INTRODUCTION

Minimal Residual Disease (MRD) tests are important to determine prognosis and response to therapy in a.o. acute and chronic leukemia, lymphoma and myeloma patients. The most important techniques for MRD testing are flow cytometry and quantitative PCR (Q-PCR). 1-3 Whereas flow cytometry is based on aberrant immunophenotypes, the Q-PCR tests are generally based on clonal immunoglobulin (IG) or T-cell receptor (TR) gene rearrangements for lymphoid malignancies and fusion gene transcripts (RNA fusion sequences) for myeloid malignancies. 4

Although IG/TR gene rearrangements can be detected in the vast majority of patients with a lymphoid malignancy, in some patient no IG/TR rearrangements can be found or the resulting Q-PCR assays do not reach the aimed sensitivity (generally 10^-4). Furthermore, IG/TR rearrangements are not related to the malignant transformation and therefore may get lost in the course of the disease due to novel subclones that emerge and carry alternative secondary rearrangements. 5 Q-PCR tests based on the most frequent clonal sequences at diagnosis could therefore provide inaccurate information about disease load during long-term follow-up.

RNA based tests are known to be variable due to the instability of RNA and inherent variation in gene-expression levels. 6, 7

GENOMIC GENE FUSION BREAKPOINT MRD TESTING

An alternative to both IG/TR rearrangements and RNA fusions are genomic fusion/deletion breakpoint sequences. 8 These promise to present a number of advantages:

- They can be applied on patients that do not have suitable IG/TR rearrangements.
- Driving gene fusion sequences are very likely to occur in all leukemic/malignant lymphoid cells and therefore promise more reliable detection and quantification.
- Genomic breakpoint sequences (DNA level) enable more robust detection and quantification of leukemic/lymphoid cells than RNA based tests.
- They are not present in normal hematopoietic cells and therefore might generally show reduced background signals.

TLA TECHNOLOGY FOR TARGETED COMPLETE GENE & GENE FUSION SEQUENCING

The TLA Technology 9 uniquely enables the targeted complete Next Generation Sequencing of genes of interest and enables the detection of all Single Nucleotide Variants, rearrangements including deletions and gene fusions in leukemia and lymphoma. TLA thus empowers the sequencing of gene fusions and deletion breakpoint sequences at the single nucleotide level.

To further validate gene fusion breakpoints as target for Q-PCR based MRD detection, we have applied TLA retrospectively on acute lymphoblastic leukemia (ALL) and Mantle Cell Lymphoma (MCL) patient samples, identified gene fusion breakpoint sequences, and performed gene fusion breakpoint specific Q-PCR’s with newly designed patient specific primer/probes.

Generated data was compared to previously generated IG/TR rearrangement specific Q-PCR’s.
Using TLA, a TCF3-PBX1 gene fusion was identified in a pediatric ALL patient. Based on the gene fusion breakpoint sequence, a patient-specific Q-PCR was designed and reached a quantitative range of $10^{-4}$ and a sensitivity of $10^{-4.5}$. Three follow-up samples were analysed for MRD using the TCF3-PBX1 gene fusion; the obtained results showed a highly comparable pattern as compared to MRD data obtained by IG/TR analysis (Figure 1).

### Erasmus MC

**Pediatric ALL**

**TCF3-PBX1 Gene Fusion**

![Figure 1](image1.png)

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**Mantle Cell Lymphoma**

**BCL1/IGH Translocation**

TLA Technology was used to identify the BCL1/IGH translocation in a Mantle Cell Lymphoma (MCL) patient not displaying the common MTC (“Major Translocation Cluster”) breakpoint. Allele specific oligoprobes (ASO) and a consensus probe were designed on BCL1/IGH sequence obtained from TLA experiments and QPCR was set to monitor Minimal Residual Disease (MRD). ASO-QPCR, performed on three follow-up samples, reached a quantitative range and a sensitivity of $10^{-5}$ and showed the same comparable trend as IGH rearrangement based MRD analysis (Figure 2).

![Figure 2](image2.png)
Using TLA, a CRLF2 deletion junction was identified in a pediatric ALL patient. Based on the junction breakpoint sequence, a patient-specific Q-PCR was designed that reached a quantitative range and a sensitivity of 10^{-4}. Six follow-up samples were analysed for MRD using the CRLF2 deletion breakpoint; the obtained results were highly comparable to MRD values obtained by IG/TR analysis (Figure 3).

**References**

5. van der Velden VH, van Dongen JJ. MRD detection in acute lymphoblastic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. Methods Mol Biol 2009; 538:115-150.

**CONCLUSIONS**

Gene fusion breakpoint sequences identified with TLA prove to be a good basis for Q-PCR based MRD detection.

In a number of our patients the newly identified gene fusion breakpoint sequences have, in absence of a suitable IG/TR rearrangement sequence, made MRD detection possible.

In addition we are, in larger follow-up analyses, currently comparing the performance of IG/TR-based MRD tests with gene fusion breakpoint MRD tests.