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

CANCER GENETICS
Empowering Personalized Cancer Treatment

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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 (1ug) 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 Feature Extraction (Agilent Technologies) was used for data extraction.

Analytical Sensitivity/Specificity

Cell Line Dilution Studies: 30-40%
FISH: 20-25%
Detection of 11q, 13q, and 17p loss, and 12 gain by FISH at 25%
Dataset 1: sensitivity = 95%, specificity = 98% (n =23)
Dataset 2: sensitivity = 100%, specificity = 92% (n=92)

Reproducibility

Dataset 1: DNA from 30 specimens were assayed twice or thrice independently and all detected aberrations in regions confirmed by quantitative PCR (QPCR) were reproducible between assays.
Dataset 2: 67 DNAs were assayed twice or thrice independently and all aberrations in the same regions were reproducible except for two that were < 0.5 Mbp in size.

Accuracy/Precision

Aberrations detected in the following regions were confirmed by QPCR in Dataset 1, 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

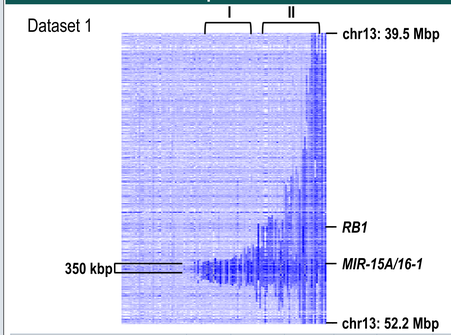
Clinical Datasets

Dataset 1: 119 IRB-approved cryopreserved mononuclear cells
Dataset 2: 194 IRB-approved CLL DNAs

CLL Datasets		1	2
Untreated (n)		81	166
Rai Stage	0	25	76
	I-II	42	48
	III-IV	5	1
	na	9	41
IGHV mutation status‡	Unmutated	37	60
	Mutated	43	100
	Non-clonal	0	6
	na	1	0
Median TTFT		87.6 mo	24.5 mo
Median OS		117.7 mo	38.6 mo
Treated (n)		38	28

‡ Tested for association with Time to First Treatment (TTFT) (p=0.0003, 0.002 respectively) and Overall Survival (OS) (p=0.0004, 0.043 respectively) using the log rank test.

13q14 Deletions



Copy Number Alteration	Set 1	TTFT	OS	Set 2	TTFT	OS
	%	p-value		%	p-value	
13q14 loss	67.9%	0.318	0.0008‡	51.2%	0.245	0.295
MIR-15A/16.1, RB1 (Type II)	34.6%	0.78	0.41	22.3%	0.025	0.081
MIR-15A/16.1 (Type I)	33.3%			28.9%		
13q14 loss (sole abnormality)	49.4%	0.066‡	0.0001‡	34.3%	0.142‡	0.007‡
MIR-15A/16.1, RB1 (Type II)	24.7%	0.40	0.56	11.4%	0.011	1
MIR-15A/16.1 (Type I)	24.7%			22.9%		

‡ Associated with longer time

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

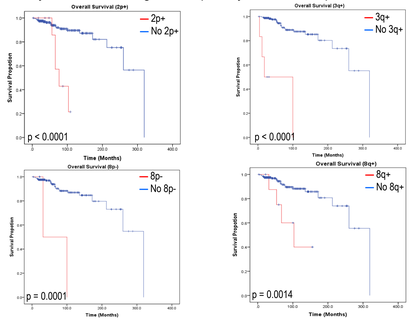
CNA Detection and Association with Time to First Treatment (TTFT) and Overall Survival (OS)

Method 1

Aberrations were detected using Genomics Workbench Lite (Agilent Technologies) with the ADM2 algorithm (thresholds 4 and 2, log ratio ≥ 0.2 for gain and ≤ -0.2 for loss). Recurrent aberrations within regions with a minimum size of 1.5 Mbp were tested for association with TTFT and OS by the log rank test. *IGK* and *IGL* loci and sites of known CNVs were excluded from further analysis. CNAs associated with TTFT or OS with p < 0.05 are listed. All exhibited association with shorter TTFT or OS, and mostly occurred at higher frequency in treated specimens.

Copy Number Alteration	Dataset 1	TTFT	OS		Dataset 2	TTFT	OS	
	Untreated	p-value		Treated	Untreated	p-value		Treated
11q22 loss (*ATM*)	12.3%	0.125	0.009	23.7%	7.2%	0.39	0.951	14.3%
17p13 loss (*TP53*)	2.5%	0.010	0.012	15.8%	4.8%	0.006	<0.0001	10.7%
2p25.3-p15 gain‡	6.2%	0.002	<0.0001	10.5%	3.0%	0.70	0.025	10.7%
8q24 gain	2.5%	0.238	0.014	7.9%	4.2%	0.561	0.007	0%
3q26-q27 gain	2.5%	<0.0001	<0.0001	5.3%	2.4%	0.85	<0.0001	7.1%
8p23-p21 loss	2.5%	0.002	0.016	10.5%	1.2%	1	<0.0001	7.1%

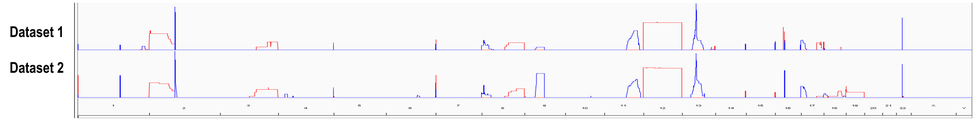
‡ Observed only in unmutated *IGHV* specimens



For visualization purposes, both datasets were combined for Kaplan Meier analysis.

Method 2

GISTIC was used to detect commonly altered regions in both datasets based on frequency and amplitude.



Recurrent regions were tested for association with TTFT and OS. Significant CNAs identified by Method 1 were confirmed plus four additional CNAs were found to associate with shorter TTFT and OS (p < 0.05). Only loss of 1q21 was found to be significant in both datasets.

Copy Number Alteration	Dataset 1	TTFT	OS		Dataset 2	TTFT	OS	
	Untreated	p-value	p-value	Treated	Untreated	p-value	p-value	Treated
1q21.1 loss	5.0%	0.017	<0.001	5.3%	13.9%	ns	0.007	14.3%
7p22.3 gain	2.5%	0.006	0.02	10.5%				
17q25.1 gain	3.7%	<0.001	<0.0001	5.3%				
16p13.3 gain					2.4%	ns	0.025	0%

Conclusions

- A targeted oligonucleotide array was designed to detect aberrations commonly altered in mature B-cell neoplasms, including CLL.
- Analytical sensitivity and specificity were 95-100% and 92-98% 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.
- Five new markers (four > 1.5Mbp and one < 500 kbp in size) 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.

Conflicts of Interest

Jane Houldsworth (Employment, Equity Ownership), Asha Guttapalli (Employment), Charles Ma (Employment), Weiyi Chen (Employment)