**Chronic Lymphocytic Leukemia**

**Detection of risk-identifying chromosomal abnormalities and genomic profiling by multiplex ligation-dependent probe amplification in chronic lymphocytic leukemia**

We performed genomic profiling using multiplex ligation-dependent probe amplification (MLPA) in 54 cases with suspected or advanced chronic lymphocytic leukemia (CLL). MLPA detected abnormalities when the percentage of mutated cells was greater than ~35%. Loss of 9p21 CDKN2A/B was revealed. MLPA is an economically attractive, powerful tool in trial-based, centralized risk-assessment for CLL.

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Chronic lymphocytic leukemia (CLL) is a common leukemia in adults. Interphase fluorescence in situ hybridization (FISH) has identified ΔATM(11q22) and ΔTP53(17p13) and +12 as adverse prognostic indicators. Blood samples of 54 patients with suspected CLL or advanced disease were referred for karyotyping and standard FISH testing. Here we describe our experience using multiplex ligation-dependent probe amplification (MLPA) for polymerase chain reaction (PCR)-based relative quantification of DNA to (i) detect risk-identifying chromosomal abnormalities; (ii) investigate potential intragenic deletions undetected by FISH; and (iii) identify additional numerical abnormalities. We used the SALSAP006 kit containing probes for 40 tumor-associated genes, including ATM, CCND2(12p13), BCL7A(12q24), RB1(13q14) and TP53(17p13) and +12 as adverse prognostic indicators. Loss of 9p21 CDKN2A/B was revealed. MLPA is an economically attractive, powerful tool in trial-based, centralized risk-assessment for CLL.

![Figure 1. MLPA demonstrating copy number changes in CLL patient. MLPA results indicating gain of RCN of MET (7q31.2), CCND2 (12p13.3) and BCL7A (12q24.3), MESDC1 (15q13), loss of TP53 (17p13.1), (two probes) and amplification of PMAIP1 and BCL2 (18q21.3), but not DCC (18q21.1) in a female patient (UPN 33). Probes targeted the genes TP53, SIM2, RAD17, FGF3, AR, MET, DNMT1, PMAIP1, KIAA0170, HIPK3, CCND1, CDKN2B, RB1, PPEF1, BAK1, ATM, BCL2, CDKN2D, BRCAl, NFkB1, CCND1, IL12A, AXIN2, TNF, SRY, MLH1, TP53, CCND2, PTPN1, CASP6, VEGF, KLK3, MESDC1, ERBB2, ABCG2, ERBB4, DCC, IER3, CTNNB1, BCL7A and EHF.](http://www.haematologica.org/journal/2006/10/1434.html)
number of 40 loci for 16 autosomes and two sex chromosomes. In UPN33 MLPA showed gain of MET(7q31.2), CCND2 and BCL7A and MESDC1(15q13) and ΔT53 resembling cytogenetics (Figure 1 and Table). MLPA discovered a marked gain of RCN for PMAIP1 and BCL2(18q21), but not of DCC, located ~7.5 Mb proximally. FISH with probe LSI1gH/BCL2 confirmed the amplification of BCL2. In UPN50 MLPA revealed gain of RCN for 18q21DCC, PMAIP1 and BCL2. FISH showed +18 and +18,t(14;18) positive cells (probes LSI1gHBCL2 and CEP18). In UPN10 MLPA exposed, beside gain of RCN of chromosome 12 CCND2 and BCL7A, gain of RCN of DNMT1 and CDKN2D (19p13.2) and KLK3 (19q13.4), suggesting a recurrent +19 in CLL, which was confirmed by FISH.5

In UPN23/25 with advanced disease, MLPA showed loss of RCN of 9p21 CDKN2B (Figure 2). FISH with LSI16p(9p21) confirmed a hemizygous deletion of >190 kb, encompassing the CDKN2A gene. The tumor suppressor genes CDKN2A/B encode the cyclin-dependent kinase inhibitors p15/16 and p14/15.6 Loss of CDKN2A/B was detected by MLPA in head and neck squamous cell carcinoma.7 Deletions of CDKN2A/B and promoter hypermethylation were demonstrated in CLL.8 Further investigations are warranted in order to determine whether ΔCDKN2A/B is a potential risk-identifier or an indicator of disease progression in CLL as it is in B-cell lymphomas.9

We demonstrated that MLPA is a new powerful technology for identifying copy number changes as risk-identifiers and new recurrent markers in CLL. Without pre-enrichment of malignant B-cells MLPA, like array CGH, is more restricted than FISH by the percentage of the leukemic clone carrying the aberration. Although not observed in our series, MLPA with more gene-specific probes may allow identification of intragenic aberrations that could go undetected using interphase FISH, or avoid targeting single nucleotide polymorphisms interfering with MLPA data interpretation. Most importantly, many samples can be assayed simultaneously in a multi-well PCR format with automated data analysis at approximately one-fifth of the cost per sample of interphase FISH. Therefore, MLPA is an attractive technology in trial-based, centralized risk-assessment.

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