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Original Article

Prevalence and clinicopathologic features of CD30-positive de novo diffuse large B-cell lymphoma in Chinese patients: a retrospective study of 232 cases

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Abstract: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease that great efforts had been made in to build up molecular and immunophenotypic subgroups that could relatively accurate indicate prognosis and give clue to therapy. Recently, CD30 was reported as a useful predictor with favorable clinical outcome. However, CD30 expression patterns and the clinicopathologic features of CD30 positive DLBCL are not well described thus far, especially in Asian patients. Here, we studied 232 cases of de novo DLBCL in East China to investigate the prevalence and clinicopathological features of CD30-positive DLBCL using a panel of immunohistochemical markers. Applying a >0% threshold, CD30 was expressed in approximately 12% patients with Epstein-Barr virus (EBV) negative DLBCL, affecting younger people and showing a lower frequency of BCL2 expression and MYC/BCL2 co-expression. Patients with CD30-positive DLBCLs showed better progression-free survival and overall survival compared with patients with CD30-negative DLBCLs, although the superior outcome of CD30 positivity had minimal effects on BCL2+ DLBCL or DLBCL with MYC/BCL2 co-expression. Moreover, CD30 could express in CD5+ DLBCL. We concluded that CD30 may be useful as a prognostic marker in rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) treated DLBCLs, indicating favorable outcomes in a Chinese population. Further studies with larger samples should be performed to investigate the function of CD30 expression in BCL2+ DLBCLs, DLBCLs with MYC/BCL2 co-expression, and CD5+ DLBCLs, and to evaluate the feasibility of anti-CD30 targeted treatment in DLBCL therapy.

Keywords: Diffuse large B-cell lymphoma, CD30, MYC/BCL2 co-expression lymphoma, CD5+ DLBCL

Introduction

Diffuse large B-cell lymphoma (DLBCL), the most common type of non-Hodgkin lymphoma, is the most heterogeneous group of lymphomas with variable response to immunochemotherapy. The World Health Organization (WHO) criteria have classified DLBCL into several subtypes and a few distinct disease entities. However, still a large number of not-otherwise-specified cases of DLBCL remain biologically heterogeneous without clear and acceptable criteria for subdivision. Great efforts have been made to build up molecular and immunophenotypic subgroups that could indicate prognosis and give clues about therapy. Gene expression profiling (GEP) has revealed at least two major biologically distinct groups: germinal center B cell-like (GCB) DLBCL and activated B-cell-like (ABC) DLBCL [1]. However, as GEP is laborious and quite expensive for clinical practice, immunohistochemical panels, such as Hans, Choi, Tally, and Visco-Young algorithms, have been proposed to predict the GEP subtype [2-4]. Furthermore, special cell-surface markers, such as CD99 [5], CD23 [6], and CD5 [7, 8], have also been developed to predict the prognosis of DLBCL. Recently, CD30 was reported as another useful predictor with favorable clinical outcomes [9]. However, only few studies focus on the clinicopathologic features of CD30-positive DLBCL, especially in Asian patients. Therefore,

Table 1. Antibodies and conditions used for immunohistochemical studies

Antibody	Manufacturer	Clone	Dilution	Staining conditions
CD30	Dako, Glostrup, Denmark	Ber-H2	1:30	Ventana standard retrieval
CD5	Abcam, Cambridge, UK	EPR2953	1:100	Ventana standard retrieval
CD10	Dako, Glostrup, Denmark	56C6	1:25	Ventana standard retrieval
BCL6	Dako, Glostrup, Denmark	LN22	ready to use	Ventana standard retrieval
MUM1/IRF4	Dako, Glostrup, Denmark	MUM1p	1:100	Ventana standard retrieval
BCL2	Dako, Glostrup, Denmark	124	1:50	Ventana standard retrieval
MYC	Abcam, Cambridge, UK	Y69	1:50	Ventana standard retrieval
FOXP1	Abcam, Cambridge, UK	JC12	1:50	Ventana standard retrieval

Table 2. Baseline Characteristics of the 232 patients, including CD30+ and CD30- DLBCL

	Total	CD30+ N (%)	CD30- N (%)	P
Median age (y)	59.4	55.0	60.0	
Age ≥60 y	115/232	9/28	106/204	0.049
Sex (M:F ratio)	146:86	13:15	133:71	0.054
ECOG performance status ≥2	38/196	2/21	36/175	0.379*
Advanced Ann Arbor stage (III-IV)	123/220	14/25	109/195	1.000
Extranodal involvement ≥2	47/197	5/22	42/175	0.537*
B symptoms	89/224	8/25	81/199	0.402
Elevated Serum LDH	103/221	9/24	94/197	0.344
High IPI risk group	75/217	6/24	69/193	0.296
Treatment				0.163
CHOP	90/220	7/25	83/195	
R-CHOP	130/220	18/25	112/195	
Treatment response				0.094*
CR	86/218	12/25	74/193	
PR	65/218	10/25	55/193	
NR	67/218	3/25	64/193	

*Statistical analysis using Fisher's exact test.

this study studied 232 cases of *de novo* DLBCL to investigate the prevalence and clinicopathologic features of CD30-positive DLBCL in patients in East China.

Materials and methods

Patients

This study retrospectively analyzed consecutive cases of *de novo* DLBCL diagnosed between October 2006 and December 2013, from the Departments of Pathology of The First Affiliated Hospital of Nanjing Medical University. All cases were diagnosed according to the World Health Organization (WHO) criteria and reviewed by two senior hematopathologists. DLBCL transforming from an indolent lymphoma, DLBCL-specific subtypes (T cell/histiocyte-rich DLBCL; DLBCL of the primary central nervous system; primary cutaneous DLBCL, leg type; Epstein-Barr virus [EBV]-positive DLBCL of the elderly) or variants (primary mediastinal, chronic inflammation-associated, human herpesvirus 8 [HHV8]-associated, anaplastic lymphoma kinase [ALK]-positive, plasmablastic, primary effusion, intravascular, and lymphomatoid granulomatosis), and borderline cases between DLBCL and classical Hodgkin lymphoma or Burkitt lymphoma were excluded to minimize the confounding prognostic effects of different subtypes and variants. DLBCLs with acquired immunodeficiency (affected with anti-human immunodeficiency virus or with a history of transplantation) and those with Epstein-Barr virus-encoded RNA (EBER) detected by in situ hybridization were also excluded from the study.

Clinical data, such as age, gender, sites of involvement, Eastern Cooperative Oncology Group (ECOG) performance status, B symptoms, and serum lactate dehydrogenase (LDH) concentrations, were obtained from the archive of the pathology department and the medical record of the hematology department. Cases were staged according to the ANN Arbor staging system, and international prognostic index (IPI) risk factors were evaluated. Among these 232 patients, 130 cases were treated with

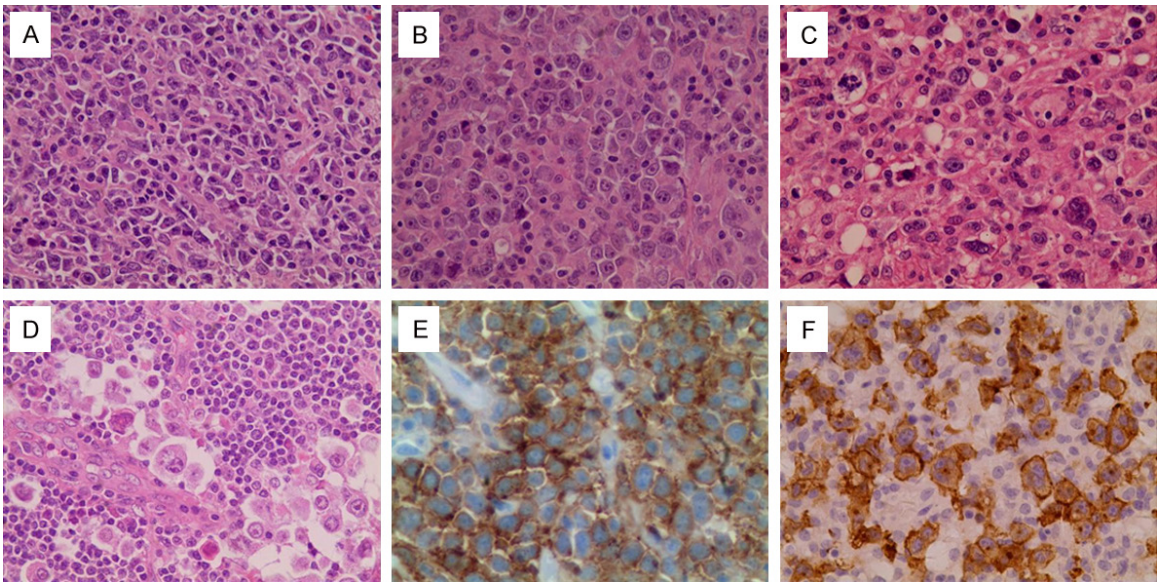


Figure 1. Morphological and immunohistochemical features of CD30+ DLBCL. Lymphoma cells were large and showed centroblastic (A), immunoblastic (B), anaplastic (C) or sinusoidal (D) feature, and CD30 were expressed on the cell membrane (E) and in the Golgi region (F). Magnification: 40×.

rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy and 90 accepted traditional cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy. Clinical follow-up was available in 218 patients and ranged from 1 to 101 months. Survival data included the progression-free survival (PFS) and the overall survival (OS). The former was defined as the time from the date of initial diagnosis to disease progression, relapse, or death from any cause, or the date of the last follow-up. The latter was defined as the time from the date of initial diagnosis to the date of death from lymphoma or the last follow-up. Only these cases dated before December 20, 2012, were included in the 2-year survival analysis. The use of these specimens and data for research purposes was approved by the Ethics Committee of the Hospital.

Immunohistochemistry

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections (4-μm-thickness) using Ventana automated stainer (BenchMark; Ventana Medical Systems, Inc, AZ, USA) according to the manufacturer's instructions. **Table 1** lists the details of all the antibodies used (CD10, BCL2, BCL6, MUM1/IRF4, FOXP1, and MYC), including their source and retrieval conditions. Normal lymph nodes with germinal center were used as positive and negative controls. For MYC immunostaining, Burkitt lymphoma was used as positive control.

Signals were interpreted by two pathologists independently. Scoring for each antibody was visually estimated in 10% increments. Disagreement >10% was resolved by joint examination under a multiheaded microscope. The cutoff value was set with reference to the literature [8, 9]. Based on the immunohistochemical findings, the cases were classified into the GCB and non-GCB subtype according to Hans and Visco-Young algorithms previously described [2].

Statistical analysis

Differences in clinical and pathological characteristics between CD30-positive and CD30-negative groups were evaluated using the Pearson χ^2 test or Fisher's exact test. PFS and OS distributions were estimated using Kaplan-Meier analysis, and the survival differences were assessed using the log-rank test. The level of significance for all statistical tests was set as $P<0.05$. Statistical evaluations were performed using the SPSS statistics software package (version 16.0; SPSS Inc., IL, USA).

Table 3. Results of the IHC stains of the 232 patients, including CD30+ and CD30- DLBCL

	Total	CD30+ N (%)	CD30- N (%)	P
CD10	59/232	4/28	55/204	0.172*
BCL6	147/232	17/28	130/204	0.756
MUM1/IRF4	150/232	20/28	130/204	0.424
FOXP1	126/232	10/28	110/204	0.071
Hans classification				0.623
GCB	83/232	6/28	77/204	
Non-GCB	149/232	22/28	127/204	
Visco-young classification				0.465
GCB	93/232	13/28	80/204	
Non-GCB	139/232	15/28	124/204	
CD5	7/232	1/28	6/204	0.599*
BCL2 (cutoff 70%)	128/232	7/28	121/204	0.001
MYC (cutoff 40%)	77/232	5/28	72/204	0.066
BCL2/MYC co-expression	53/232	1/28	52/204	0.007*
Ki67 (cutoff 70%)	148/232	17/28	131/204	0.718

*Statistical analysis using Fisher's exact test.

Results

Patient characteristics and treatment outcomes

A total of 232 cases of *de novo* DLBCL between October 2006 and December 2013 were included in the present study. These consisted of 98 primary nodal cases and 134 primary extranodal cases including 20 intestine (includes 9 small intestine, 5 ileocecum, and 6 colon), 17 stomach, 14 tonsil, 13 retroperitoneum, 10 testis, 9 spleen, 7 kidney, 6 peritoneal cavity, 6 nasal cavity/paranasal sinuses, 5 pharyngeal apparatus (includes 1 nasopharynx, and 4 oropharynx), 5 soft tissue, 5 thyroid, 4 adrenal glands, 3 breast, 2 pancreas, 2 pelvic cavity, 2 salivary glands, 2 bone, 1 liver, and 1 ureter. The baseline characteristics of the patients are listed in **Table 2**. During the median follow-up of 34.6 months, 117 (53.7%) patients experienced an event and 94 (43.1%) patients died. Among 218 patients available for evaluation for tumor response to chemotherapy, 86 (39.4%) patients achieved complete remission on initial chemotherapy and 65 (29.8%) patients experienced partly remission. The median OS and PFS were 32.3 and 26.2, respectively. Two-year OS and PFS rates were 62.4%, and 49.5%, respectively.

Morphological and immunophenotypic features of CD30-positive DLBCL

Twenty-eight patients (12.1%) showed a positive immunostaining for CD30 on the cell membrane and/or in the Golgi region (**Figure 1**). The median age was 55 years (range 19-77 years). There was a slight female predominance (1.15:1). Fifteen cases of CD30-positive *de novo* DLBCL primarily involved lymph node and 13 cases were extranodal. Among these patients, the morphological patterns were centroblastic (20), immunoblastic (2), anaplastic (5), and sinusoidal (1) types, occupying 71.4%, 7.1%, 17.9%, and 3.6% cases, respectively. Immunohistochemically, CD30 expression was seen in ≥80% of the tumor cells in eight cases (28.6%), 40%-79% in seven cases (25%), 20%-39% in seven cases (25%), and 0%-19% in six cases (21.4%). Ki67 index ranged from 40% to 90% (media 67%, compared with 74% of CD30-negative DLBCLs). The other results of the immunohistochemical stains of the individual antigens are listed in **Table 3**.

Compared with CD30-negative DLBCL, CD30-positive DLBCL affects younger populations (age <60 years), with a lower prevalence of BCL2 expression and MYC/BCL2 co-expression ($P<0.05$). Although showing female predominance and lower prevalence of FOXP1 and MYC expression, CD30-positive DLBCL do not have statistical significance compared with CD30-negative DLBCL, probably owing to the limitation of the study scale. Bivariate analysis also showed no significant difference in CD30 expression with biological subtypes (according to Hans or Visco-Young algorithm), CD10 expression, BCL6 expression, MUM1 expression, CD5 expression, or Ki67 index.

CD30 expression and survival analysis of DLBCL

Patients with CD30 expression showed significantly superior OS (P=0.007) and PFS (P=0.0-

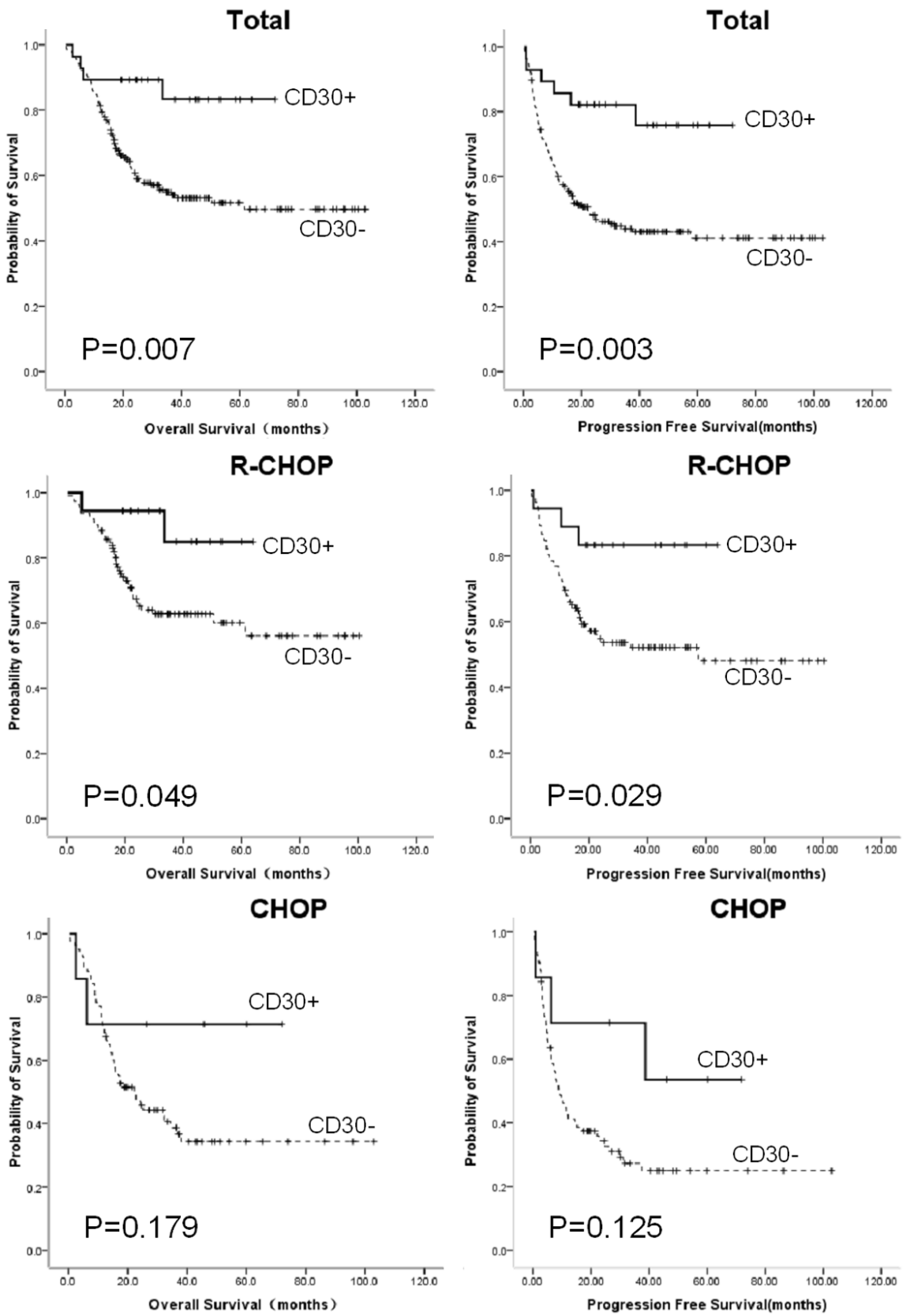


Figure 2. Kaplan-Meier plots for OS and PFS according to CD30 expression in all patients and R-CHOP treated, CHOP treated group, separately.

03) with statistical significance compared with CD30-negative cases. If these cases were separately analyzed according to their treatment protocols, the R-CHOP group had statistically significant differences in the OS (P=0.049) and PFS (P=0.029), while the CHOP group showed a tendency for superior OS (P=0.179) and PFS (P=0.125), but with no statistical significance (**Figure 2**). Bivariate analysis showed no significant difference in CD30 expression for the different ECOG performance status, Ann Arbor stage, extranodal involvement, B symptoms, serum LDH level, and IPI risk factor (**Table 2**).

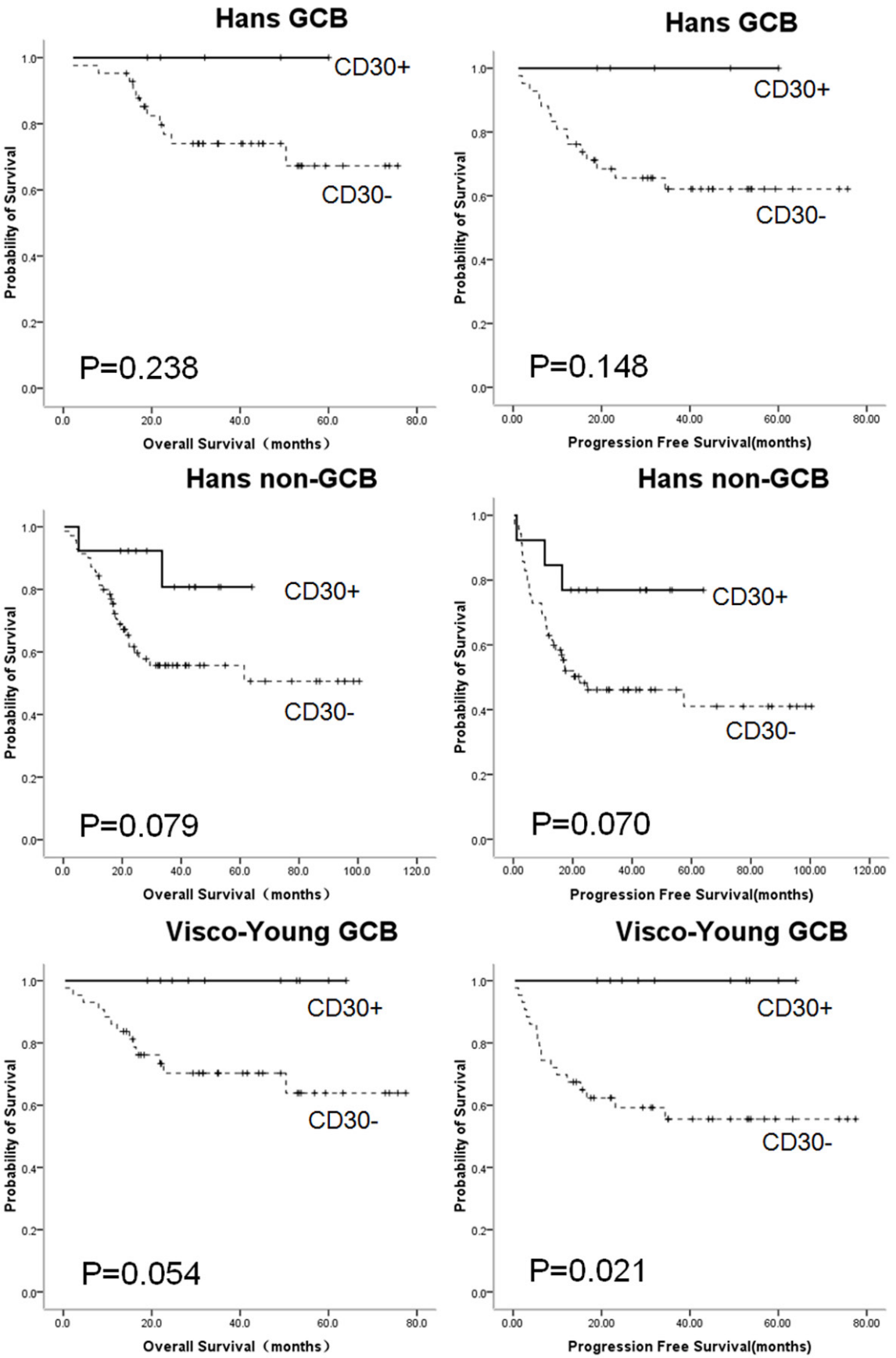
Furthermore, to minimize the prognostic influence of different biological subtypes, stratified analyses were performed to assess the prognostic effects of CD30 expression in the R-CHOP group using Hans and Visco-Young algorithms. Based on different algorithms, some cases classified into the non-GCB group in Hans's algorithm were probably reclassified into the GCB group in Visco-Young algorithm, and vice versa. Unfortunately, partly due to the lower number of cases, none of the four subgroups showed significant differences in PFS and OS (except for the GCB subtype classified according to Visco-Young algorithm; P=0.021 for PFS) between patients with CD30+ and CD30- DLBCLs (**Figure 3**).

Stratified analyses were also conducted to assess the prognostic effects of CD30 expression in BCL2-positive and BCL2-negative groups among R-CHOP-treated patients to minimize the prognostic influence of BCL2. However, there were only two cases expressing CD30 in the BCL2-positive group. NO difference was found in OS (P=0.298) and PFS (P=0.571) between patients with CD30+ and CD30- DLBCLs in the BCL2-positive group. In the BCL2-negative group, patients with CD30+ DLBCLs showed superior OS (P=0.032) and PFS (P=0.035) with statistical significance (**Figure 4**). Simultaneously, patients with CD30+ DLBCL with no MYC/BCL2 co-expression demonstrated superior outcomes compared with patients with DLBCL with MYC/BCL2 co-expression (**Figure 4**).

Discussion

CD30, which is clinically used as an important marker for the diagnosis of Hodgkin lymphoma and anaplastic large cell lymphoma, is a type I transmembrane glycoprotein and a member of the tumor necrosis factor (TNF) receptor super family [10]. CD30 is expressed in a subset of activated B and T lymphocytes and natural killer (NK) cell tumors, such as primary mediastinal large B-cell lymphoma, EBV-positive lymphomas (lymphomatoid granulomatosis, EBV-positive DLBCL of the elderly and primary effusion lymphoma), large-cell transformation of mycosis fungoides [11], and some cases of extranodal NK/T-cell lymphoma, nasal type [12, 13], as w ell as in some conditions such as infectious mononucleosis. In addition, CD30 expression can also be detected in non-hematopoietic neoplasms including embryonal carcinoma, seminomas, and a subset of melanomas and mesotheliomas.

Recently, CD30 expression has been detected in a subset of DLBCL not otherwise specified (NOS). The recent International DLBCL Rituximab-CHOP Consortium Program Study (IDCP) study [9] reported a 14% prevalence ofCD30 expression in 903 cases of *de novo* DLBCLs, using the 20% cutoff threshold. Campuzano-Zuluaga et al [14] found that 21% of 167 *de novo* DLBCL cases contained 20% or more CD30+ tumor cells. It was a retrospective study based on a predominately Latin American population that included patients with a high infection rate (27.5%) of EBV status and unknown human immunodeficiency virus (HIV) status. Another study from British Columbia [15] comprising definite HIV-negative patients demonstrated 11% cases containing 20% or more CD30+ tumor cells and 25% cases interpreted as CD30 positive if employing a >0% cutoff for CD30 positivity. This study found 12% cases of *de novo* DLBCL showing CD30 positivity using >0% cutoff threshold. If >20% cutoff was set as threshold for CD30 positivity, 9.5% cases were positive with CD30 expression. Those with only scattered reactive CD30+ immunoblasts in the background area were interpreted as negative. The relatively low positive rate of CD30 in this study could be due in part to the underlying patient demographic differences as well as the variations in study population imparted by different sampling strategies. It was reported that EBV-positive DLBCLs more frequently showed CD30 expression than EBV-negative DLBCL [9, 15]. Furthermore, patients with DLBCL with concurrent CD30 expression and EBV infection showed markedly worse outcomes than those with CD30+EBER- or CD30-EBER+ DLBCL, indi-


Hans GCB
CD30+
CD30-
P=0.238
Probability of Survival
Overall Survival（months）
Hans GCB
CD30+
CD30-
P=0.148
Probability of Survival
Progression Free Survival(months)
Hans non-GCB
CD30+
CD30-
P=0.079
Probability of Survival
Overall Survival（months）
Hans non-GCB
CD30+
CD30-
P=0.070
Probability of Survival
Progression Free Survival(months)
Visco-Young GCB
CD30+
CD30-
P=0.054
Probability of Survival
Overall Survival（months）
Visco-Young GCB
CD30+
CD30-
P=0.021
Probability of Survival
Progression Free Survival(months)

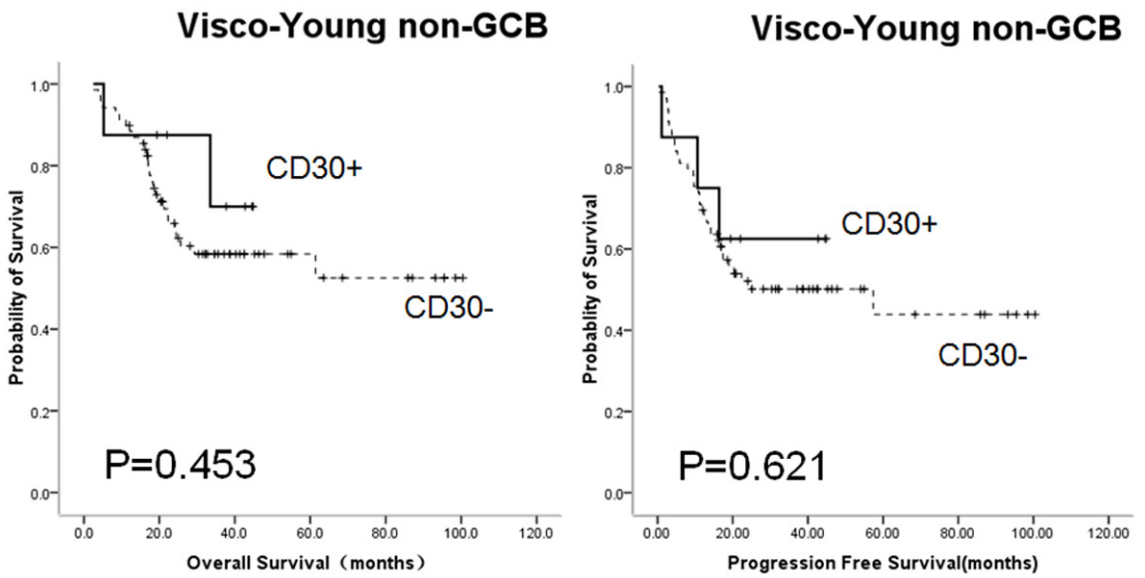


Figure 3. Kaplan-Meier plots for OS and PFS according to CD30 expression for GCB or non-GCB subtype classified by Hans's algorithm and Vsico-Young algorithm.

cating CD30+EBV+ DLBCL is a unique subset of lymphoma with aggressive clinical course [9, 15]. Hence, this study excluded EBV+ DLBCL cases. If EBV-positive cases were excluded from Graham's study [15], the prevalence of CD30 positivity was 9.0% (20% cutoff) and 22.9% (0% cutoff).

Younger patients with CD30-positive DLBCL showed a lower frequency of BCL2 expression and MYC/BCL2 co-expression, and demonstrated better outcomes, compared with CD30-negative DLBCL. The results were consistent with the IDCP study. However, Campuzano-Zuluaga's study demonstrated a higher frequency of BCL2 co-expression in CD30+ DLBCLs. He gave an explanation of CD30+ DLBCL and BCL2+ DLBCL both having predilection to younger patients and non-GCB phenotype, resulting in their correlation [14]. Conversely, the present study reported that the lower frequency of BCL2 expression and MYC/BCL2 co-expression could be a synergistic factor contributing to the favorable prognosis of patients with CD30+ DLBCL. However, CD30+ DLBCL had superior OS and PFS in the BCL2- group and the group with no MYC/BCL2 co-expression, indicating that CD30 could be an independent prognostic factor in DLBCL.

Moreover, this study tried to investigate the cumulative influence of CD30 and different biological subtypes on the patients' outcomes. However, no statistically significant difference was found between patients with CD30+ and CD30- DLBCL in the GCB or non-GCB subgroup, whether using Hans or Visco-Young algorithm, owing to the limited number of the DLBCL cases in the R-CHOP treatment group. It is speculated that CD30+ DLBCL with the GCB subtype predicted the best outcome in patients, especially using Visco-Young algorithm. From a different perspective, although Hans and Visco-Young algorithms are both GEP-related immunohistochemistry algorithms [2], the study also showed their difference and limitation for excellent concordance with the GEP subdivision. The drawback of this retrospective study was not having enough fresh tissues to subject to the GEP study. Despite this, the sample size was adequate to assess the overall frequency of CD30 expression and its correlation with other clinical pathological features, as well as its prognostic value.

Notably, there were two interesting cases in the CD30+ *de novo* DLBCL group. First, case No. 98, a 58-year-old male, presenting with a palpable mass in the supraclavicular fossa, clinically III B stage and age-adjusted IPI 2, was diagnosed as DLBCL, a non-GCB phenotype. Tumor cells had centroblastic morphology (CD30 positive in 70% tumor cells, BCL2 in >70% tumor cells, and also MYC in >40% tumor

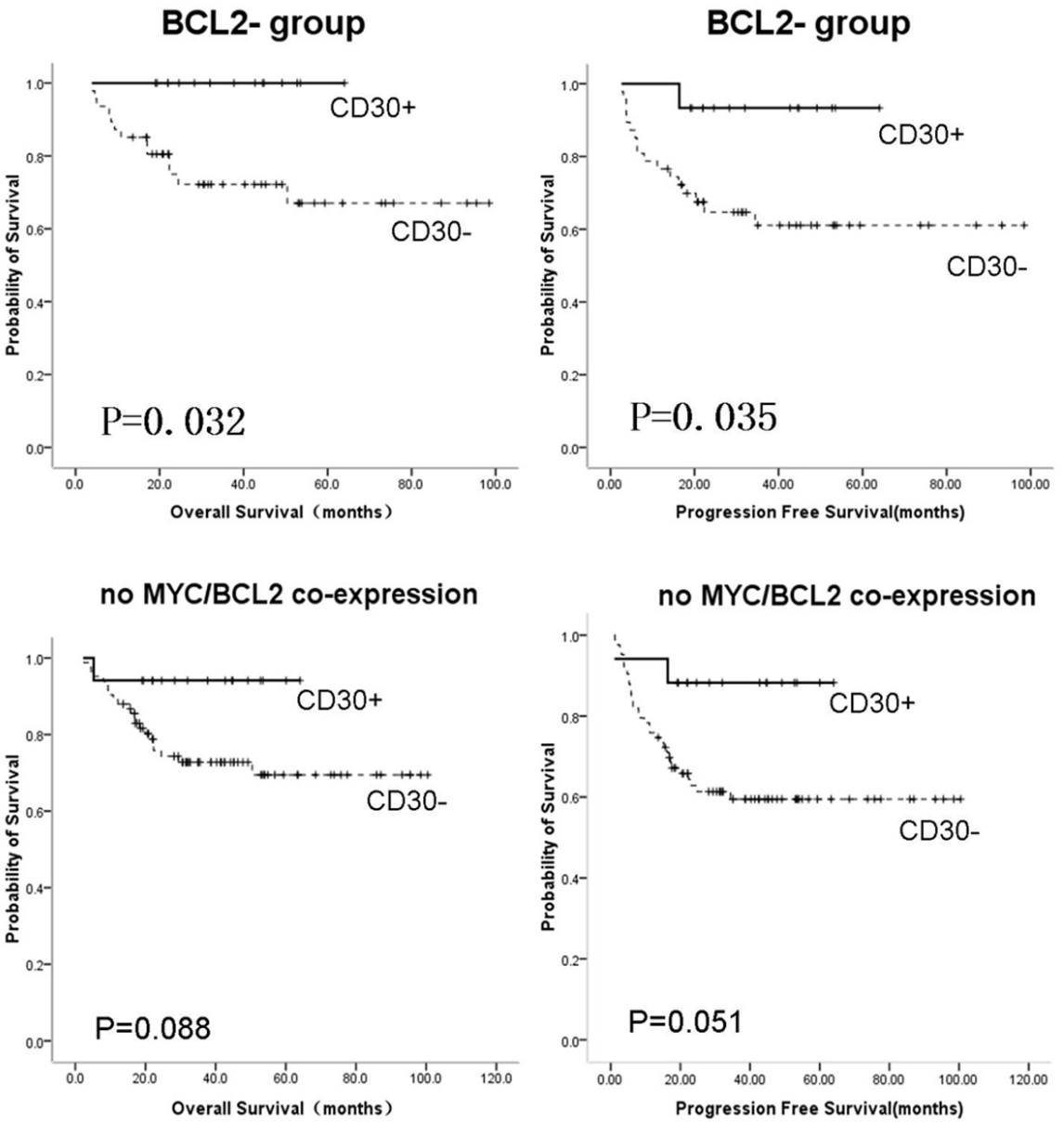


Figure 4. Kaplan-Meier plots for OS and PFS according to CD30 expression in BCL2 negative group and no MYC/BCL2 co-expression group among R-CHOP treated patients.

cells) (Supplementary Figure 1). It could be classified into the group of lymphomas with MYC/BCL2 co-expression, an expanded concept of double-hit lymphomas [16]. The patient accepted CHOP chemotherapy plus rituximab, experienced a partial remission, relapsed in 10.6 months, and died in 33.5 months. Another 29 cases of DLBCLs with CD30-MYC/BCL2 co-expression were treated with R-CHOP therapy in this study, with median PFS and OS being 14.1 and 20.4 months, respectively. The IDCP study observed that CD30+ DLBCL with MYC/BCL2 co-expression showed similar survival to CD30- DLBCL without MYC/BCL2 co-expression due to the antagonistic prognostic impact of CD30 expression and MYC/BCL2 double positivity. The present study did not find any prognostic difference between patients with CD30+ and CD30- DLBCL with MYC/BCL2 co-expression. More efforts should be made to investigate the function of CD30 expression in double-hit lymphomas or DLBCL with MYC/BCL2 co-expression.

Another interesting case was case No. 142, a 29-year-old female who presented with a huge

retroperitoneum mass, advanced Ann Arbor stage (IVA), elevated serum LDH level (978 U/L), and high age-adjusted IPI score (3). Immunohistochemically, tumor cells were positive for CD5, CD30, BCL2, and MUM1, but negative for CyclinD1 and CD23, which excluded the possibility of mantle cell lymphoma or small lymphocytic lymphoma (Supplementary Figure 2). EBER in situ hybridization stain for EBV was also negative. The patient experienced an aggressive progression with no remission to chemotherapy, and died 5.2 months after the diagnosis. In our cohort, another six cases of CD5+ DLBCL were identified; the clinicopathologic details of the CD5+ DLBCLs are listed in Supplementary Tables 1 and 2. The median PFS and OS were 8.5 and 14.9, markedly worse than the overall data (26.8 and 32.5, respectively). It seems that CD5+ DLBCL with adverse prognoses cannot be neutralized by CD30 expression. Studies [9] revealed that CD30 may participate in the upregulation of genes encoding negative regulators of NF-κB activation and lymphocyte survival as the molecular mechanism of its antiproliferative function. However, CD5 can promote B-cell survival by supporting the production of IL-10 and can exert negative feedback on B cell receptor signaling events that can contribute to cell death [17, 18]. Although the role of CD30 and CD5 in DLBCL biology is not completely understood, their effects may follow different paths.

In the era of targeted therapy for DLBCL, CD30 has emerged as an important molecular target. Brentuximab vedotin (a drug combining an anti-CD30 monoclonal antibody and the antitubulin agent monomethyl auristatin E), efficacious in the treatment of relapsed classical Hodgkin lymphoma and systemic anaplastic large-cell lymphoma, was reported to achieve an objective clinical response in patients with CD30+ DLBCL [19], showing a promising approach that may increase the response rate and prolong the survival time in patients with relapsed or refractory CD30+ DLBCL.

CD30 is expressed in approximately 12% patients with EBV-negative *de novo* DLBCL in East China, affecting younger patients and showing a lower frequency of BCL2 expression and MYC/BCL2 co-expression. CD30 immunohistochemistry may be useful as a prognostic marker in R-CHOP and CHOP treated DLBCL, as it is associated with a trend toward a better outcome. However, the superior outcome of CD30 positivity had minimal effects in DLBCL with BCL2+ or CMY/BCL2 co-expression. Further studies with larger samples should be performed to investigate the function of CD30 expression in BCL2+ DLBCLs, DLBCLs with MYC/BCL2 co-expression, and CD5+ DLBCLs, where in CD30+ cases may have poor clinical outcomes and need the salvage therapy of anti-CD30 targeted treatment.

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Disclosure of conflict of interest

None.

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References

[1] Menon MP, Pittaluga S, Jaffe ES. The histological and biological spectrum of diffuse large B-cell lymphoma in the World Health Organization classification. Cancer J 2012; 18: 411-420.

[2] Hwang HS, Park CS, Yoon DH, Suh C, Huh J. High concordance of gene expression profiling-correlated immunohistochemistry algorithms in diffuse large B-cell lymphoma, not otherwise specified. Am J Surg Pathol 2014; 38: 1046-1057.

[3] Culpin RE, Sieniawski M, Angus B, Menon GK, Proctor SJ, Milne P, McCabe K, Mainou-Fowler T. Prognostic significance of immunohistochemistry-based markers and algorithms in immunochemotherapy-treated diffuse large B cell lymphoma patients. Histopathology 2013; 63: 788-801.

[4] Visco C, Li Y, Xu-Monette ZY, Miranda RN, Green TM, Li Y, Tzankov A, Wen W, Liu WM, Kahl BS, d'Amore ES, Montes-Moreno S, Dybkær K, Chiu A, Tam W, Orazi A, Zu Y, Bhagat G,

Winter JN, Wang HY, O'Neill S, Dunphy CH, Hsi ED, Zhao XF, Go RS, Choi WW, Zhou F, Czader M, Tong J, Zhao X, van Krieken JH, Huang Q, Ai W, Etzell J, Ponzoni M, Ferreri AJ, Piris MA, Møller MB, Bueso-Ramos CE, Medeiros LJ, Wu L, Young KH. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Leukemia 2012; 26: 2103-2113.

[5] Hong J, Park S, Park J, Jang SJ, Ahn HK, Sym SJ, Cho EK, Shin DB, Lee JH. CD99 expression and newly diagnosed diffuse large B-cell lymphoma treated with rituximab-CHOP immunochemotherapy. Ann Hematol 2012; 91: 1897-1906.

[6] Linderoth J, Jerkeman M, Cavallin-Ståhl E, Kvaløy S, Torlakovic E; Nordic Lymphoma Group Study. Immunohistochemical expression of CD23 and CD40 may identify prognostically favorable subgroups of diffuse large B-cell lymphoma: a Nordic Lymphoma Group Study. Clin Cancer Res 2003; 9: 722-728.

[7] Yamaguchi M, Seto M, Okamoto M, Ichinohasama R, Nakamura N, Yoshino T, Suzumiya J, Murase T, Miura I, Akasaka T, Tamaru J, Suzuki R, Kagami Y, Hirano M, Morishima Y, Ueda R, Shiku H, Nakamura S. De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood 2002; 99: 815-821.

[8] Niitsu N, Okamoto M, Tamaru JI, Yoshino T, Nakamura N, Nakamura S, Ohshima K, Nakamine H, Hirano M. Clinicopathologic characteristics and treatment outcome of the addition of rituximab to chemotherapy for CD5-positive in comparison with CD5-negative diffuse large B-cell lymphoma. Ann Oncol 2010; 21: 2069-2074.

[9] Hu S, Xu-Monette ZY, Balasubramanyam A, Manyam GC, Visco C, Tzankov A, Liu WM, Miranda RN, Zhang L, Montes-Moreno S, Dybkær K, Chiu A, Orazi A, Zu Y, Bhagat G, Richards KL, Hsi ED, Choi WW, Han van Krieken J, Huang Q, Huh J, Ai W, Ponzoni M, Ferreri AJ, Zhao X, Winter JN, Zhang M, Li L, Møller MB, Piris MA, Li Y, Go RS, Wu L, Medeiros LJ, Young KH. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2013; 121: 2715-2724.

[10] Sotomayor EM, Young KH, Younes A. Clinical roundtable monograph: CD30 in lymphoma: its role in biology, diagnostic testing, and targeted therapy. Clin Adv Hematol Oncol 2014; 12: 1-22.

[11] Romero M, Haney M, Desantis E, Zlotoff B. Mycosis fungoides with focal CD30 transformation in an adolescent. Pediatr Dermatol 2008; 25: 565-568.

[12] Pongpruttipan T, Sukpanichnant S, Assanasen T, Wannakrairot P, Boonsakan P, Kanoksil W, Kayasut K, Mitarnun W, Khuhapinant A, Bunworasate U, Puavilai T, Bedavanija A, Garcia-Herrera A, Campo E, Cook JR, Choi J, Swerdlow SH. Extranodal NK/T-cell lymphoma, nasal type, includes cases of natural killer cell and αβ, γδ, and αβ/γδ T-cell origin: a comprehensive clinicopathologic and phenotypic study. Am J Surg Pathol 2012; 36: 481-499.

[13] Cao Q, Huang Y, Ye Z, Liu N, Li S, Peng T. Primary spleen extranodal NK/T cell lymphoma, nasal type, with bone marrow involvement and CD30 positive expression: a case report and literature review. Diagn Pathol 2014; 9: 169.

[14] Campuzano-Zuluaga G, Cioffi-Lavina M, Lossos IS, Chapman-Fredricks JR. Frequency and extent of CD30 expression in diffuse large B-cell lymphoma and its relation to clinical and biologic factors: a retrospective study of 167 cases. Leuk Lymphoma 2013; 54: 2405-2411.

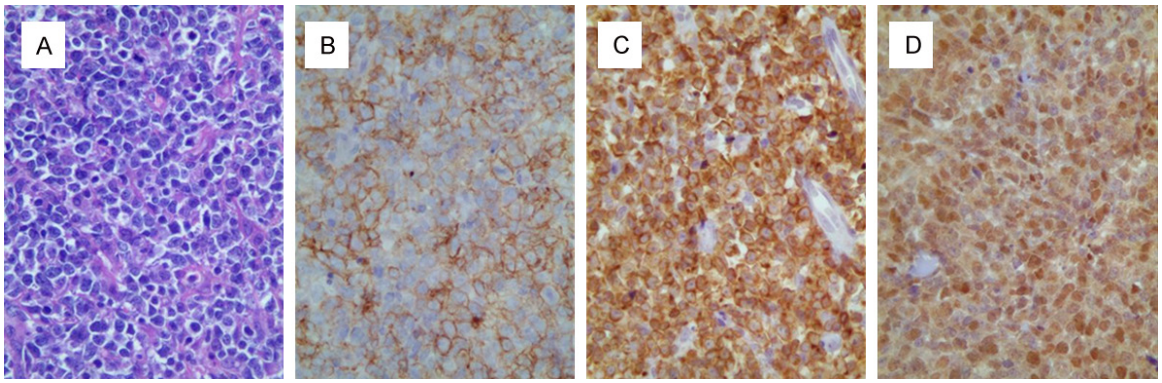
[15] Slack GW, Steidl C, Sehn LH, Gascoyne RD. CD30 expression in de novo diffuse large B-cell lymphoma: a population-based study from British Columbia. Br J Haematol 2014; 167: 608-617.

[16] Cheah CY, Oki Y, Westin JR, Turturro F. A clinician's guide to double hit lymphomas. Br J Haematol 2015; 168: 784-795.

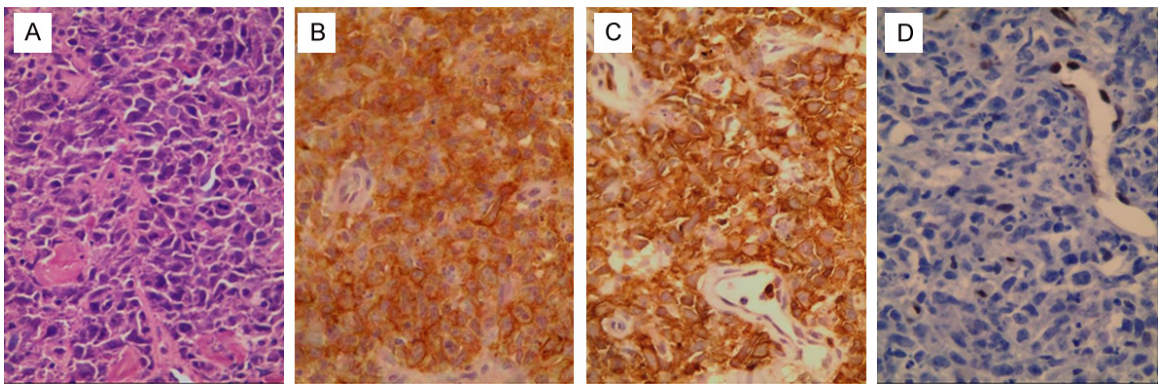
[17] Gary-Gouy H, Harriague J, Bismuth G, Platzer C, Schmitt C, Dalloul AH. Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. Blood 2002; 100: 4537-4543.

[18] Dalloul A. CD5: a safeguard against autoimmunity and a shield for cancer cells. Autoimmun Rev 2009; 8: 349-353.

[19] Hill BT, Tubbs Do RR, Smith MR. Complete Remission of CD30 Positive Diffuse Large B-Cell Lymphoma (DLBCL) in a Patient with Post-Transplant Lymphoproliferative Disorder (PTLD) and End-Stage Renal Disease Treated with Single Agent Brentuximab Vedotin. Leuk Lymphoma 2015; 56: 1552-1553.



Supplementary Figure 1. CD30+ DLBCL with MYC/BCL2 co-expression (Case No. 98). Tumor cells were centroblastic morphology (A), expressing CD30 (B), BCL2 (C) and MYC (D).



Supplementary Figure 2. CD5+ DLBCL with CD30 expression (Case No. 142). Tumor cells showed scanty cytoplasm, moderate to severe nuclear atypia, and mitotic activity. Apoptotic debris was also seen (A). Tumor cells were positive for CD30 (B) and CD5 (C) expression and negative for CyclinD1 (D), while endothelial cells were positive as inner control.

Supplementary Table 1. Clinical Features of the CD5+ DLBCL patients

Case no.	Age	Sex	Stage	IPI/aaIPI	LDH (u/L)	Regimen	Prognosis	PFS	OS
018	59	Male	IVB	3	332	R-CHOP	Died	2.5	2.5
112	73	Fe	IIIB	3	527	R-CHOP	Died	17.4	20
133	67	Fe	IIA	1	144	R-CHOP	Alive	2.9	36.4
142	29	Fe	IVA	3	978	CHOP	Died	1.1	5.2
168	50	Fe	IIIA	2	410	CHOP	Died	9.6	13.3
207	72	Fe	IIIB	3	272	CHOP	Died	12.2	12.2
224	78	Fe	IVB	3	154	R-CHOP	Died	14.2	19.3

Supplementary Table 2. Pathological Features of the CD5+ DLBCL patients

Case no.	Morphological pattern	Immunohistochemical stains								Phenotype
		CD30	CD10	BCL6	MUM1	FOXP1	BCL2	CMYC	Ki67	
018	Intravascular	-	-	-	-	-	+	+	40%	non-GCB
112	centroblastic	-	+	+	+	+	+	-	70%	GCB
133	centroblastic	-	-	+	+	-	-	-	70%	non-GCB
142	centroblastic	+	-	-	+	+	+	-	90%	non-GCB
168	centroblastic	-	-	-	+	+	+	+	90%	non-GCB
207	centroblastic	-	-	-	+	+	+	+	60%	non-GCB
224	centroblastic	-	-	+	+	+	+	-	90%	non-GCB