Bridging the gap between the north and south of the world: the case of treatment response in childhood acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. It accounts for approximately 25% of all childhood cancers and almost 75% of childhood leukemias. Treatment results in childhood ALL are one of the true success stories of modern clinical oncology with an overall cure rate currently approaching more than 85% in the developed world, mainly through the application of intensive multiagent chemotherapeutic regimens.[1,2] The therapeutic intensity of these regimens is usually adjusted according to prognostic factors associated with the risk of treatment failure. Continuing research on the clinical, biological, immunological and genetic aspects of ALL has identified numerous features with prognostic potential including patient- and leukemia-associated characteristics [e.g., age at diagnosis, white blood cell count at diagnosis, the chromosomal translocations t(9;22) and t(4;11)] as well as a variety of estimates of early response to treatment.[1,2]

Measures of treatment response in childhood acute lymphoblastic leukemia

Measures of treatment response as currently applied for therapy stratification in childhood ALL can be divided into cytomorphological and molecular approaches (see Table 1 for examples). The cytomorphological evaluation of leukemic cell reduction in the peripheral blood or bone marrow at defined time points has served as a gold standard for response evaluation for decades. However, especially during the last 10 years, molecular methods have increasingly gained importance for ALL therapy stratification strategies world-wide.

The Berlin-Frankfurt-Münster (BFM) Study Group started as early as 1983 to incorporate the so-called prednisone response in their clinical trials.[3,4] Prednisone response is characterized by the peripheral blood leukemic blast count on day 8 of treatment initiation after 7 days of monotherapy with prednisone and one intrathecal dose of methotrexate on treatment day 1. Good response is defined as a peripheral blood blast count of less than 1×10^9/L while poor response is characterized by a count of 1×10^9 blasts/L or more. Instead of analyzing peripheral blood smears, investigators of the former Children's Cancer Group have utilized early response measured in the bone marrow on day 7 and day 14 of induction.[5-7] The early response to therapy in the bone marrow is rated M1, M2 or M3. M1 represents a bone marrow aspirate displaying less than 5% residual leukemic blasts and signs of recovering hematopoesis. M2 refers to a bone marrow aspirate with the presence of leukemic blasts in the range of 5% to 25%, while an M3 rating describes all bone marrow aspirates in which the percentage of leukemic blasts exceeds 25%.

Big advantages of the cytomorphological evaluation of treatment response are its wide applicability and the

Table 1. Characteristics of clinically applied measures of treatment response in childhood acute lymphoblastic leukemia treated according to BFM strategies.[a]

	Material	Favorable prognostic information and its approximate incidence (%)	Unfavorable prognostic information and its approximate incidence (%)
Morphological assessment			
Prednisone response[b] in peripheral blood	Microscopic smear	$<1\times10^9$/L blood blasts on treatment day 8 (90%)	$\geq1\times10^9$/L blood blasts on treatment day 8 (10%)
Early bone marrow response	Microscopic smear	<5% blasts (M1) on day 15 of induction treatment (60%)	>25% blasts (M3) on day 15 of induction treatment (15%)
Remission status after induction therapy in the bone marrow	Microscopic smear	<5% blasts (M1) after 4 to 5 weeks of induction treatment (98%)	≥5% blasts (M2 or M3) after 4 to 5 weeks of induction therapy (2%)
Molecular assessment			
Quantification of immunoglobulin/T-cell receptor gene rearrangements or quantification of aberrant immunophenotype by flow cytometry (bone marrow)	DNA or cells	$<10^{-4}$ blasts after 5 weeks of induction treatment (40%)	$\geq10^{-3}$ blasts after 12 weeks of treatment (induction and consolidation) (10%)

[a]Prognostic value is treatment dependent and, therefore, the selection presented in the table above cannot be entirely comprehensive; [b]after 7 days of induction with daily prednisone and a single intrathecal dose of methotrexate on treatment day 1.

low costs associated with this method which made and still make it an attractive measure also in countries facing economic restrictions. Regarding the pros and cons of using either peripheral blood or bone marrow, it may be argued that, if performed in a centralized setting, response evaluation of peripheral blood smears yields highly reproducible results, while bone marrow morphology is more susceptible to bias introduced through, for example, technical variability related to the marrow aspiration procedure. Nevertheless, the prednisone response also has limitations. One of these concerns the population of the patients with an initial leukemic blast count of less than 1×10^9/L (~ 15% of the patients). Even though the kinetics of the leukemic cell reduction in these patients cannot, arguably, be evaluated accurately, the patients' assignment to the group of prednisone good-responders (defined by blast counts < 1×10^9/L on treatment day 8) does not result in a difference in treatment results when compared to *true* prednisone good-responders.[8] In contrast, patients with very high initial blast counts and impressive leukemic cell mass reduction under prednisone (to blast counts ≥ 1×10^9/L on treatment day 8) may be subject to overtreatment.

The submicroscopic molecular assessment of treatment response in childhood ALL by analyzing minimal residual disease (MRD) is approximately 1,000 to 10,000-fold more sensitive compared to methods based on cytomorphological detection.[9-12] Such excellent levels of sensitivity can be reached mainly by employing two different techniques, the monitoring of either leukemia-specific genetic features or aberrant expression of leukemic cell surface and intracellular proteins. The monitoring of genetic features can be further divided into DNA- and RNA-based methods. DNA-based monitoring of genetic features has a sensitivity of 10^{-4} to 10^{-5} and mostly encompasses the quantification of patient and/or leukemia-specific immunoglobulin and/or T-cell receptor gene rearrangements as well as, in specific subgroups of patients, recurrent chromosomal translocation/fusion gene breakpoints (e.g., *MLL/AF4* in infant ALL). The advantages of monitoring immunoglobulin and/or T-cell receptor gene rearrangements in childhood ALL clearly lie in its patient-specificity, wide applicability - more than 90% of patients have at least one marker with a minimum sensitivity of 10^{-4} - and, due to its robustness, the feasibility of using it in cooperative multicenter clinical trials - the standard of care for childhood ALL in many countries world-wide. RNA-based monitoring mainly relies on the quantification of fusion gene transcripts (e.g., *ETV6/RUNX1*, *BCR/ABL*) generated as a consequence of recurrent chromosomal translocations [e.g., t(12;21), t(9;22)] or transcripts specifically expressed at high levels by leukemic cells (e.g., *TAL1* in T-cell ALL).[13] Sensitivity levels for this method are in the range of 10^{-4} to 10^{-6}. However, due to its reliance on fusion genes or specifically elevated transcripts, this method is not patient-specific and currently less widely applicable in childhood ALL in comparison to the DNA-based monitoring of immunoglobulin and/or T-cell receptor gene rearrangements. The molecular monitoring of aberrant expression of leukemic cell surface and intracellular proteins is based on analysis by multiparametric flow cytometry and allows the detection of leukemic cells at a sensitivity level of up to 10^{-4}.[11,12] As for the monitoring of immunoglobulin and/or T-cell receptor gene rearrangements, this method is widely applicable in childhood ALL and is particularly suited for the analysis at early time points given that the development of patient-specific DNA-based assays for monitoring immunoglobulin and/or T-cell receptor gene rearrangements takes weeks to establish. Although most of the experience on MRD in clinical settings has been gained through DNA-polymerase chain reaction (PCR)-based detection of leukemic clone-specific immunoglobulin and/or T-cell receptor gene rearrangements, it has been shown that flow cytometry-based analyses by detection of specific antigen patterns of the leukemic clone yield results comparable to those provided by DNA-PCR-based methods.[11,12,14,15]

Unfortunately, PCR- and flow cytometry-based MRD assays are not only costly but technically complex, and in a routine setting are usually conducted in highly specialized laboratories. This raises important issues regarding the comparability of MRD results between different MRD laboratories which can only be ensured by standardization and quality control measures. During the last years, the world-wide operating European Study Group on MRD detection in ALL (ESG-MRD-ALL) has developed guidelines for the interpretation of DNA-PCR-based MRD data. These guidelines ensure identical interpretation of MRD data between different laboratories of the same MRD-based clinical protocol and, at the same time, facilitate the comparison of MRD data obtained on different treatment protocols.[16] Similarly, RNA-based approaches for monitoring MRD in ALL have been subject to standardization.[17] In the case of MRD analyses by flow cytometry, comparable initiatives to standardize reagents and procedures are under way. However, most of the knowledge in the field of flow cytometry-based MRD monitoring has been acquired in single center studies with far less being known on multicenter diagnostic settings in comparison to PCR-based methods.[18] In this issue of Haematologica, Irving and colleagues add an important piece of information to this subject for precursor B cell ALL by reporting on a four-color, flow cytometric protocol established and validated by the UK ALL Flow MRD group, a network of six laboratories.[19] In this study, the applied protocol demonstrated high sensitivity and technical applicability as well as good concordance with DNA-PCR-based monitoring of immunoglobulin gene rearrangements. In addition, the protocol was shown to be highly reproducible between laboratories across different platforms. As already mentioned above, international cooperative multicenter clinical trials and their associated diagnostic laboratory networks are the standard of care for childhood ALL in many countries world-wide. This further underscores the importance of the investigation reported by Irving and colleagues and the need for additional research addressing similar questions when implementing flow cytometry-based MRD in cooperative studies on the treatment of childhood ALL.

The clinical value of assessing treatment response in childhood acute lymphoblastic leukemia

Since 1986, the BFM group has used a cytomorphological response evaluation as measured by the *in vivo* response to prednisone for stratifying patients. In their long-term experience, including more than 5000 patients with childhood ALL from 1983 to 1999, this prognostic factor has consistently been one of the strongest predictors of treatment outcome.[3,4,20] Event-free survival rates between prednisone good- and poor-responders differ significantly (roughly 80% *vs.* 35 to 55%). Regarding the cytomorphological bone marrow evaluation at treatment days 7 and 14 as described by the former Children's Cancer Group,[5-7] an M1 rating at both time points (day 7 and day 14) confers a good prognosis, while M2 and M3 ratings are associated with a poorer prognosis. The group of patients with M2 or M3 marrows on day 7 can be further separated into patients with an intermediate or poor prognosis by using the day 14 marrow score. Those with a M2 or M3 marrow on day 14 are a subset of patients with a poor prognosis. Of importance, the value of quantifying the early reduction of the leukemic cell burden by a cytomorphological evaluation of peripheral blood or bone marrow at defined time points has been confirmed by several other study groups in the context of different treatment strategies.[21-23]

However, despite their prognostic value, conventional methods of risk classification relying on cytomorphological evaluation of either peripheral blood or bone marrow at defined time points during the clinical course of childhood ALL do not appear to be sufficient for identifying the patient at true risk of relapse. Although a cytomorphologically assessed poor early response to induction therapy is highly predictive of treatment failure, the majority of recurrences in studies applying such cytomorphological measures for treatment stratification occur in the large group of ALL patients with an adequate cytomorphological response to treatment.[20] Thus, cytomorphological response evaluation fails to identify a majority of patients at high risk of relapse who might benefit from treatment intensification. In addition, cytomorphological response evaluation also fails in identifying patients with an excellent prognosis who might be eligible for treatment de-escalation. As the importance of a prognostic marker's precision, accuracy, sensitivity, and specificity cannot be overstated, the above observations made it desirable to develop more sensitive and specific markers of treatment response.

In 1991, the International BFM Study Group (I-BFM-SG) initiated a prospective study evaluating the value of MRD analysis. Patients from Austria, Germany, Italy, and the Netherlands were enrolled.[9] Treatment was based on the strategy of the ALL-BFM 90 protocol. The results of this multicenter trial showed that the individual response to treatment, as measured by MRD analysis by DNA-PCR-based detection of leukemic clone-specific immunoglobulin and/or T-cell receptor gene rearrangements, was by far the strongest predictor of outcome. In parallel with the I-BFM-SG MRD study, two similar studies were conducted at St. Jude Children's Research Hospital and by the European Organization for Research and Treatment of Cancer-Childhood Leukemia Cooperative Group.[10,11] Altogether, these studies demonstrated, for the first time, that it was possible to identify patients with a very low risk of relapse and to define patients who had a more than 70% probability of disease recurrence while on current treatment protocols. The recently published update on the I-BFM-SG MRD study demonstrated that the initially observed results for event-free survival rates remained stable at 10 years, that is, being 93% for the standard risk group (MRD-SR), 74% for the intermediate risk group (MRD-IR), and 16% for the high risk group (MRD-HR).[24]

As the molecular evaluation of leukemic cell reduction during the early phases of treatment is being increasingly used to guide therapeutic decisions in clinical trials in developed countries, there was and is growing concern that the complexity of MRD diagnostics may exclude less developed countries from this clinical progress and contribute to a further separation of the world along the poles of technical diagnostic developments in the field of leukemia. In order to circumvent such a situation, it is important to further simplify MRD technologies and thereby guarantee their implementation in larger parts of the world. In another important study on MRD in childhood ALL in this issue of the journal, Scrideli and colleagues address this issue by performing MRD analyses in 229 children with ALL treated at three different centers in Brazil.[25] MRD analysis was performed in bone marrow samples at diagnosis and on days 14 and 28 by conventional homo/heteroduplex PCR using a simplified approach with consensus primers for immunoglobulin and T-cell receptor gene rearrangements. MRD on days 14 and 28 discriminated three prognostic groups: patients negative on days 14 and 28, those positive on day 14 but negative on day 28, and patients positive on both day 14 and day 28. Five-year event-free survival rates were 85%, 75.6%, and 27.8%, respectively ($p<0.0001$). Multivariate analysis demonstrated that MRD on day 28 was the most significant prognostic factor.

Despite the deficiencies associated with the applied technique – which are well recognized by the authors – Scrideli and colleagues have to be applauded for their study employing a simplified MRD technique in a three-center setting in Brazil as they clearly show its feasibility and prognostic potential and demonstrate that MRD assessment can become reality even when financial resources are limited. This study is, therefore, an important first step on the way to implementing MRD as a stratification tool in clinical decision making in Brazil. It is of particular interest in the context of the study by Scrideli and colleagues, who through their approach primarily identified patients at a high risk of relapse, to provide information on a St. Jude Children's Research Hospital-based study making use of a panel of three monoclonal antibodies for flow cytometry-based MRD detection on treatment day 19. This assay identified a high proportion of children with precursor B-cell ALL who have an excellent early treatment response and a high likelihood of cure at relatively low costs.[26] Taken together, the above mentioned studies indicate that different MRD assessment approaches are well under way

to increasing the access to the potential benefits of evaluating MRD to a larger population of children with ALL.

Future perspectives

MRD analyses provide strong prognostic information and can be used to identify a majority of patients with childhood ALL at a high risk of relapse. With only a few exceptions (e.g., no diagnostic and/or follow-up material available, no reliable MRD marker identifiable) MRD analyses can be applied to a majority of patients. However, MRD analyses also have disadvantages as it takes several weeks to provide the clinician with the results and – with a few exceptions from flow cytometric analyses[19] – no direct insight into the mechanism of treatment response or resistance is obtained through the procedure. In addition, relying on MRD analysis complicates therapeutic changes in the early treatment phases because the specificity of response evaluation might vary with the composition of the induction regimen and the time point of response evaluation. Of interest with regard to these limitations, several recent studies demonstrate that comprehensive genetic information as provided, for example, by microarray-based genome-wide gene expression profiling can provide molecular information that identifies distinct leukemic features and allows discrimination of resistant ALL samples from sensitive ones.[27] As the analysis of altered gene regulatory pathways through gene expression profiling mostly represents an indirect measure of the genetic alterations in leukemic cells, its combination with MRD analyses and genome-wide fine mapping arrays may also be helpful for a more precise determination of clinically relevant genetic aberrations in such cells. Besides the potential relevance of MRD analysis as a *phenotype* for the molecular dissection of treatment response at the level of the leukemic cell, MRD analyses may also aid in evaluating the clinical relevance of host factors such as genetic variation in drug metabolizing enzymes. Thus, MRD analysis for *phenotypic* characterization offers the possibility of discerning, at a molecular level, clinically relevant differences that may be important for a better understanding of leukemias but also for advancing treatment strategies. MRD analysis in combination with a comprehensive evaluation of leukemia and host characteristics holds the potential to further improve treatment by leading to an even more exact and earlier characterization of the patient at true risk of relapse and at the same time to identify those patients who may be allowed to benefit from treatment reduction. Both comprehensive molecular characterization and early identification of these patients will be essential in future clinical trials in order to utilize optimal therapy in the first treatment cycles and, for those in need of it, to ensure the timely introduction of potential targeted treatment based on individual molecular characteristics of leukemic cells. Of importance, all future approaches should be evaluated in the context of *classical* risk-adapted treatment strategies and molecular monitoring of treatment response. Finally, it is hoped that all currently performed analyses, including high-throughput approaches, will lead to a condensed panel of clinically relevant information which in the end may also be applicable in patients living outside the well-developed world.

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Chronic lymphocytic leukemia microenvironment: shifting the balance from apoptosis to proliferation

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Gene expression profiling (GEP) provides a complete picture of the transcriptome, which reflects the specific activation/differentiation states of a given cell population. The advent of GEP in the field of hematology has allowed significant strides to be made in defining the molecular taxonomy of leukemias and lymphomas. Tumors that are otherwise morphologically identical can now be classified according to molecular patterns predictive of distinct clinical outcomes. Success with such applications has led to the development and implementation of diagnostic and prognostic strategies based solely on microarray data.[1]

Our current understanding of the pathogenesis, maintenance and progression of chronic lymphocytic leukemia (CLL) has been greatly enhanced by GEP data. It is well established that CLL is a heterogeneous disease: some patients experience a slowly progressive clinical course, but most will eventually enter an advanced phase requiring repeated treatment. A significant number of CLL patients exhibit an active form of the disease from the early stages, characterized by refractoriness to treatment, infectious and autoimmune complications and a relatively rapid fatal outcome.[2] One of the long-term goals of the hematologic community is to provide a molecular explanation for the marked clinical heterogeneity of CLL. A number of descriptive parameters characterizing aggressive CLL were defined in the 1980s and 1990s, but a significant breakthrough came in 1999, when two independent groups showed that patients could be placed in distinct prognostic groups according to the presence (good prognosis) or absence (poor prognosis) of somatic mutations in the immunoglobulin variable region (IgV) genes.[3,4]

The potential of GEP was immediately exploited to answer the long-standing questions concerning the origin of the CLL cell and its relationship with normal B lymphocytes. It has also been used to explore whether the clinical heterogeneity of the disease might depend on the genetic origin of the neoplastic cells. Recent studies on this topic by several groups, including Stamatopoulos and colleagues in this issue of the journal,[5] rely on GEP to classify CLL cells according to their molecular markers and to identify the corresponding genetic signatures. Overall, the results of these studies generally concur that CLL cells are unexpectedly homogeneous in terms of their genetic signature.[6,7] However,