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ACUTE LYMPHOCYTIC LEUKEMIA (R. MESA, SECTION EDITOR)

MRD in ALL: Optimization and Innovations

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Abstract

Purpose of Review Measurable residual disease (MRD) is an important monitoring parameter that can help predict survival outcomes in acute lymphoblastic leukemia (ALL). Identifying patients with MRD has the potential to decrease the risk of relapse with the initiation of early salvage therapy and to help guide decision making regarding allogeneic hematopoietic cell transplantation. In this review, we discuss MRD in ALL, focusing on advantages and limitations between MRD testing techniques and how to monitor MRD in specific patient populations.

Recent Findings MRD has traditionally been measured through bone marrow samples, but more data for evaluation of MRD via peripheral blood is emerging. Current and developmental testing strategies for MRD include multiparametric flow cytometry (MFC), next-generation sequencing (NGS), quantitative polymerase chain reaction (qPCR), and ClonoSeq. Novel therapies are incorporating MRD as an outcome measure to demonstrate efficacy, including blinatumomab, inotuzumab ozogamicin, and chimeric antigen receptor T (CAR-T) cell therapy.

Summary Understanding how to incorporate MRD testing into the management of ALL could improve patient outcomes and predict efficacy of new therapy options.

Keywords Acute lymphoblastic leukemia · Measurable residual disease

Introduction

Measurable residual disease (MRD) status is important because it can predict risk of relapse and can impact treatment and overall outcome of acute lymphoblastic leukemia (ALL). Even if patients achieve complete remission (CR) by assessing morphology, the bone marrow could still contain up to 10^{10} leukemic cells after induction therapy [1–5]. Achieving MRD negativity ($\leq 10^{-4}$ leukemic cells) is correlated with improved overall survival (OS). In patients with ALL achieving MRD negative disease, the 10-year disease free survival (DFS) is 64% in adult and 77% in pediatric patients compared to only 21% and 32% in patients with MRD positive disease in adults and pediatric, respectively [5–6•]. MRD negativity is associated with significantly improved survival outcomes in a variety of treatment strategies, including hyperCVAD, the CALGB protocols, and the addition of tyrosine kinase inhibitors for Philadelphia chromosome positive (Ph-positive) disease [7–9]. MRD status is also incorporated into approved indications for drugs, such as the Food and Drug Administration (FDA) approval of blinatumomab for MRD positive ALL ($\geq 0.1\%$) [10–11]. Due to the increasing recognition of MRD significance, it is important to understand which patients should undergo MRD testing and which MRD monitoring strategies should be utilized.

Who Should Undergo MRD Testing?

MRD testing has evolved over the past 30 years. Early studies focused primarily on childhood ALL and showed that MRD detection was associated with inferior prognosis [12–14]. The first studies evaluating MRD in adult ALL

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confirmed the prognostic significance of this technique [15–16]. In a cohort study by Brisco et al. published in 1996, patients with lower levels of MRD detected in CR after first induction by quantitative polymerase chain reaction (PCR) had longer duration of remission [15]. Additional studies in adults have shown that outcomes are improved when MRD negative status is achieved early in the treatment course [17–19]. In a US-based trial published in 2016, among 323 adult patients with B-cell ALL who achieved CR after induction with hyperCVAD, negative MRD status assessed by multi flow cytometry (MFC) (sensitivity 1×10^{-4}) was associated with improved DFS and OS [18]. The prognostic value of MRD in all types of adult and childhood ALL was further evaluated in a meta-analysis by Berry et al. of 13,637 patients from 39 studies (16 adult studies representing 2076 adult patients) [6•]. Among both children and adults, MRD negative status after induction was associated with improved event free survival (EFS) and OS (for adults; EFS hazard ratio for achieving MRD negativity was 0.28) (95% Bayesian credible interval (BCI) of 0.24–0.33), and OS hazard ratio for achieving MRD negativity was 0.28 (95% BCI 0.20–0.39)) [6•].

MRD Monitoring for Standard Risk ALL

Although patients with standard risk ALL generally have better outcomes and increased response to chemotherapy, a large portion of them experience disease relapse [2]. MRD has emerged valuable in identifying those patients with standard risk ALL who are likely to experience relapse of their disease after achievement of CR [20–25]. Thus, patients with MRD negative disease may be spared the additional toxicity associated with allogeneic hematopoietic cell transplantation (allo-HCT). Serial monitoring of MRD in this patient population could help identifying patients who are at risk of relapse and may benefit from early salvage therapy [26].

MRD Monitoring for High-Risk Philadelphia Negative ALL

High-risk Ph-negative ALL is another patient population who may benefit from MRD testing [27]. In the PETHEMA ALL-AR-03 trial, 179 adolescent and adult patients with high risk, Ph-negative ALL who achieved CR and completed early consolidation were assigned to allo-HCT if they had positive MRD detected with MFC (sensitivity of 5×10^{-4}) or delayed consolidation chemotherapy followed by maintenance treatment for 2 years after CR. The 5-year DFS for allo-HCT and chemotherapy were 32% and 55%, respectively. The 5-year OS for the two groups were 37% and 59%, respectively. When analysis was performed by actual treatment (only 59 of 71 patients in allo-HCT group underwent transplantation), median time to relapse was shorter for the patients receiving allo-HCT compared to chemotherapy (10.3 vs 13.8 months respectively), and 5-year DFS was higher for the patients receiving chemotherapy (37% vs 48%). The results of this study show that patients with high-risk Ph-negative ALL can be stratified to different intensity of treatment based on MRD status [24]. A more recent trial (PETHEMA ALL-HR-11) analyzed 218 patients who were allocated to delayed consolidation and maintenance chemotherapy based on MRD negativity by MFC after induction (sensitivity $<1\times10^{-3}$). One hundred and six patients did not achieve CR or had detectable MRD after induction and underwent allo-HCT. In the intention to treat analysis, 5-year cumulative incidence of relapse was similar between groups (45% vs 40%; $p=0.43$), but 5-year OS was higher in the chemotherapy group compared to the allo-HCT group (59% vs 38%; $p<0.001$). Better outcomes were seen overall in this trial despite treatment group compared to the ALL-AR-03 trial, which were attributed to a reduction in treatment-related deaths and transplant associated mortality [28]. These results indicate that MRD analysis can be used to identify patients with high-risk Ph-negative ALL who have good early response to treatment and can avoid the therapy-related toxicities associated with allo-HCT.

MRD Monitoring for Philadelphia Positive ALL

The Philadelphia chromosome, t(9;22), identified in 20–30% of adult patients with ALL, is associated with a poor prognosis and higher rates of relapse after treatment [29–30]. Standard of care for this high-risk group involves remission induction with tyrosine kinase inhibitors (TKIs) followed by allo-HCT [27, 30]. The BCR-ABL mRNA transcript is detected with RT-PCR and can be used to evaluate MRD status. BCR-ABL transcript levels have been shown to correlate with relapse free survival (RFS) and OS [8, 31–32]. In one study, compared to patients with undetectable MRD, patients with MRD detectable to a cutoff of 10^{-5} and 10^{-3} were respectively 6.3 and 9.1 times more likely to relapse [33]. Other studies evaluate the use of TKIs for the treatment of Ph-positive ALL where MRD was frequently used; MRD positivity was an independent predictor of relapse and poor survival [34–37].

MRD Monitoring Prior to Allogeneic Hematopoietic Cell Transplantation

Detection of MRD prior to allo-HCT has been associated with an increased risk of post-transplant relapse and poor outcomes [38–39]. In a meta-analysis of 21 studies of

children and adults with ALL undergoing allo-HCT, detection of MRD prior to transplant was associated with a higher rate of relapse (HR 3.26; 95% CI 2.23–4.75), lower EFS (HR 4.77; 95% CI 3.31–6.87), and lower OS (HR 1.98; 95% CI 1.40–2.80) [40•]. The association of pre-transplant MRD detection by quantitative PCR with worse post-transplant outcomes has also been seen in the high-risk subgroup of Ph-positive ALL [41–42•]. MRD negativity after a TKI containing induction correlated with improved long-term outcomes after allo-HCT [43–48]. These findings support the achievement of MRD negativity for patients with Ph-positive ALL prior to allo-HCT.

In an attempt to reduce transplant related mortality, two studies incorporated MRD analysis with the use of a reduced intensity conditioning (RIC) prior to allo-HCT [49–50]. Banchova et al. showed inferior outcomes compared to myeloablative conditioning (MAC) if MRD was detected at CR, but among patients treated with TKIs and achieving MRD negativity, RIC was associated with improved OS compared to MAC [50]. This was felt to be driven in large part by a reduction in transplant-related mortality (TRM) with RIC [50]. Akahoshi et al. evaluated elderly patients (age >50 years old) with negative MRD disease at CR and compared outcomes with RIC versus MAC. They found no difference in OS, hematologic response, and relapse morality between the two groups. However, among a subgroup of patients with poor performance status or those at high risk of morbidity with allo-HCT, there was an improved OS with RIC [49]. This would suggest a role for MRD in determining the intensity of conditioning prior to allo-HCT in older patients at an increased risk for TRM.

MRD Monitoring After Allogeneic Hematopoietic Cell Transplantation

MRD monitoring in the post-HCT setting has also been shown to be a strong predictor for disease recurrence with higher sensitivity and specificity than chimerism analysis [51–53]. Studies of MRD monitoring in Ph-positive ALL have shown similar results with longer DFS with post-allo-HCT MRD negativity [54–57]. Outcomes were improved if MRD negativity was achieved before transplant and persisted following transplant [58]. The utility of MRD to guide post-allo-HCT TKI maintenance therapy with imatinib in Ph-positive ALL was evaluated in two studies [59–60]. Chen et al. showed that using an MRD-guided approach for initiation of post-transplant imatinib (starting imatinib when MRD was detected) was associated with improved DFS and OS [59]. Pfeifer et al. compared prophylactic imatinib following allo-HCT versus initiation of imatinib upon MRD detection. Although prophylactic imatinib reduced the incidence of relapse, the 5-year OS was similar between the groups [60]. In a retrospective analysis evaluating outcomes associated with MRD monitoring both before and after allo-HCT, Zhou et al. found that post-HCT MRD detection was a strong predictor of relapse, whereas pre-HCT MRD detection had a nonsignificant trend toward shorter progression free survival [53]. These results may indicate that MRD detection post allo-HCT has stronger clinical correlation than pre-allo-HCT monitoring.

MRD Monitoring with Novel Therapies

Blinatumomab is a bispecific antibody targeting CD3 on T cells and CD19 found on B cells [61]. Blinatumomab was approved for use in adults with relapsed or refractory B-cell precursor ALL after the TOWER trial showed improved OS compared with chemotherapy [62]. Achievement of negative MRD status was evaluated as a secondary outcome in this trial and was more prevalent among patients who achieved CR with blinatumomab than chemotherapy (76 vs 48 percent of patients). Other studies evaluating the efficacy of blinatumomab adopted MRD as a primary outcome and found that it correlated with longer survival [63–66]. Gokbuget et al. evaluated the use of blinatumomab for patients with B-cell precursor ALL who achieved CR after multiagent therapy but had detectable MRD. Seventy-eight percent of patients achieved MRD negativity with blinatumomab, which correlated to improved OS and RFS [67•]. This trial led to the approval of blinatumomab for patients with B-cell precursor ALL with MRD positive disease.

Inotuzumab ozogamicin is an anti-CD22 monoclonal antibody conjugated to the cytotoxic molecular calicheamicin [68]. In the randomized phase 3 INO-VATE ALL trial, inotuzumab was superior to standard intensive chemotherapy for the primary outcomes of CR and OS among patients with relapsed or refractory ALL (both Ph-positive and Ph-negative diseases were included). MRD negativity among patients achieving CR was a secondary outcome of the trial and was higher in the inotuzumab group [69]. A follow-up analysis of the INO-VATE ALL trial and a prior phase 1/phase 2 study showed improved MRD negativity with inotuzumab versus chemotherapy, but significant improvement in OS and PFS with inotuzumab was not observed. This discrepancy was attributed to a need for more sensitive MRD analysis as the cutoff for MRD negativity used in these studies <0.01% [70]. Nonetheless, MRD analysis played an important role in the evaluation of efficacy of inotuzumab in the treatment of ALL.

Chimeric antigen receptor T cells therapy (CAR-T) is approved for treatment of relapsed or refractory ALL [71–72]. After long-term follow-up in a phase 1 study of CD19 targeted CAR-T cells in 53 adults (median age of 44) with relapsed or refractory CD19+ B cell ALL, Park et al. show that MRD negative CR after infusion of cells

resulted in significantly improved EFS and OS [71]. In the ZUMA-3 trial, MRD negativity was attained in 76% of all patients treated with CAR-T, and 97% of those achieving CR. Median OS was not reached at the time of publication among responders suggesting a durable remission, which correlated with the degree of MRD negativity seen [72]. Additional studies of CAR-T cell therapy are likely to utilize MRD status as an outcome due to its correlation with long-term outcomes.

Different Types of MRD Monitoring

Broadly, approaches to MRD testing include assessment of DNA, RNA, and protein antigens and can identify extremely low numbers of cancer cells, as few as one cancer cell in one million normal cells. This technique well exceeds the limits of classical microscopy techniques. Current and developmental techniques for MRD include MFC, next-generation sequencing (NGS), qPCR, and ClonoSeq [73]. These high sensitivity methods are either able to detect targeted genetic abnormalities (PCR techniques and NGS) or tumor-associated immunologic profiles (MFC) [74] (Table 1).

A. Multiparametric Flow Cytometry (MFC)

Definition

MFC is a method for detecting multiple immunophenotypic features of cells and represents a technological advance from basic 4-color flow cytometry. Presently, MFC utilizes 6–8 colors to label antigens or other cellular features associated with target cells, including lymphoblasts, and thus generating an immunophenotypic profile. In recent years, there has been significant progress in the sensitivity of MFC-based MRD monitoring of acute leukemias, with reported sensitivities of 10^{-4} to 10^{-5} in B- and T-cell ALL and applicability to >99% of cases [74].

How It Works

MFC quantifies the expression of antigens by the strength of the signal emitted by monoclonal antibodies which have been tagged with a specific fluorochrome. The antigens selected are known to be associated with the development of T- and B-cells. Prior to therapy, leukemia-associated immunophenotypes (LAIPs) must be identified so that they can later be used for MRD detection. This is done by comparing the surface, nuclear, or cytoplasmic antigens from known leukemic cells to those of bone marrow reference samples by exposing them to a series of conjugated monoclonal antibodies.

Table 1 MRD testing methodologies and characteristics

MRD methodology	Multiparametric flow cytometry (MFC)	*Next-generation sequencing (NGS)*	*Quantitative PCR (qPCR)*	*ClonoSeq*
Advantages	-High applicability	-Can identify neoplasms without previously identified genetic anomalies.	-Has become the standard for MRD ALL	-Can provide information on clonal changes throughout the treatment process and therefore help guide treatment
	-Ease of quantification	-More sensitive than other modalities and can use peripheral blood samples		-Can be used to assess plasma circulating tumor DNA in lymphomas
	-Information on cell viability and normal cells	-More specific for predicting relapse		
	-Rapid turn-around			
	-Low cost			
Limitations	-Lower specificity compared to other techniques	-Not widely available	-Limited use in assessing relapse	-Different MRD assays may not be interchangeable and make generalizability difficult
	-Only available at a few specialized laboratories	-Prohibitively expensive	-Junctional regions of each leukemia must be already identified	-This assay may overestimate MRD frequencies near the limit of detection
		-Not as standardized as other techniques	-Only detect dominant genetic rearrangements, so subclonal anomalies may go undetected	
		-Picks up both clinically relevant mutations and passenger mutations		

An alternative to identifying LAIPs at the time of diagnosis is to use the "different from normal" (DFN) analytic approach, which compares the antigen profile of a sample to a bone marrow sample of comparable cells in terms of maturation and lineage. This technique has the advantage of not requiring the identification of LAIPs at diagnosis but does require more standardization.

For B-ALL, the most common approach is to use phenotypic markers which are commonly present in aberrant patterns in leukemic cells such as CD34, CD19, CD10, and CD20 as well as markers which are more commonly expressed in pathologic cells such as CD7, CD33, and CD58. The markers which make up the backbone of the MFC assessment include CD34, CD19, CD20, CD38, and CD45 [75]. It has been suggested that in addition to these six markers, CD81 and either CD66/CD123 or CD73/CD394 may allow for MRD measurements comparable to PCR methodologies with sensitivities up to 10^{-5} with >90% concordance in >98% of B-ALL patients if the volume of BM cells is >4 million [76].

For T-ALL, typical MFC panels consist of antibodies for maturation markers CD3, CD5, CD7, CD2, CD8, CD34, and CD45, which may be expressed asynchronously in malignancy. They may also detect thymic antigens which may be expressed ectopically in cases of T-ALL.

Advantages

Compared to molecular diagnostics, which are used to characterize the tumor genome, MFC is used to profile the tumor phenotype. While these techniques are complementary, MFC has several distinct advantages over molecular diagnostics including high applicability, ease of quantification, and ability to yield information on cell viability and normal cells. Additional practical advantages include relative ease of use, low cost, and rapid turn-around (analysis of large volumes of cells in a short amount of time) [77].

Limitations

Only a few specialized laboratories have the capability of maximizing the use of this technique in terms of sensitivity and reproducibility. Guidelines have been established for the standardization of practice of this technique. Other limitations include lower specificity compared to molecular techniques and the possibility of immunophenotypic shifts during the course of disease [77].

Current Usage: (PubMed Search of "Multiparametric Flow Cytometry" AND "Acute Lymphoblastic Leukemia")

In B- and T-cell ALL, MRD using MFC is an important prognostic factor used in monitoring treatment efficacy, relapse risk, and guiding decisions regarding treatment intensification and allo-HCT [78]. The majority of large studies using MFC for MRD were performed in pediatric populations. Definitive studies investigating use of MFC for MRD monitoring in adult ALL populations are sparser [74].

In B- and T-cell ALL, RQ-PCR of Ig/TCR rearrangements and gene fusion products remains the fundamental approach to MRD measurement, though there is a growing role for MRD using MFC. A 2017 study by Theunissen et al. used a standardized Euroflow 8-color antibody panel to measure MRD in 319 BCP-ALL patients with a sensitivity of $\leq 10^{-5}$, a result comparable to validated RQ-PCR techniques. This strategy was found to be applicable to >98% patients [76]. A 2020 study by Modvig et al. used existing MFC MRD to risk stratify 1487 patients with BCP-ALL enrolled in NOPHO ALL2008 protocol and found this to be clinically useful [79].

B. Next-Generation Sequencing (NGS)

Definition

NGS is a collective descriptor for a variety of technologies used to sequence nucleic acids and analyze molecular variants using high-throughput methodologies [80]. NGS can be used to provide information on DNA sequence including rearrangements, insertions, and deletions [81]. The quantity of reads used to cover a known reference base is referred to as "sequencing coverage" and correlates to accuracy. NGS is now commonly used for measurement of MRD in ALL, with inexpensive and rapid commercial technologies becoming increasingly available. In B- and T-cell ALL, NGS is utilized to evaluate both epigenetic and clonal changes [82].

How It Works

NGS consists of breaking up a chromosome, using PCR amplification to clone the fragments, then sequencing these fragments in parallel using light emission from each sequencing reaction to record the process one nucleotide at a time. Through this process, the sequence can then be reassembled to demonstrate an entire genomic sequence. Because the sequences are cloned, the same portion of DNA is sequenced many times over, a quality known as coverage. The greater the coverage, or number of times a known portion of genetic material has been read, the greater the accuracy of the sequence. Typically, 10×–30× is the depth of coverage for mutations such as those measured in MRD. NGS is different from other sequencing modalities in that it sequences millions of samples at once in a parallel fashion to obtain a sequence more quickly. NGS, unlike qPCR, can discover new sequences, whereas other methodologies can only detect sequences that have already been identified. This is because it does not require a known patient-specific primer, as RT-PCR does.

NGS can identify gene fragments corresponding to Ig and TCR variations as well as other gene rearrangements such as insertion, deletions, and fusion genes. NGS is specific enough that it can detect MRD presence below 10^{-5} to a limit to 10^{-7}.

Advantages

NGS can identify neoplasms that do not have the classical or previously identified genetic anomalies [83]. This technique can also further subcategorize a patient's given disease to provide more targeted management. NGS is more sensitive than other modalities and may be able to use peripheral blood samples. In a 2017 study of 32 adults with B-ALL, 17% of cases had MRD detected by NGS, but not by MFC [84]. NGS may also be more specific for predicting relapse as it can provide more information about changes in genetic expression throughout the treatment process [85].

Limitations

The infrastructure for this technique is not readily available at all institutions, and the workflow has not been standardized to the extent that other techniques have been. NGS can be prohibitively expensive, with average cost per sample being approximately $1000 [86]. Because of the genetic detail which can be assessed by NGS, it is important to decipher the difference between those mutations associated with malignancy versus polymorphisms and passenger mutations [87]. Therefore, in order to use NGS effectively, cancer-specific databases must be optimized. In order for this optimization to occur, interdisciplinary teams including physicians, genetic counselors, and engineers will need to review these databases and interpret the multitude of mutations contained within them.

Standardization of NGS for MRD identification for Ig and TCR is underway [88].

Current Usage (PubMed Search "Next-Generation Sequencing AND Acute Lymphoblastic Leukemia"

Using the RNA sequencing workflow of NGS, Rellick et al. were able to create an in vitro co-culture model which may be used to detect a transcription signature of MRD [89]. NGS has also been used to help stratify risk groups based on identified ferroptosis-related genes in patients with Ph-negative B-ALL [90]. NGS has been used in this patient population for reasons other than detecting MRD.

C. Quantitative PCR (qPCR)

Definition

Real-time quantitative PCR (RQ-PCR) uses fluorescence probes which are emitted during the PCR cycle to quantify the presence of specific target sequences. Reverse transcription PCR (RT-PCR) uses gene transcripts which are processed through mRNA reverse transcription to form cDNA exons that can then be amplified through PCR. It is during the PCR cycles that the targets are quantified, as above.

How It Works

Generally considered a standard for MRD measurement in ALL, this method uses PCR primers to detect and amplify genetic alterations in IgH and TCR of B and T cells [91]. The initial analysis used by this technique to identify these genetic rearrangements was developed by BIOMED-1 and BIOMED-2. The measurement is made by comparing the qPCR samples to a standard dilution curve made from DNA from the diagnostic sample. This typically leads to a quantitative range (limit for reproducible MRD results) of 10^{-4} and sensitivity of 10^{-5}.

Current Usage

The most common targets for quantified MRD analyses are the Ig/TCR gene rearrangements which lead to the variable (V), diversity (D), and joining (J) segments of the heavy chains and TCR of progeny cells. Because recombination continues throughout cancer therapy, PCR analysis uses multiple independent Ig/TCR targets. In addition to sequences corresponding to these cellular markers, fusion gene can also be used as primer targets for MRD [80]. A recent study assessing the use of MRD in pediatric ALL patients to predict event-free survival after chemotherapy found that real-time quantitative PCR demonstrating an MRD $>10^{-3}$ was a better poor prognostic factor than MRD by multiparametric flow cytometry [92]. Overall, there are two type of quantitative PCR methods for MRD one that targets clonal IgH or TCR rearrangements and one that targets fusion transcripts. One study found that two have strong MRD concordance in TCF3-PBX1 ALL, while Ig/TCR technique was stronger for BCR-ABL1 ALL [93].

Advantages

RT-qPCR has become the standard for MRD assessment in patients with ALL [94].

Limitations

Although RT-PCR is widely used in MRD assessment, IgH or TCR genetic rearrangements cannot be identified in 8–12% of patients with ALL [95]. Additionally, this technique may have limited use in assessing relapse as one study found that only 71% of the rearrangements initially

detected were preserved following relapse [96]. RT-PCR transcripts used in MRD are not patient-specific, therapeutic drugs may affect the level of expression of certain targets and may cause RNA instability.

Because this technique is reliant on the development of patient-specific PCR assays targeted to their leukemia, the junctional regions of each leukemia must be previously identified [95]. Additionally, this technique only targets the dominant genetic rearrangements, meaning subclones may go undetected if they were only a small portion of the leukemic cells at diagnosis [91].

D. ClonoSeq: a Combination of NGS and Multiplex PCR

Definition

ClonoSeq is a commercially available assay which uses multiplex polymerase chain reaction and NGS techniques to identify cancer cell immunoglobulin rearrangements. Although RT-PCR and MFC remain gold standard methodologies for measurement of MRD in B- and T-cell ALL, Clonoseq is increasingly utilized to detect immunoglobulin rearrangements and follow the biological activity of disease in these patients.

How It Works

ClonoSeq is used to measure MRD by detection of DNA sequences on cancer cell B- and T-cell receptors. According to the Adaptive ClonoSeq Assay Technical Information, Clonoseq is able to identify and quantify IgH, IgK, and IgL receptor gene sequences and translocated BCL1/IgH in small bone marrow samples from patients with B-cell ALL as well as patients with multiple myeloma and chronic lymphocytic leukemia. The technology utilizes a standardized protocol and large pool of synthetic primers. It was the first assay licensed for MRD measurement in ALL and multiple myeloma [97].

Advantages

Because Clonoseq can provide more information on clonal changes throughout the treatment process, it can be used to adjust treatment to better target a given patient's malignancy.

According to a recent study comparing the financial burden of multiple myeloma patients in Germany with ClonoSeq MRD measurement to those without MRD measurement, this method could save 18,396 Euros per patient in 1 year and 77,140 in 10 years [98].

Limitations

According to the Adaptive ClonoSeq Assay Technical Information, there are several limitations to the use of ClonoSeq in MRD measurement in ALL. MRD values using different assays may not be interchangeable. Assay results may vary according to sample time or sample location. ClonoSeq may overestimate MRD frequencies near the limit of detection. According to Clonoseq Assay Technical Information, sample quality may affect the ability of the methodology to detect low levels of disease. At this time, ClonoSeq is a relatively expensive commercial product.

Current Usage (PubMed Search "ClonoSeq AND Acute Lymphoblastic Leukemia"

A 2021 study by Hussaini et al. investigated the feasibility of incorporating Clonoseq for MRD and diagnosis of cutaneous T-cell lymphoma. They noted that because this assay includes primers able to detect incomplete IgH and DJ rearrangements, it can detect malignancies associated with precursor B- and T-cells and that the assay can track sequences despite alterations in V(D) J [99].

Peripheral Blood Versus Bone Marrow Aspirate MRD Monitoring

MRD has been conventionally measured in bone marrow (BM) samples. In recent years, there has been significant investigation into whether peripheral blood (PB) can be used instead of BM. The collection of PB is easier and less invasive than the collection of BM. Current research suggests that MRD is variably distributed in patients with T-lineage ALL as compared to patients with B-lineage ALL. In T-lineage ALL, PB and BM MRD levels are closely correlated suggesting that PB sampling may safely and effectively replace BM sampling [100•]. However, in patients with B-lineage ALL, BM MRD levels have been demonstrated to be considerably higher than PB MRD levels with the implication that BM testing remains preferred over PB testing [100•].

Early studies comparing MRD in BM and PB samples in B-lineage and T-lineage ALL were performed almost exclusively in pediatric populations [100•]. A study comparing BM and PB samples from 15 pediatric patients with B-cell ALL demonstrated that mean MRD levels in BM were approximately 12 times higher than MRD levels in PB [101]. A subsequent study compared 532 paired BM-PB samples from 62 children with precursor B-ALL and 149 paired BM-PB samples from 22 children with T-ALL. This study found enormous variation in MRD detectability and levels

in B-cell ALL samples with MRD levels in BM being up to 1000 times higher in BM than corresponding PB; however, it demonstrated strongly correlated BM and PB MRD levels in T-cell ALL samples [102]. The consensus conclusion of research in pediatric populations was that MRD levels in BM and PB may be comparable in children with T-cell ALL but not B-cell ALL.

At the present time, there has been relatively less investigation into comparison of MRD in PB and BM samples in adult populations than in pediatric populations but these results from analysis of adult populations appear to reinforce the results from pediatric studies.

A 2021 prospective observational study by Muffly et al. evaluated the concordance of BM and PB MRD in patients ≥18 years with ALL receiving allo-HCT and CAR-T using an NGS platform. This study examined 126 paired PB and BM samples and a strong correlation between PB and BM MRD ($r = 0.87$, $p < 0.001$) [103].

A 2020 study by Kotrova et al. analyzed MRD positivity in 1219 sample pairs from 377 B-cell precursor ALL and 632 sample pairs from 161 T-ALL patients using EuroMRD allele-specific oligonucleotide RQ-PCR and EuroMRD guidelines [100•]. Among 632 T-ALL sample pairs, 302 (48%) were found to be MRD-negative, while 270 (43%) were positive in both BM and PB. Of the MRD positive T-ALL pairs, 186 (68.9%) were measurably positive within both BM and PB with relatively strong correlation between MRD results ($R^2 = 0.72$). In contrast, among 1219 BCP-ALL sample pairs, 647 (53.1%) were negative in both BM and PB, 458 were (37.6%) were positive in both BM and PM, but among MRD positive pairs only 332 (27.2%) were measurable in both BM and PB with relatively weak correlation between results ($R^2 = 0.48$). This data reinforced results from studies of pediatric populations demonstrating tighter correlation of MRD in BM and PB in T-cell ALL when compared to B-cell ALL.

A 2018 study by Keegan et al. used six-color flow cytometry to evaluate for MRD in 76 matched BM and PB samples from ALL patients. This study found MRD positivity in 18/76 (24%). Of BM MRD positive samples, 4/18 (22%) matched PB samples were also found to be MRD positive [104]. However, two cases were found to be MRD positive in PB but not BM suggesting PB sampling in addition to MRD sampling may provide an additional detection benefit.

Value of Serial MRD Monitoring

Although a comparative paucity of studies exists regarding the use of serial MRD monitoring during and after treatment, MRD evaluation is routinely performed after induction therapy in patients with Ph-negative and Ph-positive ALL [27]. Additional MRD testing is considered every 3–6 months as clinically indicated for at least 5 years [27].

A prospective study in one hundred and five standard-risk adult ALL patients in the post-consolidation setting evaluated the use of quantitative PCR MRD monitoring at 3-month intervals as an indicator of impending disease relapse [26]. All patients who were included were in hematologic remission, had received multi-agent chemotherapy, and were MRD negative at the time of study entry. Over an approximate 2-year period, 27% of patients had converted to MRD positivity with 61% of those patients developing clinical relapse. The median time from MRD positivity to clinical relapse was 9.5 months. The results of this study indicated a subset of patients with adult standard-risk ALL will ultimately convert to MRD positivity in the early post-consolidation phase [26]. A separate study evaluated the significance of MRD positivity as detected by MFC after induction and consolidation therapies. Five hundred forty-six patients were included and had achieved MRD negative status following induction and consolidation therapy with hyperCVAD or augmented BFM regimens. A total of fifty-five patients developed MRD positivity with median time to MRD relapse of 14 months. Eighty percent of patients who became MRD positive went on to develop morphologic relapse. Median DFS from time of MRD relapse was 3.6 months [105]. Overall, these studies provide evidence to support a relatively high risk for morphological disease relapse following MRD relapse.

Specific to patients with Ph-positive disease, serial MRD monitoring is recommended for patients in complete molecular remission no more than every 3 months while receiving TKI maintenance therapy after allo-HCT [27]. An increase in frequency of MRD monitoring can be considered in the setting of detectable MRD [27]. MRD persistence or reappearance in Ph-positive patients may indicate TKI resistance, which may warrant change in therapy or consideration of other treatment strategies.

In patients who underwent allo-HCT, a common approach is to utilize both serial donor chimerism and MRD monitoring. MRD has been shown to be a strong predictor for disease recurrence with higher sensitivity and specificity than chimerism analysis [51]. MRD assessment post-transplant can pose unique challenges such as shared immunophenotypes between regenerating hematogones and ALL blasts and less predictable kinetics of leukemia cell eradication which has the ability to complicate clinical interpretation [106].

Targeting MRD in ALL

Improved OS and RFS with use of blinatumomab for patients with B-cell precursor ALL with MRD positive disease provided evidence of clinical benefit for targeting MRD [67•]. There are several ongoing clinical trials evaluating therapeutic strategies to target patients who are MRD positive after receiving first-line

treatment. NCT03876769 is an ongoing phase II clinical trial including pediatric and young adult B-ALL patients (up to age 25) who received first-line treatment with MRD positivity by multi-parameter flow cytometry at the end of consolidation therapy that is evaluating the efficacy and safety of tisagenlecleucel. The primary outcome of the study is DFS with key secondary outcomes including overall survival, quality of life assessment, percentage of patients achieving MRD negativity at month 3, and percentage of patients who are disease free without allogeneic stem cell transplant. In addition, NCT03441061 is an ongoing phase II clinical trial including adult patients who are MRD positive by multicolor flow cytometry, PCR, or NGS at least 3 months after first-line treatment for B-ALL that is evaluating the efficacy of inotuzumab ozogamicin. The primary outcome of the study is relapse-free survival with key secondary outcomes including overall survival, safety, and MRD negativity rate.

Conclusion

Evaluation of MRD has become a useful tool in predicting prognosis and guiding treatment decisions for adults with ALL. Regardless of whether patients have standard-risk, high-risk Ph-negative disease, or Ph-positive ALL, achieving MRD negative disease is correlated with improved long-term outcomes including increased DFS and OS. The optimal timing of MRD evaluation in the treatment course of ALL has not been established. Studies have varied protocols as to when during induction or consolidation MRD evaluation should be performed. Despite this heterogeneity, improved outcomes are seen in patients who achieve early MRD negative disease.

MRD evaluation may also have a role in intensification or de-escalation of therapy. Patients with disease detectable by MRD at the time of CR may benefit from additional induction chemotherapy prior to proceeding to allo-HCT and patients with standard risk disease without MRD may be able to forgo allo-HCT or, among those with high-risk disease, receive reduced intensity conditioning. MRD can also serve as an early warning sign of impending relapse and be used to guide re-initiation of therapy. MRD has also been an important outcome measure in trials evaluating novel therapies for the treatment of ALL such as blinatumomab, inotuzumab ozogamicin, and CAR-T cell therapy. As an outcome measure, it is readily available and shows good correlation with long-term outcomes.

Current techniques for measuring MRD include MFC, NGS, qPCR, and ClonoSeq with MFC and qPCR being the most commonly used. The sensitivity of these techniques has improved in recent years with MRC and qPCR achieving MRD detection to 10^{-4} to 10^{-5}. The newer techniques of NGS and ClonoSeq have the ability to detect malignant clones with even higher sensitivity, but as of yet they are not the predominant technique used in clinical practice or research studies. One major limitation of these varied techniques is the lack of an agreed upon definition for MRD. Trials evaluating MRD utilize different techniques and sensitivities making comparison of results between trials difficulty and creating confusion for applying this technique in clinical practice.

MRD analysis has historically been carried out on BM samples versus PB samples. However, recent studies in adults have shown that analysis of MRD in PB and BM samples correlates in T-cell ALL, a finding which had been previously been established in the pediatric literature. Meanwhile, the results of BM and PB MRD analysis in B-cell ALL are discordant. These results suggest that MRD monitoring in T-cell ALL may be possible on easily accessible PB samples while in B-cell ALL BM samples may still be required. However, additional studies are needed to confirm these results.

Given the high correlation between MRD relapse and morphological relapse in patients with ALL who achieve MRD negativity, there appears to be a benefit to serial MRD monitoring. NCCN guidelines recommend MRD testing every 3–6 months after induction therapy for at least 5 years with a role for testing following allo-HCT. MRD relapse can serve as an early indicator for consideration of alternative treatment strategies or closer monitoring for morphological relapse.

Although techniques for MRD analysis are varied and there is no standardized definition, MRD is a powerful technique for the treatment of patients with ALL. MRD testing provides early prognostic information, which can guide treatment decisions and be used to monitor for relapse.

Declarations

Conflict of Interest The authors declare no competing interests.

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.

References

Papers of particular interest, published recently, have been highlighted as: • Of importance

1. Bassan R. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). Blood. 2009;113(18):4153–62. https://doi.org/10.1182/blood-2008-11-185132.
2. Brüggemann M. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood. 2006;107(3):1116–23. https://doi.org/10.1182/blood-2005-07-2708.

3. Mortuza FY. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol. 2002;20(4):1094–104. https://doi.org/10.1200/JCO.2002.20.4.1094.
4. Holowiecki J. Status of minimal residual disease after induction predicts outcome in both standard and high-risk Ph-negative adult acute lymphoblastic leukaemia. The Polish Adult Leukemia Group ALL 4-2002 MRD Study. Br J Haematol. 2008;142(2):227–37. https://doi.org/10.1111/j.1365-2141.2008.07185.x.
5. Patel B. Minimal residual disease is a significant predictor of treatment failure in non T-lineage adult acute lymphoblastic leukaemia: final results of the international trial UKALL XII/ECOG2993. Br J Haematol. 2010;148(1):80–9. https://doi.org/10.1111/j.1365-2141.2009.07941.x.
6.• Berry DA. Association of minimal residual disease with clinical outcome in pediatric and adult acute lymphoblastic leukemia: a meta-analysis. JAMA Oncol. 2017;3(7):e170580. https://doi.org/10.1001/jamaoncol.2017.0580. **This meta-analysis including 39 studies showed significantly improved event-free survival and overall survival for adult and pediatric patients with ALL who achieved MRD negativity following induction therapy.**
7. Cassaday RD. Description and prognostic significance of the kinetics of minimal residual disease status in adults with acute lymphoblastic leukemia treated with HyperCVAD. Am J Hematol. 2018;93(4):546–52. https://doi.org/10.1002/ajh.25030.
8. Ravandi F. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. Blood. 2013;122(7):1214–21. https://doi.org/10.1182/blood-2012-11-466482.
9. Stock W. A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403. Blood. 2019;133(14):1548–59. https://doi.org/10.1182/blood-2018-10-881961.
10. Samra B. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. J Hematol Oncol. 2020;13(1):70. https://doi.org/10.1186/s13045-020-00905-2.
11. Blinatumomab [package insert]. Thousand Oaks, CA: Amgen Inc.; 2022. https://www.pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/blincyto/blincyto_pi_hcp_english.pdf. Accessed 24 May 2022.
12. Cavé H. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer—Childhood Leukemia Cooperative Group. N Engl J Med. 1998;339(9):591–8. https://doi.org/10.1056/NEJM199808273390904.
13. Foroni L. Investigation of minimal residual disease in childhood and adult acute lymphoblastic leukaemia by molecular analysis. Br J Haematol. 1999;105(1):7–24.
14. van Dongen JJJM. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet. 1998;352(9142):1731–8. https://doi.org/10.1016/S0140-6736(98)04058-6.
15. Brisco J. Relationship between minimal residual disease and outcome in adult acute lymphoblastic leukemia. Blood. 1996;87(12):5251–6.
16. Foroni L. Molecular detection of minimal residual disease in adult and childhood acute lymphoblastic leukaemia reveals differences in treatment response. Leukemia. 1997;11(10):1732–41. https://doi.org/10.1038/sj.leu.2400841.
17. Krampera M. Outcome prediction by immunophenotypic minimal residual disease detection in adult T-cell acute lymphoblastic leukaemia. Br J Haematol. 2003;120(1):74–9. https://doi.org/10.1046/j.1365-2141.2003.03974.x.
18. Ravandi F. Minimal residual disease assessed by multiparameter flow cytometry is highly prognostic in adult patients with acute lymphoblastic leukaemia. Br J Haematol. 2016;172(3):392–400. https://doi.org/10.1111/bjh.13834.
19. Vidriales MB. Minimal residual disease in adolescent (older than 14 years) and adult acute lymphoblastic leukemias: early immunophenotypic evaluation has high clinical value. Blood. 2003;101(12):4695–700. https://doi.org/10.1182/blood-2002-08-2613.
20. Beldjord K, Chevret S, Asnafi V, Huguet F, Boulland ML, Leguay T, Thomas X, Cayuela JM, Grardel N, Chalandon Y, Boissel N. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood. 2014;123(24):3739–49. https://doi.org/10.1182/blood-2014-01-547695.
21. Gökbuget N. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. Blood. 2012;120(9):1868–76. https://doi.org/10.1182/blood-2011-09-377713.
22. Gökbuget N. Minimal residual disease level predicts outcome in adults with Ph-negative B-precursor acute lymphoblastic leukemia. Hematology. 2019;24(1):337–48. https://doi.org/10.1080/16078454.2019.1567654.
23. Nagafuji K. Monitoring of minimal residual disease (MRD) is useful to predict prognosis of adult patients with Ph-negative ALL: results of a prospective study (ALL MRD2002 Study). J Hematol Oncol. 2013;6:14. https://doi.org/10.1186/1756-8722-6-14.
24. Ribera JM. Treatment of high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in adolescents and adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial. J Clin Oncol. 2014;32(15):1595–604. https://doi.org/10.1200/JCO.2013.52.2425.
25. Salah-Eldin. Clinical significance of minimal residual disease in young adults with standard-risk/Ph-negative precursor B-acute lymphoblastic leukemia: results of prospective study. Med Oncol. 2014;31(5):938. https://doi.org/10.1007/s12032-014-0938-z.
26. Raff T. Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials. Blood. 2007;109(3):910–5. https://doi.org/10.1182/blood-2006-07-037093.
27. Brown PA. Acute lymphoblastic leukemia, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2021;19(9):1079–109. https://doi.org/10.6004/jnccn.2021.0042.
28. Ribera JM. Chemotherapy or allogeneic transplantation in high-risk Philadelphia chromosome-negative adult lymphoblastic leukemia. Blood. 2021;137(14):1879–94. https://doi.org/10.1182/blood.2020007311.
29. Götz G. Clinical and prognostic significance of the Philadelphia chromosome in adult patients with acute lymphoblastic leukemia. Ann Hematol. 1992;64(2):97–100. https://doi.org/10.1007/BF01715353.
30. Liu-Dumlao T. Philadelphia-positive acute lymphoblastic leukemia: current treatment options. Curr Oncol Rep. 2012;14(5):387–94. https://doi.org/10.1007/s11912-012-0247-7.
31. Dombret H. Outcome of treatment in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia—results of the prospective multicenter LALA-94

trial. Blood. 2002;100(7):2357–66. https://doi.org/10.1182/blood-2002-03-0704.
32. Pane F. Significant reduction of the hybrid BCR/ABL transcripts after induction and consolidation therapy is a powerful predictor of treatment response in adult Philadelphia-positive acute lymphoblastic leukemia. Leukemia. 2005;19(4):628–35. https://doi.org/10.1038/sj.leu.2403683.
33. Kim DY. Nilotinib combined with multiagent chemotherapy for newly diagnosed Philadelphia-positive acute lymphoblastic leukemia. Blood. 2015;126(6):746–56. https://doi.org/10.1182/blood-2015-03-636548.
34. Chiaretti S. A sequential approach with imatinib, chemotherapy and transplant for adult Ph+ acute lymphoblastic leukemia: final results of the GIMEMA LAL 0904 study. Haematologica. 2016;101(12):1544–52. https://doi.org/10.3324/haematol.2016.144535.
35. Foà R. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2011;118(25):6521–8. https://doi.org/10.1182/blood-2011-05-351403.
36. Ottmann OG. Imatinib compared with chemotherapy as front-line treatment of elderly patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL). Cancer. 2007;109(10):2068–76. https://doi.org/10.1002/cncr.22631.
37. Rousselot P. Dasatinib and low-intensity chemotherapy in elderly patients with Philadelphia chromosome-positive ALL. Blood. 2016;128(6):774–82. https://doi.org/10.1182/blood-2016-02-700153.
38. Bassan R. Different molecular levels of post-induction minimal residual disease may predict hematopoietic stem cell transplantation outcome in adult Philadelphia-negative acute lymphoblastic leukemia. Blood Cancer J. 2014;4:e225. https://doi.org/10.1038/bcj.2014.48.
39. Spinelli O. Clearance of minimal residual disease after allogeneic stem cell transplantation and the prediction of the clinical outcome of adult patients with high-risk acute lymphoblastic leukemia. Haematologica. 2007;92(5):612–8. https://doi.org/10.3324/haematol.10965.
40.• Shen Z. Influence of pre-transplant minimal residual disease on prognosis after Allo-SCT for patients with acute lymphoblastic leukemia: systematic review and meta-analysis. BMC Cancer. 2018;18(1):755. https://doi.org/10.1186/s12885-018-4670-5. **This meta-analysis including 21 studies showed patients with positive MRD prior to allogeneic stem cell transplantation was a significant negative predictor of relapse-free survival, event-free survival, and overall survival as compared to those with negative MRD.**
41. Akahoshi Y. Minimal residual disease (MRD) positivity at allogeneic hematopoietic cell transplantation, not the quantity of MRD, is a risk factor for relapse of Philadelphia chromosome-positive acute lymphoblastic leukemia. Int J Hematol. 2021;113(6):832–9. https://doi.org/10.1007/s12185-021-03094-x.
42.• Li S. Different effects of pre-transplantation measurable residual disease on outcomes according to transplant modality in patients with Philadelphia chromosome positive ALL. Front Oncol. 2020;10:320. https://doi.org/10.3389/fonc.2020.00320. **This retrospective study evaluated the effects of MRD positivity prior to allogeneic HCT for management of Philadelphia chromosome positive ALL and revealed that haploidentical HCT was related to higher probability of survival and lower incidences of relapse as compared to matched sibling donor HCT in this patient population.**
43. Lee S. The extent of minimal residual disease reduction after the first 4-week imatinib therapy determines outcome of allogeneic stem cell transplantation in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia. Cancer. 2009;115(3):561–70. https://doi.org/10.1002/cncr.24026.
44. Lee S. Impact of minimal residual disease kinetics during imatinib-based treatment on transplantation outcome in Philadelphia chromosome-positive acute lymphoblastic leukemia. Leukemia. 2012;26(11):2367–74. https://doi.org/10.1038/leu.2012.164.
45. Lou Y. Efficacy and prognostic factors of imatinib plus CALLG2008 protocol in adult patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Front Med. 2017;11(2):229–38. https://doi.org/10.1007/s11684-017-0506-y.
46. Lussana F. Achieving molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: impact on relapse and long-term outcome. Biol Blood Marrow Transplant. 2016;22(11):1983–7. https://doi.org/10.1016/j.bbmt.2016.07.021.
47. Mizuta S. Prognostic factors influencing clinical outcome of allogeneic hematopoietic stem cell transplantation following imatinib-based therapy in BCR-ABL-positive ALL. Blood Cancer J. 2012;2(5):e72. https://doi.org/10.1038/bcj.2012.18.
48. Nishiwaki S. Impact of MRD and TKI on allogeneic hematopoietic cell transplantation for Ph+ALL: a study from the adult ALL WG of the JSHCT. Bone Marrow Transplant. 2016;51(1):43–50. https://doi.org/10.1038/bmt.2015.217.
49. Akahoshi Y. Reduced-intensity conditioning is a reasonable alternative for Philadelphia chromosome-positive acute lymphoblastic leukemia among elderly patients who have achieved negative minimal residual disease: a report from the Adult Acute Lymphoblastic Leukemia Working Group of the JSHCT. Bone Marrow Transplant. 2020;55(7):1317–25. https://doi.org/10.1038/s41409-020-0951-0.
50. Bachanova V. Ph+ ALL patients in first complete remission have similar survival after reduced intensity and myeloablative allogeneic transplantation: impact of tyrosine kinase inhibitor and minimal residual disease. Leukemia. 2014;28(3):658–65. https://doi.org/10.1038/leu.2013.253.
51. Terwey T. Comparison of chimerism and minimal residual disease monitoring for relapse prediction after allogeneic stem cell transplantation for adult acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2014;20(10):1522–9. https://doi.org/10.1016/j.bbmt.2014.05.026.
52. Zhao X. Monitoring MRD with flow cytometry: an effective method to predict relapse for ALL patients after allogeneic hematopoietic stem cell transplantation. Ann Hematol. 2012;91(2):183–92. https://doi.org/10.1007/s00277-011-1285-1.
53. Zhou Y. The effect of peritransplant minimal residual disease in adults with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation. Clin Lymphoma Myeloma Leuk. 2014;14(4):319–26. https://doi.org/10.1016/j.clml.2014.01.002.
54. Miyamura K. Detection of Philadelphia chromosome-positive acute lymphoblastic leukemia by polymerase chain reaction: possible eradication of minimal residual disease by marrow transplantation. Blood. 1992;79(5):1366–70.
55. Radich J. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. Blood. 1997;89(7):2602–9.
56. Stirewalt DL. Predictors of relapse and overall survival in Philadelphia chromosome-positive acute lymphoblastic leukemia after transplantation. Biol Blood Marrow Transplant. 2003;9(3):206–12. https://doi.org/10.1053/bbmt.2003.50025.
57. Zhao X. Comparative analysis of flow cytometry and RQ-PCR for the detection of minimal residual disease in Philadelphia chromosome-positive acute lymphoblastic leukemia after hematopoietic stem cell transplantation. Biol Blood Marrow

Transplant. 2018;24(9):1936–43. https://doi.org/10.1016/j.bbmt.2018.03.015.
58. Candoni A. Outcome of allogeneic hematopoietic stem cell transplantation in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia in the era of tyrosine kinase inhibitors: a registry-based study of the Italian Blood and Marrow Transplantation Society (GITMO). Biol Blood Marrow Transplant. 2019;25(12):2388–97. https://doi.org/10.1016/j.bbmt.2019.07.037.
59. Chen H. Administration of imatinib after allogeneic hematopoietic stem cell transplantation may improve disease-free survival for patients with Philadelphia chromosome-positive acute lymphobla stic leukemia. J Hematol Oncol. 2012;5:29. https://doi.org/10.1186/1756-8722-5-29.
60. Pfeifer H. Randomized comparison of prophylactic and minimal residual disease-triggered imatinib after allogeneic stem cell transplantation for BCR-ABL1-positive acute lymphoblastic leukemia. Leukemia. 2013;27(6):1254–62. https://doi.org/10.1038/leu.2012.352.
61. Löffler A. A recombinant bispecific single-chain antibody, CD19 x CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. Blood. 2000;95(6):2098–103.
62. Kantarjian H. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017;376(9):836–47. https://doi.org/10.1056/NEJMoa1609783.
63. Martinelli G. Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia following treatment with blinatumomab: results from a phase II, single-arm, multicenter study. J Clin Oncol. 2017;35(16):1795–802. https://doi.org/10.1200/JCO.2016.69.3531.
64. Topp MS. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol. 2011;29(18):2493–8. https://doi.org/10.1200/JCO.2010.32.7270.
65. Topp MS. Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL. Blood. 2012;120(26):5185–7. https://doi.org/10.1182/blood-2012-07-441030.
66. Zugmaier G. Long-term survival and T-cell kinetics in relapsed/refractory ALL patients who achieved MRD response after blinatumomab treatment. Blood. 2015;126(24):2578–84. https://doi.org/10.1182/blood-2015-06-649111.
67.• Gökbuget N. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood. 2018;131(14):1522–31. https://doi.org/10.1182/blood-2017-08-798322. **This prospective, single-arm, phase II study showed that a majority of patients in hematologic remission with MRD positivity after chemotherapy achieved a complete MRD response to blinatumomab and a significant OS improvement in MRD responders**.
68. DiJoseph JF. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. Blood. 2004;103(5):1807–14. https://doi.org/10.1182/blood-2003-07-2466.
69. Kantarjian HM. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med. 2016;375(8):740–53. https://doi.org/10.1056/NEJMoa1509277.
70. Stock W. Efficacy of inotuzumab ozogamicin in patients with Philadelphia chromosome-positive relapsed/refractory acute lymphoblastic leukemia. Cancer. 2021;127(6):905–13. https://doi.org/10.1002/cncr.33321.
71. Park JH. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med. 2018;378(5):449–59. https://doi.org/10.1056/NEJMoa1709919.
72. Shah BD. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet. 2021;398(10299):491–502. https://doi.org/10.1016/S0140-6736(21)01222-8.
73. van der Velden VH. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007;21(4):604–11. https://doi.org/10.1038/sj.leu.2404586.
74. Riva G. Multiparametric flow cytometry for MRD monitoring in hematologic malignancies: clinical applications and new challenges. Cancers (Basel). 2021;13(18):4582. https://doi.org/10.3390/cancers13184582.
75. DiGiuseppe JA. Applications of flow cytometric immunophenotyping in the diagnosis and posttreatment monitoring of B and T lymphoblastic leukemia/lymphoma. Cytometry B Clin Cytom. 2019;96(4):256–65. https://doi.org/10.1002/cyto.b.21833.
76. Theunissen P. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017;129(3):347–57. https://doi.org/10.1182/blood-2016-07-726307.
77. Al-Mawali A. The role of multiparameter flow cytometry for detection of minimal residual disease in acute Myeloid Leukemia. Am J Clin Pathol. 2009;131(1):16–26. https://doi.org/10.1309/AJCP5TSD3DZXFLCX.
78. Brüggemann M. Has MRD monitoring superseded other prognostic factors in adult ALL? Blood. 2012;120(23):4470–81. https://doi.org/10.1182/blood-2012-06-379040.
79. Modvig S. Value of flow cytometry for MRD-based relapse prediction in B-cell precursor ALL in a multicenter setting. Leukemia. 2021;35(7):1894–906. https://doi.org/10.1038/s41375-020-01100-5.
80. Alekseyev YO. A next-generation sequencing primer—how does it work and what can it do? Acad Pathol. 2018;5:2374289518766521. https://doi.org/10.1177/2374289518766521.
81. Kruse A. Minimal residual disease detection in acute lymphoblastic leukemia. Int J Mol Sci. 2020;21(3):1054. https://doi.org/10.3390/ijms21031054.
82. Faham M. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2012;120(26):5173–80. https://doi.org/10.1182/blood-2012-07-444042.
83. Panuzzo C. Revealing the mysteries of acute myeloid leukemia: from quantitative PCR through next-generation sequencing and systemic metabolomic profiling. J Clin Med. 2022;11(3):483. https://doi.org/10.3390/jcm11030483.
84. Sala Torra O. Next-generation sequencing in adult B cell acute lymphoblastic leukemia patients. Biol Blood Marrow Transplant. 2017;23(4):691–6. https://doi.org/10.1016/j.bbmt.2016.12.639.
85. Pulsipher MA. IgH-V(D) J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients. Blood. 2015;125(22):3501–8. https://doi.org/10.1182/blood-2014-12-615757.
86. Kotrova M. Is next-generation sequencing the way to go for residual disease monitoring in acute lymphoblastic leukemia? Mol Diagn Ther. 2017;21(5):481–92. https://doi.org/10.1007/s40291-017-0277-9.
87. Zhang J. Identifying driver mutations from sequencing data of heterogeneous tumors in the era of personalized genome sequencing. Brief Bioinform. 2014;15(2):244–55. https://doi.org/10.1093/bib/bbt042.

88. Brüggemann M. Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study. Leukemia. 2019;33(9):2241–53. https://doi.org/10.1038/s41375-019-0496-7.
89. Rellick SL. Co-culture model of B-cell acute lymphoblastic leukemia recapitulates a transcription signature of chemotherapy-refractory minimal residual disease. Sci Rep. 2021;11(1):15840. https://doi.org/10.1038/s41598-021-95039-x.
90. Hong Y. Identification of immune subtypes of Ph-neg B-ALL with ferroptosis related genes and the potential implementation of Sorafenib. BMC Cancer. 2021;21(1):1331. https://doi.org/10.1186/s12885-021-09076-w.
91. Merli P. Minimal residual disease prior to and after haematopoietic stem cell transplantation in children and adolescents with acute lymphoblastic leukaemia: what level of negativity is relevant? Front Pediatr. 2021;9:777108. https://doi.org/10.3389/fped.2021.777108.
92. Tüfekçi Ö. Assessment of minimal residual disease in childhood acute lymphoblastic leukemia: a multicenter study from Turkey. J Pediatr Hematol Oncol. 2022;44(2):e396–402. https://doi.org/10.1097/MPH.0000000000002419.
93. Huang YJ. Comparison of two quantitative PCR-based assays for detection of minimal residual disease in B-precursor acute lymphoblastic leukemia harboring three major fusion transcripts. J Mol Diagn. 2021;23(10):1373–9. https://doi.org/10.1016/j.jmoldx.2021.07.008.
94. Flohr T. Minimal residual disease-directed risk stratification using real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements in the international multicenter trial AIEOP-BFM ALL 2000 for childhood acute lymphoblastic leukemia. Leukemia. 2008;22(4):771–82. https://doi.org/10.1038/leu.2008.5.
95. van der Velden VH. MRD detection in acute lymphoblastic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. Methods Mol Biol. 2009;538:115–50. https://doi.org/10.1007/978-1-59745-418-6_7.
96. Germano G. Clonality profile in relapsed precursor-B-ALL children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. Leukemia. 2003;17(8):1573–82. https://doi.org/10.1038/sj.leu.2403008.
97. Monter A. ClonoSEQ assay for the detection of lymphoid malignancies. Expert Rev Mol Diagn. 2019;19(7):571–8. https://doi.org/10.1080/14737159.2019.1627877.
98. Walzer S. A cost impact analysis of clonoSEQ. Oncol Ther. 2021;9(2):607–19. https://doi.org/10.1007/s40487-021-00169-x.
99. Hussaini MO. Assessment of clonotypic rearrangements and minimal residual disease in lymphoid malignancies. Arch Pathol Lab Med. 2022;146(4):485–93. https://doi.org/10.5858/arpa.2020-0457-OA.
100.• Kotrova M. Comparison of minimal residual disease levels in bone marrow and peripheral blood in adult acute lymphoblastic leukemia. Leukemia. 2020;34(4):1154–7. https://doi.org/10.1038/s41375-019-0599-1. **This retrospective study evaluated bone marrow and peripheral blood sample pairs from B-ALL and T-ALL patients using EuroMRD allele-specific oligonucleotide RQ-PCR and EuroMRD guidelines and revealed stronger correlation of MRD in bone marrow and peripheral blood in T-cell ALL when compared to B-cell ALL**.
101. Brisco MJ. Monitoring minimal residual disease in peripheral blood in B-lineage acute lymphoblastic leukaemia. Br J Haematol. 1997;99(2):314–9. https://doi.org/10.1046/j.1365-2141.1997.3723186.x.
102. van der Velden VH. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. Leukemia. 2002;16(8):1432–6. https://doi.org/10.1038/sj.leu.2402636.
103. Muffly L. Concordance of peripheral blood and bone marrow measurable residual disease in adult acute lymphoblastic leukemia. Blood Adv. 2021;5(16):3147–51. https://doi.org/10.1182/bloodadvances.2021004234.
104. Keegan A. Flow cytometric minimal residual disease assessment of peripheral blood in acute lymphoblastic leukaemia patients has potential for early detection of relapsed extramedullary disease. J Clin Pathol. 2018;71(7):653–8. https://doi.org/10.1136/jclinpath-2017-204828.
105. Pemmaraju N. Significance of recurrence of minimal residual disease detected by multi-parameter flow cytometry in patients with acute lymphoblastic leukemia in morphological remission. Am J Hematol. 2017;92(3):279–85. https://doi.org/10.1002/ajh.24629.
106. Spyridonidis A. How I treat measurable (minimal) residual disease in acute leukemia after allogeneic hematopoietic cell transplantation. Blood. 2020;135(19):1639–49. https://doi.org/10.1182/blood.2019003566.

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