

## **Abstract**

Acute leukemias (AL) comprise a heterogeneous group of hematologic malignancies, and individual patient responses to treatment can be difficult to predict. Monitoring of minimal residual disease (MRD) is thus very important and holds great potential for improving treatment strategies. Common MRD targets include immunoglobulin heavy chain or T-cell receptor gene rearrangements, recurrent cytogenetic abnormalities and mutations in important hematological genes. Whereas in the majority of adult acute lymphoblastic leukemia patients a suitable MRD target can be identified, in adult acute myeloid leukemia patients well-characterized targets are found in only half of cases. The identification of new specific molecular markers of leukemic blasts for MRD assessment, particularly in AML patients, is therefore highly desirable.

Our aim was to develop a flexible strategy for mapping of cytogenetically identified unique clone-specific abnormalities down to the single nucleotide level and, based on the sequence, design a specific real-time PCR assay for MRD assessment in AL patients without any previously described MRD marker.

Using a combination of cytogenetic (chromosome banding, chromosome microdissection), molecular cytogenetic (mFISH, mBAND) and molecular biological (next-generation sequencing, long-range PCR, Sanger sequencing, real-time PCR) techniques we were able to characterize the DNA sequence flanking unique chromosomal breakpoints. Finally, we designed a specific real-time PCR assays for sensitive MRD monitoring in AL patients.

With the use of above mentioned techniques, we mapped derivative chromosomes in 5 AL patients and performed real-time PCR quantification of unique MRD markers for each patient. A comparison of these newly-designed assays to a standard assay used in clinical practice shows that our technical approach is suitable for the identification of novel molecular MRD markers in AL patients.

Our work shows that rapidly moving from the chromosomal level to the nucleotide level is feasible, opening new vistas in the characterization of unique chromosomal breakpoints as well as providing new MRD markers for eligible AL patients.

**Keywords:** Acute leukemia, chromosome microdissection, molecular marker, minimal residual disease, personalized medicine