Diffuse Large B-Cell Lymphoma With A Complex Genome Exhibit p53 But Not MYC Expression And Have Inferior Survival

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Introduction

Genomic complexity in diffuse large-B-cell lymphoma has recently been reported to have strong prognostic value in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab-based immunochemotherapy (PMID:22975378). In that study, the presence of gain/loss of at least one of the following nine genomic markers along the CDKN2A-TP53-RB-E2F axis was used to define cases with "complex" genomes.

Target Gene	Aberration	Coordinates (hg18)
MDM4	Gain 1q23.3	Chr1:159,738,009-159,946,881
CCND3	Gain 6p21.32	Chr6:32,798,809-32,867,250
CDK6	Gain 7q22.1	Chr7:100,846,670-101,680,144
CDK2	Gain 12q15	Chr12:67,637,703-69,005,611
BCL2L12	Gain 19q13.42	Chr19:60,201,501-60,985,512
CDKN2A	Loss 9p21.3	Chr9:21,953,431-21,978,391
RB1	Loss 13q14.2	Chr13:47,878,937-47,858,483
RBL2	Loss 16q12.2	Chr16:52,040,758-52,209,001
TP53	Loss 17p13.1	Chr17:7,483,385-7,964,774

Genomic imbalance at these specific loci were suggested to functionally contribute toward cell cycle deregulation resulting in increased overall genomic instability.

Other clinicopathologic studies have clearly demonstrated that inferior survival is also associated with elevated expression of p53, which serves as a surrogate for *TP53* mutation (PMID:24619762). To date, there have been very few studies, if any, that have examined the relationship between genomic imbalance and complexity, and clinicopathologic features in DLBCL, in particular with relevance to TP53.

Materials and Methods

All studies were performed with IRB approval.

DNA was extracted from sections of formalin-fixed paraffin-embedded biopsy specimens (more than 50% tumor burden) from 85 patients diagnosed with de novo DLBCL at a single institution (PMID:24619762).

DNAs were submitted to array-CGH using a custom designed oligonucleotide array (Agilent Technologies) with an equimixture of normal male:female DNA (Promega) as reference (essentially as described in PMID:24047479).

Each specimen was scored for the presence of each of 50 aberrations according to criteria established on overlapping copy number alterations found in at least 2 of 3 genomic profiled publically available DLBCL datasets. **See Abstract 2993 for details.**

Gains			Losses		
MCR	Abr #	Aberration	MCR	Abr #	Aberration
1	1-3	A1q22.1-q25.1	20	28	D1p36.32-36.31
2	4	A1q31.3-q32.1	21	29	D1p13.1
3	5	A2p16.1-p15	22	30	D2q22.3
4	6	A3q27.3-q29	23	31	D2q24.2
5	7	A5p15.33	24	32	D3p21.31-p21.2
6	8	A6p21.32-p21.2	25	33	D3p14.2
7	9	A7p22.2	26	34	D6p21.33
8	10	A8q24.3	27	35-39	D6q11.1-q27
9	11	A9q34.3	28	40	D8p22-21.3
10	12	A11p15.4	29	41	D9p24.1
11	13	A11q23.3	30	42	D9p21.3
12	14	A12q13.11-q13.12	31	43	D10q23.31
13	15	A12q14.2-q21.1	32	44	D13q14.13-q14.3
14	16	A13q31.3	33	45-46	D15q15.1-q21.1
15	17	A16q24.3	34	47	D16q12.1-q12.2
16	18-24	A18p11.21-18q23	35	48-49	D17p13.3-p11.2
17	25	A19p13.3	36	50	D19p13.3
18	26	A19q13.33-q13.43			
19	27	A21q22.3			

Results

Clinical Characteristics of 85 De Novo DLBCL

Variable	Value (%)	Variable	Value (%)
Race/ethnicity		**IPI**	
Hispanic	58 (71)	0	16 (20)
African American	4 (5)	1	21 (26)
Asian	9 (11)	2	15 (19)
White	11 (13)	3	10 (13)
NA	3	4	15 (19)
Sex		5	3 (4)
Female	34 (40)	NA	5
Male	51 (60)		
Treatment			
R-CHOP or RCHOP-like	68 (80)		
Palliative	6 (8)		
NA	11		

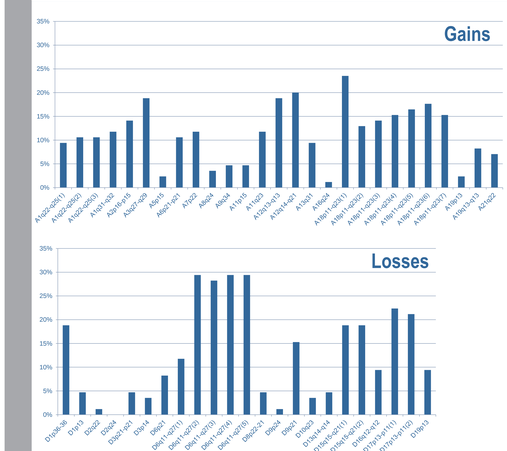
Patients were biopsied as part of their routine care at the Los Angeles County plus University of Southern California Medical Center (PMID: 24619762)

Immunopathologic Features of 85 De Novo DLBCL (PMID:24619762)

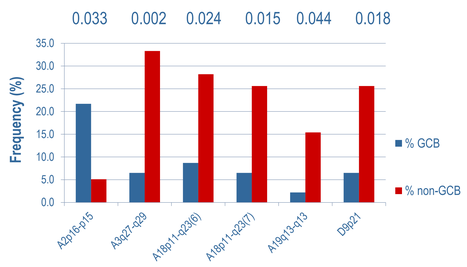
Variable	Value	Variable	Value
p53 expression		**MYC/BCL2 co-expression**	
0 (low)	8	No	66
1 (low)	42	Yes	17
2 (high)	27	NA	2
3 (high)	8	**MYC/p53 co-expression**	
MYC expression		MYC low, p53 low	43
< 40%	62	MYC high, p53 low	7
≥ 40%	23	MYC low, p53 high	19
BCL2 expression		MYC high, p53 high	16
< 70%	31	**Cell of Origin Subtype (Hans)**	
≥ 70%	52	GCB	46
NA	2	non-GCB	39

No "double-hit" DLBCL were included in the dataset

Genomic Imbalance in 85 De Novo DLBCL



Genomic Aberrations Enriched in Cell-of-Origin Subtypes



Aberrations Associated with p53, MYC, and BCL2 Expression

Expression	# Samples	Aberration
		D17p13.3
Low p53	50	8*
High p53	35	10*
		A8q24.3
Low MYC	62	3
High MYC	23	0
		A18p11.21-18q23
Low BCL2	31	0
High BCL2	52	15**

* Not significant by Exact t-test
**All cases with gain of the 18q21 aberration with a peak closest to *BCL2* (Abr 23) exhibited elevated BCL2 expression.

Genomic Aberrations Associated with Patient Outcome

Ten aberrations had previously been found in other datasets to univariately associate with shorter overall survival (OS) (**See Abstract 2993 for details**).
These were tested for association using the log rank statistic in both the entire dataset and in those treated with RCHOP or an RCHOP-like regimen.

Aberration	All (n=85)		RCHOP-treated (n=68)	
	Cases	*P*-value	Cases	*P*-value
A12q13.11-q13.12	16 (19%)	ns	14 (21%)	ns
A12q14.2-q21.1	17 (20%)	ns	13 (19%)	ns
A16q24.3	1 (1%)	ND	1 (1%)	ND
A19q13	7 (8%)	ns	6 (9%)	ns
D2q24.2	0	ND	0	ND
D6q21q11.1	24 (28%)	ns	22 (32%)	ns
D8p22-p21.3	4 (5%)	ns	3 (4%)	ns
D9p21.3	13 (15%)	ns	9 (13%)	ns
D15q15.1-q21.2	16 (19%)	ns	15 (22%)	ns
D17p13.3-p11.2	18 (21%)	0.010	11 (16%)	0.103

Conflicts of Interest

CM and JH are employees of Cancer Genetics, Inc., and are stock/stock option holders.

IS, YX, and AT have no conflicts of interest.

Genomic Complexity

METHOD 1
Complex genome = Sample exhibits any one of aberrations of the CDKN2A-TP53-RB-E2F axis:

Gain 1q23.3	Loss of 9p21.3
Gain 6p21.32	Loss of 13q14.2
Gain 7q22.1	Loss of 16q12.2
Gain 12q15	Loss of 17p13.1
Gain 19q13.42	

COMPLEX = 53 (62%) **CLEAN = 32 (38%)**

METHOD 2
Complex genome = Sample exhibits > 1 MCR aberration (median of >300 DLBCL)

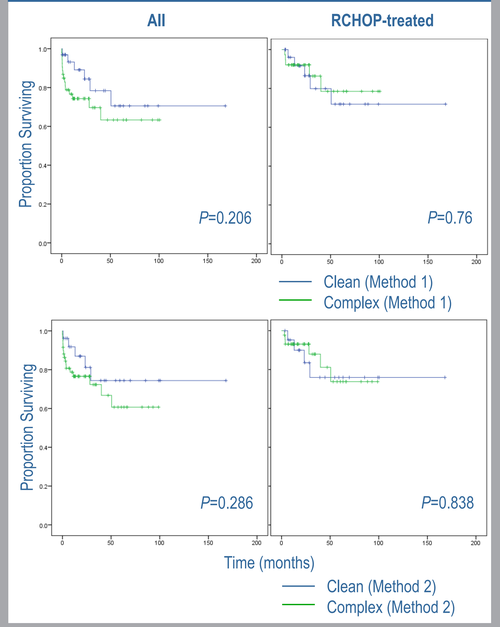
COMPLEX = 59 (69%) **CLEAN = 26 (31%)**

Correlation of Immunopathologic Features with Genomic Complexity

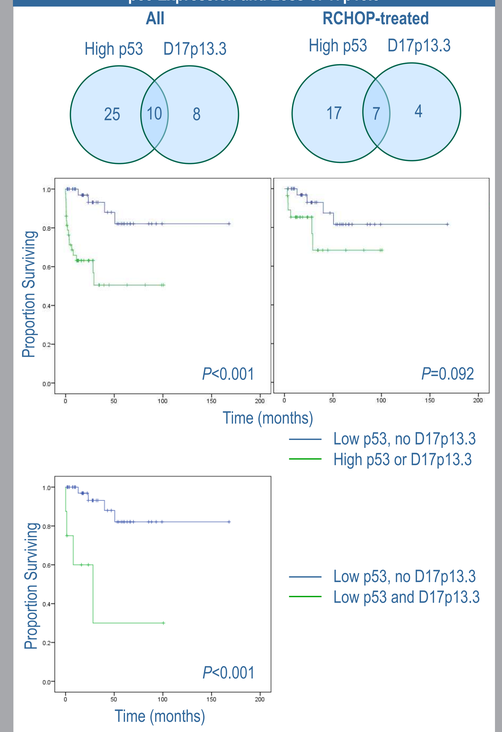
Feature	Method 1		Method 2	
	Complex (%)	Clean (%)	Complex (%)	Clean (%)
High p53	26 (49)	9 (28)	27 (46)	8 (31)
High MYC	17 (32)	6 (19)	18 (31)	5 (19)
High BCL2	32 (63)	20 (63)	37 (64)	15 (60)
Non-GCB	25 (47)	14 (44)	29 (49)	10 (38)
High IPI (>2)	20 (40)	8 (27)	23 (42)	5 (20)

Exact t-test *P*-value = 0.05-0.1

Correlation of Genomic Complexity with Patient Outcome



p53 Expression and Loss of 17p13.3



Conclusions

- In a single institution series of 85 de novo DLBCL, every specimen was scored for the presence of 50 genomic gains/losses commonly detected in DLBCL using robust platform-agnostic criteria.
- Pathogenomic correlations confirmed the association of genomic aberrations with cell-of-origin subtype, and showed that all cases with gain of 18q21 also had elevated expression of BCL2.
- Genomic complexity, as assessed by aberrations in the CDKN2A-TP53-RB-E2F axis or number of aberrations, did not correlate with patient overall survival.
- Loss of 17p13.3 positively correlated with adverse survival and appeared to mark another smaller subset of DLBCL patients with inferior survival who exhibited low p53 expression.

References

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