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MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA

Dario Campana

Departments of Oncology and Pathology, St. Jude Children's Research Hospital; and Department of Pediatric, University of Tennessee Health Science Center, Memphis, TN

Abstract

In patients with acute lymphoblastic leukemia (ALL), monitoring of minimal residual disease (MRD) offers a way to precisely assess early treatment response and detect relapse. Established methods to study MRD are flow cytometric detection of abnormal immunophenotypes, polymerase chain reaction (PCR) amplification of antigen-receptor genes, and PCR amplification of fusion transcripts. The strong correlation between MRD levels and risk of relapse in childhood ALL is well established; studies in adult patients also support its prognostic value. Hence, results of MRD studies can be used to select treatment intensity and duration, and estimate the optimal timing for hematopoietic stem cell transplantation. Practical issues in the implementation of MRD assays in clinical studies include determining the most informative time point to study MRD, the levels of MRD that will trigger changes in treatment intensity, as well as the relative cost and informative power of different methodologies. The identification of new markers of leukemia and the use of increasingly refined assays should further facilitate routine monitoring of MRD and help clarifying the cellular and biologic features of leukemic cells that resist chemotherapy in vivo.

Introduction

Three independent studies published ten years ago conclusively demonstrated that minimal residual disease (MRD) is a powerful prognostic indicator in newly diagnosed childhood acute lymphoblastic leukemia (ALL).^{1–3} These studies represented the culmination of efforts of many investigators (reviewed in ⁴), and stimulated the design of treatment protocols in which risk assignment was largely based on MRD measurements

Research to develop methods for detecting small numbers of leukemic cells had begun to intensify approximately two decades earlier. In 1980, Janossy and colleagues reported the use of antibody staining of terminal deoxynucleotidyl transferase (TdT) to differentiate leukemic lymphoblasts from normal lymphocytes in the cerebrospinal fluid of patients with ALL;⁵ a year later, they reported that anti-TdT in combination with an anti-T cell antibody could identify MRD in the bone marrow of patients with T-ALL in morphologic remission.⁶ The advent of flow cytometers subsequently widened and improved the use of antibodies to identify leukemic cells.^{7–9} About the same time, polymerase chain reaction (PCR)-based methods to amplify fusion transcripts in ALL cells were developed,^{10,11} and the use of PCR amplification of antigen-receptor genes to track ALL cells was reported.^{12–15} Numerous studies that followed

Correspondence: D. Campana, M.D. Ph.D., Department of Oncology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis TN 38105; Telephone 901-595 2528; FAX 901-595 5947; E-mail: dario.campana@stjude.org.

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(reviewed in ^{16,17}) refined and expanded this pioneering work, ultimately producing methods that are sufficiently sensitive, precise and reliable to guide treatment decisions.

Clinical applications of MRD studies

Selection of patients for treatment intensification

MRD studies revealed that many patients who achieve morphologic remission harbored residual disease, a finding that is associated with a higher risk of relapse.^{16,17} MRD can also identify patients with a higher risk of relapse among those with specific ALL subtypes defined by presenting features.^{18–20} Moreover, among patients with first-relapse ALL who achieve a second remission, MRD predicts a significantly worse outcome.^{21,22} Hence, the most immediate application of MRD testing is the identification of patients who are candidates for treatment intensification.

Levels of MRD are proportional to the risk of relapse. We found that MRD equal or greater than 1% at the end of remission induction therapy (in patients who were morphologically in remission) associated with a dismal outcome,¹⁸ prompting the design of protocols which recommended transplant in first remission for these patients.²³ Investigators of the International Berlin-Frankfurt-Munster (I-BFM) Study Group found that patients with MRD levels of 0.1% or higher on both day 33 and day 78 of treatment had a relapse rate of 75%,³ prompting treatment intensification for this group of patients.²⁴

Selection of patients for treatment deintensification

MRD studies revealed that remission induction therapy can produce dramatic leukemia cytorreduction and undetectable MRD after only 2–3 weeks of therapy in a proportion of patients. In a recent analysis of 402 patients with B-lineage ALL, we found that 183 (45.5%) were MRD-negative on day 19, defined as having <0.01% leukemic cells in bone marrow.²⁵

Patients with early clearance of leukemic cells typically remain MRD-negative, and their prognosis is excellent with current treatment protocols.^{26,27} Should treatment deintensification be considered for these patients? One argument against is that early MRD-negativity might be a good prognostic feature *only* in the context of intensive therapy. Thus, if therapy is deintensified, the risk of relapse of MRD-negative patients might increase significantly. The counterargument is that some patients with ALL must be curable with less intensive therapy than in prevailing in contemporary regimens. Indeed, 36% of patients were cured in St Jude treatment protocols from 1967 to 1979 (much less intense than today's regimens), and 53% in those from 1979 to 1983 (including limited treatment intensification).²⁸ Moreover, in a study where all treatment stopped 1 year after diagnosis, the mean 5-year event-free survival approached 60%.²⁹ If some forms of ALL can be cured with less intensive and/or shorter therapy, an excellent response to initial therapy should be a feature of these leukemias. The best way to apply MRD results for treatment deintensification has not yet been defined. However, this option appears to be especially attractive in when intensive therapy confers a high risk of serious toxicities (as at centers with limited resources, or in older adult patients), and the potential benefits of treatment deintensification might outweigh the risk of relapse.

MRD postremission and timing of hematopoietic stem cell transplantation

In patients who attain first or second remission, sequential MRD monitoring may identify relapse before its detection by morphology or cytogenetics. Sequential MRD monitoring is particularly useful in those patients who are in remission but who remain MRD-positive at the end of remission induction therapy. Conversion to MRD-negativity is associated with a favorable outcome, while persistence or increase in levels of MRD carries a high hazard of

relapse.¹⁸ Vigorous lympho-hematopoietic regeneration mimicking relapse may occur in patients who are not compliant with post-remission therapy. In these cases, MRD studies can quickly clarify the nature of the morphologically suspect cells.

Based on the observation that detection of MRD before allogeneic hematopoietic stem cell transplantation (HSCT) is associated with an increase risk of relapse post-HSCT,^{30–35} MRD assays increasingly are being used to select the timing of transplant. Thus, patients with MRD-positivity who are candidates for transplant may receive additional courses of chemotherapy in efforts to reduce the levels of MRD, possibly below detection threshold, before HSCT. After transplant, detection of MRD can serve as an indicator for decreasing immunosuppressive therapy and/or administering donor lymphocyte infusions.

Methodologies to detect MRD

Leukemic cells differ from normal hematopoietic cells in several genetic and cellular features.³⁶ One leukemia-associated property is the expression of abnormal cell marker profiles which can be detected with flow cytometry.¹⁷ With rare exceptions, leukemic lymphoblasts have immunophenotypes sufficiently distinct to allow the detection of 1 leukemic cells among 10,000 normal cells (0.01%).^{27,37} Another leukemia-associated characteristic is the clonal rearrangement of immunoglobulin (IG) and T-cell receptor (TCR) genes. This rearrangement results in unique molecular signatures which can be detected by PCR in most cases, with a sensitivity of 0.01% to 0.001%.³⁸ A third leukemia-associated feature is represented by chromosomal abnormalities and their corresponding gene fusions, such as *BCR-ABL*, *MLL-AF4*, *E2A-PBX1*, and *TEL-AML1*.^{39,40} Less than one-third of patients with ALL have leukemic cells with genetic abnormalities that can be studied with the typical assays performed in molecular pathology laboratories, allowing the detection of MRD with a sensitivity ranging from 0.1% to 0.001%.

Strengths of flow cytometry-based assays include accurate quantification of MRD and the capacity to examine the status of normal hematopoietic cell maturation simultaneously. Accurate quantification of MRD is also a property of PCR amplification of antigen-receptor genes, in addition to its high sensitivity. A strength of PCR amplification of fusion transcripts is the stable association between the molecular abnormality and the leukemic clone, regardless of cellular changes caused by therapy or clonal selection.

MRD assays are complex and necessitate considerable expertise to be executed well. For example, flow cytometric studies require specific knowledge of the immunophenotypic profiles of bone marrow and peripheral blood under a variety of conditions, and experience in selecting the best markers to use in each case.³⁷ The interpretation of the data should take into account the fact that chemotherapy may alter the phenotype.^{2,41} PCR amplification of antigen-receptor genes also requires considerable expertise. Careful consideration must be given to the presence of minor clones, which might be undetected at diagnosis and can that become predominant during the course of the disease.^{42,43} Targeting two or more different rearrangements has been recommended to avert this problem,⁴⁴ but multiple sensitive probes are not identifiable in approximately 30% of cases.^{24,45} Finally, a pitfall of PCR amplification of fusion transcripts is imprecise quantification of MRD, as the number of transcripts per leukemic cell varies from patient to patient with the same genetic subtype of ALL and might also be affected by therapy.⁴⁰

Clinically informative MRD levels

The 0.01% threshold is commonly used to define MRD positivity, simply because this represents the typical limit of detection for routine flow cytometric and molecular assays. Nevertheless, it is possible to achieve a routine sensitivity of 0.001% by PCR in clinical

samples. With improvements in technology, it is likely that such threshold could also soon be achieved by flow cytometry. The current 0.01% threshold has proven to be clinically informative. For example, we found that patients who had MRD of 0.01% or higher in bone marrow at any time point during treatment had a significantly higher risk of relapse.^{2,18,27} Likewise, the Children's Oncology Group (COG) found that the presence of MRD (0.01% or higher) on day 29 predicted a poorer outcome and was the strongest prognostic indicator.⁴⁶ Depending on the protocol and on the time point at which MRD is examined, other threshold levels can also be informative. Cave et al.¹ found a cut-off level of 0.1% at the end of remission induction and thereafter to be particularly informative. Investigators of the I-BFM Study Group reported that patients with 0.1% or higher MRD on days 33 and 78 had a particularly high relapse rate;^{3,24} those of the Austrian BFM group also reported that the cut-off level of 0.1% on day 33 was particularly informative.⁴⁷ For the Dana-Farber Cancer Institute ALL Consortium, an MRD threshold of 0.1% best predicted relapse hazard.⁴⁸

Flow cytometry and PCR amplification of IGH and TCR genes should yield similar estimates whenever MRD is 0.01% or higher.^{49,50} Whether the results of these methods coincide with those of PCR amplification of fusion transcripts is not yet established. It is possible that in some cases preleukemic clones⁵¹ might be detected by PCR targeting of the fusion transcript but remain undetected by flow cytometric methods or PCR targeting antigen receptor genes.

Prognostic significance of MRD in adult ALL

There is increasing evidence that MRD also has strong prognostic significance in adult patients with ALL. In 85 patients with Philadelphia (Ph) chromosome-negative B-lineage ALL, the presence of MRD correlated with an adverse clinical outcome, particularly when measured 3–5 months after induction therapy.⁵² In 196 standard-risk patients, Bruggeman et al.⁵³ identified 10% of patients with a rapid MRD decline to lower than 0.01% on day 11 and day 24 (low-risk group) who had a 3-year relapse rate of 0%; 23% of patients had MRD 0.01% or higher until week 16 (high-risk) and had a relapse rate of 94%; the remaining patients (intermediate-risk) had a relapse rate of 47%. The same group also reported a prospective analysis of post-consolidation samples in 105 patients who were in hematologic remission, had completed first-year chemotherapy, and had tested MRD-negative prior to enrollment in the study. Twenty-eight patients converted to MRD positivity and 17 of these had relapsed, with a median time from molecular to clinical relapse of 9.5 months. By contrast, only 5 of the 77 continuously MRD-negative patients had relapsed.⁵⁴ A recent study assessed MRD in 116 patients with Ph-negative ALL and indicated that MRD equal or greater than 0.1% after induction was an independent predictor for relapse in both standard- and high-risk groups.⁵⁵

MRD monitoring of BCR-ABL fusion transcripts predicted the outcome of allogeneic or autologous HSCT in adult patients with Ph-positive ALL.⁵⁶ In 27 patients who received imatinib upon detection of MRD after HSCT, BCR-ABL transcripts became undetectable in 14, after a median of 1.5 months.⁵⁷ These patients remained in remission for the duration of imatinib treatment; 3 relapsed after imatinib was discontinued. By contrast, 12 of the 13 patients who failed to achieve molecular remission relapsed. MRD status after allogeneic bone marrow transplantation was also found to be an important predictor of outcome in adults with Ph-negative ALL,⁵² while MRD detected in bone marrow samples taken prospectively from patients with ALL before initiating the conditioning regimen was a significant predictor of outcome.⁵⁸ In 43 adult patients with ALL undergoing HSCT, the cumulative incidence of relapse at 36 months was 0% for the 12 patients who were MRD-negative before transplantation compared to 46% for MRD-positive patients.⁵⁹

Practical issues for clinical application

What are the most informative time points for MRD testing? MRD measurements during remission induction therapy are highly informative in childhood ALL. In general, measurements during remission induction therapy (typically 2 weeks after diagnosis) provide an early identification of good responders and of very poor responders, which can be further refined by assessing MRD at the end of induction therapy and during the early phases of continuation therapy. At St Jude Children's Research Hospital, we currently use MRD on day 15 and day 42 for treatment assignment. Patients with MRD of 1% or higher on day 15 receive intensified remission induction therapy; further intensification is reserved for patients with 5% or more leukemic cells. Conversely, patients with undetectable 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 0.01% or higher on day 42 are reclassified as high-risk. Any patient with MRD of 1% or higher at this time point is a candidate for HSCT in first remission.

What is the best method to study MRD? Both flow cytometry and PCR amplification of antigen-receptor genes yield similar results when MRD is at levels of 0.01% or above,^{49,50} and both methods can produce MRD estimates within 24 hours of sample collection. In our experience, the overall cost of the two methods is similar but others have estimated PCR to be more expensive.⁶⁰ Flow cytometry is more likely to be readily available (flow cytometers and methods for leukemia immunophenotyping are used at virtually every cancer center) and, for studies at early time points during therapy, like day 15, has an advantage over PCR, as the development of a patient-tailored PCR assay typically requires more than two weeks. PCR might be preferable for studies post-HSCT or at the end of therapy because of its high sensitivity. Eventually, the type of expert laboratory available to a cancer center or a cooperative group could be the most important factor in determining which method should be used.

Can MRD be determined in peripheral blood? In patients with B-lineage ALL, MRD is usually present at higher levels in bone marrow than in peripheral blood.^{61–63} Such is not the case in T-ALL, where MRD levels in peripheral blood are similar to those in bone marrow.^{62,63} In these patients, sequential MRD testing can be performed in blood, which is our current practice.

Concluding remarks

Molecular assessment of treatment response is increasingly used to guide therapeutic decisions. MRD measurements can also be used to identify molecular features of leukemic cells associated with treatment response in vivo. To this end, comparisons of gene expression profiles of lymphoblasts in patients with and without MRD allowed the identification of several prognostic genes.^{64–66}

MRD studies are relatively expensive in comparison to other diagnostic assays, but considering their potential clinical benefits, the investment appears justified. As was eloquently argued by Goulden et al.,⁶⁰ MRD analysis should pay for itself. To widen the use of MRD, it is important to further simplify the relevant technologies. To this end, we modified flow cytometry to study MRD in patients with B-lineage ALL during remission induction therapy.⁶⁷ When applied to samples collected on day 19 of treatment, the results of the simplified assay correlated well with those of the more complex flow cytometric assay and those of PCR amplification of antigen-receptor genes. This assay is well suited for measurements of early treatment response. Because of its low costs and simplicity, it should facilitate the implementation of MRD measurements in centers with limited resources.

The identification of new markers and the further refinement of techniques should also increase applicability of MRD testing. MRD assays provide new opportunities for renewing the classical design of phase II studies, by using changes in MRD levels to rapidly identify the most effective new anti-leukemic agents. Finally, the direct probing of the biology of MRD cells and further understanding of the features that distinguish leukemic cells that do not respond to treatment from those that respond should provide clues to improve effectiveness of therapy.

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