JOURNAL OF HEMATOLOGY & ONCOLOGY

LETTER TO THE EDITOR Open Access

The addition of rituximab to CHOP therapy alters the prognostic significance of CD44 expression

Xiaolei Wei[1†], Meng Xu[1†], Yongqiang Wei[1], Fen Huang[1], Tong Zhao[2], Xiangzhao Li[2], Ru Feng[1*] and B Hilda Ye[3*]

Abstract

Expression of CD44 splice isoforms has been previously reported to correlate with inferior outcomes in DLBCL patients treated with CHOP therapy. However, it is unclear whether this observation remains valid in the R-CHOP era. In this study, we correlated CD44H and CD44v6 status with survival outcomes among DLBCL patients with an emphasis on the comparison between CHOP- and R-CHOP-treated subgroups. Our results suggest that rituximab has significantly decreased the prognostic value of CD44H. We also observed that the therapeutic benefit of rituximab is largely restricted to CD44H-positive cases in this cohort.

Keywords: DLBCL, Prognosis, CHOP, Rituximab, CD44 variant isoforms, Bone marrow involvement

To the Editor

Although incorporation of rituximab into CHOP (R-CHOP) has dramatically improved the outcome of DLBCL [1-5], approximately 40% of patients still succumb to the disease [6]. One of the prognostic markers studied in the CHOP era is CD44, a transmembrane glycoprotein with many alternative splicing isoforms [7]. Variations in its extracellular domain lead to isoform-specific activities of CD44 in cell adhesion, lymphocyte homing, and cell signaling [7]. In general, CD44 plays a positive role in cell survival and invasiveness, and it is implicated in cancer stem cell maintenance in certain solid tumors [8]. The objective of the current study is to compare the prognostic significance of CD44 isoforms in the CHOP and R-CHOP treatment groups.

This study enrolled 117 de novo DLBCL patients among whom 53 were treated with CHOP and 64 were treated with R-CHOP (Additional file 1; Additional file 2: Table S1). As expected, the incorporation of rituximab markedly improved the overall survival (OS) and event-free survival (EFS) rates (not shown). We used immunohistochemistry (IHC) to examine the expression of CD44H (the standard isoform) and CD44v6 (isoforms containing the variant exon 6) in diagnostic specimens (Additional file 3: Figure S1). Expression of CD44H and CD44v6 was detected in 65.0% and 34.2% of patients, respectively, with strong correlation to each other (Spearman's correlation, $r = 0.423$, $p < 0.001$). The baseline clinical features were not significantly different between the CD44H+ and CD44H- patients. The CD44v6+ and CD44v6- cases were also very comparable (Additional file 2: Table S2).

In the entire cohort of 117 patients, CD44H positivity strongly correlated with poor OS ($p = 0.002$, Figure 1A) and EFS ($p = 0.011$, Figure 1B) outcomes. Specifically, the 5-year OS rates in the CD44H+ and CD44H- subgroups were 82% and 41%, respectively. CD44v6 positivity also correlated with poor prognosis, although the trend was only marginally significant (OS: $p = 0.050$; EFS: $P = 0.058$, Figure 1C and D). Nevertheless, because CD44v6 showed an IPI-independent survival impact in multi-variable analysis (Additional file 2: Table S3), the relatively weak survival association based on the Kaplan-Meier estimates likely reflects the low frequency of CD44v6 expression and hence a greater sample size requirement. CD44v6 did not show any prognostic value when the cohort was divided into treatment subgroups (not shown). The negative prognostic value for CD44H detected among all patients could also be observed in the CHOP subgroup (OS: $p = 0.021$; EFS: $P = 0.044$, Figure 1E and F), but not the R-CHOP subgroup (OS: $p = 0.095$; EFS: $P = 0.211$, Figure 1G and H). Because the OS response was very similar among all R-CHOP-treated cases and CHOP-treated CD44H- patients,

* Correspondence: ruth1626@hotmail.com; hilda.ye@einstein.yu.edu
†Equal contributors
[1]Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, China
[3]Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, USA
Full list of author information is available at the end of the article

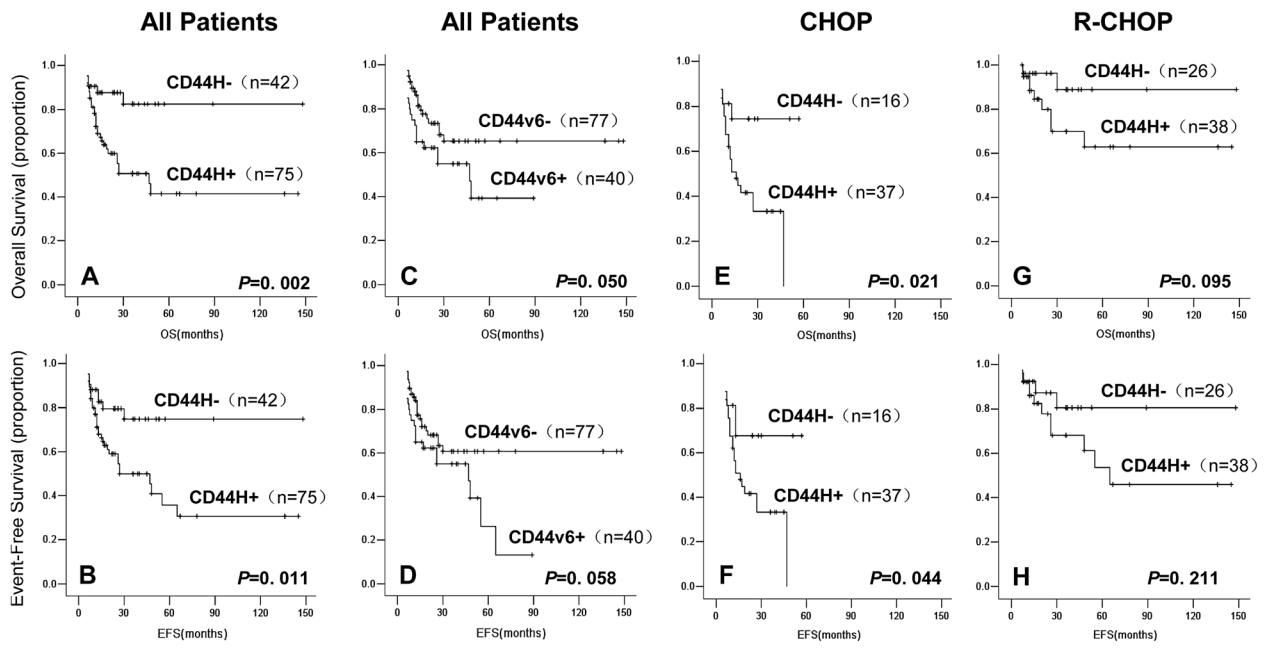

Figure 1 Overall survival (OS) and event-free survival (EFS) according to CD44H and CD44v6 expression status and type of therapy. The Kaplan-Meier method was used to estimate the OS and EFS distributions with the log-rank test performed to compare the survival curves. OS **(A and C)** and EFS **(B and D)** of all patients were analyzed based on their CD44H **(A and B)** and CD44v6 **(C and D)** status. OS **(E and G)** and EFS **(F and H)** according to CD44H expression status were also analyzed in the CHOP **(E and F)** and R-CHOP **(G and H)** treatment groups separately.

we reasoned that the extremely unfavorable response to CHOP among CD44H-positive patients may have been specifically ameliorated by rituximab. To test this notion, the rituximab-associated survival benefit was examined in patient subgroups of different CD44 expression status. For CD44H, although rituximab substantially improved the outcome for CD44H+ patients (OS: $p < 0.001$; EFS: $P = 0.001$, Figure 2A and B), the impact of this agent was insignificant for the CD44H- cases (OS: $p = 0.093$; EFS: $P = 0.183$, Figure 2C and D). Interestingly, this phenomenon

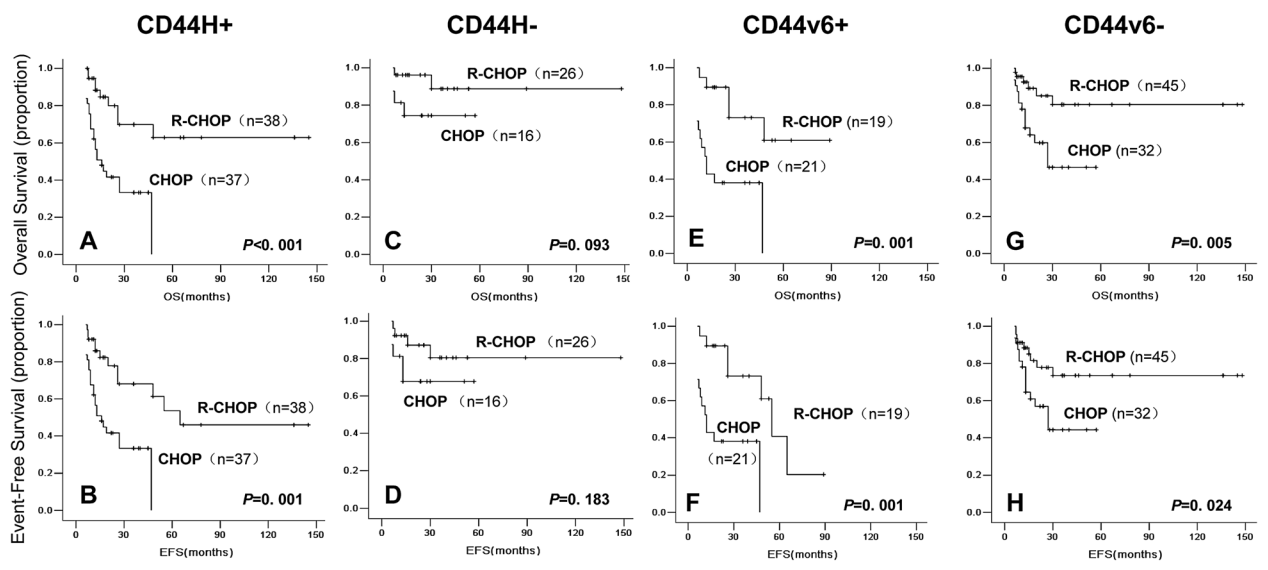

Figure 2 Rituximab-associated survival benefits based on CD44H and CD44v6 expression status. The Kaplan-Meier method was used to estimate the OS and EFS distributions with the log-rank test performed to compare the survival curves. For cases that were either CD44H+ **(A and B)** or CD44H- **(C and D)**, survival outcomes after CHOP and R-CHOP treatments were compared for OS **(A and C)** and EFS **(B and D)**. CHOP versus R-CHOP comparison was also made for OS **(E and G)** and EFS **(F and H)** among the CD44V6+ **(E and F)** and CD44v6- **(G and H)** subgroups.

appeared to be specific to CD44H because the rituximab-associated survival benefit was significant irrespective of the CD44v6 status (Figure 2E to H).

Possibly due to the use of different antibodies and different IHC staining/scoring methods, there have been some controversial observations on the prognostic importance of CD44 in CHOP-treated DLBCL patients. In agreement with the majority of published studies [9-11], we have observed a negative survival impact of CD44H and CD44v6 expression in our entire study cohort (Figure 1 and Additional file 2: Table S3) as well as the CHOP treatment group (Figure 1E and F), although there were differences between these two markers. As the first study aimed to examine CD44 isoform expression in the R-CHOP era, our data suggest that rituximab has decreased the prognostic significance of CD44H, while the impact of rituximab on CD44v6 awaits future studies of larger cohorts. We also observed that the rituximab-associated survival benefit was profound among CD44H-positive cases but fairly limited among the CD44H-negative subgroup.

Additional files

Additional file 1: Information on Patients and Methods.

Additional file 2: Table S1. Clinical features and CD44 variant expression in the CHOP and RCHOP groups. **Table S2.** Patient characteristics according to CD44H and CD44v6 expression status. **Table S3.** Prognostic factors and multivariable survival analysis.

Additional file 3: Figure S1. Representative immunohistochemical staining of DLBCL samples for CD44H and CD44v6 expression. (A, C), negative control stain using isotype-matched Abs. (B) CD44H staining in apositive case. (D) CD44v6 staining in a positive case.

Competing interests

The authors declare no conflicts of interest.

Authors' contributions

FR and BHY designed the study and analyzed and interpreted the data. WXL and XM collected and analyzed data. WYQ and HF collected data. ZT and LXZ provided study material and helped with the IHC staining. BHY, WXL and FR wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 81028013), the Science and Technology Project of Guangdong Province of China (Grant No. 2010B050700020), the Science and Technology Project of Guangzhou City (Grant No. 12C22121553), and a pilot grant from CFAR, the Center for AIDS Research at the Albert Einstein College of Medicine.

Author details

[1]Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, China. [2]Department of Pathology, Nanfang Hospital, Southern Medical University, Guangzhou, China. [3]Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, USA.

Received: 21 February 2014 Accepted: 24 March 2014
Published: 16 April 2014

References

1. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, Morel P, Van Den Neste E, Salles G, Gaulard P, Reyes F, Lederlin P, Gisselbrecht C: **CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma.** *N Engl J Med* 2002, **346**(4):235–242.
2. Habermann TM, Weller EA, Morrison VA, Gascoyne RD, Cassileth PA, Cohn JB, Dakhil SR, Woda B, Fisher RI, Peterson BA, Horning SJ: **Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma.** *J Clin Oncol: Official J Am Soc Clin Oncol* 2006, **24**(19):3121–3127.
3. Pfreundschuh M, Schubert J, Ziepert M, Schmits R, Mohren M, Lengfelder E, Reiser M, Nickenig C, Clemens M, Peter N, Bokemeyer C, Eimermacher H, Ho A, Hoffmann M, Mertelsmann R, Trümper L, Balleisen L, Liersch R, Metzner B, Hartmann F, Glass B, Poeschel V, Schmitz N, Ruebe C, Feller AC, Loeffler M; German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL): **Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60).** *Lancet Oncol* 2008, **9**(2):105–116.
4. Pfreundschuh M, Trumper L, Osterborg A, Pettengell R, Trneny M, Imrie K, Ma D, Gill D, Walewski J, Zinzani PL, Stahel R, Kvaloy S, Shpilberg O, Jaeger U, Hansen M, Lehtinen T, López-Guillermo A, Corrado C, Scheliga A, Milpied N, Mendila M, Rashford M, Kuhnt E, Loeffler M; MabThera International Trial Group: **CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group.** *Lancet Oncol* 2006, **7**(5):379–391.
5. Cang S, Mukhi N, Wang K, Liu D: **Novel CD20 monoclonal antibodies for lymphoma therapy.** *J Hematol Oncol* 2012, **5**:64.
6. Sehn LH: **Paramount prognostic factors that guide therapeutic strategies in diffuse large B-cell lymphoma.** *Hematology Am Soc Hematol Educ Program* 2012, **2012**:402–409.
7. Ponta H, Sherman L, Herrlich PA: **CD44: from adhesion molecules to signalling regulators.** *Nat Rev Mol Cell Biol* 2003, **4**(1):33–45.
8. Guo W, Frenette PS: **Alternative CD44 splicing in intestinal stem cells and tumorigenesis.** *Oncogene* 2013.
9. Horst E, Meijer CJ, Radaszkiewicz T, Ossekoppele GJ, Van Krieken JH, Pals ST: **Adhesion molecules in the prognosis of diffuse large-cell lymphoma: expression of a lymphocyte homing receptor (CD44), LFA-1 (CD11a/18), and ICAM-1 (CD54).** *Leukemia* 1990, **4**(8):595–599.
10. Inagaki H, Banno S, Wakita A, Ueda R, Eimoto T: **Prognostic significance of CD44v6 in diffuse large B-cell lymphoma.** *Mod Pathol* 1999, **12**(5):546–552.
11. Drillenburg P, Wielenga VJ, Kramer MH, van Krieken JH, Kluin-Nelemans HC, Hermans J, Heisterkamp S, Noordijk EM, Kluin PM, Pals ST: **CD44 expression predicts disease outcome in localized large B cell lymphoma.** *Leukemia* 1999, **13**(9):1448–1455.

doi:10.1186/1756-8722-7-34
Cite this article as: Wei *et al.*: The addition of rituximab to CHOP therapy alters the prognostic significance of CD44 expression. *Journal of Hematology & Oncology* 2014 **7**:34.

Additional file 1, Wei et al

Information on Patients and Methods

Patient Population

Included in this retrospective study are 120 patients diagnosed with de novo DLBCL between 1998 and 2008 in Nan Fang Hospital, Guangzhou, China. Patients were included in the study if they were ≥ 18 years of age with a biopsy-proven diagnosis of DLBCL according to the current WHO criteria [1], had unilateral BM aspirate and biopsy at the time of diagnosis and had complete clinical data. Patients were excluded if they were HIV-positive, or had various other types of lymphoma, including primary mediastinal, central nervous system, intravascular and testicular lymphomas. All patients were staged based on the Ann Arbor staging system [2] and assigned IPI scores using the original definition [3]. Among the 120 patients, 53 were treated with CHOP, 64 were treated with R-CHOP, either alone or in combination with surgery and/or involved-field radiation. Three patients were treated with other types of therapies and were included in the CD44 expression analysis but excluded from prognostic studies. The study was approved by the institutional review board of the Nan Fang Hospital.

Immunohistochemistry (IHC)

Sections from formalin-fixed and paraffin-embedded diagnostic samples were collected for histological review and immunohistochemical analysis. IHC was carried out using a peroxidase-conjugated labeled dextran polymer method as previously described [4]. Rabbit polyclonal antibody for BCL6 (N3) was from Santa Cruz Biotechnology. The mouse monoclonal antibody (mAb) for CD10 (Clone 56C6) was from Novocastra, mAb for MUM1 (clone MUM1p) was from Dako, and mAbs for CD44H (clone 156-3C11) and CD44v6 (clone VVF-7) were from ZSGB-BIO, Beijing, China. CD44 isoform expression was scored using a semiquantitative method as described by Fromowitz et al [5] Briefly, 500 cells in 5 well-preserved areas were scored for overall staining intensity and the percentage of the positively stained cells. Intensity was graded as negative (0) (no evidence of staining), low (1) (weak positive staining), moderate (2) (positive staining), and intense (3) (strong positive staining). Proportional staining was classified as negative (0): < 5%, (1): 5-25%, (2): 26-50%, (3): 51-75%, (4) : >75%. A composite score ranging from 0 to 7 was then calculated as the sum of intensity and percentage scores. The cut-off for CD44H positivity was >= 1 while the cut-off for CD44v6 was >= 2. For COO (GCB/non-GCB) sub-classification, CD10, BCL6, and Mum1 positivity was defined according to Hans et al [6].

Statistical analysis

Statistical analyses were performed using the SPSS software, version 13.0. The Spearman test was used to analyze relationships between the CD44H and CD44v6 expression. The Mann-Whitney U-test was applied to assess the difference between group means. Event free survival (EFS) was calculated from the date of diagnosis to the date of documented disease progression, relapse or death from any cause or to the date on which the study was stopped. Overall survival (OS) was calculated from the date of diagnosis until death from any cause or the last follow-up. The Kaplan-Meier method was used to estimate the OS and EFS distributions with the log-rank test performed to compare the survival curves. Univariable and multivariable modeling of OS and EFS was based on the Cox regression analysis. $P < 0.05$ was considered to indicate statistical significance.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, IARC: **WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.** In. Geneva, Switzerland: World Health Organization; 2008
2. Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, Rosenberg SA, Coltman CA, Tubiana M: **Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting.** *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1989, **7:**1630-1636.
3. Project. TIN-HsLPF: **A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project.** *The New England journal of medicine* 1993, **329:**987-994.
4. Li HL, Huang XP, Zhou XH, Ji TH, Wu ZQ, Wang ZQ, Jiang HY, Liu FR, Zhao T: **Correlation of seven biological factors (Hsp90a, p53, MDM2, Bcl-2, Bax, Cytochrome C, and Cleaved caspase3) with clinical outcomes of ALK+ anaplastic large-cell lymphoma.** *Biomedical and environmental sciences : BES* 2011, **24:**630-641.
5. Fromowitz FB, Viola MV, Chao S, Oravez S, Mishriki Y, Finkel G, Grimson R, Lundy J: **ras p21 expression in the progression of breast cancer.** *Human pathology* 1987, **18:**1268-1275.
6. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Muller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, et al: **Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray.** *Blood* 2004, **103:**275-282.

Wei et al.

Additional file 2.

Table S1. Clinical features and CD44 variant expression in the CHOP and R-CHOP groups.

		CHOP		R-CHOP		
Characteristics	*N*	n	%	n	%	*P*
Gender						0.380
Female	50	25	47.2	25	39.1	
Male	67	28	52.8	39	60.1	
Age(years)						0.487
<60y	71	34	64.2	37	57.8	
≥60y	46	19	35.8	27	42.2	
Systemic symptoms						0.360
A	78	33	62.3	45	70.3	
B	39	20	37.3	19	29.7	
BM involvement						0.004
Yes	22	16	30.2	6	9.4	
No	95	37	69.8	58	90.6	
LDH levels						0.015
Normal	71	26	47.2	45	70.3	
High	46	27	52.8	19	29.7	
Ann Arbor stage						0.884
I or II	45	20	37.3	25	39.1	
III or IV	72	33	62.3	39	60.9	
No. of extranodal sites						0.498
0-1	81	35	66.0	46	71.9	
≥2	36	18	34.0	18	28.1	
IPI score						0.104
0-1	54	20	37.7	34	53.1	
2	26	13	24.5	13	20.3	
3	18	7	13.2	11	17.2	
4-5	19	13	24.5	6	9.4	
COO subtype						0.707
GCB	42	20	37.7	22	34.4	
NON-GCB	75	33	62.3	42	65.6	
CD44H						0.243
-	42	16	30.2	26	40.6	
+	75	37	69.8	38	59.4	
CD44v6						0.261
-	77	32	60.4	45	70.3	
+	40	21	39.6	19	29.7	

Mann-Whitney U test was applied to assess the difference in distribution between the CHOP and R-CHOP groups.

Additional file 2.

Table S2.Patient characteristics according to CD44H and CD44v6 expression status.

Characteristics	*n*	CD44H		*P*	CD44v6		*P*
		—	+		—	+	
Gender				0.173			0.113
Female	53(44.2%)	15	38		39	14	
Male	67(55.8%)	27	40		40	27	
Age(years)				0.276			0.309
<60y	72(60.0%)	28	44		50	22	
≥60y	48(40.0%)	14	34		29	19	
Systemic symptoms				0.887			0.782
A	81(67.5%)	28	53		54	27	
B	39(32.5%)	14	25		25	14	
IPI score				0.130			0.642
0-2	83(72.5%)	33	50		55	28	
3-5	37(27.5%)	9	28		24	13	
Bone marrow involvement				0.014			0.365
Yes	23(19.2%)	3	20		17	6	
No	97(80.8%)	39	58		62	35	
Lactate dehydrogenase				0.218			0.193
Normal	74(61.7%)	29	45		52	22	
High	46(38.3%)	13	33		27	19	
Ann Arbor stage				0.756			0.309
or	48(40.0%)	16	32		29	19	
or	72(60.0%)	26	46		50	22	
No. of extranodal sites				0.421			0.273
0-1	83(69.2%)	31	52		52	31	
≥2	37(30.8%)	11	26		27	10	
Cell-of-origin subtype				0.116			0.499
GCB	43(35.8%)	19	24		30	13	
NON-GCB	77(64.2%)	23	54		49	28	
Treatment				0.243			0.261
CHOP	53(44.2%)	16	37		32	21	
R-CHOP	64(53.3%)	26	38		45	19	

Note: Three patients that were treated with therapies other than CHOP and R-CHOP were included in this table but excluded from the prognostic studies.

Additional file 2.

Table S3.Prognostic factors and multivariable survival analysis.

Prognostic factors	Multivariable analysis		
	RR	95%CI	*P*
Analysis of the entire cohort(n=117)			
Overall survival			
IPI (High:3~5 vs Low:0~2)	2.9	2.0-4.1	**<0.001**
CD44H (PosvsNeg)	1.3	0.48-3.4	0.618
CD44v6 (PosvsNeg)	2.4	1.2-4.7	**0.017**
Event-free survival			
IPI (High:3~5 vs Low:0~2)	2.4	1.8-3.3	**<0.001**
CD44H (Pos vs Neg)	1.1	0.47-2.5	0.861
CD44v6 (Pos vs Neg)	2.2	1.1-4.1	**0.019**
Analysis of the CHOP group (n=53)			
Overall survival			
IPI (High:3~5 vs Low:0~2)	2.6	1.7-4.0	**<0.001**
CD44H (PosvsNeg)	0.93	0.25-3.4	0.909
CD44v6 (PosvsNeg)	2.1	0.9-5.3	0.098
Event-free survival			
IPI (High:3~5 vs Low:0~2)	2.5	1.7-3.8	**<0.001**
CD44H (PosvsNeg)	0.76	0.22-2.6	0.664
CD44v6 (PosvsNeg)	2.1	0.87-5.2	0.096
Analysis of the R-CHOP group (n=64)			
Overall survival			
IPI (High:3~5 vs Low:0~2)	2.9	1.5-5.5	**0.002**
CD44H (PosvsNeg)	1.6	0.3-8.7	0.579
CD44v6 (PosvsNeg)	2.3	0.6-8.5	0.226
Event-free survival			
IPI (High:3~5 vs Low:0~2)	2.0	1.5-4.5	**0.003**
CD44H (PosvsNeg)	1.3	0.38-4.4	0.691
CD44v6 (PosvsNeg)	2.0	0.69-5.9	0.201

Additional File 3, Wei et al.

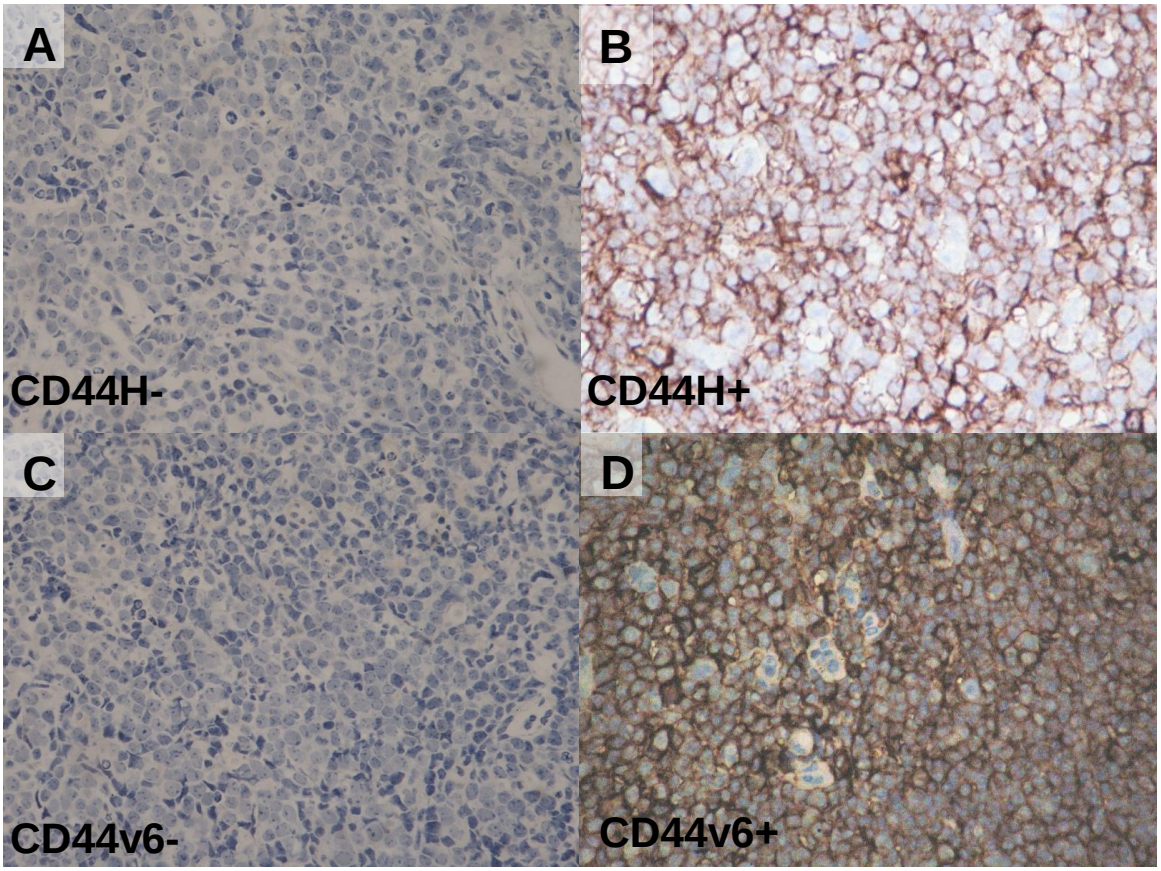

Figure A1. Representative immunohistochemical staining of DLBCL samples for CD44H and CD44v6 expression. (**A, C**), negative control stain using isotype-matched Abs. (**B**) CD44H staining in a positive case. (**D**) CD44v6 staining in a positive case.