

# Measurable residual disease in acute lymphoblastic leukemia: methods and clinical context in adult patients

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## Abstract

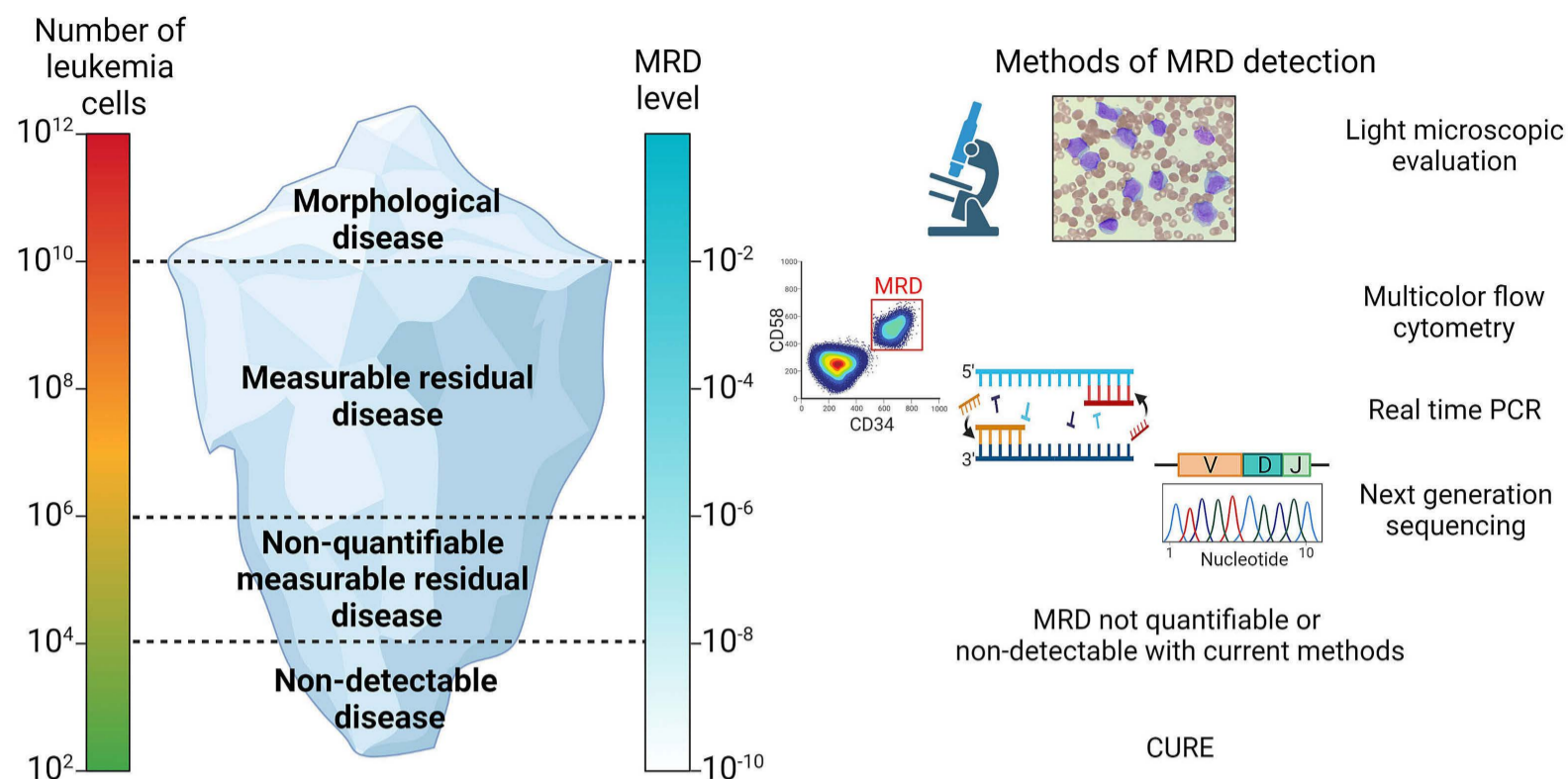
Measurable residual disease (MRD) is the most powerful independent predictor of risk of relapse and long-term survival in adults and children with acute lymphoblastic leukemia (ALL). For almost all patients with ALL there is a reliable method to evaluate MRD, which can be done using multi-color flow cytometry, quantitative polymerase chain reaction to detect specific fusion transcripts or immunoglobulin/T-cell receptor gene rearrangements, and high-throughput next-generation sequencing. While next-generation sequencing-based MRD detection has been increasingly utilized in clinical practice due to its high sensitivity, the clinical significance of very low MRD levels ( $<10^{-4}$ ) is not fully characterized. Several new immunotherapy approaches including blinatumomab, inotuzumab ozogamicin, and chimeric antigen receptor T-cell therapies have demonstrated efficacy in eradicating MRD in patients with B-ALL. However, new approaches to target MRD in patients with T-ALL remain an unmet need. As our MRD detection assays become more sensitive and expanding novel therapeutics enter clinical development, the future of ALL therapy will increasingly utilize MRD as a criterion to either intensify or modify therapy to prevent relapse or de-escalate therapy to reduce treatment-related morbidity and mortality.

## Introduction

Multi-agent chemotherapy and risk-adapted protocols revolutionized the management of pediatric acute lymphoblastic leukemia (ALL), and transformed this historically fatal disease into a curable entity in ~90% of children.<sup>1</sup> Although outcomes continue to be inferior for adults with ALL relative to children, the successful adoption of pediatric-inspired intensive chemotherapy in the treatment of young adults (<40 years) with ALL has significantly improved overall survival with rates approaching 65–80%.<sup>2,3</sup> Furthermore, novel immunotherapies such as the CD19-directed bi-specific T-cell engager blinatumomab, the anti-CD22 antibody-drug conjugate inotuzumab ozogamicin, and chimeric antigen receptor (CAR) T-cell therapies offer new treatment options for B-lineage ALL and are now being explored in the front-line setting.<sup>4–6</sup> As our therapeutic armamentarium expands, accurate risk stratification at critical time points is essential to deliver optimal treatment to improve outcomes in adults with ALL. After standard multi-agent chemotherapy, the majority of adults will achieve complete morphological remission (CR).<sup>7,8</sup> However, response assessment is further

refined by the evaluation of subclinical (not detected morphologically) measurable (also known as minimal) residual disease (MRD), which strongly and independently associates with outcomes across ALL studies and patient cohorts.<sup>9–12</sup> Furthermore, MRD is increasingly incorporated into risk-adapted protocols in adult ALL populations, in which MRD may inform therapeutic decisions by identifying patients at high risk of relapse who may benefit from novel treatment approaches and/or allogeneic hematopoietic cell transplant (HCT), or patients with chemosensitive disease at lower risk of relapse who are likely to do well in the absence of HCT in first complete remission.<sup>13,14</sup> The prognostic significance of MRD in adult ALL has been shown in both younger adults (<40 years) and older adults (>40 years).<sup>15,16</sup>

MRD is defined as measurable leukemia in a sample that is devoid of leukemia cells by morphological assessment with a light microscope. It is estimated that a patient presents with  $10^{12}$  (a trillion) bone marrow leukemic blasts at diagnosis, and morphological assessment can detect approximately 1% blasts or more (Figure 1). Therefore, MRD theoretically represents anything less than  $10^{10}$  cells (<1% blasts). In clinical practice, MRD is often used to describe



**Figure 1. Measurable residual disease assessment in acute lymphoblastic leukemia.** Schematic representation of disease levels in acute lymphoblastic leukemia with corresponding measurable residual disease levels. This can resemble an “iceberg”, with different detection methods offering different levels of sensitivity and breadth of detecting residual disease. MRD: measurable residual disease; PCR: polymerase chain reaction.

any detectable leukemia below the traditional remission definition of 5% blasts by morphological assessment. However, as MRD detection assays have become more sensitive, it is generally recognized that an appropriate assay for the detection of ALL MRD in the clinic should be validated and reproducible at a sensitivity threshold of at least  $10^{-4}$ , or 0.01% leukemia cells in the bone marrow. The fundamental idea behind MRD interpretation is simple: the rate of decline in disease burden in response to systemic therapy is of prognostic value and a measure of risk for relapse, and intervening on lower levels of disease should result in improved outcomes. Herein, we review commonly used methods of MRD detection in ALL and provide clinical context and guidance to practising clinicians on how to interpret and intervene on MRD in adult ALL.

## Methods and technical aspects of measurable residual disease assessment in acute lymphoblastic leukemia

All MRD detection methods leverage features that are present exclusively in leukemic blasts to differentiate them from normal cells. Commonly used techniques include multicolor flow cytometry (MFC) to detect leukemic cells by immunophenotypic aberrancies, real-time quantitative polymerase chain reaction (qPCR) for detection of recurrent gene fusions (e.g., *BCR-ABL1*) or rearranged immunoglobulin (IG) and T-cell receptor (TCR) genes.<sup>17</sup> A more recent tech-

nique which relies on high-throughput next-generation sequencing (NGS) may offer a more sensitive approach to detect IG and TCR rearrangements in ALL blasts.<sup>18</sup> The main advantages and disadvantages of these MRD assessment methods are summarized in Table 1.

### Multicolor flow cytometry

MFC is a fast and relatively inexpensive method that is broadly applicable to most ALL cases. It distinguishes leukemic cells based on their aberrant immunophenotype or leukemia-associated immunophenotype. A leukemia-associated immunophenotype can include antigen over- or under-expression, asynchronous antigen expression, cross-lineage antigen expression, and ectopic phenotypes.<sup>16</sup> It is necessary to obtain information about the immunophenotype at diagnosis in order to track it throughout the clinical course of an individual patient. However, these features may change under therapeutic pressure, and antigens may be lost or new antigens may be over-expressed as the leukemia evolves. To overcome this challenge, an alternative flow-based MRD approach named “different from normal” has been widely utilized.<sup>19</sup> The “different from normal” approach involves a standardized panel of several markers that are used to distinguish leukemic cells from normal hematopoietic cells (Figure 2). The most common markers used to identify leukemic B lymphoblasts include CD10, CD19, CD45, CD34 and CD38. Leukemic cells often have high CD10 and low CD38 expression, which may distinguish them from hematogones. Aberrant myeloid marker expression (e.g., CD33, CD13, and CD15), or expression of CD9, CD73, and CD81 may also be

**Table 1.** Comparison of techniques to measure residual disease in acute lymphoblastic leukemia.

	Multi-color flow cytometry	qPCR for fusion genes	ASO-qPCR for IG/TR genes	High-throughput NGS
Sensitivity	10 <sup>-4</sup>	10 <sup>-4</sup> to 10 <sup>-5</sup>	10 <sup>-4</sup> to 10 <sup>-5</sup>	10 <sup>-6</sup>
Applicability	>90%	40-50%	90-95%	>90%
Advantages	<ul style="list-style-type: none"> <li>- Rapid</li> <li>- Relatively inexpensive</li> <li>- DfN method does not require access to diagnostic specimen</li> </ul>	<ul style="list-style-type: none"> <li>- Sensitive</li> <li>- Standard primers used for specific fusions</li> </ul>	<ul style="list-style-type: none"> <li>- Sensitive</li> <li>- Applicable to most patients</li> <li>- Standardized guidelines in Europe</li> </ul>	<ul style="list-style-type: none"> <li>- Very sensitive</li> <li>- Applicable to almost all patients</li> <li>- Clone-unbiased (can track multiple clones and evolution)</li> <li>- Only US FDA-approved assay (ClonoSEQ)</li> <li>- Data for MRD use in peripheral blood</li> </ul>
Limitations	<ul style="list-style-type: none"> <li>- Variable sensitivity</li> <li>- Requires technical expertise</li> <li>- Fresh cells required</li> <li>- Less standardized</li> <li>- Immunophenotypic shifts can lead to false negative results</li> </ul>	<ul style="list-style-type: none"> <li>- Not applicable to all patients</li> </ul>	<ul style="list-style-type: none"> <li>- Time-consuming</li> <li>- Expensive</li> <li>- Relies on pre-treatment sample</li> <li>- Requires extensive experience and labor</li> </ul>	<ul style="list-style-type: none"> <li>- Expensive</li> <li>- Longer turn-around time than MFC</li> <li>- Requires diagnostic pre-treatment sample</li> </ul>

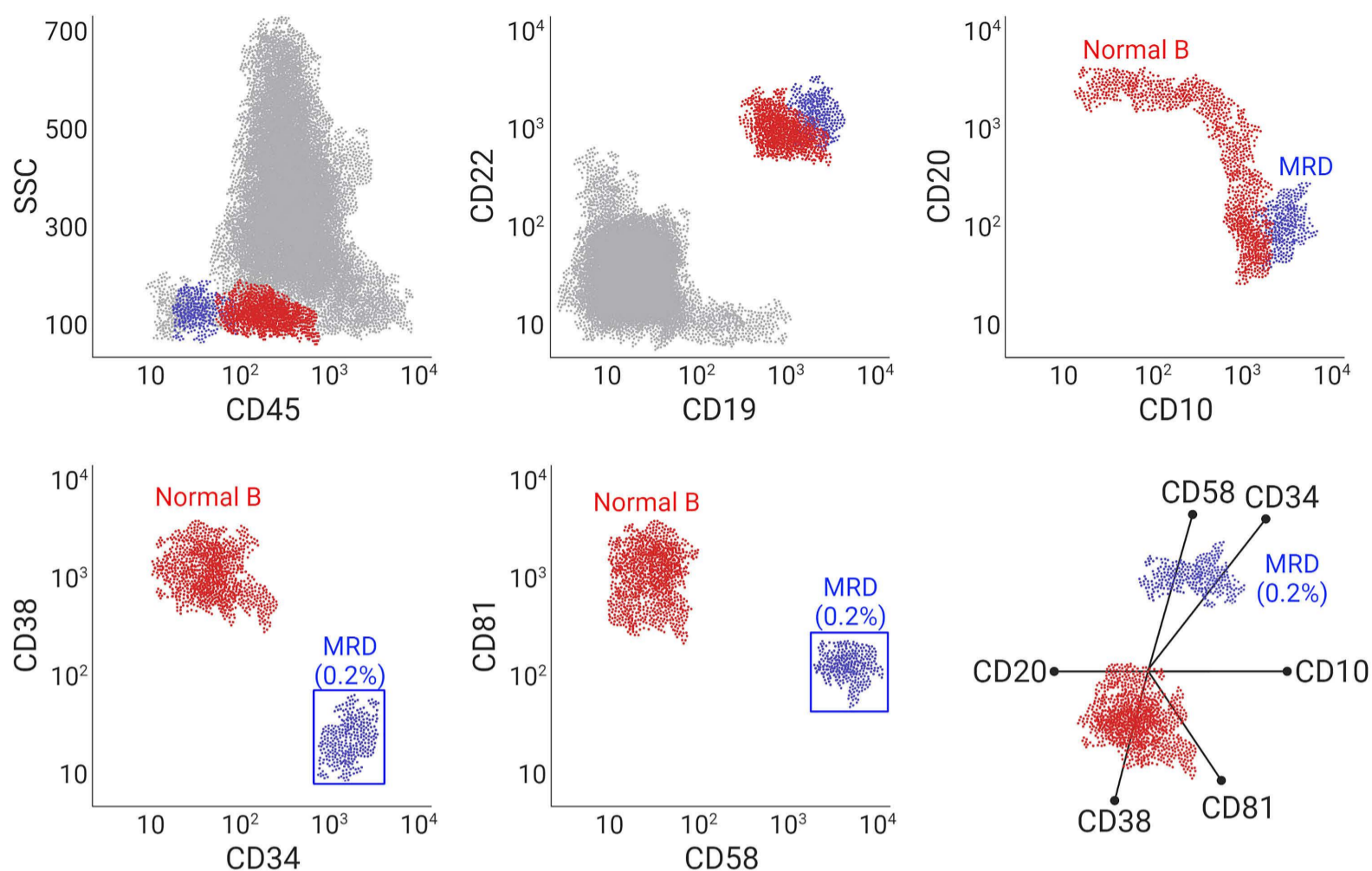
ALL: acute lymphoblastic leukemia; ASO: allele-specific oligonucleotide; DfN: different-from-normal; FDA: Food and Drug Administration; IG: immunoglobulin; MFC: multicolor flow cytometry; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; TCR, T-cell receptor.

helpful to define B-lymphoblasts.<sup>20</sup> Clinical laboratories should be informed if the patient has received CD19-, CD20- or CD22-targeted therapies, as these markers may no longer be reliable in MRD detection for these patients. It is important to identify CD19 antigenic escape with flow MRD, since these patients would not benefit from further CD19-targeted therapies. In patients receiving anti-CD19 agents, other B-cell markers, such as CD20, CD22, and CD79a, can be used to identify the CD19-negative leukemia population, but can also lead to misidentification of normal B-cell precursors.<sup>21</sup> Therefore, flow MRD should be used in conjunction with other methods of MRD evaluation (such as qPCR or NGS-based methods) for patients who have received anti-CD19 therapies. Clinicians and MRD laboratories should also be aware of the rare event of myeloid lineage switch after anti-CD19 therapies, which has been reported in both children and adults treated with blinatumomab or CAR T-cell therapies.<sup>22,23</sup> This is more commonly observed in patients with *KMT2A*-rearranged ALL, but has also been seen in ALL with *BCR-ABL1* translocation.<sup>23</sup> In these cases, flow cytometry may identify blasts expressing myeloid as opposed to lymphoid markers. Since these cases persistently harbor their cytogenetic rearrangement at the time of myeloid relapse, complementing flow MRD with reverse transcriptase PCR (RT-PCR)-based MRD assessment can enable accurate diagnosis of this entity. Similarly, NGS-based MRD assessment may be helpful when flow-based MRD assessment

has limitations in these cases. Finally, another recently described rare entity called switch ALL (swALL) may pose challenges for flow-based MRD detection.<sup>24</sup> These precursor B-ALL arise from CD2<sup>+</sup> lymphoblasts that do not harbor *KMT2A* rearrangements but have upregulated CEBP $\alpha$  activity. These cases are characterized by a switch between precursor B (CD19<sup>+</sup> CD14<sup>-</sup>) and monocytoid (CD19<sup>-</sup> CD14<sup>+</sup>) immunophenotypes through a transdifferentiation mechanism involving alterations in the expression of CEBP $\alpha$ , PAX5, PU1 and GM-CSFR.<sup>25</sup> This disease can be tracked by using IG gene rearrangements that are preserved throughout different switch states.

CD34, TdT, CD7, cytoplasmic CD3, and CD1a are commonly utilized markers for flow-based MRD detection in T-ALL; however, MFC for residual T-lymphoblast detection is less developed compared to that for B-ALL.<sup>26</sup>

Although the sensitivity of MRD MFC from ALL bone marrow aspirate sampling approaches 10<sup>-4</sup>, results are dependent upon the quality of sample obtained, and the laboratory operator's experience.<sup>27</sup> The results of MFC-based MRD assessment may be optimized by treating samples with EDTA or heparin anti-coagulation and using 2-5 mL from the first pull of bone marrow aspirate. Since the degree of cellularity in the sample will affect the recorded number of events, up to 5 mL may be required for hypocellular remission samples. There is no evidence for unequal distribution of ALL cells in different parts of the bone marrow compartment as shown in studies of bilat-



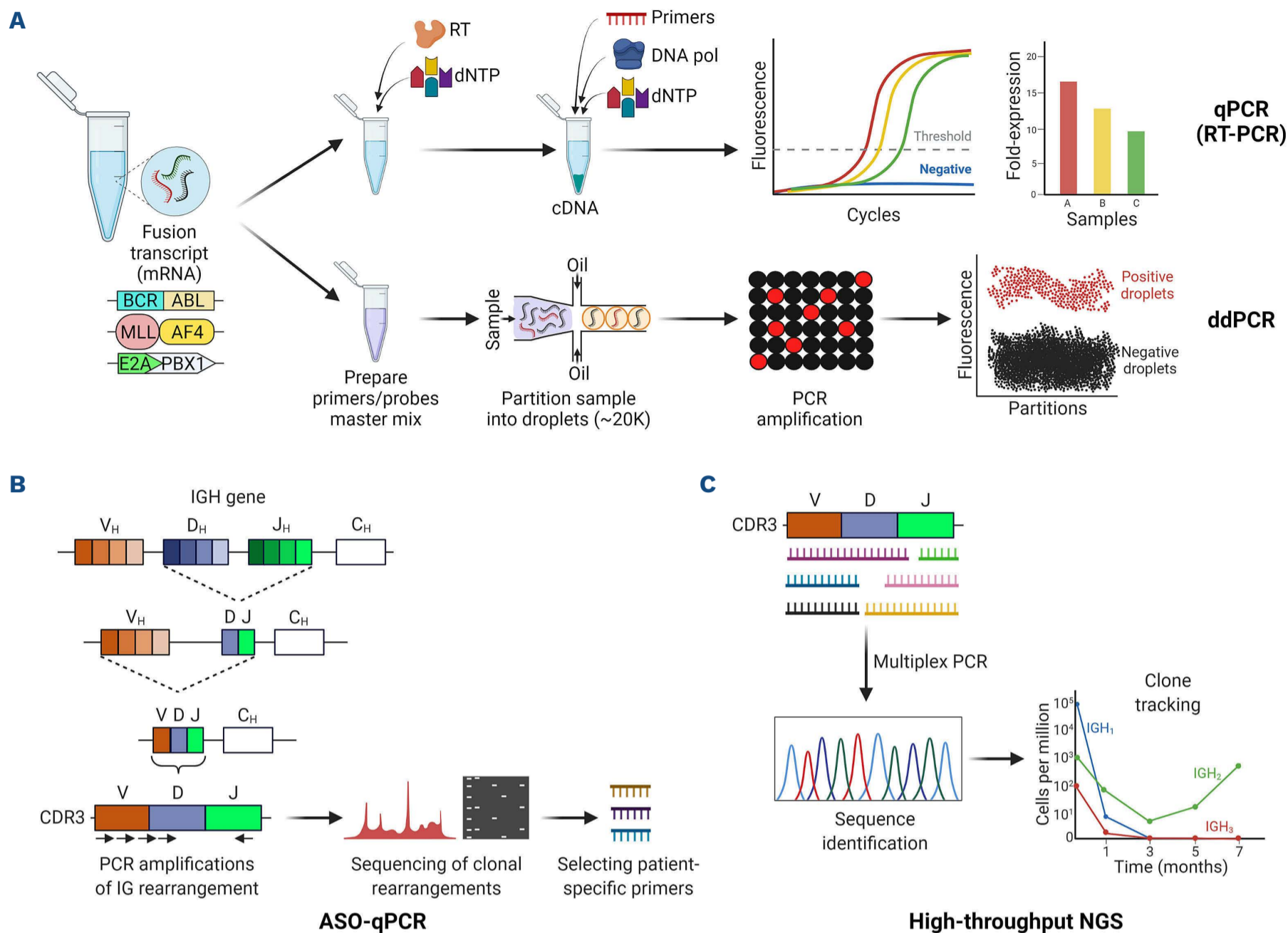
**Figure 2. Multicolor flow cytometry as a method of measurable residual disease detection in acute lymphoblastic leukemia.** An example showing multicolor flow cytometry of a bone marrow specimen, obtained after induction with chemotherapy plus rituximab (anti-CD20) in a patient with B-cell acute lymphoblastic leukemia (B-ALL). The measurable residual disease (MRD) population (0.2% of total events) is shown in blue, and distinguished from normal B cells and hematogones by its overexpression of CD10, CD58, and CD34, and underexpression of CD38 and CD81. CD20 expression is lost in leukemic blasts as a result of rituximab therapy. The radar plot visualization easily distinguishes the B-ALL MRD population.

eral bone marrow sampling for MRD detection.<sup>28</sup> Fresh samples sent for MFC should be processed within 24-48 hours of collection, and an advantage of MFC is the rapid reporting time for clinical results which may be returned to the clinician within 3 days of collection. Since MFC assays try to identify rare events, the number of events needed to be collected depends on the desired assay sensitivity and the optimal coefficient of variation as dictated by Poisson statistics.<sup>29</sup> For ALL MRD, the recommended current threshold for clinical decisions is 0.01% ( $10^{-4}$ ) sensitivity. Thus, to obtain a coefficient of variation of 10%,  $10^6$  events must be acquired. Select reference laboratories can reach high sensitivity, as was reported in the PETHEMA study under the EuroFlow MRD Consortium.<sup>13,30</sup> By using an optimized erythrocyte bulk-lysis protocol, bone marrow samples containing more than  $10^7$  cells can be lysed and resuspended in a small volume of buffer (~100  $\mu$ L), with which one can achieve the 0.01-0.001% ( $10^{-4}$  to  $10^{-5}$ ) sensitivity.<sup>19</sup> This bulk-lysis protocol for sample preparation was combined with a two-tube next-generation flow approach by the EuroFlow Consortium, which also benefits from an optimized combination of fluorochromes and antibody reagents to increase specificity at very low MRD levels.<sup>31</sup>

Although MFC may be performed using peripheral blood, the ability to use blood as a source for MRD detection by MFC is limited by reduced sensitivity.

### Real-time quantitative polymerase chain reaction

Chimeric gene fusions are major oncogenic drivers that can be found in ~40% of B-ALL, and some T-ALL cases. Since these rearrangements are oncogenic, they are often stable throughout the disease course, making them good targets for MRD assessment (Figure 3A). RT-PCR can be used to track *BCR-ABL1*, *E2A-PBX1*, *KMT2A* and *CRLF2* rearrangements in B-ALL, as well as *TAL1*, *TLX1*, and *TLX3* fusion transcripts in T-ALL.<sup>32</sup> The advantage of fusion transcript detection is the availability of standardized universal primers specific for each fusion transcript, which simplifies the MRD detection process while offering a sensitivity of  $10^{-4}$  to  $10^{-5}$ .<sup>33</sup> Quantification of *BCR-ABL1* transcripts is relatively straightforward, and p210 transcript quantification can be reported using an international standard (IS) because of the work done in chronic myeloid leukemia, while the IS score cannot be applied to the more common (in ALL) p190 transcript, so standard quantification of the *BCR-ABL/ABL* ratio is used. Fusion transcript detection with RT-PCR is an essential



**Figure 3. Molecular methods of measurable residual disease detection in acute lymphoblastic leukemia.** (A) Quantitative polymerase chain reaction (qPCR) and digital droplet PCR (ddPCR) can be used to detect fusion mRNA transcripts in acute lymphoblastic leukemia (ALL). (B) Allele-specific oligonucleotide qPCR (ASO-qPCR) leverages IG/TR gene rearrangements to detect measurable residual disease in ALL. It relies on identification of V(D)J sequences in a diagnostic sample, followed by the design of patient-specific primers for the sequences. (C) High-throughput next-generation sequencing (NGS) also targets specific V(D)J rearrangements, but it is fast and clone-unbiased as it uses multiplex PCR and does not require development of patient-specific primers.

method of MRD assessment for patients with *BCR-ABL* and *KMT2A* rearrangements, since these patients may experience immunophenotypic shifts or myeloid lineage switch under the influence of CD19-directed antibody and CAR T-cell therapies.<sup>23</sup>

Digital droplet PCR (ddPCR) is a third-generation PCR technology, which is based on separation of the sample into at least 20,000 water-oil emulsion droplets, followed by PCR amplification in each droplet. This technique is highly sensitive with improvement in the limit of detection and does not require a reference curve. Early studies suggest that ddPCR may have utility as a more sensitive MRD detection tool in Philadelphia chromosome-positive (Ph<sup>+</sup>) B-ALL.<sup>34,35</sup> During early stages of lymphocyte development, B- and T-cell progenitors undergo somatic recombination of the variable (V), joining (J), and in some cases, diversity (D) gene segments of their IG and TCR genes, respectively.

Leukemic blasts arising from these precursor lymphoid cells contain clonal V(D)J rearrangements in more than 80% of cases.<sup>33</sup> Given the specificity of the rearranged IG/TR DNA sequence for identification of leukemic cells, qPCR-based methods have been developed to track MRD. Allele-specific qPCR (ASO-qPCR) is a labor-intensive procedure, which relies on the identification of “fingerprint-like” IG/TR V(D)J sequence(s) in a diagnostic leukemia sample, followed by development of patient-specific primers to assess for the presence of these specific sequence(s) in remission samples (Figure 3B).<sup>36</sup> The initial characterization requires a panel of screening PCR using established primers for the complementarity-determining region 3 (CDR3), which is the hypervariable heavy chain region of IG/TR genes. This is then followed by Sanger sequencing of products to identify the clones and develop patient-specific primers. Depending on template avail-

ability, primer selection, and the amount of DNA in a given specimen, this patient-specific assay can have a sensitivity between  $10^{-4}$  and  $10^{-5}$ .<sup>37</sup> IG/TR-based methods are applicable to more than 90% of ALL cases, with reduced sensitivity in early T-precursor ALL, since the latter often arises from more immature progenitors that have not undergone TCR rearrangement. In addition to its laborious and time-consuming methodology, ASO-qPCR may be limited by the loss or emergence of new V(D)J sequences, leading to false negative MRD results.<sup>38</sup>

In a study in which both *BCR-ABL1* level and IG/TR ASO-qPCR were monitored in the bone marrow of Ph<sup>+</sup> B-ALL patients, overall concordance between the two methods was ~70%,<sup>39</sup> but IG/TR was found to be more reliable at predicting outcomes. However, another study investigating the discrepancy between the two methods showed that some patients with persistent *BCR-ABL1* transcript levels may in fact contain the fusion gene in myeloid cells, indicating a chronic myeloid leukemia-like stem cell disease, which may be missed by ASO/qPCR.<sup>40</sup> In the GRAAPH-2014 study, 38% of adults with *de novo* Ph<sup>+</sup> ALL had residual *BCR-ABL1* positivity during treatment, related to *BCR-ABL1* “clonal hematopoiesis”.<sup>41</sup> The presence of *BCR-ABL1* clonal hematopoiesis was not associated with poorer outcome, and patients with residual clonal hematopoiesis did not benefit from allogeneic HCT. Therefore, RT-PCR and IG/TR MRD techniques may complement each other and should be monitored simultaneously in Ph<sup>+</sup> ALL patients in order to guide therapeutic decisions.

Several studies compared the sensitivity of MFC- and qPCR-based MRD assessment in peripheral blood versus bone marrow samples. In B-ALL, MRD can be up to three-logs lower in the peripheral blood than in bone marrow, indicating the importance of marrow assessments when these methods are used to track MRD.<sup>42</sup> In contrast, MRD levels in T-ALL are roughly equivalent between the two sources, which may in part be explained by the thymic origin of this disease.<sup>43</sup>

### High-throughput next-generation sequencing

Recent advances in NGS have led to a great interest in developing NGS-based MRD methods, which can potentially increase the sensitivity of detection. These assays include sequencing the IG/TR gene V(D)J rearrangements as in ASO-qPCR, but have the capability of simultaneously amplifying multiple combinations of rearranged IG/TR genes by multiplex PCR without the need for patient-specific primers (Figure 3C).<sup>44</sup> Leveraging the high capacity of NGS, a larger picture of the IG/TR repertoire can be interrogated in one experiment, enabling the detection of clones that are at lower frequency at diagnosis but expand later in the disease trajectory.<sup>45</sup> Unlike ASO-qPCR, NGS-based IG/TR MRD is not clone-biased, meaning that new clones may be identified as they emerge in remission and relapse samples if the necessary clonal evolution and new clone identifica-

tion tools are included in the assessment.<sup>46</sup> The sensitivity of NGS MRD approximates 0.0001% ( $10^{-6}$ ) in the bone marrow;<sup>47</sup> however, the significance of MRD below the traditional threshold of  $10^{-4}$  has not been defined across different clinical scenarios. Similar to flow MRD, the sensitivity of NGS MRD depends on the amount of input, which is the amount of DNA in this method. The analysis of NGS MRD data also requires a bioinformatic pipeline. The U.S. Food and Drug Administration approved the use of ClonoSEQ NGS technology (Adaptive Biotechnologies, Seattle, WA, USA) as an MRD assessment method in ALL.<sup>48</sup> Studies have demonstrated concordance of NGS-based ALL MRD assessment using bone marrow and peripheral blood; thus, NGS may potentially enable MRD quantification from peripheral blood without the need for frequent bone marrow sampling.<sup>49,50</sup> In addition to RT-PCR and ASO-qPCR, NGS-based MRD may represent another platform to track the disease in the setting of immunophenotypic shifts or myeloid lineage switch. Since it is an IG/TR-based method, NGS MRD technology may have lower sensitivity in early T-precursor-ALL, as was the case for ASO-qPCR.

### Emerging methods

Routine clinical MRD assessment for a given patient often includes at least two methods described above (e.g., flow MRD plus RT-PCR and/or IG/TR-based methods), which provide complementary information and are advantageous for overcoming the limitations of one method. EuroClonality-NGS DNA capture (EuroClonality-NDC) is an emerging assay that is designed as an integrated tool to characterize IG/TR rearrangements, chromosomal translocations, copy number alterations, and somatic mutations through a standardized pipeline.<sup>51</sup> This assay is a robust tool providing a single workflow for detection of B- and T-cell clonality, as well as fusion transcripts and sequence variants.

As the technology advances, several innovative approaches are promising to improve the technical aspects of current MRD assessment methods, including new flow MRD methods (e.g., spectral flow cytometry which allows the simultaneous analysis of multiple surface markers), novel MFC panels, cell-free DNA-based methods, and novel NGS strategies (e.g., ALL-specific mutation detection). However, none of these techniques has robust clinical validation in ALL yet, therefore we will not discuss them within the scope of this review.

## Clinical significance of measurable residual disease in adult acute lymphoblastic leukemia

Prognostication in ALL has historically relied on baseline leukemia characteristics including white blood cell count at diagnosis, immunophenotype, and cytogenetic abnor-

malities.<sup>52</sup> Increasingly, studies have demonstrated that MRD response to front-line therapy outweighs traditional prognostic parameters and has emerged as the strongest independent predictor of ALL outcomes.<sup>30,53-55</sup> In a meta-analysis of 39 publications, comprising 13,637 patients of all ages and ALL subtypes, achieving MRD negativity (by flow or qPCR) was associated with hazard ratios of 0.25 (0.24-0.33) for event-free survival and 0.28 (0.19-0.41) for overall survival.<sup>56</sup> The authors also showed the significant prognostic utility of MRD at varying time points of assessment regardless of the MRD detection method used.

### Monitoring measurable residual disease during front-line multi-agent chemotherapy

The addition of MRD to the response assessment following front-line multi-agent chemotherapy is standard of care in the management of ALL.<sup>57</sup> Given that the traditional therapeutic course of ALL tends to include up to 12 months of intensive chemotherapy followed by 1.5-2.5 years of maintenance, understanding the optimal timing and frequency of MRD assessment is important to the delivery of high quality ALL care. Few studies have actually reported the utility of ongoing interval MRD monitoring in patients achieving MRD-negative response. The German Multicenter Study Group on Adult ALL (GMALL) evaluated a cohort of adults with ALL in first complete remission and found that the median time between emergence of MRD by ASO-qPCR and clinical relapse was approximately 4 months, suggesting that the interval between MRD assessment time-points should likely be 3 months or less, and that ongoing MRD monitoring may provide a potential window for clinical intervention prior to fulminant disease relapse.<sup>58</sup> Given that the majority of ALL relapses occur within the first 3 years following diagnosis,<sup>59</sup> MRD monitoring for early relapse detection is likely most critical during this time period.

Typically, MRD is first assessed at the completion of remission induction, which occurs 2-4 weeks following therapy initiation depending on the treatment regimen utilized. Patients who achieve MRD negativity (at least  $<10^{-4}$ ) at this early time point are considered to have chemosensitive disease, which is associated with excellent disease-free survival, and this information guides the initiation of consolidation therapy.<sup>2</sup> For these patients, we generally recommend that MRD be assessed both following induction therapy and following consolidation therapy. For adults who receive a pediatric inspired regimen, current data suggest that persistence of MRD following consolidation therapy (usually 12-16 weeks into treatment) may be the time-point with greatest relevance for making a change in treatment. Alternative therapy for MRD persistence includes blinatumomab in patients with B-ALL, given the high rates of reported MRD clearance in the BLAST trial among patients with persistent MRD and good outcomes for responding patients, the majority of whom proceeded to allogeneic transplant.<sup>60</sup> Currently, the U.S. In-

tergroup A041501 study (NCT03150693) is evaluating the incorporation of inotuzumab ozogamicin into a pediatric inspired regimen with the aim of early MRD eradication following induction therapy to improve event-free survival. Unfortunately, there is currently no proven MRD “eraser” for patients with T-ALL. For these patients, persistence of MRD following consolidation or within 3 months of therapy initiation is a signal to escalate therapy and to strongly consider allogeneic HCT in transplant-eligible adults. MRD should be assessed at shorter intervals, if possible, in patients with persistent, or newly detectable MRD, as impending clinical relapse is often likely.

NGS MRD offers a new and sensitive platform for MRD detection, yet we have limited data for the prognostic significance of low-level (between  $10^{-4}$  and  $10^{-6}$ ) MRD after multi-agent chemotherapy. The recent GMALL 07/2003 study looked at the outcomes of patients who had persistent NGS MRD below the  $10^{-4}$  detection level after consolidation therapy.<sup>61</sup> Patients with undetectable NGS-MRD had significantly better overall survival when compared to patients with positive NGS-MRD. Similarly, a recent retrospective analysis looking at 84 Ph-negative B-ALL patients in complete remission by flow MRD showed that 38% were MRD-positive by the ClonoSEQ NGS method.<sup>62</sup> Patients who were MRD negative by the NGS method had significantly lower risk of relapse, which also translated into longer overall survival.

Monitoring of MRD for Ph<sup>+</sup> ALL may be performed by a variety of methods, but RT-PCR for *BCR-ABL1* transcripts is convenient and commonly used in the USA. For adult patients with Ph<sup>+</sup> ALL receiving intensive chemotherapy in combination with second- or third-generation tyrosine kinase inhibitors, MRD response at 3 months following therapy has been shown to be highly informative. In a retrospective study conducted by the group at the MD Anderson Cancer Center, among 85 adult Ph<sup>+</sup> ALL patients who received hyper-CVAD plus a tyrosine kinase inhibitor, those who achieved complete molecular remission by RT-PCR at 3 months had an excellent overall survival even without HCT.<sup>63</sup> A phase II study of hyper-CVAD plus ponatinib in Ph<sup>+</sup> B-ALL demonstrated higher MRD-negative complete remission rates when compared to the hyper-CVAD plus dasatinib historic control group.<sup>64</sup> The role of MRD response following chemotherapy-free/light regimens for Ph<sup>+</sup> ALL remains unclear but MRD response will likely also be important in identifying patients in need of intensification and/or additional therapeutic interventions.

### Measurable residual disease and allogeneic hematopoietic cell transplantation

Allogeneic HCT is currently indicated for adults with relapsed or refractory ALL or patients who demonstrate persistent MRD following frontline induction/consolidation therapy. Several studies have shown that achieving an MRD-negative state prior to allogeneic HCT improves relapse-free survival

and overall survival. However, the depth of MRD response prior to traditional myeloablative allogeneic HCT may be important. For example, in an international study of 616 ALL patients who underwent allogeneic HCT, patients with low MRD had similar outcomes to those without MRD pre-transplant.<sup>65</sup> MRD positivity after allogeneic HCT was more important than pre-HCT MRD positivity, thus close monitoring with sensitive techniques is warranted. In a smaller study of adult patients with ALL, the presence of pre-transplant MRD at  $>10^{-4}$  detected by an NGS-based method predicted a 7.7-times higher risk of relapse after transplantation.<sup>66</sup> The prognostic impact of MRD has been demonstrated across transplant donor source and conditioning regimens.<sup>67,68</sup> It is clear that further therapy should be considered to deepen response prior to allogeneic HCT if MRD is present at  $>10^{-4}$ ; an open question remains whether additional clearance of MRD present at  $<10^{-4}$  is necessary to achieve successful transplant outcomes.

Serial tracking of MRD following allogeneic HCT also predicts for impending disease relapse. In a study of 43 adult patients who underwent HCT for ALL, those with detectable qPCR-based MRD on day +100 had an 80% relapse risk compared to a 7% risk in patients with undetectable post-HCT MRD.<sup>69</sup> In another small study of adult ALL HCT recipients, detection of MRD by NGS at any level following HCT heralded clinical relapse; the median time between MRD detection and relapse was 3 months.<sup>66</sup> Finally, MRD tracking using peripheral blood following HCT in adult ALL has been shown to be feasible and convenient, to correlate closely with bone marrow NGS-based MRD assessment, and to predict for clinical relapse.<sup>49</sup>

### Measurable residual disease in relapsed/refractory acute lymphoblastic leukemia and novel therapies

In addition to the robust evidence in newly diagnosed ALL, prognostic value of MRD for patients in second complete remission and beyond has also been demonstrated.<sup>70</sup> MRD has also played an important role in establishing eligibility and monitoring treatment response in key studies of novel therapies such as blinatumomab, inotuzumab ozogamicin, and CAR T-cell therapy.<sup>4,5,71</sup> Not only did MRD-positive ALL define the inclusion criteria for the BLAST trial, leading to an acceptance of MRD as an indication for blinatumomab in B-ALL, but MRD was also shown in secondary analyses to predict for relapse-free survival and overall survival in patients with relapsed/refractory ALL achieving complete remission following blinatumomab.<sup>72</sup> In the landmark phase III INO-VATE trial comparing inotuzumab ozogamicin versus standard chemotherapy in relapsed/refractory B-ALL, the MRD-negative complete remission rate, assessed by MFC ( $10^{-4}$  sensitivity), was 46% in patients treated with inotuzumab.<sup>73</sup> Patients who had MRD response had longer overall survival and progression-free survival than patients with per-

sistent MRD. Among children receiving CAR T-cell therapy, real-world datasets have demonstrated improved survival and reduced toxicity following CAR T-cell therapy when administered to patients with lower level/MRD-positive disease, as opposed to patients in full clinical relapse. Although the MRD-negative response rate following CAR T-cell therapy in adult ALL is very high (approximated at 81% in a meta-analysis including 489 adults receiving CAR T-cell therapies across published trials),<sup>74</sup> whether early MRD response to CAR T-cell therapy translates into long-term durable remissions in adult ALL patients remains unclear.

Re-emergence or persistence of MRD in patients with Ph<sup>+</sup> B-ALL might be associated with the selection of clones harboring ABL1 kinase domain mutations (e.g., T315I).<sup>75</sup> It is often difficult to identify the specific resistance mutation given the low level of the transcript in patients without morphological relapse. Blinatumomab has been shown to be effective in eradicating MRD in Ph<sup>+</sup> ALL.<sup>76</sup> A common strategy would be to combine blinatumomab with ponatinib, since the latter has activity against most of the common resistance mutations of ABL1.<sup>64</sup>

## Conclusions

Incorporation of MRD assessment is a fundamental component of risk-directed therapy in ALL. For almost all patients with ALL there is a reliable method to assess residual disease, which remains the most powerful independent predictor of outcomes. Blinatumomab, inotuzumab ozogamicin and CAR T cells are highly effective immunotherapy approaches in B-ALL; each is currently being explored alone and in combinations as a mechanism for MRD eradication earlier in the course of disease. The development of novel, active agents for T-ALL patients with persistent MRD remains an unmet need. As MRD detection assays become increasingly more sensitive and additional novel therapeutics enter clinical development, the future of ALL management in adults will require nuanced and evidence-based, risk-adapted treatment approaches to guide intensification or de-escalation of therapy.

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*No conflicts of interest to disclose.*

### Contributions

*All the authors wrote the review article and approved its final version.*

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