Seminars in Hematology 57 (2020) 142–148

Contents lists available at ScienceDirect

Seminars in Hematology

journal homepage: www.elsevier.com/locate/seminhematol

Review

Monitoring MRD in ALL: Methodologies, technical aspects and optimal time points for measurement

Jack Bartram[a,b,*], Bela Patel[c], Adele K. Fielding[d]

[a] Department of Haematology, Great Ormond Street Hospital for Children, London, UK
[b] Cancer Section, DBC Programme, University College London, London, UK
[c] Barts Cancer Institute, London, UK
[d] UCL Cancer Institute, London, UK

ARTICLE INFO

Keywords:
Acute lymphoblastic leukemia
Minimal residual disease

ABSTRACT

The accurate determination of minimal or measurable residual disease (MRD) during the early months of therapy in acute lymphoblastic leukemia is well established as the most important independent prognostic biomarker, predicting response to combination chemotherapy. Stratification based on MRD maximizes treatment effectiveness while minimizing adverse effects. Allele-specific real-time quantitative PCR of clone-defining immunoglobin/T-cell receptor gene rearrangements in the patients' leukemic clones and/or multiparametric flow cytometric tracking of leukemia-associated immunophenotypes are considered standard of care. Following recent advances in high throughput sequencing (HTS; next generation sequencing), much attention has been devoted to the development of HTS-based MRD assays, which can increase sensitivity; theoretically only limited by the number of cells input into the assay. Knowledge of the methods and limitations of each technology, along with awareness of the sensitivity and specificity of MRD at particular treatment time points is important in interpretation of the MRD value. MRD negativity at pre-established protocol-appropriate time points guides continuance with consolidation/maintenance chemotherapy, whereas positivity leads to a change to a biological therapy such as blinatumomab and intensification of therapy to allogeneic stem cell transplant. Positivity after maintenance may herald impending relapse enabling treatment intervention. MRD has been integral to the introduction of novel agents and cellular therapies into clinical trials and standard of care, but the long-term predictive value of MRD on outcome of novel therapies is not yet established. Integration of somatic genetics with MRD may further improve accurate identification of patients with the lowest and highest risk of relapse.

Introduction

The majority of adults with acute lymphoblastic leukemia (ALL) achieve remission with the use of multiagent chemotherapy, however half of patients will relapse [1-3]. Accurate risk stratification is essential to deliver optimal treatment to improve outcomes. The assessment of treatment response through the measurement of minimal or measurable residual disease (MRD) is the most powerful factor-predicting outcome after combination chemotherapy, and has been standard of care for well over a decade [4-8]. Integration of MRD monitoring into risk-adapted protocols has been used to successfully guide therapy intensification and reduction [5,9-11]. The use of MRD in adult ALL is equally as predictive of outcome as in pediatric practice and a recent survey of adult practice in Europe confirms MRD testing is standard of care in the treatment of adult ALL [12-15]. The clinical management of patients with ALL relies on accurate prediction of relapse hazard to determine the intensity of therapy. Novel therapeutic approaches and allogenic stem cell transplant (alloSCT) must target patients who will derive the most benefit whilst consideration to reducing therapy (and associated toxicity) may be appropriate in patients with the most chemotherapy-sensitive disease. Here we review MRD methodologies and technical aspects for interpretation.

What is MRD?

MRD, simply defined, is "measurable leukemia in samples apparently devoid of leukemia cells." It is the low-level disease, which is not detectable by morphological assessment with a light microscope. Given the estimated 10^{12} (a trillion) blasts present at diagnosis, MRD represents anything less than 10 billion cells

* Corresponding author. Jack Bartram, MD, Department of Haematology, Great Ormond Street Hospital for Children, Great Ormond Street, London WC13 3JH, UK.
E-mail address: jack.bartram@gosh.nhs.uk (J. Bartram).

https://doi.org/10.1053/j.seminhematol.2020.06.003
0037-1963/

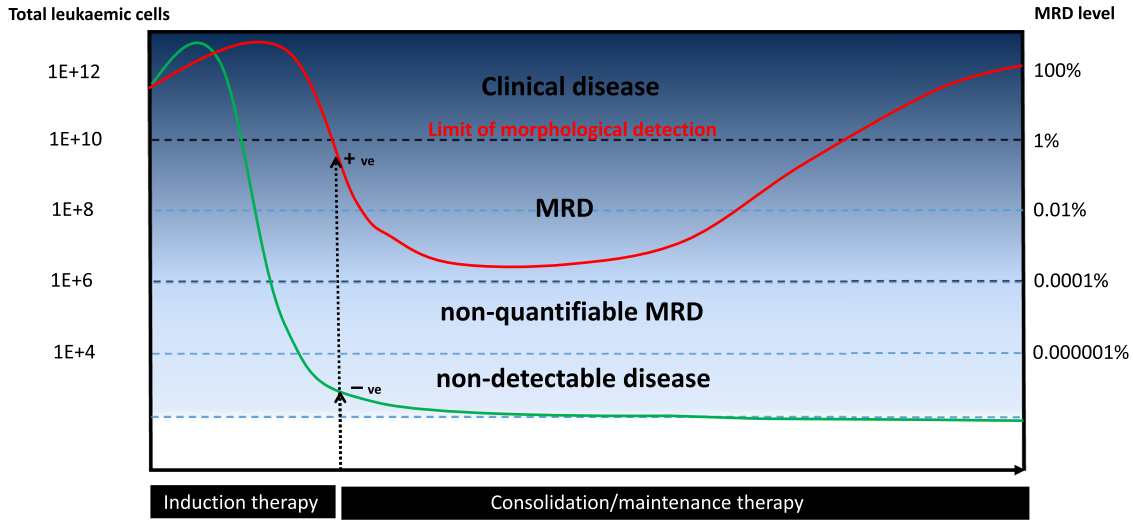

Fig. 1. MRD assessment in ALL. Schematic diagram of disease levels in ALL, with equivalent MRD levels. Two example of patients assessed after induction chemotherapy are shown. The patient represented by the green line is MRD negative after induction chemotherapy and is essentially cured with a very low risk of relapse. The patient represented by the red line is MRD positive and destined to relapse.

Table 1
Characteristic of MRD techniques.

MRD technique	Sensitivity	Applicability	Benefits	Limitations
ASO-RQPCR IG/TCR	0.001% (10^{-5})	BCP-ALL: 95% T-ALL: 90-95%	-Applicable to majority of patients -Sensitive -Highly standardized with QA scheme	-Time consuming -Expensive -Requires pre-treatment sample -Requires extensive experience
MFC for LAIP	0.01% (10^{-4})	BCP-ALL: >90% T-ALL: >90%	-Rapid -Cheap (relatively) -Information about whole sample cellularity	-Variable sensitivity -Need technical expertise (especially low level disease) -Lack of standardization, no QA -Fresh cells required
RQ-PCR fusion genes	0.01% to 0.0001% (10^{-4} to 10^{-6})	<50%	-Sensitive -Standardized primers	-Not applicable to all (absence of targets in >50% cases) -Risk contamination
HTS IG/TCR	0.0001% (10^{-6}) –potentially lower (depending on amount of DNA analyzed)	Potentially 100%	-Applicable to all patients -Highly sensitive (only limited by cells input) -Clone unbiased -revealing persisting or evolving clones (even if not defining clones at presentation) -Whole repertoire assessment	-Lack of standardization -Complex bioinformatics pipelines -Only clinically available in US -Minimal clinical validation -Risk contamination (if no barcoded primers are used)

(10^{10}). With a maximal sensitivity of 5 logs MRD negative represents anything below 10 million cells (10^6), hence the use of terminology "measurable" replacing "minimal" by many groups. The driving concept behind MRD is really quite simple; the rate of decline in disease quantity in the face of systemic chemotherapy is of prognostic value and is a measure of relapse prediction, examples of this are shown in Fig. 1.

The human eye (down a microscope) can detect leukemic blasts at a maximal sensitivity of 1%. At the end of induction therapy, the majority of patients are in clinical remission with less than 5% blasts by morphologic examination of a bone marrow (BM) smear. However, morphological assessment of regenerating post therapy BM is prone to error [16,17]. Sensitive measurement of response to treatment is achieved through powerful molecular and flow cytometry methods to more precisely monitor disease.

Methodologies/technical aspects of MRD

All methods of MRD detection exploit features present exclusively in leukemic cells to differentiate them from normal cell. In current clinical practice this includes use of gene fusions, highly variable junctional regions of immunoglobulin (IG), and T-cell receptor (TCR) gene rearrangements and aberrant leukemia-associated immunophenotypes (LAIP). The principles and the advantages and disadvantages of these MRD techniques are summarized in Table 1 and Fig. 2.

Allele-specific real-time quantitative (ASO-RQ) PCR of clone-defining IG/TCR gene rearrangements in the patients' leukemia can be used in >80% of adults with ALL although the applicability of this technology does decline with increasing age [18]. Initial characterization of clonal IG/TCR in performed in diagnostic samples with a panel of screening PCRs which target the rearranged VDJ (variable, diversity, joining) of the IG/TCR genes, which encode the hypervariable complementarity determining region 3 domain. This is then followed by Sanger sequencing or high throughput sequencing (HTS) of products to identify the clones [19-23]. ASO-RQ PCR then requires assays tailored to each individual patients clonal rearrangements and, depending on template availability and primer selection, has a maximal sensitivity of $1{:}1 \times 10^{-5}$ (1 leukemic cell in 100,000 normal cells) due to nonspecific background amplification [20]. This method has been highly standardized by the EuroMRD Consortium (http://www.euromrd.org)

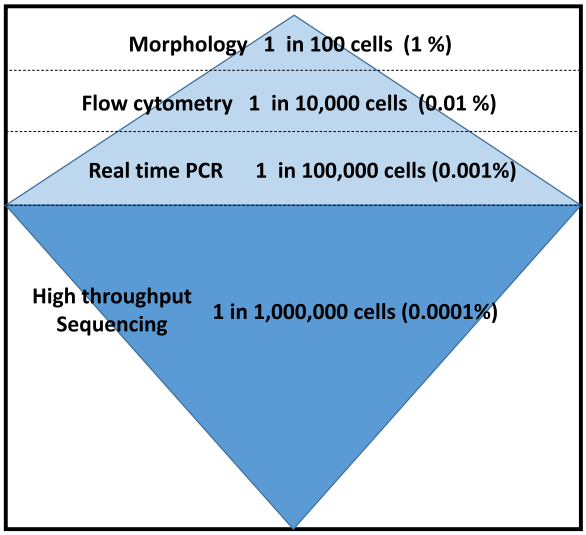

Fig. 2. Comparison of relative sensitivities of residual disease assessment methods. This can be thought of as an "iceberg" - with HTS technologies potentially offering the power to increase sensitivity and breadth of detection.

with laboratories all over the world participating in shared quality assurance testing twice a year working to the same protocols/guidelines. The ASO-RQ PCR MRD method requires expertise, a large amount knowledge about IG/TCR gene rearrangement and is both laborious and time consuming. Detection and sequencing of IG/TCR rearrangements and design of the corresponding ASO primers typically takes up to 4 weeks, whereas the analysis of follow-up samples (actual MRD assessment) takes less than a week.

IG/TCR rearrangements are not directly related to the oncogenic process and can clonally evolve, which may lead to loss of leukemia specific sequences and false negative results. Clonal architecture is dynamic. When disease relapse occurs, it can involve clones that were not identified, or only viewed as minor clones, at diagnosis and therefore were not tracked [24].

Multiparamter flow cytometric (MFC) tracking of LAIP approach evaluates ALL at diagnosis with a specific reagent panel. This defines regions where leukemic events are present outside of those seen during normal maturation for the relevant lineage (the LAIP). These LAIP regions are then employed for analysis of subsequent samples and events appearing in the predefined regions counted as residual disease [25,26]. This is more rapid than ASO-RQ PCR, allowing prompt reporting of results. The LAIP method is applicable to >90% of patients; however sensitivity is often a log lower than ASO-RQ PCR. Standardization of flow cytometric tracking is difficult, requires an experienced operator, and inter-operator variation can lead to inconsistent reporting [26]. The method also assumes stability of immunophenotype for both the leukemia and the background normal and regenerating BM populations. Phenotypic shifts commonly occur in MRD and normal cells during therapy consequently can give rise to both false positive and negative results. Immunotherapy and cellular therapies targeting antigens on leukemic blasts (such as CD19, CD20, or CD22) may hamper classic flow cytometry gating strategies.

Although highly predictive of outcome, these methods are not applicable to all patients. Following recent advances in HTS (next generation sequencing), much attention has been devoted to the development of HTS-based MRD assays, which can increase sensitivity; theoretically only limited by the number of cells input into the assay. Highly parallel HTS is employed to sequence the rearranged VDJ of the IG/TCR genes. Combined with the high capacity of HTS, this allows a single, clone-unbiased, and highly sensitive test to be applied to all patients, revealing persisting or evolving clones, potentially even if these were not the defining clones at presentation. Importantly, HTS generates exact nucleotide sequences for all clones, which can be traced through subsequent follow-up analysis [27,28]. HTS technology has the advantage of being able to visual a larger picture of the IG/TCR repertoire in one experiment and therefore the ability of detecting clones that are low frequency at diagnosis, but become major players at later stage [29]. HTS has been widely introduced for the screening of diagnostics samples and increases applicability [19,22,23]. The clonoSEQ HTS technology (Adaptive Biotechnologies, Seattle, WA) is the first assay to be approved by the US Food and Drug Administration for MRD detection [30]. Clinical application of this assay has been demonstrated by improving stratification compared to MFC in B-ALL [31-34]. However, before implementation of HTS-MRD detection into routine clinical practice, several issues must be addressed, direct comparison to ASO-RQ PCR is required and workflows standardized, which will require large multicentre trials [35].

Practical consideration for MRD data interpretation

Source and quality of material for assessment

MRD levels in T cell ALL are roughly equivalent (generally less than a log lower) in peripheral blood (PB) and BM, however in precursor B cell (PBC) ALL MRD can be up to three logs lower in the PB than in BM, so hence BM is a prerequisite [36-39]. HTS has an enhanced sensitivity that could potentially enable MRD quantification from PB, this has been explored in both the adult and pediatric setting but requires larger studies to confirm its clinical utility [33,34].

Attention must be given to the quality of the sample obtained. MRD should be measured in the first 2.5 mL of BM aspirated from one puncture site and the procedure performed by experienced and carefully instructed operator, as MRD may be underestimated in large PB contaminate BM aspirates [40]. There is however no evidence for unequal distribution of ALL cells in different parts of the BM compartment [41].

Finally, sufficiently regenerated BM is required for the assessment of morphological BM remission and in order to fulfill qualitative and quantitative criteria for MRD analysis. However, this has to be balanced by the effect of BM aspiration delay. Delay has been shown to have a significant impact on measured MRD levels with inaccurate risk group allocation in over 20% of patients [42].

Limits of detection and how to define "MRD negativity"

The definition of MRD negativity is a misnomer, differs by technology used and is related to different definitions of sensitivity. MRD negative does not mean complete absence of disease. As mentioned previously with a maximal sensitivity of 5 logs "MRD negative" represents anything below 10 million cells present. Therefore like other quantitative assays consideration to the lower limit of detection and lower limit of quantification are crucially important when interpreting results as well as the clinical time point at which the test is applied. MRD negativity implies that no MRD is detected with high certainty, using an MRD technique that can truly measure low residual disease levels with quantitative range $\leq 10^{-4}$ thereby identifying patients with a comparatively lower risk of relapse than those remaining MRD positive. However, MRD negative states still associate with an appreciable risk of relapse (>20%-30%) highlighting a current limitation in assaying measurable disease. The use of HTS technologies claim to reach sensitivities down to 10^{-7} potentially enabling a status of MRD negativity to be assigned more robustly [27,33]. However, to

Table 2
Summary of characteristic of international study group of MRD in adult ALL.

Study group	MRD technique	Time point	Cut-off value
GMALL [44,46]	ASO-PCR	Day 11, day 24, week 16	1×10^{-4}
MRC UKALL XII/ECOG2993 [13]	ASO-PCR	Week 8	1×10^{-4}
PETHEMA [48]	MFC	Week 16-18	5×10^{-4}
GRALL [47]	ASO-PCR	Week 6	1×10^{-4} (1×10^{-3} HR)
MD Anderson [49]	MFC	CR, 3 months, 6 months	1×10^{-4}
NILG [45]	ASO-PCR	Week 16, week 22	1×10^{-4}

achieve these levels of sensitivity experiments require a sufficient amount of DNA (cells) input, which ignore the cellularity limits of BM samples, especially aplastic samples during treatment.

MRD assessment is not possible in all patients because of technical limitations such as lack of identification of patient specific clonal markers or insufficient diagnostic or follow-up sample. These limitations are more prevalent in the advanced age setting [18]. HTS has been shown to address some of these limitations, although further data is required in the adult setting [19,34].

Optimal time points and prognostic significance

The rate of decline in disease quantity in the face of systemic chemotherapy varies by patient, giving each patient a unique relapse prediction. MRD interpretation is dependent on the time point taken in treatment, and interpretation of different time points gives different relapse predictions. As well as for risk classification and prediction MRD is increasingly being utilized for post remission treatment decision making [43]. When comparing MRD studies several important considerations influence the predictive impact of MRD, including treatment regime used, sensitivity of MRD assay, timing of assessment and the rate of SCT in the cohort.

Use of MRD during chemotherapy

In adults with T-cell ALL or Philadelphia (Ph)-negative PBC ALL, initial MRD response is a highly relevant prognostic marker, reported by all major study groups [13,44-49]. Study groups use different cut-off values, depending on the MRD technique used and timing of MRD analysis, this is summarized in Table 2. Apart from these differences, all study groups confirmed the strong independent predictive value of chemotherapy response in adult ALL. This is further confirmed by a recent meta-analysis of 16 studies (studies evaluated MRD at the end of induction or early consolidation, using ASO-PCR or MFC) comprising 2076 adult ALL patients, which concluded 10-year disease free survival rate of 64% for patients who achieved MRD negativity, compared with 21% for those who were positive [12].

The most commonly used time point for conducting the first MRD assessment is at the completion of induction chemotherapy which is approximately 2 to 3 months into therapy. For patients who are MRD negative, therapy is often guided to start consolidation. In contrast, MRD positivity leads to a change to blinatumomab and alloSCT. There is no clear guidance on the frequency of monitoring post remission, however, a German multicentre adult ALL study demonstrated that nearly 90% of patient relapsed with a median interval of 4 months, suggesting that it would be prudent to monitor at a minimum of 3 monthly intervals in order to intercede before frank relapse [50]. A recent survey of European practice reported an average of four to five tests per patient in the 12-months after initial negative MRD test. In MRD positive patients, the number of tests was reported to be lower, possibly reflecting intensification with SCT in these patients and frequency of MRD testing not specified by protocols; alternatively it may reflect poorer survival. The use of MRD in second remission to monitor for progression and offer additional therapeutic options including informing on SCT was also reported [14].

MRD and alloSCT

Several studies have confirmed that poor early MRD response identifies adult patients who may benefit from intensification of therapy with myeloablative alloSCT in adult ALL [45,46,48,51]. However, many adults above the age limit for this approach and it remains unclear whether reduced intensity conditioned (RIC) alloSCT is an effective salvage therapy for adult patients with MRD prior to alloSCT. The UKALL14 study prospectively evaluated a RIC alloSCT and the data from several hundred patients indicate a substantial difference in outcome when patients are MRD positive prior to the procedure [52]. MRD is, of course, a continuous quantitative variable and MRD levels correlate with post SCT outcomes [45,46,53]. Patients with very high level MRD $\geq 10^{-3}$ pre alloSCT have worse outcome than those with levels $<10^{-3}$ irrespective of when MRD is measured (end of induction or immediately prior to SCT) [33,51,54,55]. Such patients would now be recommended to receive blinatumomab prior to alloSCT. On the basis of the BLAST study, the agent is licensed for patients with residual high level MRD [56]. Serial MRD measurements after RIC alloSCT are likely to be critical, allowing early identification of impending relapse and important clinical window to act on MRD to prevent overt clinical relapse [33,55,57]. It is hoped that the sensitivity of HTS may allow frequent PB testing to supplement BM sampling, to allow this clinical window to be detected in a timely and more feasible fashion than repeated BM assessment [34,55,58]. Interventions that may have value in this context include donor lymphocyte infusions, tyrosine kinase inhibitors in Ph+ ALL and novel agents [52].

MRD in the context of novel therapies

The rapid development and use of immune and cellular therapies has offered effective alternatives for treatment in patients with relapsed or refractory ALL who previously would have had salvage cytotoxic chemotherapy, or no viable treatment option. MRD has played a key role in defining eligibility and monitoring response in key studies of inotuzomab ozogamicin, blinatumomab, and chimeric antigen receptor T-cells (CART) therapy [59-61]. MRD is now considered standard of care assessment of response to these therapies, planning further therapy and assessing eligibility for SCT. With the increasing use of these therapies, the number of MRD assessment per patient will potentially increase on contemporary protocols. Long term data on the prognostic value of MRD in these therapies is still preliminary, however compared to MRD response data from first line chemotherapeutic approaches, relapse rate is high even in patients reaching MRD negativity [62,63]. Therefore, caution has to be used when extrapolating interpretation of MRD values after these novel therapies from upfront treatment MRD response long-term outcome data. These data highlight the treatment/protocol dependency of MRD diagnostics as a tool for clinical prediction. Attention must also be given to the MRD method used to monitor these novel therapies. Antigen negative (eg, CD19-negative) relapse in PBC ALL is observed after immunotherapy and in up to 20% of patients after CD19-directed CART immunotherapy [64-66]. Monitoring blasts in patients with CD19-negative relapse by MFC is challenging as CD19 is often used as a parameter to quantify MRD and diagnose relapse. MRD detected by PCR provides reliable results independently from CD19 expression; however, it does not identify the important change of CD19-negative relapse,

which may in itself have therapeutic implications. The ability to accurately monitor ALL progression during treatment and after CD19-negative relapse by MFC requires identifying additional B-lineage or other specific markers consistently expressed on B cells. Using a combination of MFC, ASO-RQ PCR as well as tracking CART persistence and B cell recovery ensures impending relapse is detected [67].

MRD in the context of Ph+ ALL

The Philadelphia chromosome is found in approximately 25% of adults with. MRD in Ph+ ALL can be assessed by quantifying BCR-ABL transcripts for p190 or p210, as well as the methods already discussed. BCR-ABL transcript quantification (using RNA) is relatively straightforward assay technically and, for p210 transcript quantification can be carried out and reported to international standards due to the work done in CML. However, the reports produced for CML characterize the molecular response on a scale which is well validated for CML but not validated as suitable for Ph+ ALL. P190 quantification is less commonly available and is much less standardized. International standardization of p190 quantification has been led by Heike Pfeifer on behalf of the EuroMRD consortium with 35 laboratories participating [68]. At the lowest levels of MRD there was still variation between labs and an appreciable false negative rate. The extremely detailed laboratory recommendations are a considerable move forward in p190 quantification.

Although there is general agreement in the literature that patients in whom BCR-ABL1 does not significantly diminish during initial therapy after second generation TKI are at higher risk for a poor outcome [69-72] the relationship between BCR-ABL1 levels and outcome the predictive value of BCR-ABL monitoring is not so great for that of Ig/TCR monitoring in Ph- disease. Where BCR-ABL1 and Ig/TCR monitoring have both been monitored in a childhood ALL study, overall concordance between the two methods was only 69%. Ig/TCR appeared more reliable at predicting outcome. An early MRD response was highly predictive of a favorable outcome [73]. The discrepancies between the two methods were investigated by Hovorkova et al by flow-sorting different subsets of cells. A BCR-ABL1-positive clonal haematopoiesis emanating from early progenitors, closely resembling a CML-like disease was shown, suggesting that at least some of the biological heterogeneity of BCR-ABL1-positive ALL may result from this [74].

In practical terms, wherever possible, BCR-ABL1 should be monitored early and monitored often and if possible, both patient specific Ig/TCR re-arrangements and BCR-ABL1 should be monitored.

Combining genotype profiling with MRD for more accurate prognostication in ALL

MRD alone is not sufficient to fully predict outcome. Somatic genetic abnormalities define fundamentally distinct biological subgroups with important prognostic and predictive significance. MRD is a continuous variable, however it has traditionally been used in a dichotomized way, using the same “cut-off” for all patients. When MRD is examined as a continuous variable it is log normally distributed but the shape of the distribution depends on genetic subtype; confirming differential MRD kinetics. Using a single MRD threshold does not reflect the biological heterogeneity that is a fundamental feature of the disease [75,76]. These observations clearly demonstrate the need for tailoring MRD cut-offs to different genetic subgroups. HTS technologies with deeper levels of detection may further influence these integrated risk groups. The future of stratification in ALL lies in the integration of clinical risk factors with high-sensitivity MRD and detailed genetics. Prognostic index models have been developed to include all these variables and provide an accurate method for predicting outcome, but also allow greater flexibility for defining risk groups [77].

Summary

MRD is clearly fundamental to risk directed therapy for ALL. It is accepted as the most powerful independent prognostic indicator in both adult and childhood ALL. The rapid introduction of novel therapies, particularly blinatumomab, inotuzomab ozogamicin, and CART therapies are playing an increasing role in MRD eradication and long-term data on prognostic significances of the depth and kinetics of MRD clearance are eagerly awaited. Development of ultra-sensitive HTS assays may allow for further refinement of risk stratification. However, multicentre standardization for all phases of analysis including use of control quantification material, quality assessment and informatics analysis of HTS data is still lacking and this is a priority topic for the EuroClonality NGS Consortium. Other sensitive technologies including digital/droplet PCR are also being widely assessed. To allow correct interpretation of the MRD result, MRD reports must provide information on the MRD technique used and on MRD markers as well as the theoretical limits of detection and the limits of quantification of the assay.

Recent studies have suggested that other genetic factors influence MRD kinetics and MRD cut-offs. MRD is a continuous variable and optimal cut-offs vary by genetics subgroup so the current “one size fits all” may not be utilizing the data efficiently as dichotomization of continuous variables leads to the loss of statistical power [75]. To develop true precision medicine strategies, integrated prognostic index models should be considered in future clinical trial design to refine patient stratification.

These factors all serve as a framework for integration of MRD as a diagnostic and research tool in to future adult ALL studies.

Conflicts of interest

The authors have no conflicts of interest to disclose.

References

[1] Fielding AK, Rowe JM, Buck G, et al. UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. Blood 2014;123(6):843–50.
[2] Gokbuget N, Stanze D, Beck J, et al. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. Blood 2012;120(10):2032–41.
[3] Sive JI, Buck G, Fielding A, et al. Outcomes in older adults with acute lymphoblastic leukaemia (ALL): results from the international MRC UKALL XII/ECOG2993 trial. Br J Haematol 2012;157(4):463–71.
[4] Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 2008;111(12):5477–85.
[5] Vora A, Goulden N, Wade R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol 2013;14(3):199–209.
[6] Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. N Engl J Med 2015;373(16):1541–52.
[7] Coustan-Smith E, Sancho J, Hancock ML, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 2000;96(8):2691–6.
[8] Bartram J, Wade R, Vora A, et al. Excellent outcome of minimal residual disease-defined low-risk patients is sustained with more than 10 years follow-up: results of UK paediatric acute lymphoblastic leukaemia trials 1997–2003. Arch Dis Child 2016;101(5):449–54.
[9] Vora A, Goulden N, Mitchell C, et al. Augmented post-remission therapy for a minimal residual disease-defined high-risk subgroup of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukaemia (UKALL 2003): a randomised controlled trial. Lancet Oncol 2014;15(8):809–18.
[10] Pieters R, de Groot-Kruseman H, Van der Velden V, et al. Successful therapy reduction and intensification for childhood acute lymphoblastic leukemia based on minimal residual disease monitoring: study ALL10 from the Dutch Childhood Oncology Group. J Clin Oncol 2016;34(22):2591–601.

[11] Pui CH, Pei D, Coustan-Smith E, et al. Clinical utility of sequential minimal residual disease measurements in the context of risk-based therapy in childhood acute lymphoblastic leukaemia: a prospective study. Lancet Oncol 2015;16(4):465–74.
[12] Berry DA, Zhou S, Higley H, et al. Association of minimal residual disease with clinical outcome in pediatric and adult acute lymphoblastic leukemia: a meta–analysis. JAMA Oncol 2017;3(7):e170580.
[13] Patel B, Rai L, Buck G, et al. 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.
[14] Pigneux A, Montesinos P, Cong Z, et al. Testing for minimal residual disease in adults with acute lymphoblastic leukemia in Europe: a clinician survey. BMC Cancer 2018;18(1):1100.
[15] Bassan R, Bruggemann M, Radcliffe HS, Hartfield E, Kreuzbauer G, Wetten S. A systematic literature review and meta-analysis of minimal residual disease as a prognostic indicator in adult B-cell acute lymphoblastic leukemia. Haematologica 2019;104(10):2028–39.
[16] Gupta S, Devidas M, Loh ML, et al. Flow-cytometric vs. -morphologic assessment of remission in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group (COG). Leukemia 2018;32(6):1370–9.
[17] O'Connor D, Moorman AV, Wade R, et al. Use of minimal residual disease assessment to redefine induction failure in pediatric acute lymphoblastic leukemia. J Clin Oncol 2017;35(6):660–7.
[18] Alapi KM RJ, Naughton T, Muirhead RCJ, et al. Minimal residual disease testing on the UKALL14 trial; an overview of the UK's first use of MRD-based risk stratification in adult acute lymphoblastic leukaemia. Br J Haematol 2018;181(S1):5–211.
[19] Wright G, Watt E, Inglott S, Brooks T, Bartram J, Adams SP. Clinical benefit of a high-throughput sequencing approach for minimal residual disease in acute lymphoblastic leukemia. Pediatr Blood Cancer 2019;66(8):e27787.
[20] van der Velden VH, Cazzaniga G, Schrauder A, et al. 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.
[21] van Dongen JJ, Langerak AW, Bruggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003;17(12):2257–317.
[22] Bruggemann M, Kotrova M, Knecht H, et al. 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.
[23] Knecht H, Reigl T, Kotrova M, et al. Quality control and quantification in IG/TR next-generation sequencing marker identification: protocols and bioinformatic functionalities by EuroClonality-NGS. Leukemia 2019;33(9):2254–65.
[24] Szczepanski T, van der Velden VH, Raff T, et al. Comparative analysis of T–cell receptor gene rearrangements at diagnosis and relapse of T-cell acute lymphoblastic leukemia (T-ALL) shows high stability of clonal markers for monitoring of minimal residual disease and reveals the occurrence of second T-ALL. Leukemia 2003;17(11):2149–56.
[25] Wood BL. Flow cytometry in the diagnosis and monitoring of acute leukemia in children. J Hematopathol 2015;8(3):191–9.
[26] Kalina T, Flores-Montero J, van der Velden VH, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 2012;26(9):1986–2010.
[27] Faham M, Zheng J, Moorhead M, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 2012;120(26):5173–80.
[28] Bartram J, Mountjoy E, Brooks T, et al. Accurate sample assignment in a multiplexed, ultrasensitive, high-throughput sequencing assay for minimal residual disease. J Mol Diagn 2016;18(4):494–506.
[29] Bartram J, Goulden N, Wright G, et al. High throughput sequencing in acute lymphoblastic leukemia reveals clonal architecture of central nervous system and bone marrow compartments. Haematologica 2018;103(3):e110–e1e4.
[30] ClonoSEQ cleared for residual cancer testing. Cancer Discov 2018;8(12) Of6.
[31] Wood B, Wu D, Crossley B, et al. Measurable residual disease detection by high throughput sequencing improves risk stratification for pediatric B-ALL. Blood 2017.
[32] Wu D, Emerson RO, Sherwood A, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. Clin Cancer Res 2014;20(17):4540–8.
[33] Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post- allo-transplant defines very low and very high risk ALL patients. Blood 2015.
[34] Sala Torra O, Othus M, Williamson DW, et al. Next-generation sequencing in adult B cell acute lymphoblastic leukemia patients. Biol Blood Marrow Transplant 2017;23(4):691–6.
[35] Monter A, Nomdedéu JF. ClonoSEQ assay for the detection of lymphoid malignancies. Expert Rev Mol Diagn 2019;19(7):571–8.
[36] Brisco MJ, Sykes PJ, Hughes E, et al. Molecular relapse can be detected in blood in a sensitive and timely fashion in B-lineage acute lymphoblastic leukemia. Leukemia 2001;15(11):1801–2.
[37] Coustan-Smith E, Sancho J, Hancock ML, et al. Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. Blood 2002;100(7):2399–402.
[38] van der Velden VH, Jacobs DC, Wijkhuijs AJ, et al. 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.
[39] Kotrova M, Volland A, Kehden B, et al. Comparison of minimal residual disease levels in bone marrow and peripheral blood in adult acute lymphoblastic leukemia. Leukemia 2020;34(4):1154–7.
[40] Helgestad J, Rosthoj S, Johansen P, Varming K, Ostergaard E. Bone marrow aspiration technique may have an impact on therapy stratification in children with acute lymphoblastic leukaemia. Pediatr Blood Cancer 2011;57(2):224–6.
[41] van der Velden VH, Hoogeveen PG, Pieters R, van Dongen JJ. Impact of two independent bone marrow samples on minimal residual disease monitoring in childhood acute lymphoblastic leukaemia. Br J Haematol 2006;133(4):382–8.
[42] Zuna J, Moericke A, Arens M, et al. Implications of delayed bone marrow aspirations at the end of treatment induction for risk stratification and outcome in children with acute lymphoblastic leukaemia. Br J Haematol 2016;173(5):742–8.
[43] Short NJ, Jabbour E, Albitar M, et al. Recommendations for the assessment and management of measurable residual disease in adults with acute lymphoblastic leukemia: A consensus of North American experts. Am J Hematol 2019;94(2):257–65.
[44] Bruggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood 2006;107(3):1116–23.
[45] Bassan R, Spinelli O, Oldani E, et al. 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.
[46] Gokbuget N, Kneba M, Raff T, et al. 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.
[47] Beldjord K, Chevret S, Asnafi V, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood 2014;123(24):3739–49.
[48] Ribera JM, Oriol A, Morgades M, et al. 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.
[49] Ravandi F, Jorgensen JL, O'Brien SM, et al. Minimal residual disease assessed by multi-parameter flow cytometry is highly prognostic in adult patients with acute lymphoblastic leukaemia. Br J Haematol 2016;172(3):392–400.
[50] Raff T, Gokbuget N, Luschen S, et al. 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):910–15.
[51] Dhedin N, Huynh A, Maury S, et al. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. Blood 2015;125(16):2486–96 quiz 586.
[52] Okasha D, Kirkwood AA, Copland M, et al. Fludarabine, melphalan and alemtuzumab conditioned reduced intensity (RIC) allogeneic hematopoietic cell transplantation for adults aged >40 years with de novo acute lymphoblastic leukemia: a prospective study from the UKALL14 Trial (ISRCTN 66541317). Blood 2015;126(23):733.
[53] Bassan R, Spinelli O, Oldani E, et al. 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.
[54] Bachanova V, Burke MJ, Yohe S, et al. Unrelated cord blood transplantation in adult and pediatric acute lymphoblastic leukemia: effect of minimal residual disease on relapse and survival. Biol Blood Marrow Transplant 2012;18(6):963–8.
[55] Logan AC, Vashi N, Faham M, et al. Immunoglobulin and T cell receptor gene high-throughput sequencing quantifies minimal residual disease in acute lymphoblastic leukemia and predicts post-transplantation relapse and survival. Biol Blood Marrow Transplant 2014;20(9):1307–13.
[56] Gokbuget N, Dombret H, Bonifacio M, et al. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood 2018;131(14):1522–31.
[57] Spinelli O, Peruta B, Tosi M, et al. 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):612–18.
[58] Kotrova M, van der Velden VHJ, van Dongen JJM, et al. Next-generation sequencing indicates false-positive MRD results and better predicts prognosis after SCT in patients with childhood ALL. Bone Marrow Transplant 2017;52(7):962–8.
[59] Kantarjian H, Stein A, Gokbuget N, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med 2017;376(9):836–47.
[60] Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: final report and long-term survival follow-up from the randomized, phase 3 INO–VATE study. Cancer 2019;125(14):2474–87.
[61] Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med 2016;375(8):740–53.
[62] Issa GC, Kantarjian HM, Yin CC, et al. Prognostic impact of pretreatment

cytogenetics in adult Philadelphia chromosome-negative acute lymphoblastic leukemia in the era of minimal residual disease. Cancer 2017;123(3):459–67.
[63] Jabbour E, Short NJ, Jorgensen JL, et al. Differential impact of minimal residual disease negativity according to the salvage status in patients with relapsed/refractory B-cell acute lymphoblastic leukemia. Cancer 2017;123(2):294–302.
[64] Mejstrikova E, Hrusak O, Borowitz MJ, et al. CD19-negative relapse of pediatric B-cell precursor acute lymphoblastic leukemia following blinatumomab treatment. Blood Cancer J 2017;7(12):659.
[65] Orlando EJ, Han X, Tribouley C, et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. Nat Med 2018;24(10):1504–6.
[66] Ruella M, Maus MV. Catch me if you can: leukemia escape after CD19-directed T cell immunotherapies. Comput Struct Biotechnol J 2016;14:357–62.
[67] Ghorashian S, Kramer AM, Onuoha S, et al. Enhanced CAR T cell expansion and prolonged persistence in pediatric patients with ALL treated with a low-affinity CD19 CAR. Nat Med 2019;25(9):1408–14.
[68] Pfeifer H, Cazzaniga G, van der Velden VHJ, et al. Standardisation and consensus guidelines for minimal residual disease assessment in Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL) by real-time quantitative reverse transcriptase PCR of e1a2. Leukemia 2019.
[69] Rousselot P, Coude MM, Gokbuget N, et al. Dasatinib and low-intensity chemotherapy in elderly patients with Philadelphia chromosome-positive ALL. Blood 2016;128(6):774–82.
[70] Yoon JH, Yhim HY, Kwak JY, et al. Minimal residual disease-based effect and long-term outcome of first-line dasatinib combined with chemotherapy for adult Philadelphia chromosome-positive acute lymphoblastic leukemia. Ann Oncol 2016;27(6):1081–8.
[71] Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med 2013;369(19):1783–96.
[72] Kim DY, Joo YD, Lim SN, et al. Nilotinib combined with multiagent chemotherapy for newly diagnosed Philadelphia-positive acute lymphoblastic leukemia. Blood 2015;126(6):746–56.
[73] Cazzaniga G, De Lorenzo P, Alten J, et al. Predictive value of minimal residual disease in Philadelphia-chromosome-positive acute lymphoblastic leukemia treated with imatinib in the European intergroup study of post-induction treatment of Philadelphia-chromosome-positive acute lymphoblastic leukemia, based on immunoglobulin/T-cell receptor and BCR/ABL1 methodologies. Haematologica 2018;103(1):107–15.
[74] Hovorkova L, Zaliova M, Venn NC, et al. Monitoring of childhood ALL using BCR-ABL1 genomic breakpoints identifies a subgroup with CML-like biology. Blood 2017;129(20):2771–81.
[75] O'Connor D, Enshaei A, Bartram J, et al. Genotype-specific minimal residual disease interpretation improves stratification in pediatric acute lymphoblastic leukemia. J Clin Oncol 2018;36(1):34–43.
[76] Bartram J, O'Connor D, Enshaei A, Moorman A. Combining genotype profiling with MRD for more accurate prognostication in acute lymphoblastic leukemia. Clin Lymphoma Myeloma Leukemia 2019;19:S63–SS5.
[77] Enshaei A, O'Connor D, Bartram J, et al. A validated novel continuous prognostic index to deliver stratified medicine in pediatric acute lymphoblastic leukemia. Blood 2020;135(17):1438–46.