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 *CDNK2A/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.[1] 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)[2] for polymerase chain reaction (PCR)-based relative quantification of DNA to (i) detect risk-identifying aberrations; (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 (*see www.mrc-holland.com/mlpa/info/P006DNA-2 for probe description and methodology*). Loss-or-gain of targets was normalized using MLPA on DNA from blood donors.[3] Cut-off levels for loss or gain of relative copy numbers (RCN) were set at 0.8 and 1.2, respectively. Data obtained by MLPA were compared with cytogenetics and standard FISH (*Table 1, online version only*).

RCN by FISH and MLPA for risk-identifiers and additional abnormalities are shown in the Table 1 (*online version only*). Loss of RCN for ATM was confirmed in all six cases. A +12 was confirmed in 7/11 cases showing gain of RCN for *CCND2* and *BCL7A* (Figure 1). Two cases with RCN of 0.97 to 1.15 reflected the limitation of MLPA at detecting +12 in 20-40% of cells. Similarly, MLPA demonstrated loss of RCN of RB1 in 11/15 cases with *ΔRB1* or *ΔΔRB1*. In four cases MLPA showed RCN of 0.96 to 0.82 in samples with less than 32% of the cells with ΔRB1. Lacking a D13S319 specific probe, this set is less suitable for validating 13q14.3 abnormalities. MLPA confirmed *ΔTP53* in two samples (Figure 1). In two cases in which MLPA was not informative, the percentages of cells with *ΔTP53* were shown to be 13% and 20%. Thus, given the limits of this series with few samples with a FISH aberration in the 30-55% range, MLPA positively identified n/3n copy number changes when the percentage of mutated cells was 54% (*ΔATM*), 40% (*ΔRB1*), 54% (+12) and 56% (*ΔTP53*). A similar restriction was observed for MLPA detection of loss of 9p21 CDKN2A/ARF and CDKN2B in bladder cancer in which homozygous deletions could be detected in the presence of up to 30% of normal DNA.[4] By matrix-based comparative genomic hybridization (CGH) aberrations could be detected in CLL samples with a clonal abnormality in >53% of the cells.[5] When comparing the measured RCN by MLPA with the RCN determined by FISH the overall mean difference in RCN was 0.03±0.05 for for *ΔATM* (n=6), 0.07±0.09 for +12 (n=9), 0.03±0.07 for *ΔRB1* (n=15) and 0.03±0.06 for *ΔTP53* (n=4), demonstrating the power of MLPA for quantifying RCN. MPLA revealed additional numerical aberrations, even with the limited

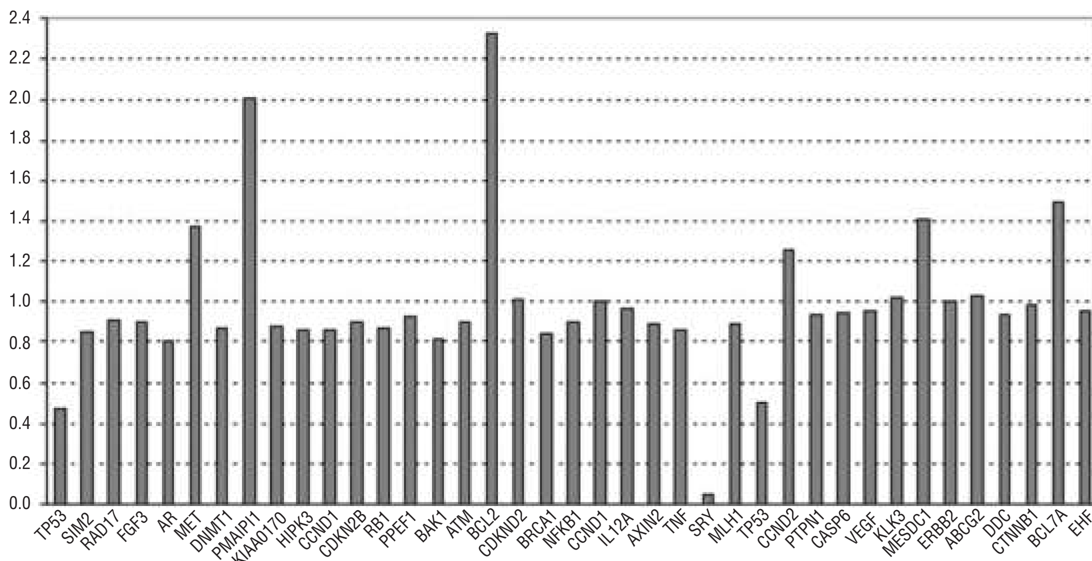

Figure 1. MLPA demonstrating copy number changes in CLL patient. MLPA results indicating gain of RCN of *MET* (7q31.2), CCND2 (12p13.3) and *BCL7A* (12q24.3), *MESDC1* (15q13), loss of *TP53* (17p13.1)(two probes) and amplification of PMAIP1 and BCL2 (18q21.3), but not DCC (18q21.1) in a female patient (UPN 33). Probes targeted the genes *TP53, SIM2, RAD17, FGF3, AR, MET, DNMT1, PMAIP1, KIAA0170, HIPK3, CCND1, CDKN2B, RB1, PPEF1, BAK1, ATM, BCL2, CDKN2D, BRCA1, NFKB1, CCND1, IL12A, AXIN2, TNF, SRY, MLH1, TP53, CCND2, PTPN1, CASP6, VEGF, KLK3, MESDC1, ERBB2, ABCG2, ERBB4, DCC, IER3, CTNNB1, BC7A* and *EHF*.

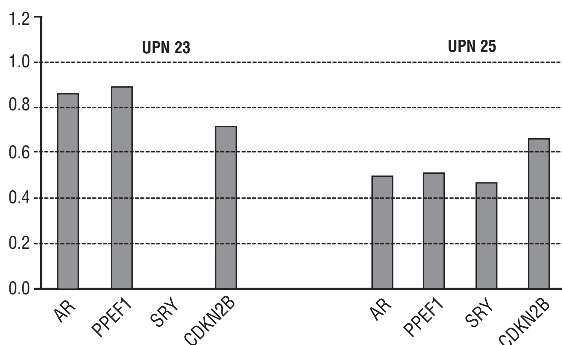

Figure 2. Recurrent loss of 9p21 *CDKN2A/B* locus detected by MLPA in two patients with CLL. Loss of RCN for chromosome 9p21 specific target *CDKN2B* (9p21) detected by MLPA in one female patient (UPN 23) and one male patient (UPN 25). The figure shows relevant targeted genes. Probes specific for *AR* and *PPEF1* (X-chromosome), and *SRY* (Y-chromosome) are shown.

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 *ΔTP53* resembling cytogenetics (Figure 1 and Table). MLPA discovered a marked gain of RCN of *PMAIP1* and *BCL2*(18q21), but not of DCC, located ~7.5 Mb proximally. FISH with probe LSIIgH/BCL2 confirmed the amplification of BCL2. In UPN50 MLPA revealed gain of RCN for 18q21*DCC*, *PMAIP1* and *BCL2*. FISH showed +18 and +18,t(14;18) positive cells (probes LSIIgHBCL2 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 LSIp16(9p21) confirmed a hemizygous deletion of >190 kb, encompassing the *CDKN2A* gene. The tumor suppressor genes *CDKN2A/B* encode the cyclin-dependent kinase inhibitors $p15^{INK4b}$ and $p16^{INK4a}/p14^{ARF}$.[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.[8,9] 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.[10]

We demonstrated that MPLA 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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