Minimal Residual Disease in Hematological Malignancies

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Do you prefer quality to quantity? This question has been asked for many years. It is well known that mathematics is the science of pure quantity but what about medicine? For many years, counting cells and identifying them by microscopic inspection have determined the number of normal or abnormal cells in hematological and nonhematological malignancies. During the last decade, several studies have shown that detection and quantification of residual tumor cells significantly correlate with clinical outcome in several types of hematological malignancies. In particular the quantitative measurement of the decrease of the leukemic cell load during the first phases of treatment has a high prognostic value [1]. Detection of minimal residual disease (MRD) is now becoming routinely implemented in treatment protocols and is increasingly used for guiding therapy and for evaluation of new treatment modalities [2].

Methods to detect MRD include technologies designed to detect residual malignant cells beyond the sensitivity of conventional approaches like for example morphology and banding cytogenetics in leukemia. A wide variety of techniques have been developed. The choice of the best method for the particular clinical situation certainly depends on the biology of the individual malignancy, i.e. on the determination of specific markers, which are useful to differentiate between leukemic cells and normal hematopoiesis in leukemic patients. These markers include leukocyte differentiation antigens, fusion transcripts, transcripts overexpressed by mutated or nonmutated genes,

rearranged genes, and individual markers, like polymorphic repetitive DNA sequences.

In this issue we sought to provide a comprehensive overview on the major technologies for the detection of MRD and their clinical applications. Campana and Cous-tan-Smith critically discuss advantages and disadvantages of flow cytometry methods in acute leukemias. A special direction of MRD detection is the differentiation between donor and recipient hematopoiesis after allogeneic stem cell transplantation as discussed by Thiede et al. The authors also reviewed the technical issues, advantages and limitations of the methods currently used for chimerism analysis. Adding the morphological analysis of small populations of cells to malignant or recipient-associated markers may improve the accuracy of chimerism and MRD testing, and delineate their clinical significance. Trakhtenbrot et al. introduce the simultaneous analysis of morphology, immunophenotyping and FISH on the same cell, i.e. a multiparametric scanning system. For the comparability of results, an agreement should be reached between various laboratories and different multicenter studies on sample source and volumes obtained (or minimum cell counts) for RT-PCR analyses and regarding the general methods of cell purification, RNA extraction and cDNA synthesis. Rigorous, internationally accepted controls need to be implemented. Müller et al. investigated the impact of preanalytical factors and their standardization. Their review introduces some important considerations for the implementation of RT-PCR-based MRD

endpoints into clinical trials. Specific aspects of childhood acute lymphoblastic leukemia (ALL) are elucidated by Izraeli and Waldman. Fusion transcripts or overexpressed genes, like the Wilms tumor gene WT1, represent a common target for MRD analysis in acute myelogenous leukemia (AML). Current standards and their clinical implications are demonstrated by Raanani and Ben-Bassat as well as the dilemmas and unresolved issues in the interpretation of MRD in AML patients. Recently, the genomic structure of acute promyelocytic leukemia (APL) has been described. Structural factors for the pathogenesis of APL and their implications for MRD monitoring are reviewed by Reiter et al. FLT3 length mutations in AML are considered as prognostic markers as well as markers for MRD studies and are also targets for novel treatment modalities. The current view in this respect is summarized by Schnittger et al. Reduced intensity stem cell transplantation techniques are based on individualized additional immunotherapy depending on exact and rapid MRD monitoring. Shimoni and Nagler outline clinical and methodological aspects of MRD in nonmyeloablative transplantations. MRD monitoring in multiple myeloma is part of purging studies of stem cell aphaeresis and evaluation of novel therapies for this disease. The clinical considerations and molecular data are summarized by Rasmussen et al. The clinical need for molecular endpoints has become even more apparent with the introduction of imatinib in the therapy of chronic myelogenous leukemia (CML) and BCR-ABL-positive ALL. A systematic quantitative PCR monitoring emphasized the prognostic impact of residual BCR-ABL transcripts in CML patients [3, 4]. Paschka et al. discuss the clinical applications and standardized approaches for treatment surveillance of CML patients in the imatinib era. The majority of malignant lymphoproliferative disorders display clonal rearrangement of the antigen receptor genes suitable for detection by PCR. According to Brüggemann et al. MRD-based intensification, modification of treatment and guidance of maintenance treatments should be the goal of current clinical studies to determine the prognostic significance of MRD in chronic lymphocytic leukemia and non-Hodgkin's lymphomas. The WT1 gene is considered a new universal marker for MRD detection and quantification in myeloid malignancies and myelodysplastic syndromes. Cilloni and Saglio review molecular studies using WT1 for the follow-up of these patients.

Currently, PCR-based methods represent the most widely accepted technologies for MRD detection. Over the past 15 years, PCR techniques and implications have been optimized and improved. In view of the limited val-

ue of qualitative PCR for treatment surveillance, quantitative PCR assays were developed. Competitive RT-PCR was employed to monitor CML patients after stem cell transplantation [5, 6] or treatment with interferon alpha [7], and patients with acute leukemias harboring specific fusion transcripts (CBF β -MYH11 [8]; AML1-ETO [9, 10]). Despite considerable specificity and sensitivity, these methods were cumbersome and time-consuming and were therefore only employed in specialized laboratories.

With the introduction of real-time PCR methodologies and machines quantification of residual disease has been simplified. Results of real-time PCR were readily comparable with competitive PCR data [11, 12]. Thus, quantification of residual disease has been developed as a major diagnostic tool in most studies focusing on treatment optimization in leukemias characterized by fusion transcripts. A variety of real-time PCR instruments are available and different approaches can be applied. To reach reproducible, sensitive and standardized quantitative PCR data, important prerequisites should be considered: (1) The sensitivity that can be obtained in RT-PCR analyses depends on the number of cells and the total amount of RNA analyzed and on the use of a single or nested PCR approach. (2) Unstabilized anticoagulated blood can be processed even in multicenter trials, when processing is guaranteed within 36 h. As an alternative, bedside RNA stabilization could be made available for multicenter studies with central analysis [13]. (3) The level of target gene transcripts per volume cDNA should be related to the expression of a standard gene.

However, the heterogeneity of the preanalytical environment of PCR machines and methods, and of the methods to calculate the final results causes confusion among patients, treating physicians and laboratories. The universal acceptance of real-time PCR urgently demands standardization of nomenclature and technologies. At present, at least seven different systems are commercially available using three different methods of fluorescence labeling [2]. However, adjustment of the protocols, standardization of preanalytical considerations and of the methods to calculate the final transcript ratio should result in comparable data.

In an attempt to reach an agreement on the minimal requirements for standardization of real-time PCR analyses in leukemias, new international studies have recently been launched. A significant step forward in this direction has been achieved by the recent creation of the European LeukemiaNet. A particular work package within this network has been assigned to MRD analysis in leukemias.

Standardized PCR protocols will serve as a methodological reference [14, 15] and the progress in the development of PCR machines, software and biochemical assays will certainly lead to further improvement.

Methodological aspects and clinical applications of MRD monitoring and standardization strategies are the

major topics of this issue – ‘from bench to bedside’. We tried to give a comprehensive overview on the significance of MRD in the evaluation, treatment and follow-up of hematological malignancies. We are most grateful to our international colleagues for their major contributions to the subject.

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