Published in final edited form as:

Best Pract Res Clin Haematol. 2010 September ; 23(3): 347–358. doi:10.1016/j.beha.2010.07.007.

IMMUNOLOGIC MINIMAL RESIDUAL DISEASE DETECTION IN ACUTE LYMPHOBLASTIC LEUKEMIA: A COMPARATIVE APPROACH TO MOLECULAR TESTING

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Abstract

The generation of antisera directed against leukocyte differentiation antigens opened the possibility of studying minimal residual disease (MRD) in patients with acute lymphoblastic leukemia (ALL). During the three decades that followed the pioneering studies in this field, great progress has been made in the development of a wide array of monoclonal antibodies and of flow cytometric techniques for rare event detection. This advance was accompanied by an increasingly greater understanding of the immunophenotypic features of leukemic and normal lymphoid cells, and of the antigenic differences that make MRD studies possible. In parallel, molecular methods for MRD detection were established. The systematic application of immunologic and molecular techniques to study MRD in clinical samples has demonstrated the clinical significance of MRD in patients leading to the use of MRD to regulate treatment intensity in many contemporary treatment protocols. In this article, we discuss methodologic issues related to the immunologic monitoring of MRD and the evidence supporting its clinical significance, and compare the advantages and limitations of this approach to those of molecular monitoring of MRD.

Keywords

acute lymphoblastic leukemia; minimal residual disease; flow cytometry; polymerase chain reaction

INTRODUCTION

After considerable experimental and pre-clinical testing, the results of minimal residual disease (MRD) assays have become a determining factor in the treatment of patients with acute lymphoblastic leukemia (ALL). Underlying the increasing reliance on MRD monitoring are two well-established principles, namely that a) treatment intensity should be proportional to the aggressiveness of the disease and to the relative drug resistance of leukemic cells, and b) ALL cell clones vary in their growth potential and sensitivity to chemotherapy. MRD testing provides a direct measurement of the residual leukemic burden

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Conflict of interest statement

There are no conflicts to declare.

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as well as the rate of leukemia clearance by chemotherapy. Studies of MRD, a term commonly interpreted as leukemia undetectable by morphology, are gradually supplanting morphologic studies of treatment response in contemporary clinical practice.

The first direct demonstration that bone marrow samples from patients with ALL in morphologic remission could conceal leukemic lymphoblasts was obtained by applying immunologic techniques. Using fluorescence microscopy and polyclonal antisera against leukocyte antigens that were developed to identify leukemia subtypes, Janossy and colleagues were able to identify leukemic cells in remission marrow samples of patients with T-lineage ALL [1]. Immunologic techniques to study MRD have steadily improved over the three decades following this discovery. In this article, we discuss their methodological principles and clinical applications in parallel to those of MRD assays based on polymerase chain reaction (PCR).

IMMUNOLOGIC DETECTION OF ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

Selection of markers

ALL cells display markers that disclose their affiliation with the B- or the T-lymphoid lineage as well as their immature stage of development. In cases of T-lineage ALL, the expression of markers characteristic of T-cell precursor is generally sufficient to identify leukemia cells. Thus, MRD can be monitored in most cases by searching for cells expressing terminal deoxynucleotidyl transferase (TdT) or, less commonly, CD34 in combination with T-cell antigens, such as CD3 and/or CD5; these immunophenotypes are not expressed in peripheral blood or bone marrow of non-T-ALL patients or healthy individuals. For a better discrimination between leukemic and normal cells, these combinations can be further enriched by additional T-cell markers, such as CD99 [2], and by markers that are usually not expressed by T-ALL blasts, such as CD19 and CD33 [3].

To monitor MRD in B-lineage ALL, searching for cells with immature B-cell features is not sufficient because B-cell progenitors are normally present in the bone marrow. In fact, bone marrow samples from young children, or samples from patients obtained after or during a pause in chemotherapy, may contain high proportions of such cells (e.g., 10% or more) [4,5], and they may even be found in blood. These normal cells are occasionally misclassified as leukemic, leading to erroneous conclusions about MRD or impending relapse. To monitor MRD in B-lineage ALL cells, it is imperative to determine the expression of additional markers which can distinguish ALL cells from their normal counterparts. The expression of such markers contributes to characteristic immunophenotypes which are called “asynchronous”, “aberrant” or “leukemia-associated” [3,6–9]. Aberrant immunophenotypes can be identified in over 95% of B-lineage ALL cases (Fig. 1). The discovery of leukemia-associated immunophenotypes has traditionally been made by painstakingly comparing the immunophenotype of leukemic cells to that of normal progenitor cells [8,9,17]. An alternative approach is to use gene expression arrays to screen for genome-wide differences between normal and leukemic cells and then validate the findings using specific antibodies. This strategy identified CD58 as a useful marker for MRD studies in B-lineage ALL [18–22].

Established leukemia-associated immunophenotypes must be identified at diagnosis (or relapse) in each patient. The combinations used for MRD studies typically contain a “backbone” set of CD19, CD10 and CD34 (or anti-TdT) antibodies to identify B-cell precursors (Fig. 1). The immunophenotypes used to distinguish leukemic cells from normal cells must be expressed only in leukemic cells. Some immunophenotypes are apparently restricted to leukemic cells when compared to results obtained in bone marrow samples from

healthy adult donors but their lack of specificity becomes clear once they are tested on bone marrow samples recuperating after chemotherapy [3]. Some investigators have reported results of MRD studies obtained without prior knowledge of the immunophenotype of the leukemic cells, a process that involves searching for abnormal profiles in follow-up samples with a large panel of antibody combinations [23]. This approach may be the only option when patients are referred from another center for continuation therapy or transplant. In such cases, a negative MRD result would be clearly difficult to interpret.

A simplified panel to study early treatment response in B-lineage ALL

As stated above, when performing MRD studies in B-lineage ALL it is critical to use markers that can distinguish leukemic cells from normal B-cell progenitors. However, if the bone marrow sample is devoid of lymphoid progenitors, then the presence of cells with features of immature B cells should denote residual leukemia. This is precisely the case when studies are performed during the first 2–3 weeks of remission-induction therapy, when normal B-cell progenitors are typically absent owing to their extreme sensitivity to chemotherapy [24]. We therefore hypothesized that the detection of CD19⁺ cells expressing CD10 and/or CD34 on day 19 of treatment in patients with B-lineage ALL indicates the presence of MRD. We tested this prediction by comparing the results of this simplified MRD assay with a more complex flow cytometric test based on “leukemia-associated” immunophenotypes defined at diagnosis, and PCR amplification of antigen-receptor gene rearrangements (Fig. 2). In comparisons between the simplified assay and the more complex flow cytometric evaluation, discordant results were seen in only 4 of the 376 samples. In comparisons with PCR, using a threshold detection level of 0.01%, 3 of 149 samples were MRD-negative by the simplified flow cytometric assay but MRD-positive by PCR, although at very low levels (<0.02%). Ten additional samples had $\geq 0.01\%$ lymphoblasts by the simplified assay but had MRD levels <0.01% by PCR [24].

The main advantages of the simplified test are its low cost and ease of interpretation. Because the main clinical use of the test is to identify patients that clear leukemia rapidly for possible inclusion in chemotherapy regimens of reduced intensity, false-positive results should not be a major concern as they would not trigger any change in therapy. It is important to stress that the use of the assay at other time points during or after treatment, when normal B-cell progenitors are most likely to be present, will inevitably yield false-positive results. At these time points, more complex flow cytometric and molecular MRD assays are necessary.

Methodologic issues regarding flow cytometric monitoring of MRD

Most published MRD studies relied on the detection of 4-marker combinations. Current instruments that allow the detection of more than 4 markers facilitate the distinction between normal and leukemic cells and can potentially increase the sensitivity of MRD detection. With additional markers, it should also be possible to study other features of the MRD cells, including their cell cycle, expression of drug-resistance molecules, and DNA repair activity [25].

The sensitivity of MRD detection depends not only on the degree of ‘aberrancy’ of the targeted immunophenotypic profile but also on the number of cells that can be analyzed. A realistic sensitivity for clinical samples is around 1 target cell in 10^4 to 10^5 cells [3]. Because MRD positivity may trigger treatment intensification and increase the potential for iatrogenic toxicities, it is imperative to avoid false-positive results. Besides sample mislabeling and contamination, false “MRD” results can be caused by reagents containing impurities, a less-than-perfectly clean fluidics system in the flow cytometer, and non-specific antibody binding to Fc receptors [3]. The reproducibility of MRD analysis can also

be affected by changes between different batches of antibodies and by instrument instability. Therefore, MRD studies demand extremely meticulous procedures for cell collection, staining and analysis.

CLINICAL SIGNIFICANCE OF IMMUNOLOGIC DETECTION OF MRD

Childhood ALL

Many studies have demonstrated the prognostic importance of measuring MRD by flow cytometry in childhood ALLs [10,14,26–34]. In early studies with patients enrolled in the Total XIII study at our institution, MRD levels $\geq 0.01\%$ on days 19, 46 or following time points during treatment were strongly associated with a higher risk of relapse [10,14,26]. MRD remained significantly prognostic after adjusting for adverse presenting features, including levels of peripheral blood lymphoblasts on day 7 and 10 of remission-induction therapy, and in analyses limited to patients with high-risk or low-risk presenting features [26]. Notably, the cumulative incidence of relapse among patients with MRD at the end of the induction phase was 7% if MRD became undetectable on week 14 of continuation therapy but increased to 68% if it remained $\geq 0.01\%$ [26]. MRD measurements in peripheral blood were equally informative to those obtained in bone marrow in patients with T-lineage ALL [35], a finding that, as we have recently shown [36], applies also to measurements of disease dissemination in patients with T-cell lymphoblastic lymphoma.

The clinical importance of immunologic detection of MRD in children and adolescents with newly diagnosed ALL was definitively demonstrated by large multicentric studies. The reference laboratory at the Johns Hopkins Medical Institutions studied day-29 bone marrow samples from 2086 patients with B-lineage ALL enrolled on 9900 series treatment protocols of the Children's Oncology Group (COG) [30]. In only 4% of cases, sample cellularity was too low or the immunophenotype of the leukemic cells (determined at diagnosis) was not sufficiently distinct to allow a sensitivity of detection of 0.01% [30]. Among the 1971 patients studied on day 29, those with undetectable MRD ($n = 1588$) had a 5-year event-free survival of $88\% \pm 1\%$, as compared to $59\% \pm 5\%$ for those with MRD levels between 0.01% and 0.1% ($n = 175$), $49\% \pm 6\%$ for 0.1% to 1% MRD ($n = 141$) and $30\% \pm 8\%$ for MRD $\geq 1\%$ ($n = 67$). MRD was the most important prognostic factor in multivariate analysis. The reference laboratory at the University of Padua (Italy) studied day-15 bone marrow samples from 830 patients enrolled in the Italian cooperative group Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) [32]. Flow cytometric analysis identified 3 risk groups: standard ($< 0.1\%$ MRD; 42%), intermediate ($0.1\% - < 10\%$ MRD; 47%), and high ($\geq 10\%$; 11%) with a 5-year cumulative incidence of relapse of 8%, 18%, and 47%, respectively [32].

Flow cytometric studies of MRD also proved to be useful in the setting of relapse ALL. We studied 35 children with first-relapse ALL who were in second morphologic remission; 19 (54%) had MRD $\geq 0.01\%$ [29]. Two-year cumulative incidence of second leukemia relapse was 70% for the MRD-positive patients and 28% for the 16 MRD-negative patients. Time of first relapse and MRD were the only two significant predictors of outcome in a multivariate analysis. Investigators of the COG AALL01P2 study measured MRD by flow cytometry in 124 patients with first-relapse ALL [31]. One year-event-free survival was 58% for patients with MRD $\geq 0.01\%$ at the end of block 1 and 80% for those with negative MRD [31]. Elorza et al. [34] recently reported results of MRD measurements performed at a median of 10 days before hematopoietic stem cell transplantation in 31 children with ALL. In 10 patients, MRD was $\geq 0.01\%$ and the estimated event-free survival at 2 years was 20% as compared to 74% for the remaining patients.

Adult ALL

MRD studies by flow cytometry could also be clinically helpful in adult patients with ALL [8,37–39]. Krampera et al. found that over 95% of adult B-lineage ALL samples at diagnosis had abnormal phenotypes [40]. They also performed correlative studies between MRD and outcome in 53 patients with T-lineage ALL enrolled in the Italian co-operative GIMEMA LAL0496 protocol [37]: the 2-year probability of relapse was 81.5% for MRD-positive and 38.9% for MRD-negative patients when MRD was measured at preconsolidation, 54.5% and 15.8% pre-third reinduction and 50.0% and 16.4% pre-sixth reinduction. Vidriales et al. [38] studied MRD in 102 adolescent and adults with ALL. MRD < 0.05% on day 35 of treatment predicted a significantly longer relapse-free survival (42 months versus 16 months) and was the most important prognostic factor in a multivariate analysis. Holowiecki et al. [39] monitored MRD in 116 patients with Philadelphia-negative ALL out of 132 potentially eligible enrolled in the Polish Adult leukemia Group ALL 4–2002 MRD study. They found that MRD $\geq$ 0.1% after remission-induction therapy was an independent predictor for relapse and that patients with standard-risk features and MRD < 0.1% after remission-induction therapy had a 3-year risk of relapse of 9% versus 71% for the remaining patients.

MOLECULAR MONITORING OF MRD

A detailed discussion of the methodological aspects of molecular monitoring of MRD is beyond the scope of this review; we refer the readers to the recent article by Bruggemann et al. [41] and to the references therein. Molecular assays of MRD target clonal rearrangements of immunoglobulin (IG) and T-cell receptor (TCR) genes, and chimeric mRNA transcripts resulting from gene fusions such as *BCR-ABL1*, *MLL-AFF1*, *TCF3-PBX1*, and *ETV6-RUNX1*. These targets are most frequently studied by “real-time” quantitative PCR (RQ-PCR) [42], using mixtures of diagnostic leukemic and normal DNA to gauge the sensitivity of the assays.

Several studies using PCR amplification of IG and TCR receptor genes lead to conclusions about the clinical significance of MRD in childhood ALL similar to those discussed for flow cytometric studies [41,43–58]. A recent study from our laboratory in 455 patients with B-lineage ALL, confirmed the adverse prognostic impact of MRD as detected by PCR amplification of IG/TCR genes at the end of remission induction therapy and demonstrated that patients with low levels of MRD (i.e., 0.001%–<0.01%; $n = 63$) had a significantly higher risk of relapse than those with findings below 0.001% ($n = 319$): patients with this low level of MRD had a 13% cumulative risk of relapse at 5 years, compared with 5% for those with lower or undetectable MRD [58]. The presence of low levels of MRD could not be predicted by presenting clinical and biologic features of ALL, but patients with higher levels of MRD on day 19 were significantly more likely to have MRD, including low levels, at the end of induction therapy (Fig. 3).

Studies in adult patients confirmed the clinical importance of MRD testing [59–67]. For example, in a recent study of 161 patients with non T-lineage, *BCR-ABL1*-negative ALL enrolled in the UKALL XII trial, MRD $\geq$ 0.01% in patients treated with chemotherapy alone predicted a shorter relapse-free survival at several time-points during the first year of therapy [65]. MRD prior to transplant was associated with relapse risk in patients receiving autologous transplant but not in those receiving allogeneic transplant in first complete remission [65].

IMMUNOLOGIC VERSUS MOLECULAR MONITORING OF MRD

Relative sensitivity of different methods and their concordance

In general, the sensitivity of detection afforded by PCR is approximately one log better than that of flow cytometry although it is possible to monitor MRD at the 0.01% levels in most patients with either method. Interestingly, in a recent study of bone marrow samples collected at the end of remission-induction therapy (day 43) from patients with B-lineage ALL enrolled at our institution, 24 of 60 (40%) with MRD 0.001%–<0.01% by PCR amplification of antigen-receptor genes, also had MRD detectable by 4-color flow cytometry [58]. With improvements in flow cytometric methods, it might be possible to achieve such high sensitivity in an increasing proportion of such patients but with current techniques, most cases with PCR signals below 0.01% are negative by flow cytometry.

Above the 0.01% threshold, flow cytometry and PCR amplification of antigen-receptor genes yield remarkably close measurements, if MRD is present at a $\geq 0.01\%$ level [68–70]. Our current strategy is to use preferentially flow cytometry to monitor MRD during remission induction therapy, and develop a PCR assay for antigen-receptor genes (or fusion transcripts) only if a suitable immunophenotype cannot be identified.

Advantages and disadvantages

Flow cytometric detection of aberrant immunophenotypes and PCR amplification of antigen-receptor genes can be used to study MRD in the vast majority of patients with ALL. In addition to its capacity for accurate quantification of MRD, specific advantages of flow cytometry include the potential for analyzing the status of normal hematopoietic cells while searching for MRD, thus obtaining information about the degree of hemodilution in the samples studied and on the degree of lympho-hematopoietic recovery. It is also possible to analyze multiple biologic features of ALL cells while detecting MRD, and to identify markers than can be used to sort leukemic cells for biologic and genetic studies. Amplification of antigen-receptor genes by RQ-PCR also yields precise MRD estimates [42,61,71]. Another advantage of this method is that the results are, in general, definite and easily interpretable.

Each method has potential weaknesses. In the case of flow cytometry, fluctuations in the expression of the markers used to monitor MRD may occur during therapy. For example, Gaipa et al. [20] observed a decrease in the expression of CD10 and CD34, and an increase in CD19, CD20, CD45RA, and CD11a in samples collected during remission induction therapy of childhood ALL; expression of CD58 remained stable. Recently, this group of investigators reported that changes in CD10, CD34, CD20 and CD45 were primarily related to exposure to glucocorticoid-containing therapy chemotherapy and reverted to initial levels later, while CD11a changes were recorded later during treatment [72]. In our experience, it is possible to avoid the potentially erroneous results caused by these immunophenotypic shifts with the use of multiple combinations of markers. Perhaps an even more significant source of errors is lack of specific expertise in interpreting results of MRD assays. This risk is particularly high for flow cytometry as immunophenotyping of leukemia is currently performed at nearly every leukemia center, tempting investigators to venture in this area despite the lack of specific training.

IG and TCR genes in ALL might undergo continuing or secondary rearrangements, resulting in subclones with distinct rearrangements [73,74]. It is unclear whether this phenomenon impacts the reliability of MRD detection to great extent, particularly at early stages of treatment. Targeting fusion transcripts has the risk of yielding imprecise estimate of the percentage of leukemic cells present owing to the fact that the amount of transcripts per leukemic cell may vary from patient to patient with the same genetic subtype of ALL, and

could be affected by chemotherapy and by the cell integrity when the sample is examined [75].

Application in clinical studies of MRD

Routine monitoring of MRD in the majority of patients using immunologic or molecular techniques is feasible, and results can be provided in a timely fashion. Thus, most major childhood ALL protocols and an increasing number of adult ALL trials now include MRD for risk stratification. In the St Jude Total XV trial for children with newly diagnosed ALL, we used flow cytometry and/or PCR amplification of antigen-receptor genes to monitor MRD in children enrolled in the St Jude Total XV trial [76]. Flow cytometry was applied in patients with B-lineage ALL and T-ALL whereas PCR studies were performed only in patients with B-lineage ALL; 482 of 492 patients (98%) were monitored by flow cytometry, and 403 of 492 (82%) by PCR [76]. The two methods combined could study 491 of 492 patients (99.8%) of the patients. The single patient with no available immunophenotypic or antigen-receptor gene rearrangements was monitored by RQ-PCR targeting the *MLL-MLLT3* fusion transcript. According to the requirements of the protocol, MRD results on day 19 were provided within 24 hours, while those at later time points were released within 1 week of sample receipt.

Time points selected for MRD studies are usually mandated by the treatment protocol design, and the levels of MRD used for risk assignment vary in different protocols [41]. We currently test MRD levels on day 15 and day 42 for treatment assignment. We use primarily flow cytometry, reserving PCR amplification of IG/TCR genes if suitable immunophenotypes are not identified or the result of the flow cytometric analysis is difficult to interpret. Patients with MRD $\geq 1\%$ on day 15 receive a more intensive remission-induction regimen, with further intensification for patients with $\geq 5\%$ leukemic cells. Patients with MRD $<0.01\%$ on day 15 receive a slightly less intensive reinduction therapy and lower cumulative doses of anthracyclin. Patients with standard-risk ALL who have MRD of $\geq 0.01\%$ on day 42 are reclassified as high-risk, and patients with MRD $\geq 1\%$ are eligible for transplant in first remission. MRD monitoring ceases on day 42 for patients with B-lineage ALL who are MRD negative, while it continues in patients who are MRD positive, and in any patient with T-lineage ALL (with peripheral blood). The current COG protocol for children with standard risk B-lineage ALL includes MRD measurements at the end of remission induction therapy (day 29) and the cut-off levels used to assign risk are 0.1% and 1% [77].

Our team also uses MRD to guide treatment for patients with first-relapse ALL who achieve a second remission. Those with persistent MRD are candidates for transplant whereas those who achieve MRD negativity (in the context of other favorable clinical features) are eligible for continuing chemotherapy. For patients who undergo transplant, additional courses of chemotherapy may be administered in efforts to reduce MRD levels before transplant.

CONCLUSIONS AND FUTURE PERSPECTIVE

MRD assays have overcome the uncertainties of morphologic studies of treatment response, offering a powerful new parameter to implement risk-adapted therapy of ALL. MRD studies have also provided a compelling demonstration that the degree of early treatment response is directly related with risk of relapse. Even very low levels of MRD (i.e., 0.001%–0.01%) at the end of remission induction have an adverse prognostic impact [58], an unexpected finding if one considers that they would correspond to an estimated total leukemic burden of only 10^7 to 10^8 cells (i.e., 0.1 gram or less of tissue) and that patients received nearly 2 years of reinduction/consolidation and continuation therapy.

In addition to its use as a prognostic factor, MRD tests can recognize leukemia before overt relapse, estimate the leukemia burden before transplant, and measure the efficacy of a treatment regimen in relation to that of its predecessor. Hence, MRD studies can play multiple roles in patient clinical management. As discussed in this review, all methods have advantages and disadvantages and, ideally, all should be available but this is likely to be impossible at most centers. Flow cytometric detection of aberrant immunophenotypes and RQ-PCR amplification of IG/TCR genes can be applied to most patients with ALL, are highly quantitative, and have been shown to yield clinically informative results. The selection of one of these two methods depends on the existing expertise and facilities.

Most cases of childhood ALL with *ETV6-RUNX1*, hyperdiploidy (>50 chromosomes) and *TCF3-PBX1* ALL show a steep decline in MRD levels, while in cases of *BCR-ABL1* (pre-imatinib), *IKZF1*-deleted and ETP-ALL, persistent MRD and higher MRD levels are more common [78–80]. Gene expression profiles of ALL cells [81–83], and germline or leukemia-associated gene polymorphisms [84] have also been related to distinct gradients of leukemia cytorreduction. Despite these associations, MRD has been consistently shown to be an independent prognostic factor. It is likely that the combined information of MRD testing and genetic features of leukemia will be increasingly used for risk assignment [76,85].

Skeptics of the utility of MRD may raise several theoretical caveats. One is that leukemia might be distributed so heterogeneously that any quantitation of MRD would be uninformative. Another is that small amounts of MRD could perhaps be controlled by microenvironmental and/or immunologic mechanisms, nullifying its clinical threat. More recently, some reports have elicited a concern that some of the markers used to monitor MRD may not be expressed on leukemia-initiating cells, a notion that remains rather controversial [86]. Although these arguments are worthy of serious consideration, the strong correlation between MRD levels and relapse has rendered them more academic than practical. It is now clear that MRD levels (at least during the early phases of treatment) closely reflect the degree of persistent disease, and that the residual leukemic cell population revealed by MRD testing has the potential of causing ALL recurrence.

PRACTICE POINTS

- Immunologic testing of MRD relies on “leukemia-associated” immunophenotypes which can be identified in 95% or more of ALL cases.
- The sensitivity of MRD detection that can be achieved in routine assays by flow cytometry is 0.01%. The development of more powerful flow cytometric methods may extend the sensitivity to 0.001% for most cases.
- Molecular methods for MRD detection rely on PCR amplification of antigen-receptor gene rearrangements (present in approximately 95% of cases) and fusion transcripts (present in approximately 40% of cases). The sensitivity is typically 0.01% to 0.001%.
- Both immunologic and molecular MRD assays have relative advantages and disadvantages but yield generally concordant results in samples with MRD 0.01% or higher.
- Results of MRD testing provide unique and clinically important information, and are now used in clinical protocols for risk-assignment.

RESEARCH AGENDA

1. Because erroneous results in flow cytometric MRD determination can result from immunophenotypic changes due to therapy, e.g., clonal selection, multiple combinations of markers need to be tested in MRD setting to decrease the chances of missing low-levels of leukemic cells.
2. The most significant source of errors is a lack of specific expertise in interpreting results of MRD assays.

Acknowledgments

This work was supported by grants CA60419 and CA21765 from the National Cancer Institute, and by the American Lebanese Syrian Associated Charities (ALSAC)

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Figure 1. Percentage of cases of B-lineage ALL in which the marker combination indicated was used to monitor MRD with a sensitivity of 10^{-4} . Data are from a series of 414 pediatric patients with newly diagnosed B-lineage ALL. Percentage of markers used for cases with MLL gene rearrangement (typically CD10-negative) are shown separately.

Figure 2.

Relation between results of the simplified flow cytometric assays with those of more complex MRD assays. Bone marrow samples collected on day 19 of remission induction therapy from children with B-lineage ALL were examined for the presence of CD19⁺ cells co-expressing CD10 and/or CD34. Results were compared with those obtained with several sets of 4-antibody combinations in 376 patients (A), and with those of PCR amplification of immunoglobulin and T-cell receptor genes in 149 patients (B). Results of Spearman rank correlations are shown. This research was originally published in *Blood*, Coustan-Smith E, Ribeiro RC, Stow P, Zhou Y, Pui CH, Rivera GK, Pedrosa F, Campana D. A simplified flow cytometric assay identifies children with acute lymphoblastic leukemia who have a superior clinical outcome. *Blood* 2006; 108: 97–102. © the American Society of Hematology [24].

Figure 3.

Day 19 MRD levels by flow cytometry in 3 patient groups defined by day-46 MRD levels as measured by PCR amplification of antigen receptor genes. Patient number in each group is shown. Horizontal bars indicate median values. Levels of MRD on day 19 were significantly different in the 3 groups ($P < 0.0001$ by Kruskal-Wallis one-way analysis-of-variance test). This research was originally published in *Blood*, Stow P, Key L, Chen X, Pan Q, Neale GA, Coustan-Smith E, Mullighan CG, Zhou X, Pui CH, Campana D. Clinical significance of low levels of minimal residual disease at the end of remission induction therapy in childhood acute lymphoblastic leukemia. *Blood* 2010; 115(23):4657-4663. © the American Society of Hematology [58].