Application Note

Targeted Locus Amplification Technology

TLA-based targeted NGS sequencing & genomic gene fusion breakpoint sequence specific MRD testing in leukaemia & lymphoma

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Summary

- Minimal residual disease (MRD) tests are important to determine prognosis and response to therapy in, among others, acute and chronic leukaemia, lymphoma and myeloma patients.
- TLA empowers the sequencing of gene fusions and deletion breakpoint sequences at the single nucleotide level.
- We have applied TLA retrospectively on acute lymphoblastic leukaemia (ALL) and mantle cell lymphoma (MCL) patient samples, identified gene fusion breakpoint sequences, and performed gene fusion breakpoint specific qPCRs with newly designed patient specific primer/probes.
- Gene fusion breakpoint sequences identified with TLA prove to be a good basis for qPCR-based MRD detection.

Introduction

Minimal residual disease (MRD) tests are important to determine prognosis and response to therapy in, among others, acute and chronic leukaemia, lymphoma and myeloma patients. The most important techniques for MRD testing are flow cytometry and quantitative PCR (qPCR).1-3

Whereas flow cytometry is based on aberrant immunophenotypes, the qPCR 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 qPCR 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 sub clones that emerge and carry alternative secondary rearrangements.5 qPCR 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.5,7

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 leukaemic/malignant lymphoid cells and therefore promise more reliable detection and quantification.
- Genomic breakpoint sequences (DNA level) enable more robust detection and quantification of leukaemic/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 and gene fusion sequencing

The TLA technology9 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 leukaemia 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 qPCR-based MRD detection, we have applied TLA retrospectively on acute lymphoblastic leukaemia (ALL) and mantle cell lymphoma (MCL) patient samples, identified gene fusion breakpoint sequences, and performed gene fusion breakpoint specific qPCRs with newly designed patient specific primer/probes.

Generated data was compared to previously generated IG/TR rearrangement specific qPCRs.
Erasmus MC: Pediatric ALL - TCF3-PBX1 Gene Fusion

Using TLA, a TCF3-PBX1 gene fusion was identified in a pediatric ALL patient. Based on the gene fusion breakpoint sequence, a patient-specific qPCR was designed and reached a quantitative range of $10^{-4}$ and a sensitivity of $10^{-4}$. 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).

Figure 1: An ALL patient was monitored using two IGH rearrangements (red and blue) as well as with the TCF3-PBX1 gene fusion (green). The quantitative ranges were $10^{-4}$, $10^{-4}$, and $10^{-4}$, respectively, whereas the sensitivities were $10^{-5}$, $10^{-5}$, and $10^{-4}$, respectively. The IGH rearrangements and TCF3-PBX1 gene fusion gave highly comparable MRD data.

University of Torino: 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 major translocation cluster (MTC) breakpoint. Allele specific oligoprimers (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: MRD was monitored in a MCL patient using IGH rearrangement (blue) and BCL1/IGH translocation (green). For both markers, the quantitative ranges were $5\times10^{-5}$ and the sensitivities were $10^{-5}$ and $5\times10^{-5}$, respectively. The results showed that the BCL1/IGH sequence obtained by TLA was as useful in MRD monitoring as the IGH rearrangement detected using classic sequencing techniques. Moreover, in the FU1 sample the tumour burden quantification by TLA BCL1/IGH was even higher than the IGH results.

University Children's Hospital Zurich: Pediatric ALL - CRLF2 Deletion

Using TLA, a CRLF2 deletion junction was identified in a pediatric ALL patient. Based on the junction breakpoint sequence, a patient-specific qPCR 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).

Figure 3: An ALL patient was monitored using an IGH rearrangement (blue) and a TRD (red) rearrangement as well as the junction region of a CRLF2 deletion (green). The quantitative ranges were $5\times10^{-4}$ (TCRD) and $1\times10^{-4}$ (IGH and CRLF2), whereas the sensitivities were $10^{-4}$ (CRLF2) and $10^{-5}$ (TRD and IGH). The IGH and TRD rearrangements and CRLF2 deletion junction gave highly comparable MRD data. Relapse refers to an extramedullary relapse.
Conclusion

Gene fusion breakpoint sequences identified with TLA prove to be a good basis for qPCR-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.

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.


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