- and post-SCT settings. Gene fusions and IG/TR gene molecular rearrangements are used as targets to identify residual leukemic cells in ALL with/without newer markers for therapeutic actionable purposes and to also improve the evaluable numbers. Multicolor FCM and real time quantitative-PCR are broad platforms for MRD assessment and monitoring provided

limitations are overcome. PCR, NGS, and next-generation FCM, making them standard of care is yet to be proven that could be proven as an important way to identify MRD.

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### Conflicts of Interest

There are no conflicts of interest.

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