Targeted Locus Amplification (TLA): A Novel Next Generation Sequencing (NGS) Technology to Detect New Molecular Markers and Monitoring Minimal Residual Disease (MRD) in Mantle Cell and Follicular Lymphoma

KEYWORDS:
NGS, Minimal residual disease, t(11;14), t(14;18)

INTRODUCTION AND AIMS
Minimal residual disease (MRD) monitoring by PCR methods is a strong and standardized predictor of clinical outcome in mantle cell (MCL) and follicular (FL) lymphoma\textsuperscript{1-2}. However, some technical limitations hamper its feasibility in daily clinical routine. First of all, current techniques allow the identification of a molecular marker only in 75-80\% of MCL and 55-60\% of FL patients. All the other “no marker” cases cannot be studied for MRD purposes. Next generation sequencing (NGS) tools might overcome these limitations. Among them, the recently developed targeted locus amplification (TLA) technology\textsuperscript{3} that selectively amplifies and sequences entire genes on the basis of the crosslinking of physically proximal DNA loci, was able to identify novel candidate oncogenes and gene fusions in acute lymphoblastic leukemia\textsuperscript{4}.

The aim of this study was to test the TLA approach in MCL and FL diagnostic samples, with at least one translocation confirmed by FISH or a failure in molecular marker identification, with the aim to increase the rate of success in marker screening for MRD purposes. Moreover, the performances of the newly identified molecular markers were compared to the standardized IGH quantitative PCR (qPCR) approach for MRD monitoring.

Methods: Genomic DNA was extracted from bone marrow (BM) and peripheral blood (PB) samples of MCL and FL patients, enrolled in prospective clinical trials of the Fondazione Italiana Linfomi (FIL). All patients provided written informed consent for the research use of the biological samples. Libraries for TLA (Cergentis, Utrecht) were prepared using only one couple of antisense primers targeting the immunoglobulin heavy chain (IGH) enhancer locus, as previously described\textsuperscript{5} and sequenced on MiSeq platform (Illumina, San Diego). MRD monitoring was carried out by qPCR designing allele specific oligonucleotides (ASO) and consensus probes based on the new breakpoint sequences obtained from TLA NGS. Finally, the efficiency of TLA novel markers to track MRD was compared to MRD data obtained from clonal IGH rearrangements previously detected and tracked by standardized ASO qPCR approaches, following the Euro MRD guidelines\textsuperscript{6}.

Results: TLA was firstly tested on 21 t(11;14)-positive, BCL-1/IGH major translocation cluster (MTC) negative MCL baseline samples (8 BM and 13 PB): in 86\% of the cases (18/21) a novel BCL-1/IGH breakpoint was identified by NGS. In these cases, TLA described new rearrangements between “no-MTC” BCL1 loci and JH1, JH4, JH5 or JH6 regions on chromosome 14. Therefore, additional 8 FL baseline BM samples, resulting t(14;18) FISH positive and BCL-2/IGH MBR/mcr/“minor rearrangements”-negative by PCR, were tested obtaining a 62\% (5 out of 8 samples) success rate. Also in these cases, new and not yet described BCL2/IGH rearrangements, were found. Additionally, to assess the specificity of the technology, the MBR-positive control sample (DOHH2 cell line) was tested and correctly sequenced, as well. Interestingly among the new TLA positive FL cases, in one sample, the NGS technology highlighted the breakpoint on the DH region of chromosome 14, describing an unusual t(14;18) translocation. Otherwise, TLA failed in 5 out of 29 (17\%) mainly characterized by a low tumor infiltration, estimated <5\% by flow cytometry (4.4\%, 0.03-8\% range). Secondly, NGS sequences validation was performed by ASO primers design on the new TLA breakpoints and MRD was monitored by ASO qPCR.
Overall, in the 14 MCL cases where also a classical, IGH – based marker was available the MRD results were superimposable between the two markers (Fig. 1A), with good and overlapping performances in terms of overall correlation ($r^2=0.86$; Fig 1B). In FL samples, ASO qPCR confirmed the high tumor burden of the analyzed tissues (Fig 1C). Finally, an extension of the analyzed samples series and MRD validation is currently ongoing among additional FL patients.

**Conclusions:** The TLA NGS technology allowed to identify a translocation-based, novel molecular marker in FISH positive t(11;14) and t(14;18) MCL and FL baseline samples, where the classical Sanger sequencing failed a marker identification. These new breakpoints can reliably be used for the design of ASO qPCR primers for MRD detection in previously not traceable patients.


