MatBA: A Targeted Oligonucleotide Array for Assessment of Genomic Copy Number Alterations for Risk Stratification in Chronic Lymphocytic Leukemia

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Introduction

Risk stratification in chronic lymphocytic leukemia (CLL) is highly desirable and should comprise clinical features and molecular prognostic markers. Currently genomic abnormalities including loss of 17p13 (*TP53*), 11q22 (*ATM*), 13q14 (*MIR-15A/16.1*), 6q22 (*MYB*), and gain of chromosome 12 are assessed by fluorescence *in situ* hybridization (FISH) and the mutation status of the variable region of the *IGH* gene (*IGHV*) assessed by sequencing are valuable approaches. In recent years, genome-wide scanning technologies such as array-comparative genomic hybridization (array-CGH) have revealed novel and refined known copy number alterations (CNAs) in the CLL genome. In order to evaluate the potential of array-CGH in prognostication in mature B-cell neoplasms, including CLL, a targeted oligonucleotide-based microarray (MatBA™) was custom-designed.

MatBA™

eARRAY-designed Agilent oligonucleotide 4 x 44K
80 regions ranging in size from 0.3-21.3 Mbp
35 kbp regional resolution (duplicate)
1 Mbp backbone (duplicate)
5 x 301 replicates

Region	Size (Mbp)	Region	Size (Mbp)	Region	Size (Mbp)
1p36.32-p36.23	7.9	6p21.31-p21.2	2	11q25	2
1p21	13	6p21.1	2	12p13.1	2.2
1p13.2-p13.1	6	6q12	0.3	12q13.1-q13.2	11.9
1q21	10.9	6q16	12.8	12q15	3.8
1q31	19.7	6q21	2.5	13q14	12.7
1q41-q44	8	6q22	16.5	13q31	16
2p25.3	1.7	6q23.3-q24	11.9	13q33-q34	13.6
2p16.1-p15	4.6	6q25	1	14q12	9
2p11.2-q11.2	2	7p22	7.2	14q32	15
2q13-2q14.1	0.6	7p21.3-p21.2	1.7	15q21.1	1.5
2q24	14.9	7q31	19.7	15q23-q24	10
3p22	9.9	8p23	12.7	16p13.3	6.3
3p14.1-p13	3.8	8p21.3	4.5	16p13.13	2
3q12.2-q12.3	1.2	8p12-p11.23	2	16p11.1-p11.2	10.6
3q21.2	0.3	8q21.2	0.3	16q24	5.2
3q22	8.9	8q24.21	4.2	17p13	11.2
3q26.1-q26.2	11.3	9p24.2-p24.1	2	17q22-q23.1	2
3q26.31	2	9p21	12.9	17q24.2-25.1	5.8
3q27	5.2	9q22	12	18p11	16.1
4p15	9.8	9q33.2-q34.1	10	18q21	18
4q11-q12	4	10p14	5.6	18q23	0.3
4q24	2.8	10p12.31-p12.2	2.5	19p13.3-p13.2	11
4q34.3-q35	11.6	10q23.2	1.9	19q13.33-q13.43	10
5p15	10	11p13	1.5	20q13	21.3
5q13.2-5q13.3	3	11q13	13.6	21q21	15.2
5q31.3	1	11q22.1-q22.2	1.4	22q12	15.9
6p25	7	11q22.3-q23	14		

Array-CGH Methods

Test DNA was extracted from cryopreserved mononuclear cell pellets and quality and quantity confirmed (A260/A280 = 1.6-2.0, A260/A230 >2.0). An equimixture of male and female normal DNA (Promega) served as the reference DNA. Test and reference DNAs (1μg) were digested with RsaI and AluI and differentially enzymatically-labeled with Cy5 and Cy3-dUTP respectively. Purified labeled DNAs were mixed and hybridized to MatBA essentially as described by the manufacturer (Agilent Technologies). After washing, the slides were scanned and Genomics Workbench Lite (Agilent Technologies) was used for aberration detection using the ADM2 algorithm (thresholds 4 and 2).

Analytical Sensitivity

Cell Line DNA dilution: 30-40% FISH: 20-25%

Represented Region	697			KMH2			SKMM-2			L-428		
	100%	40%	30%	100%	40%	30%	100%	40%	30%	100%	40%	30%
chr2: 2.4-4.1												
chr2: 59.3-63.9												
chr3: 173-175												
chr3: 184.2-189.4												
chr8: 0-12.7												
chr8: 18.7-23.2												
chr8: 127.3-131.5												
chr11: 106.7-120.7												
chr12: 12.6-14.8												
chr12: 44.6-56.5												
chr12: 66-69.8												
chr13: 39.5-52.2												
chr17: 0-11.2												

	Gain of 12			Loss of 13q			Loss of 11q			Loss of 17p		
ID	12cen	% Cells	MatBA	D13S319	% Cells	MatBA	*ATM*	% Cells	MatBA	*TP53*	% Cells	MatBA
524	Normal			Normal								
665	Normal			Abnormal	48%	Positive	Normal			Normal		
738	Normal			Abnormal	96%	Positive	Normal			Normal		
767	Abnormal	37%	Positive	Normal			Normal			Normal		
774	Normal			Abnormal	100%	Positive	Normal			Normal		
809	Normal			Abnormal	66%	Positive	Abnormal	78%	Positive	Normal		
821	Normal			Normal			Normal			Normal		
923	Normal			Abnormal	76%	Positive				Normal		
1099	Normal			Abnormal	94%	Positive	Normal			Normal		
1118							Normal			Normal		
1158	Normal			Abnormal	97%	Positive	Abnormal	48%	Positive	Normal		
1222	Normal			Abnormal	75%	Positive	Normal			Normal		
1239	Normal			Normal			Normal			Normal		
1294	Abnormal	81%	Positive	Abnormal	44%	Positive	Normal			Normal		
1299	Normal			Abnormal	81%	Positive	Normal			Normal		
1301	Abnormal	25%	Positive	Normal			Normal			Normal		
1326	Abnormal	91%	Positive	Normal			Normal			Normal		
782	Normal			Normal			Normal			Abnormal	70%	Positive
667	Normal			Abnormal	86%	Positive	Normal			Normal		
899	Normal			Normal			Normal		Positive	Normal		
1156	Normal			Abnormal	16%		Normal			Abnormal	79%	Positive
1408	Normal			Abnormal	20%		Normal			Abnormal	88%	Positive
1344	Normal			Abnormal	75%	Positive	Normal			Abnormal	87%	

Discrepancies At 25%, sensitivity = 95%, specificity = 98%

Case 899: FISH one month later was positive for the abnormality detected by MatBA.
Case 1156 and 1408: Below level of detection by MatBA.
Case 1344: Reason for lack of detection by MatBA is unknown.

Clinical Dataset

119 IRB-approved cryopreserved mononuclear cells

CLL Cases		n
Untreated		81
Rai Stage	0	25
	I-II	42
	II-IV	5
	na	9
IGHV mutation status‡	Unmutated	37
	Mutated	43
	na	1
Median TTFT		87.6 mo
Median OS		117.7 mo
Treated		38

‡ Associated with TTFT (p=0.0003) and OS (p=0.0004)

Recurrent aberrations within regions with a minimum size of 1.5 Mbp were tested for association with time to first treatment (TTFT) and overall survival (OS) by the log rank test and with *IGHV* mutation status using the Fisher's two-sided exact test. *IGK* and *IGL* loci were excluded from further analysis as was sites of known CNVs. Deletions at 13q14 less than 1.5 Mbp were also tested for clinical association.

Reproducibility

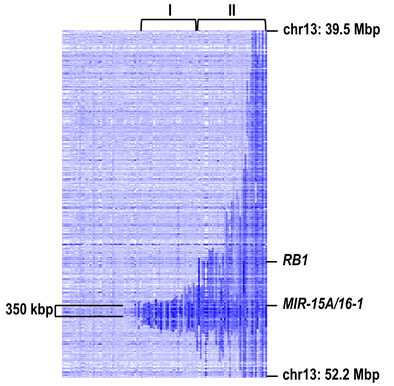
DNA from 30 specimens were assayed twice independently and all detected aberrations were reproducible between assays.

Accuracy/Precision

Aberrations detected in the following regions were confirmed by quantitative PCR (QPCR) as a second independent method of validation. Only one aberration could not be confirmed.

Aberration	Gene	Copy Number Assay
Loss of 8p	*GATA4*	Hs01297945_cn
	TNFRSF10B	Hs00098983_cn
Loss of 11q	*ATM**	Hs02355120_cn
Loss of 13q	*DLEU2^*	Hs03846573_cn
	*RB1**	Hs01344097_cn
Loss of 17p	*TP53**	Hs05506931_cn
Gain of 2p	*REL*	Hs00231626_cn
Gain of 3q	*BCL6*	Hs02145887_cn
Gain of 8q	*MYC*	Hs01764918_cn
Gain of 12	*MDM2*	Hs00738157_cn
Control (5p15)	*TERT*	Cat#4403316
Control (11p12)	*RAG2*	Hs00705088_cn

13q14 Deletions



Copy Number Alteration	Dataset 1	TTFT	OS
	Untreated	p-value	p-value
13q14 loss	67.9%	0.318	0.0008‡
MIR-15A/16.1, RB1 (Type II)	34.6%	0.78	0.41
MIR-15A/16.1 (Type I)	33.3%		
13q14 loss (sole abnormality)	49.4%	0.066‡	0.0001‡
MIR-15A/16.1, RB1 (Type II)	24.7%	0.40	0.56
MIR-15A/16.1 (Type I)	24.7%		
‡ Associated with longer time			

While loss of 13q14 was associated with a better overall outcome, no significant difference between Type I and Type II deletion with TTFT or OS was observed.

Aberrations Per Specimen

Specimen Group	# Aberrations	# Specimens (%)	TTFT	OS
			p-value	p-value
Untreated (n=81)	0	11 (13.5%)		
	1	49 (60.5%)		
	>1	21 (26.0%)	0.0073‡	0.0002‡
Treated (n=38)	0	4 (10.6%)		
	1	14 (36.8%)		
	>1	20 (52.6%)		
‡ Associated with shorter time				

Specimens with more than one aberration exhibited a poorer overall outcome than those with only one aberration or none. Following treatment, more specimens exhibited more than one aberration.

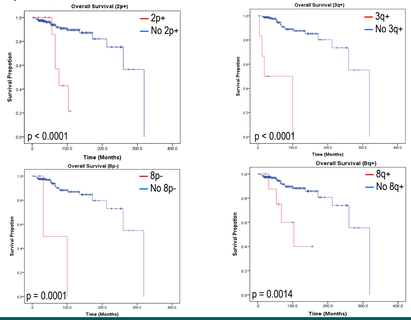
Recurrent CNAs

Recurrent aberrations associated with TTFT or OS with $p < 0.05$ are listed. All aberrations exhibited association with a shorter TTFT or OS occurred at higher frequency in treated specimens.

Copy Number Alteration	Dataset 1	TTFT	OS	
	Untreated	p-value	p-value	Treated
11q22 loss (*ATM*)	12.3%	0.125	0.009	23.7%
17p13 loss (*TP53*)	2.5%	0.010	0.012	15.8%
2p25.3-p15 gain‡	6.2%	0.002	<0.0001	10.5%
8q24 gain	2.5%	0.238	0.014	7.9%
3q26-q27 gain	2.5%	<0.0001	<0.0001	5.3%
8p23-p21 loss	2.5%	0.002	0.016	10.5%

‡ Observed only in unmutated *IGHV* specimens

In an independent dataset of 166 untreated CLL specimens, significant associations with OS was confirmed. For visualization purposes, both datasets were combined.



Conclusions

- A targeted oligonucleotide array detected aberrations in 87% of CLL MNC specimens.
- Sensitivity and specificity were 95% and 98% respectively based on 25% detection by FISH.
- No difference in TTFT or OS was found between specimens with Type I versus Type II 13q14 deletions.
- Specimens with more than one aberration exhibited a shorter TTFT and OS.
- Four additional markers of reduced overall survival were identified and validated in a second dataset.
- These data support the implementation of array-CGH into clinical practice in risk stratification of CLL patients for the detection of aberrations not routinely assayed by FISH.
- CLIA/NYS approved in November 2010/April 2011.