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Multiplex ligation-dependent probe amplification (MLPA): Utility as a first line screening tool for detection of clinically relevant genomic aberrations in CLL

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INTRODUCTION

- In Chronic lymphocytic leukemia (CLL), a strong correlation has been established between the presence of specific genetic abnormalities and the clinical outcome
  - Del (17p) and del(11q) are associated with reduced survival
  - Trisomy 12 (+12) and normal karyotype have intermediate prognosis
  - Del (13q) as a single aberration has favorable prognosis

  [Dohner et al, 2000; Reddy, 2006; Seiler et al, 2006]
- Current diagnostic screening strategies based on karyotyping or FISH for detection of chromosomal abnormalities are laborious, time-consuming, costly and have limited resolution
- An alternative to these techniques is Multiplex ligation-dependent probe amplification (MLPA) which is rapid, allows simultaneous processing of multiple samples and simultaneous detection of copy number changes of multiple loci in one simple PCR reaction

OBJECTIVES

- The present study carried out to assess the sensitivity of Multiplex ligation-dependent probe amplification (MLPA) and to detect the frequency and association of genomic aberrations with clinical outcome in Indian CLL patients

METHODS

- CLL patients were investigated for aberrations at 13q14, 11q22, 17p13 and trisomy12 by
  - iFISH (n=272; Metasystems, Germany)
  - MLPA ((n=345; MRC Holland, The Netherlands)
- Data for both iFISH and MLPA was available for 114 cases
- The survival status on the basis of FISH and/or MLPA was analyzed in 503 cases
- Overall survival (OS) was assessed in 503 cases
- Time to first treatment (TTFT) was assessed in 341 (Rai stage 0-2) cases
- The statistical analysis was carried out using Sigma Plot software (version 15.0).

RESULTS

- Chromosomal aberrations were detected in a total of 69% cases with del(13q) in 32%, del(11q) in 3.7%, trisomy 12q in 12%, and del(17p) in 8% cases as sole abnormality.
- Concomitant presence of two abnormalities in same patient was detected in 11.5% cases.
- In group with coexistence of ≥2 aberrations, del(13q) was a major clone (n=45) indicating it as a primary event followed by del(11q) (n=39) and del(17p) (n=37)
- Comparison of MLPA results with FISH (n=114), resulted in a concordance of 89% cases; discordance was observed for 2, 2, 4 and 5 cases for trisomy 12, del(11q), del (17p) and del(13q) respectively. Del(17p) discordant cases suggest inability of MLPA in detection of ≤15% positive fraction (Fig.1 &2)
- OS (n=503) and TTFT(n=341) were significantly lower in high risk [(del(17p)] and mid risk [(del(11q) and del(12q)] cases as compared to low risk cases [del(13q) and no abnormality)].

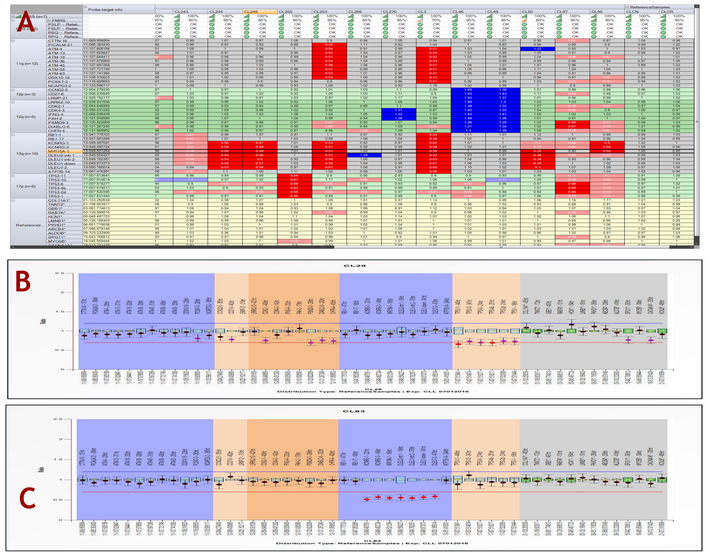
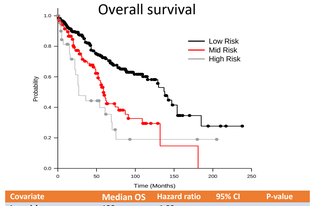
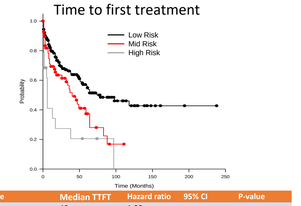


Figure 1: Representative figure for copy number changes on CLL samples by MLPA. A. The gains (Probe ratio>1.2) are highlighted in blue and deletions (Probe ratio<0.7) are highlighted in red on the heatmap. B. Patient with no identifiable genetic aberrations and C. Patient with del 13q



Covariate	Median OS	Hazard ratio	95% CI	P-value
Low risk (Del13q+No abnorm)	138	1.00		
Mid Risk (Del 11q+Trisomy12)	59	2.093	1.488-2.943	<0.001
High Risk (Del17p)	27	2.919	1.838-4.634	<0.001



Covariate	Median TTFT	Hazard ratio	95% CI	P-value
Mid Risk (Del 11q+Trisomy12)	40	1.00		
Low risk (Del13q+No abnorm)	74	0.580	0.389-0.865	0.007
High Risk (Del17p)	6	2.007	1.080-3.729	0.028

Figure 2: Kaplan-Meier survival curves representing Overall survival (A; n=503) and time to first treatment (B; n=341, Rai stage: 0-2) in patients having low risk [Del(13q)+no abnormality], mid risk [Del(11q)+Trisomy12] and high risk [Del(17p)] genomic abnormalities

Table 1 : Comparison of frequencies of genetic alterations in Indian CLL patients with existing literature

No of cases	Del 13q	Del11q	+12	Del17p	Technique	Study
503	32%	3.7%	12%	8%	MLPA+iFISH	Present study
58	33%	16%	16%	7%	MLPA	Grigore et al, 2013
86	31%	17%	19%	6%	iFISH	Giertlova et al, 2011
100	25%	3%	8%	3%	MLPA	Fabris et al, 2010
79	33%	5%	15%	8%	FISH	Durak et al, 2009
50	30%	10%	5%	6%	MLPA	Coll Mullet et al, 2008
217	54%	12%	16%	16%	FISH	Sindelarova et al, 2005
100	40%	23%	11%	12%	FISH	Glassman and Hayes, 2005
113	64%	15%	25%	8%	iFISH	Dewald et al, 2003
258	62%	11%	10%	6%	FISH	Bullinger et al, 2001
325	55%	18%	16%	7%	FISH	Dohner et al, 2000
139	51%	25%	12%	3%	FISH	Stilgenbauer et al, 1996

CONCLUSIONS

- Although the frequencies of detected aberrations in India differ slightly from reports available worldwide, the distribution pattern is similar (Table 1)
- Patients with high risk abnormalities had shorter OS and TTFT as compared to patients with 13q deletions.
- A high concordance (89%) of MLPA results with FISH (n=114), indicate that MLPA represents a high-throughput, cost-effective and comprehensible technique that can be used as a first-line screening for categorization of CLL patients into prognostically different genetic subtypes

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