The Pathogenomic Landscape of Diffuse Large B-Cell Lymphomas Reveals That MYC/TP53 Co-expression and Loss of 17p13 Are Associated With Inferior Survival

Charles Ma, PhD[1], Venkata Thodima, PhD[1], Yi Xie, MD PhD[2], Anil Tulpule, MD[3], Kshitija Desai[1], Hannah Sidoti[1], Christina Caviasco[1], Imran N. Siddiqi, MD PhD[2], and Jane Houldsworth, PhD[1]

[1] Cancer Genetics, Inc., Rutherford, NJ; [2] University of Southern California, Los Angeles, CA; [3] Medicine and [4] Hematopathology Section, University of Southern California Keck School of Medicine, Los Angeles, CA.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma diagnosed each year. Despite intense efforts to characterize biomarkers for risk stratification and clinical decision-making, results have been confounded by molecular and pathological heterogeneity displayed by this disease.

Immunohistochemistry (IHC) has helped to resolve some of this complexity and has proven to be an invaluable tool for both determining cell-of-origin and other clinical biomarkers for disease prognosis. For instance, elevated expression of BCL2, BCL6, MYC, and TP53, as assessed by IHC, have all been found to have clinical prognostic significance, but inconsistently across studies. In a recent study by us, high TP53 expression correlated with inferior outcome and co-expression of MYC had an enhanced negative effect on outcome (PMID: 24619762). Yet others have examined the clinical relevance of genomic abnormalities such as gain/loss and mutation, but mostly by studying single genes.

To date, few, if any, studies have sought to reconcile the contribution of such genomic abnormalities to IHC-generated expression profiles and their potential additive value in the context of established pathological features. Therefore in the current study, pathogenomic associations were investigated in a dataset of 85 de novo DLBCL for whom immunopathologic data were available (PMID:24619762). In addition, genomic alterations were examined in greater detail to ascertain whether any may add prognostic value, particularly in the context of elevated TP53 and TP53/MYC expression.

Materials and Methods

All studies were performed with IRB approval.

DNA was extracted from sections or cores 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.

Bi-directional Sanger's sequencing was performed for Exons 5-8 of TP53 for 80 specimens.

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			

Conflicts of Interest

CM, VT, KD and JH are employees of Cancer Genetics, Inc., and are stock/stock option holders. HS and CC were employees of Cancer Genetics, Inc. YX, AT, and IS have no conflicts of interest.

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		

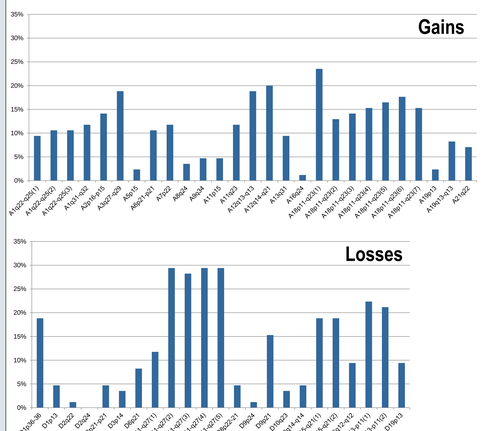
Patients were biopsied as part of their routine care at the Los Angeles County plus University of Southern California Medical Center (PMID: 24619762)

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

Variable	*Value*	*Variable*	*Value*
TP53 expression		**MYC/BCL2 co-expression**	
0 (low)	8	No	66
1 (low)	42	Yes	17
2 (high)	27	NA	2
3 (high)	8	**MYC/TP53 co-expression**	
MYC expression		MYC low, TP53 low	43
< 40%	62	MYC high, TP53 low	7
≥ 40%	23	MYC low, TP53 high	19
BCL2 expression		MYC high, TP53 high	16
< 70%	31	**Cell of Origin Subtype (Hans)**	
≥ 70%	52	GCB	46
NA	2	non-GCB	39

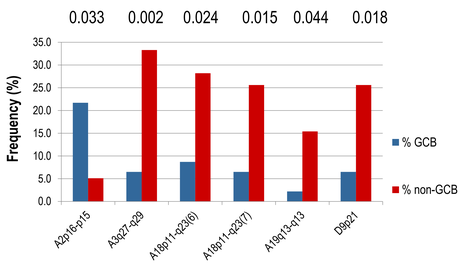
No "double-hit" DLBCL were included in the dataset

Genomic Imbalance in 85 De Novo DLBCL



Results

Genomic Aberrations Enriched in Cell-of-Origin Subtypes



Aberrations Associated with TP53, MYC, and BCL2 Expression

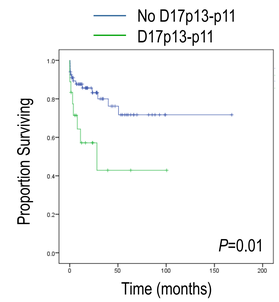
Expression	*# Samples*	*Aberration*
		D17p13.3
Low TP53	50	8*
High TP53	35	10*
		A8q24.3
Low MYC	62	3
High MYC	23	0
		A18p11.21-q23
Low BCL2	31	0
High BCL2	52	15**

* 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

Recurrent aberrations were tested for association with overall survival (OS) using the log rank statistic and plotted using the Kaplan-Meier method.

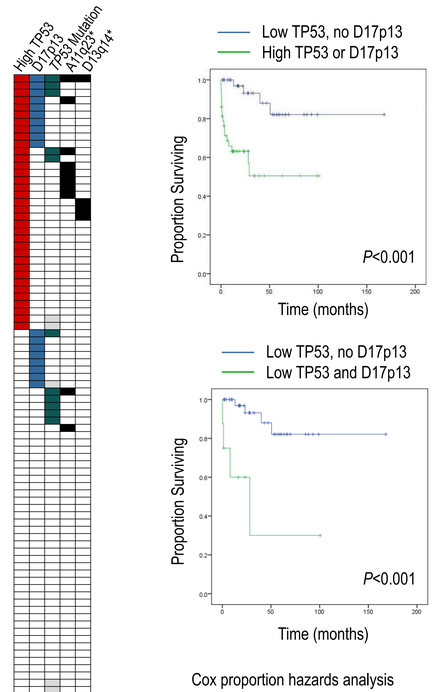
Aberration	*Abr #*	*Cases*	*P-value*
D3p21	32	4 (5%)	0.101
D3p14	33	3 (4%)	0.019
D17p13-p11 (1)	48	19 (22%)	0.021
D17p13-p11 (2)	49	18 (21%)	0.010
D19p13	50	8 (9%)	0.067



Results

Correlation of TP53 Expression with Genomic Abnormalities and Patient Outcome

Two aberrations significantly associated with elevated TP53 expression.
TP53 mutation did not significantly correlate with expression or loss.



* *P<0.05* by Exact t-test

Cox proportion hazards analysis

Variable	*P-value*	*HR*	*95% CI*
High TP53	0.031	3.64	1.45-9.12
D17p13	0.069	4.28	1.72-10.67

Correlation of MYC Expression with Genomic Abnormalities

No aberrations were significantly associated with elevated MYC expression.

Correlation of BCL2 Expression with Genomic Abnormalities

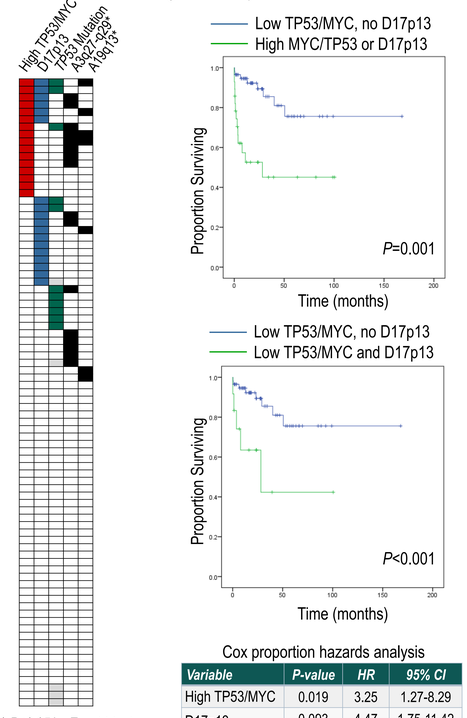
Eight aberrations were significantly associated with elevated BCL2 expression, including five mapped to 18q.

Aberration	*Abr #*	*Cases*	*P-value*
A18p11.21-q23 (3)	20	12 (14%)	0.006
A18p11.21-q23 (4)	21	13 (15%)	0.005
A18p11.21-q23 (5)	22	14 (16%)	0.002
A18p11.21-q23 (6)	23	15 (18%)	0.001
A18p11.21-q23 (7)	24	13 (15%)	0.001
D6q11.1-q27 (2)	36	25 (29%)	0.0028
D6q11.1-q27 (3)	37	24 (28%)	0.0138
D9p21.3	42	13 (15%)	0.0267

Results

Correlation of TP53/MYC Expression with Genomic Abnormalities and Patient Outcome

Two aberrations significantly associated with elevated TP53/MYC co-expression (also associated with non-GCB COO subtype, but not other non-GCB enriched gains/losses).



* *P<0.05* by Exact t-test

Cox proportion hazards analysis

Variable	*P-value*	*HR*	*95% CI*
High TP53/MYC	0.019	3.25	1.27-8.29
D17p13	0.093	4.47	1.75-11.42

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 identified aberrations associated with elevated TP53 and BCL2 expression (including 18q21 for the latter).
- 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 TP53 and low TP53/MYC co-expression but were not independent predictors of outcome.
- *TP53* mutation of Exons 5-8 by Sanger sequencing did not exhibit additional clinical relevance.

References

PMID:24619762
Xie Y, Bulbul MA, Ji L, et al. *p53 Expression is a strong marker of inferior survival in de novo diffuse large B-cell lymphoma and may have an enhanced negative effect with MYC coexpression.* Am J Clin Pathol 2014;141:593-604
PMID:24047479
Houldsworth J, Guttapalli A, Thodima V, et al. *Genomic imbalance defines three prognostic groups for risk stratification of patients with chronic lymphocytic leukemia.* Leuk Lymphoma 2014;55:920-928