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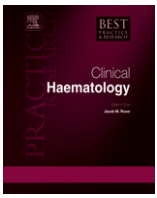

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Detection of fusion genes at the protein level in leukemia patients via the flow cytometric immunobead assay

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Nowadays, the presence of specific genetic aberrations is progressively used for classification and treatment stratification, because acute leukemias with the same oncogenetic aberration generally form a clinically and diagnostically homogenous disease entity with comparable prognosis. Many oncogenetic aberrations in acute leukemias result in a fusion gene, which is transcribed into fusion transcripts and translated into fusion proteins, which are assumed to play a critical role in the oncogenetic process. Fusion gene aberrations are detected by karyotyping, FISH, or RT-PCR analysis. However, these molecular genetic techniques are laborious and time consuming, which is in contrast to flow cytometric techniques. Therefore we developed a flow cytometric

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immunobead assay for detection of fusion proteins in lysates of leukemia cell samples by use of a bead-bound catching antibody against one side of the fusion protein and fluorochrome-conjugated detection antibody. So far, we have been able to design such fusion protein immunobead assays for BCR-ABL, PML-RARA, TEL-AML1, E2A-PBX1, MLL-AF4, AML1-ETO and CBFB-MYH11. The immunobead assay for detection of fusion proteins can be performed within 3 to 4 hours in a routine diagnostic setting, without the need of special equipment other than a flow cytometer. The novel immunobead assay will enable fast and easy classification of acute leukemia patients that express fusion proteins. Such patients can be included at an early stage in the right treatment protocols, much faster than by use of current molecular techniques. The immunobead assay can be run in parallel to routine immunophenotyping and is particularly attractive for clinical settings without direct access to molecular diagnostics.

Introduction

Nowadays, the diagnosis and classification of hematological malignancies is no longer based only on cytological and histomorphological characteristics or the immunophenotype of the malignant cells. Progressively, also the presence of specific genetic aberrations is used for classification and treatment stratification, because malignancies with the same oncogenetic aberration generally form a clinically and diagnostically homogeneous disease entity with comparable prognosis [1–3].

In leukemias, many chromosome aberrations result in an aberrant fusion gene, caused by the incorrect coupling of the upstream part of one gene to the downstream part of another gene and vice versa (reciprocal fusion gene). In most leukemias, such fusion genes are transcribed into fusion transcripts, which are translated into fusion proteins which (at least in part) have been shown to play a critical role in the pathogenesis of the leukemias. The first chromosome aberration discovered in leukemia patients was the so-called Philadelphia (Ph) chromosome, which was shown to result from the (9;22) translocation, in which the *BCR* gene is aberrantly coupled to the *ABL1* gene [4,5]. The t(9;22) is found in virtually all patients with chronic myeloid leukemia (CML) and in a fraction of the patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL), particularly in adults [6].

In BCP-ALL, also other fusion genes have been discovered such as t(1;19) with the *E2A-PBX1* fusion gene, t(4;11) with the *MLL-AF4* fusion gene, and t(12;21) with the *TEL-AML1* fusion gene. In patients with acute myeloid leukemia (AML), several types of fusion gene aberrations have been found; the most frequently occurring aberrations are t(8;21) with the *AML1-ETO* gene and inv(16) or t(16;16) with the *CBFB-MYH11* gene [2,7]. The t(15;17) with the *PML-RARA* fusion gene is found in virtually all (>95%) patients with acute promyelocytic leukemia (APL) [8].

The occurrence and relative frequency of fusion gene aberrations not only depends on the type of leukemia, but also on the age of the patient. Particularly in BCP-ALL, large differences in relative frequencies are found between different age groups (Fig. 1) [9]. For example, *MLL* fusion genes, such as *MLL-AF4*, occur at high frequency in infant ALL (~80%), whereas the *BCR-ABL* fusion gene is rare at young age (<2%) and its frequency rapidly increases with age (Fig. 1) [9].

Most chromosome aberrations have prognostic value for treatment outcome. *TEL-AML1* in childhood BCP-ALL and *AML1-ETO* or *CBFB-MYH11* in AML are associated with a good prognosis. In contrast, most *MLL* gene aberrations as well as the *BCR-ABL1* fusion gene in BCP-ALL are associated with poor prognosis, and the afflicted patients are being treated on high-risk protocols [2,9].

When it became clear that the fusion gene products contribute to the malignant transformation process, several pharmaceutical research programs focused on the development of drugs to block the fusion proteins in their function. For example, the BCR-ABL fusion protein induces increased signaling activation via the ABL tyrosine kinase domain, which appears to result in higher proliferation and