VOLUME 26 · NUMBER 3 · JANUARY 20 2008

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

LMO2 Protein Expression Predicts Survival in Patients With Diffuse Large B-Cell Lymphoma Treated With Anthracycline-Based Chemotherapy With and Without Rituximab

Yasodha Natkunam, Pedro Farinha, Eric D. Hsi, Christine P. Hans, Robert Tibshirani, Laurie H. Sehn, Joseph M. Connors, Dita Gratzinger, Manuel Rosado, Shuchun Zhao, Brad Pohlman, Nicholas Wongchaowart, Martin Bast, Abraham Avigdor, Ginette Schiby, Arnon Nagler, Gerald E. Byrne, Ronald Levy, Randy D. Gascoyne, and Izidore S. Lossos

From the Department of Pathology and Department of Medicine, Division of Oncology, Stanford University School of Medicine; Departments of Health Research and Policy and Statistics, Stanford University, Stanford, CA; Departments of Clinical Pathology and Hematologic Oncology and Blood Disorders, Cleveland Clinic Foundation, Cleveland, OH; Departments of Pathology and Medicine, University of Nebraska Medical Center, Omaha, NE; Department of Medicine, Division of Hematology-Oncology and Molecular and Cellular Pharmacology, Sylvester Comprehensive Cancer Center, and Department of Pathology, University of Miami, Miami, FL; Department of Pathology and Division of Medical Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; and Chaim-Sheba Medical Center, Tel-Aviv, Israel.

Submitted June 14, 2007; accepted October 15, 2007; published online ahead of print at www.jco.org on December 17, 2007.

Supported by Grant Nos. NIH CA109335, NIH CA34233, NIH CA 33399, and CA122105 from the National Institutes of Health, a Leukemia and Lymphoma Society SCORE grant, National Cancer Institute of Canada Terry Fox Program Project Grant No. 016003, Grant No. 06BCBG113 from the Bankhead-Coley Foundation, FL, and the Dwoskin Family Foundation.

R.D.G. and I.S.L. contributed equally to this work.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Izidore S. Lossos, MD, Sylvester Comprehensive Cancer Center, Department of Medicine, Division of Hematology-Oncology, University of Miami, 1475NW 12th Ave (D8-4), Miami, FL 33136; e-mail: ilossos@med.miami.edu.

0732-183X/08/2603-447/$20.00

DOI: 10.1200/JCO.2007.13.0690

ABSTRACT

Purpose
The heterogeneity of diffuse large B-cell lymphoma (DLBCL) has prompted the search for new markers that can accurately separate prognostic risk groups. We previously showed in a multivariate model that *LMO2* mRNA was a strong predictor of superior outcome in DLBCL patients. Here, we tested the prognostic impact of LMO2 protein expression in DLBCL patients treated with anthracycline-based chemotherapy with or without rituximab.

Patients and Methods
DLBCL patients treated with anthracycline-based chemotherapy alone (263 patients) or with the addition of rituximab (80 patients) were studied using immunohistochemistry for LMO2 on tissue microarrays of original biopsies. Staining results were correlated with outcome.

Results
In anthracycline-treated patients, LMO2 protein expression was significantly correlated with improved overall survival (OS) and progression-free survival (PFS) in univariate analyses (OS, $P = .018$; PFS, $P = .010$) and was a significant predictor independent of the clinical International Prognostic Index (IPI) in multivariate analysis. Similarly, in patients treated with the combination of anthracycline-containing regimens and rituximab, LMO2 protein expression was also significantly correlated with improved OS and PFS (OS, $P = .005$; PFS, $P = .009$) and was a significant predictor independent of the IPI in multivariate analysis.

Conclusion
We conclude that LMO2 protein expression is a prognostic marker in DLBCL patients treated with anthracycline-based regimens alone or in combination with rituximab. After further validation, immunohistologic analysis of LMO2 protein expression may become a practical assay for newly diagnosed DLBCL patients to optimize their clinical management.

J Clin Oncol 26:447-454. © 2008 by American Society of Clinical Oncology

INTRODUCTION

Gene expression profiling has been used to determine prognostic subgroups in diffuse large B-cell lymphoma (DLBCL).[1-4] The pivotal study of Alizadeh et al[1] led to the discovery that the overall survival (OS) is significantly longer in DLBCL patients with a gene expression profile similar to that of normal germinal center (GC) B cells. This result suggests that the cell of origin has an impact on clinical outcome. Several models have been developed based either on RNA or protein expression to predict survival in DLBCL patients[5]; however, a consensus on how to stratify DLBCL patients has not been achieved. To avoid the limitations of fresh tissue and for ease of use in routine clinical practice, many recent studies have also focused on the use of immunohistochemistry to identify risk groups.

From gene expression studies, *LMO2* mRNA expression emerged as the strongest single predictor of superior outcome in DLBCL patients in a multivariate model based on the expression of six genes.[6] We developed a monoclonal anti-LMO2 antibody and documented that the LMO2 protein is expressed in normal GC B cells and in a subset of GC-derived B-cell lymphomas.[7] Here, we examined whether LMO2 protein expression, as assessed in archival diagnostic biopsies from 263 DLBCL patients who were treated with anthracycline-based regimens, can predict outcome. The addition of the

anti-CD20 monoclonal antibody rituximab to anthracycline-based chemotherapy (cyclophosphamide, vincristine, doxorubicin, and prednisone [CHOP]) was recently shown to improve the survival of DLBCL patients.[8-11] The clinical applicability of a prognostic factor may depend on a specific therapy, and its usefulness should be reassessed when therapies change.[12,13] Therefore, we analyzed the impact of LMO2 protein expression in 80 DLBCL patients treated with rituximab plus CHOP (R-CHOP) to test its prognostic value in the rituximab era.

PATIENTS AND METHODS

Patient Selection

A total of 343 specimens were studied; 263 specimens were from patients treated with anthracycline-containing chemotherapy (CHOP or CHOP-like regimens) and were contributed from the British Columbia Cancer Agency, University of Nebraska Medical Center, Cleveland Clinic Foundation, University of Miami, and Chaim-Sheba Medical Center, Israel; and 80 specimens were from patients treated with R-CHOP and were contributed from the British Columbia Cancer Agency. The latter patients were treated according to the British Columbia guidelines that instituted R-CHOP as the standard therapy as of March 1, 2001, as previously described in detail,[11] and have now had clinical follow-up through March 15, 2007. The specimens were selected based on the following criteria: diagnosis of de novo DLBCL clinically presenting at stage I, II, III, or IV; availability of tissue obtained at diagnosis before the initiation of therapy; treatment with a curative intent with an anthracycline-containing regimen with or without rituximab; and availability of follow-up and outcome data at the treating institution. Patients with primary mediastinal large B-cell lymphoma or involvement of CNS at presentation were not included in this study. None of the patients in the current study were included in our previous studies of gene expression profiling that led to the derivation of the six-gene model.[6]

Institutional review board approval was obtained from all participating institutions. In all patients chosen for this study, information was available about staging of the disease by physical examination, bone marrow biopsy, and computed tomography of the chest, abdomen, and pelvis. Patients were staged according to the Ann Arbor system. Because the clinical data were collected retrospectively, criteria commonly used for prospective studies, such as normal renal and liver functions, absence of comorbid conditions, and good performance status, were not applied for patient selection. The following clinical and laboratory data were available at the time of diagnosis: age, sex, performance status, stage, number of extranodal sites involved, serum lactate dehydrogenase level, and the presence or absence of systemic ("B") symptoms. Given this information, International Prognostic Index (IPI) scores could be determined in 256 of the patients treated with anthracycline-based regimens and in all 80 patients who received R-CHOP. Patients were categorized into either a low-risk group (IPI score, 0 to 2) or a high-risk group (IPI score, 3 to 5). None of the patients had a known history of HIV infection or other forms of immunosuppression. Follow-up information was obtained from the patients' medical records and included response to initial therapy based on the Cheson criteria,[14] OS, and progression-free survival (PFS).

Histologic sections were reviewed to confirm the diagnoses and were compatible with features of DLBCL according to the WHO classification of hematopoietic tumors.[15] Pathologists from the five participating institutions (Y.N., P.F., E.D.H., C.P.H., D.G., N.W., A.A., G.S., G.E.B., and R.D.G.) were involved in the review of patients from each of their centers. One pathologist (Y.N.) reviewed all patients.

Tissue Microarrays and Immunohistochemistry

Standardized methods for tissue fixation (10% buffered formalin) and processing were used at all participating centers. Tissue microarrays (TMAs) were obtained from the British Columbia Cancer Agency, Cleveland Clinic Foundation, and University of Nebraska Medical Center. A TMA of patients from the University of Miami and Chaim-Sheba Medical Center was constructed using a tissue arrayer (Beecher Instruments, Silver Spring, MD), as previously described.[16] Two to four representative cores were selected for TMAs to maximize informative cores based on characteristic morphology without prior knowledge of immunohistologic staining results. Sections of 4 to 5 μm were cut from TMAs, placed on glass slides, and baked for 1 hour at 60°C.

Immunohistochemistry for LMO2 protein was performed in one laboratory, and staining in greater than 30% of lymphoma cells was assigned a

Table 1. Patient and Disease Characteristics

Variable	CHOP-Like Regimen (n = 263)			R-CHOP Regimen (n = 80)		
	No. of LMO2-Positive Patients (n = 140)	No. of LMO2-Negative Patients (n = 123)	P*	No. of LMO2-Positive Patients (n = 44)	No. of LMO2-Negative Patients (n = 36)	P*
Mean age, years	63.4	63.0	NS	54.0	63.2	NS
Stage						
I-II	68	60		18	12	
III-IV	72	63	NS	26	24	NS
ECOG performance status						
0-1	116	88		28	24	
2 or more	24	35	.041	16	12	NS
Lactate dehydrogenase						
Normal	74	55		24	12	
> Normal	66	68	NS	20	24	NS
No. of extranodal sites						
0-1	109	89		34	14	
> 1	31	34	NS	10	22	.001
IPI†						
0-2	80	71		29	20	
3-5	55	50	NS	15	16	NS

Abbreviations: CHOP, cyclophosphamide, vincristine, doxorubicin, and prednisone; R-CHOP, rituximab plus cyclophosphamide, vincristine, doxorubicin, and prednisone; NS, not significant; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index.

*P values were obtained using Student's *t* test for age and Pearson's χ^2 test with Yates continuity correction for all other variables; values of $P < .05$ are considered statistically significant.

†IPI scores were available for 256 of 263 CHOP patients and all 80 R-CHOP patients.

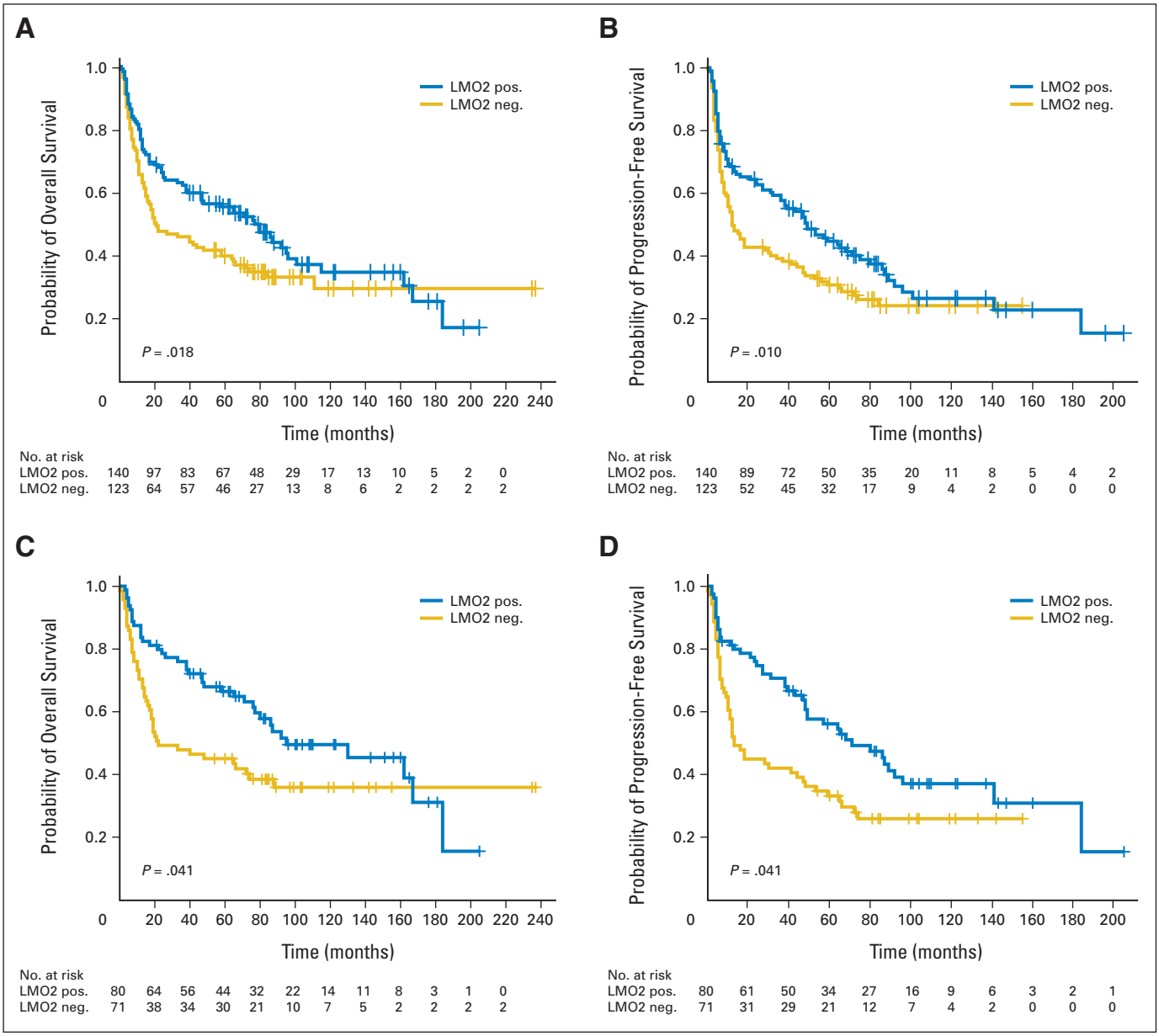

Fig 1. LMO2 protein expression correlates with overall survival (OS) and progression-free survival (PFS) in patients with diffuse large B-cell lymphoma (DLBCL) treated with anthracycline-based chemotherapy. Kaplan-Meier curves of (A) OS and (B) PFS in 263 patients with DLBCL show that LMO2 protein expression correlates with longer OS ($P = .018$) and PFS ($P = .010$); Kaplan-Meier curves of (C) OS and (D) PFS in 151 patients with DLBCL with low clinical risk (International Prognostic Index [IPI] score, 0 to 2) grouped on the basis of LMO2 protein expression show that LMO2 protein expression correlates with longer OS ($P = .041$) and PFS ($P = .041$). Pos, positive; Neg, negative.

Table 2. Multivariate Analysis of LMO2 Protein Expression With OS and PFS As Dependent Variables in DLBCL Patients Treated With Anthracycline-Based Chemotherapy and R-CHOP

	CHOP-Like Regimen				R-CHOP Regimen			
	OS		PFS		OS		PFS	
Variable	*z* Score	*P*	*z* Score	*P*	*z* Score	*P*	*z* Score	*P*
IPI	3.61	.001	3.26	.001	1.46	.140	1.96	.050
LMO2	−2.21	.027	−2.44	.015	−2.61	.009	−2.20	.028

Abbreviations: OS, overall survival; PFS, progression-free survival; DLBCL, diffuse large B-cell lymphoma; CHOP, cyclophosphamide, vincristine, doxorubicin, and prednisone; R-CHOP, rituximab plus cyclophosphamide, vincristine, doxorubicin, and prednisone; IPI, International Prognostic Index.

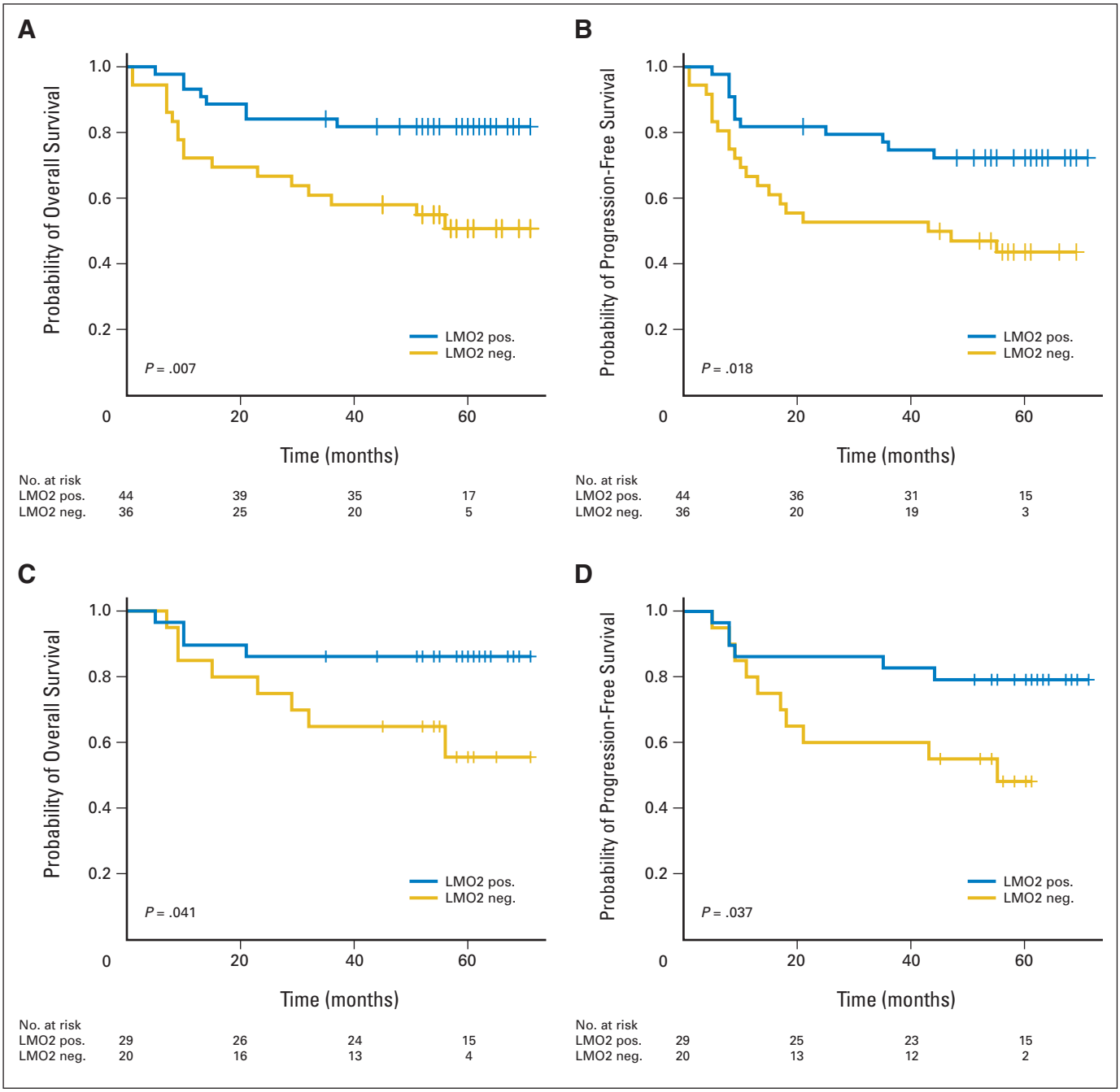

Fig 2. LMO2 protein expression correlates with overall survival (OS) and progression-free survival (PFS) in diffuse large B-cell lymphoma (DLBCL) patients treated with rituximab plus cyclophosphamide, vincristine, doxorubicin, and prednisone (R-CHOP). Kaplan-Meier curves of (A) OS and (B) PFS in 80 DLBCL patients treated with R-CHOP show that LMO2 protein expression correlates with longer OS ($P = .007$) and PFS ($P = .018$); Kaplan-Meier curves of (C) OS and (D) PFS in 49 patients with DLBCL of low clinical risk (International Prognostic Index [IPI] score, 0 to 2) grouped on the basis of LMO2 protein expression show that LMO2 protein expression correlates with longer OS ($P = .041$) and PFS ($P = .037$). Pos, positive; Neg, negative.

positive score based on our and other prior studies of immunohistologic prognostic markers in DLBCL.[7,16-18] LMO2 staining showed a robust and primarily nuclear signal, and staining intensity did not vary among normal and neoplastic lymphoid cells. The distinction between positive and negative specimens was relatively straightforward.[7] Two hematopathologists (Y.N. and D.G.) independently scored the TMAs of 263 CHOP-treated patients with a concordance rate of 96%. Discrepancies were resolved over a double-headed microscope. Two hematopathologists (Y.N. and P.F.) independently scored the TMA of 80 R-CHOP–treated patients with a concordance rate of 100%. Staining and scoring for CD10, BCL6, and MUM1/IRF4 were performed as previously described.[7]

Statistical Analysis

OS was defined as the time interval between the date of diagnoses and the date of death or last follow-up. PFS was defined as the time interval between the date of initial diagnosis and the date of disease progression or death from any cause, whichever came first, or date of last follow-up evaluation. Survival curves were estimated using the product-limit method of Kaplan-Meier and

Table 3. Log-Rank Analysis of Individual Immunohistologic Markers in DLBCL Patients Treated With Anthracycline-Based Chemotherapy

Marker	*P*
BCL6	.007
CD10	.463
MUM1/IRF4	.99

Abbreviation: DLBCL, diffuse large B-cell lymphoma.

were compared using the log-rank test. Multivariate regression analysis according to the Cox proportional hazards regression model,[19] with OS or PFS as the dependent variable, was used to adjust for the effect of immunohistologic staining and IPI. The *t* test or Pearson's χ^2 test with Yates continuity correction, as indicated, was used to compare the clinical characteristics between LMO2-positive and LMO2-negative patient groups. $P < .05$ was considered significant.

RESULTS

Patient Characteristics

For analyses of the prognostic impact of LMO2 protein expression in patients treated with anthracycline-based regimens, 263 informative DLBCL patients whose ages ranged from 18 to 93 years (median age, 66 years) were studied. The follow-up period ranged from 5 days to 237 months (median, 46 months), and 161 patients (61%) had died. For analyses of the prognostic impact of LMO2 protein expression in patients treated with R-CHOP, 80 informative DLBCL patients whose ages ranged from 20 to 82 years (median age, 58 years) were studied. The follow-up period for R-CHOP–treated patients ranged from 1 to 71 months (median, 54 months), and 24 patients (30%) had died. Patient and disease characteristics for both cohorts of patients, including the five clinical parameters that comprise the IPI, are listed in Table 1.

Immunohistologic Findings

Staining for LMO2 protein was present in 140 (53%) of 263 patients treated with CHOP-like regimens and in 44 (55%) of 80 patients treated with R-CHOP. The relationships between the expression of LMO2 protein and clinical characteristics are listed in Table 1. LMO2-positive and -negative patients treated with CHOP-like regimens differed significantly in performance status, whereas LMO2-positive and -negative patients treated with R-CHOP differed significantly in the number of extranodal sites (Table 1).

Outcome According to LMO2 Protein Expression in DLBCL Patients Treated With Anthracycline-Based Chemotherapy

The relationships between LMO2 protein expression and patient clinical outcomes were examined (Fig 1). The median OS and PFS times were 80 months (95% CI, 48 to 130 months) and 49 months (95% CI, 36 to 76 months), respectively, in LMO2-positive patients compared with 21 months (95% CI, 16 to 58 months) and 12 months (95% CI, 10 to 31 months), respectively, in LMO2-negative patients. Overall, the OS and PFS were significantly longer in DLBCL patients with positive LMO2 staining ($P = .018$ and $P = .010$, respectively). In an attempt to limit the contribution of non–lymphoma-related deaths, the analysis was repeated in patients with age less than 75 years (197 of 263 patients, 75%), and a similar difference in OS between patients with LMO2-positive and LMO2-negative tumors ($P = .009$) was found (data not shown). To examine whether the prognostic significance of LMO2 expression is independent of the current clinical gold standard for outcome prediction, a multivariate Cox regression analysis that included IPI scores and LMO2 with OS or PFS as the dependent variables was performed. Both the IPI score and LMO2 expression were independent predictors of OS and PFS (Table 2). Next, we specifically examined the prognostic power of LMO2 expression in patients with low (n = 151) and high (n = 105) IPI scores. In the subgroup with low IPI scores, patients with LMO2-positive tumors exhibited significantly longer OS and PFS ($P = .041$ for both parameters). However, no difference in OS or PFS was observed between patients with LMO2-positive and -negative tumors in the subgroup with high IPI scores.

Outcome According to LMO2 Protein Expression in DLBCL Patients Treated With R-CHOP

The relationship between LMO2 protein expression and clinical outcome was studied in DLBCL patients treated with R-CHOP. The median OS and PFS times for patients in the R-CHOP group were not yet reached. By the log-rank test, LMO2 protein expression was significantly correlated with both improved OS ($P = .007$) and PFS ($P = .018$; Figs 2A and 2B). In a multivariate Cox regression analysis that included IPI scores and LMO2 protein expression with OS and PFS as the dependent variables, LMO2 protein expression remained a significant predictor of both OS ($P = .009$) and PFS ($P = .028$; Table 2). LMO2 protein expression correlated with a longer 4-year OS rate when compared with patients who lacked LMO2 protein expression (82% *v* 56%, respectively; $P = .030$). Similarly, the 4-year PFS rate was significantly longer in patients expressing the LMO2 protein compared with those who lacked its expression (72% *v* 47%, respectively; $P = .010$). To examine whether the prognostic significance of LMO2 expression in R-CHOP–treated patients is also independent of the IPI, we performed a multivariate Cox regression analysis that included IPI scores and LMO2 with OS or PFS as the dependent variables. LMO2 expression was an IPI-independent prognostic marker for both OS and PFS, whereas IPI did not reach independent significance in this multivariate analysis (Table 2). Analysis of OS and PFS in the subgroup with low IPI scores among patients treated with R-CHOP demonstrated that patients with LMO2-positive tumors had significantly better OS ($P = .041$) and PFS ($P = .037$) compared with patients with LMO2-negative tumors (Figs 2C and 2D). A similar trend was observed in patients with high IPI scores; however, the difference in OS and PFS did not reach statistical significance, probably because of the small number of patients with a high IPI score.

Comparison of LMO2 Expression With Other Immunohistologic Markers

Previously, Hans et al[18] demonstrated that an immunohistologic algorithm based on the expression of CD10, BCL6, and MUM1/IRF4 proteins can be used to predict survival in DLBCL patients treated with CHOP-like regimens without addition of rituximab. Therefore, to compare the predictive power of LMO2 protein expression with the

aforementioned model, we tested the predictive power of the individual markers comprising the algorithm as well as the complete algorithm in our cohort of patients treated with CHOP-like regimens. The expression of BCL6 protein correlated with a superior OS ($P = .007$), but there was no significant correlation between the expression of CD10 and MUM1/IRF4 proteins and patient outcome (Table 3). There was also no significant association between patient outcome and GC and non-GC subgroups of DLBCL classified by the immunohistologic algorithm according to the CD10, BCL6, and MUM1/IRF4 model (OS, $P = .194$; PFS, $P = .455$; Figs 3A and 3B). Because BCL6 also individually predicted OS in our cohort of patients, we then addressed whether the expression of LMO2 would add value to the predictive power of BCL6 protein expression. In a multivariate Cox regression analysis that included LMO2 and BCL6 with OS or PFS as the dependent variable, both LMO2 and BCL6 were independent predictors of OS and PFS ($P < .05$ for both markers and both variables). In addition, we examined whether the combined expression of LMO2 and BCL6 proteins correlated with an improved patient

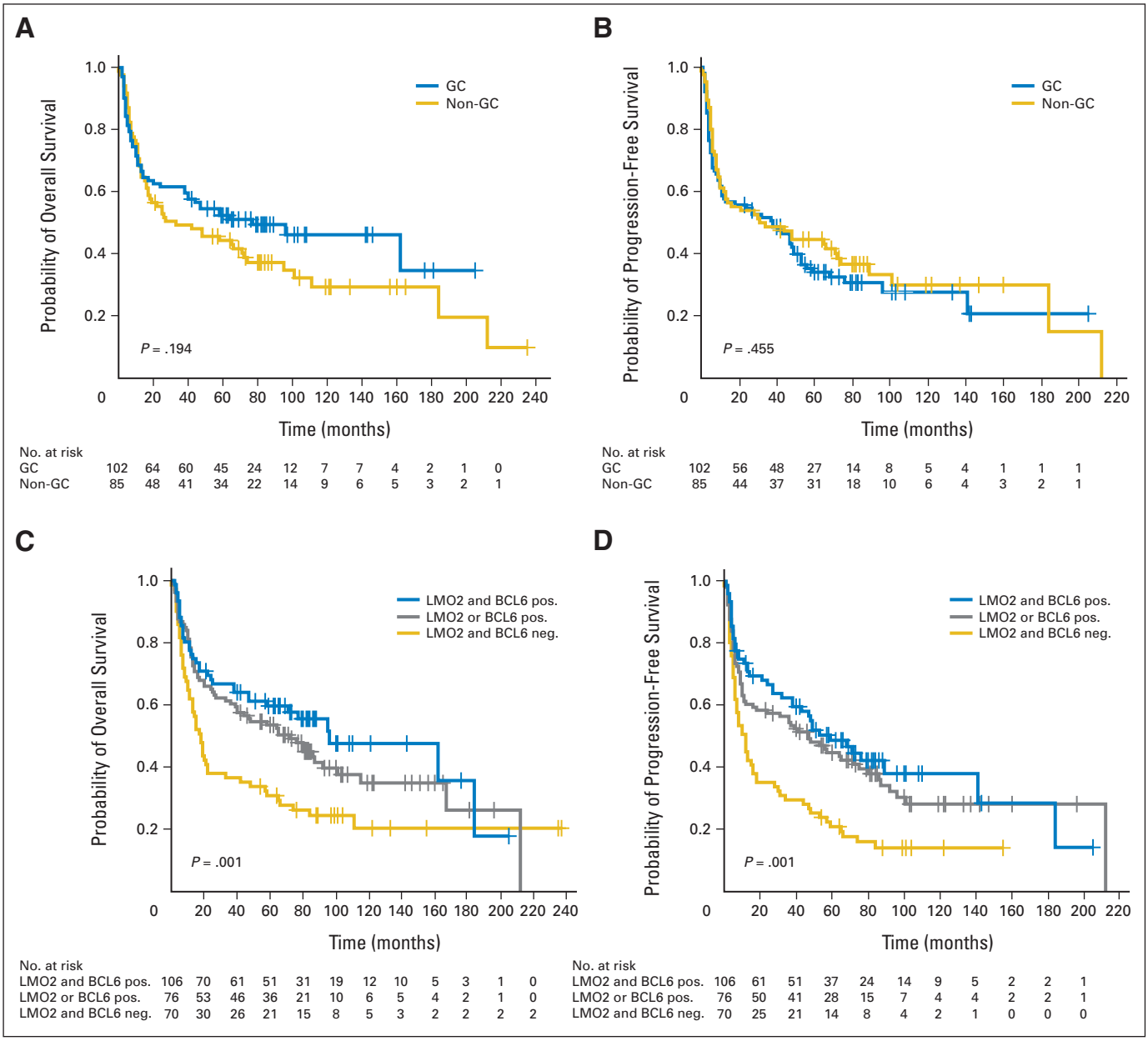

Fig 3. The expression of LMO2 and BCL6 proteins correlates with longer overall survival (OS) and progression-free survival (PFS) in patients with diffuse large B-cell lymphoma. Kaplan-Meier curves of (A) OS and (B) PFS in 187 patients treated with cyclophosphamide, vincristine, doxorubicin, and prednisone (CHOP) –like regimens who were classifiable into germinal center (GC) and non-GC subtypes based on the immunohistologic (IHC) algorithm using CD10, BCL6, and MUM1/IRF4 staining show no significant correlation with patient outcome (OS, $P = .194$; PFS, $P = .455$). Kaplan-Meier curves of (C) OS and (D) PFS in 252 patients treated with CHOP-like regimens according to the expression of both LMO2 and BCL6 proteins are shown (BCL6 staining was available in 252 of 263 patients). The overall log-rank test for the three curves had a $P = .001$. Patients with expression of either LMO2 or BCL6 (but not both) have a significantly better OS ($P = .007$) and PFS ($P = .001$) compared with patients who do not express both markers. Patients with expression of both LMO2 and BCL6 have significantly better OS ($P < .001$) and PFS ($P < .001$) compared with patients who do not express both markers. Pos, positive; Neg, negative.

outcome. The overall log-rank test for the three curves had a $P = .001$ (Figs 3B and 3C). Patients who expressed both LMO2 and BCL6 proteins had a significantly better OS ($P < .001$) and PFS ($P < .001$) compared with patients who lacked the expression of both markers. Furthermore, the expression of either LMO2 or BCL6 was also found to confer a superior OS ($P = .007$) and PFS ($P = .001$) compared with the lack of expression of both proteins.

In R-CHOP–treated patients, neither BCL6 protein expression nor GC B-cell phenotype based on the immunohistologic algorithm of Hans et al[18] was significantly correlated with OS or PFS ($P > .05$). Thus, in our cohort of R-CHOP–treated patients, LMO2 protein expression emerged as the only predictive marker among patients tested in this study.

DISCUSSION

The GC provides a microenvironment in which naïve B cells proliferate and diversify their antigen receptors to produce high-affinity antibodies.[20-23] It is well recognized that dysregulation of these steps of normal B-cell ontogeny plays an important role in the genesis of GC-derived B-cell lymphomas.[24] DLBCL with a gene expression profile similar to that of GC B cells exhibits a more favorable clinical outcome.[1] Therefore, immunohistologic markers associated with a GC phenotype are also likely to be associated with a better clinical outcome in patients with DLBCL. Indeed, this provided the basis for an immunohistologic model based on the expression of CD10, BCL6, and MUM1/IRF4 proteins.[18] Here, we investigated the impact on DLBCL prognosis of another marker of GC lymphocytes. We show that LMO2 protein expression has prognostic significance in DLBCL, as was previously noted for *LMO2* mRNA expression.[6] Furthermore, we confirmed the prognostic value of LMO2 protein expression in an independent cohort of patients with DLBCL treated with R-CHOP. The latter cohort of patients demonstrated that the prognostic impact of LMO2 is unaffected by the addition of rituximab to the treatment of DLBCL patients.

LMO2 was the strongest single predictor of superior outcome in DLBCL patients in a multivariate model that we had constructed based on the expression of six genes.[6] The *LMO2* gene encodes a transcription factor that regulates key events in erythropoiesis, angiogenesis, and embryogenesis.[25-28] Mice deficient in *LMO2* die as a result of failure of yolk sac erythropoiesis, whereas chimeric mice show that *LMO2* plays a role in the development of all bone marrow–derived hematopoietic lineages.[29] This gene is of relevance in lymphoid and myeloid leukemias resulting from the deregulated expression of *LMO2* as caused by chromosomal translocations and insertional mutations.[30-33] Subsequently, *LMO2* was shown to be overexpressed in DLBCL of the GC type.[1] Using a novel monoclonal anti-LMO2 antibody, we recently confirmed that LMO2 protein is expressed in GC-derived B-cell lymphomas, normal human bone marrow hematopoietic lineages, and leukemias.[7] To date, no acquired genetic aberrations are known that account for the overexpression of LMO2 in DLBCL; in these cases, its expression is possibly a reflection of the cell of origin or may be associated with a specific function of *LMO2* that is as yet unknown. Previous studies have demonstrated that different binding partners interact with LMO2 protein in multiprotein transcriptional complexes.[25-28] Therefore, it is likely that, in GC B cells, the LMO2 protein interacts with a discrete set of transcription factors to exert specific effects in these cells.

Protein expression studies from several institutions have explored the clinicopathologic and molecular diversity of DLBCL; however, they have yielded conflicting results.[5,17,18,34,35] Thus, validation of results in independent groups of patients and reassessment in the postrituximab era are necessary. Our findings show that LMO2 protein expression has prognostic significance in two independent cohorts of DLBCL patients treated with anthracycline-based regimens with and without rituximab. Furthermore, the predictive power of LMO2 protein expression in patients treated with CHOP-like regimens added value to the predictive power of BCL6 protein expression and was superior to the immunohistologic algorithm based on the expression of CD10, BCL6, and MUM1/IRF4 proteins previously shown to predict survival in DLBCL patients treated in the prerituximab era. The expression of the LMO2 protein, unlike the BCL6 protein, retained its predictive power in patients treated with R-CHOP. Whether the lack of BCL6 protein expression to predict outcome in patients treated with R-CHOP was a result of the small numbers of patients analyzed in this study or a result of treatment with rituximab needs further evaluation in additional large studies of R-CHOP–treated patients.

In conclusion, we show that, similar to its mRNA expression, LMO2 protein expression is significantly correlated in univariate and multivariate analyses with improved OS and PFS in DLBCL patients treated with anthracycline-based chemotherapy with and without rituximab. Multivariate analyses show that LMO2 protein expression is an IPI-independent predictor of OS and PFS. Further confirmation of these observations in independent cohorts of unselected DLBCL patients treated with R-CHOP is needed before this marker may be applied in routine clinical practice.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Yasodha Natkunam, Pedro Farinha, Eric D. Hsi, Christine P. Hans, Robert Tibshirani, Laurie H. Sehn, Dita Gratzinger, Manuel Rosado, Shuchun Zhao, Gerald E. Byrne, Ronald Levy, Randy D. Gascoyne, Izidore S. Lossos

Financial support: Ronald Levy, Randy D. Gascoyne, Izidore S. Lossos

Provision of study materials or patients: Yasodha Natkunam, Pedro Farinha, Eric D. Hsi, Christine P. Hans, Laurie H. Sehn, Joseph M. Conners, Dita Gratzinger, Brad Pohlman, Nicholas Wongchaowart, Martin Bast, Abraham Avigdor, Ginette Schiby, Arnon Nagler, Gerald E. Byrne, Ronald Levy, Randy D. Gascoyne, Izidore S. Lossos

Collection and assembly of data: Yasodha Natkunam, Pedro Farinha, Eric D. Hsi, Christine P. Hans, Laurie H. Sehn, Manuel Rosado, Shuchun Zhao, Brad Pohlman, Nicholas Wongchaowart, Martin Bast, Abraham Avigdor, Ginette Schiby, Arnon Nagler, Randy D. Gascoyne, Izidore S. Lossos

Data analysis and interpretation: Yasodha Natkunam, Pedro Farinha, Robert Tibshirani, Laurie H. Sehn, Joseph M. Conners, Manuel Rosado, Shuchun Zhao, Gerald E. Byrne, Ronald Levy, Randy D. Gascoyne, Izidore S. Lossos

Manuscript writing: Yasodha Natkunam, Robert Tibshirani, Laurie H. Sehn, Gerald E. Byrne, Ronald Levy, Randy D. Gascoyne, Izidore S. Lossos

Final approval of manuscript: Yasodha Natkunam, Pedro Farinha, Eric D. Hsi, Christine P. Hans, Robert Tibshirani, Laurie H. Sehn, Joseph M. Conners, Dita Gratzinger, Manuel Rosado, Shuchun Zhao, Brad Pohlman, Nicholas Wongchaowart, Martin Bast, Abraham Avigdor, Ginette Schiby, Arnon Nagler, Gerald E. Byrne, Ronald Levy, Randy D. Gascoyne, Izidore S. Lossos

REFERENCES

1. Alizadeh AA, Eisen MB, Davis RE, et al: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403: 503-511, 2000

2. Rosenwald A, Wright G, Chan WC, et al: The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346:1937-1947, 2002

3. Shipp MA, Ross KN, Tamayo P, et al: Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nat Med 8:68-74, 2002

4. Wright G, Tan B, Rosenwald A, et al: A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci U S A 100:9991-9996, 2003

5. Lossos IS, Morgensztern D: Prognostic biomarkers in diffuse large B-cell lymphoma. J Clin Oncol 24:995-1007, 2006

6. Lossos IS, Czerwinski DK, Alizadeh AA, et al: Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. N Engl J Med 350:1828-1837, 2004

7. Natkunam Y, Zhao S, Mason DY, et al: The oncoprotein LMO2 is expressed in normal germinal-center B cells and in human B-cell lymphomas. Blood 109:1636-1642, 2007

8. Coiffier B, Lepage E, Briere J, et al: CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235-242, 2002

9. Habermann TM, Weller EA, Morrison VA, et al: Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol 24:3121-3127, 2006

10. Pfreundschuh M, Trumper L, Osterborg A, et al: 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 7:379-391, 2006

11. Sehn LH, Donaldson J, Chhanabhai M, et al: Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. J Clin Oncol 23:5027-5033, 2005

12. Mounier N, Briere J, Gisselbrecht C, et al: Rituximab plus CHOP (R-CHOP) overcomes bcl-2–associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). Blood 101:4279-4284, 2003

13. Winter JN, Weller E, Horning SJ, et al: Rituximab (R) alters the prognostic indicator profile in diffuse aggressive non-Hodgkin lymphomas. Blood 107:4207-4213, 2006

14. Cheson BD, Horning SJ, Coiffier B, et al: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas: NCI Sponsored International Working Group. J Clin Oncol 17:1244, 1999

15. Jaffe ES, Harris NL, Stein H, et al: Pathology and Genetics of Tumors of the Hematopoietic and Lymphoid Tissues. Lyon, France, International Agency for Research on Cancer Press, 2001

16. Natkunam Y, Lossos IS, Taidi B, et al: Expression of the human germinal center-associated lymphoma (HGAL) protein, a new marker of germinal center B-cell derivation. Blood 105:3979-3986, 2005

17. Colomo L, Lopez-Guillermo A, Perales M, et al: Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood 101:78-84, 2003

18. Hans CP, Weisenburger DD, Greiner TC, et al: Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103:275-282, 2004

19. Cox DR: Regression models and life tables. J R Stat Soc B 74:187-220, 1972

20. Klein U, Tu Y, Stolovitzky GA, et al: Gene expression dynamics during germinal center transit in B cells. Ann N Y Acad Sci 987:166-172, 2003

21. McHeyzer-Williams LJ, Driver DJ, McHeyzer-Williams MG: Germinal center reaction. Curr Opin Hematol 8:52-59, 2001

22. Rajewsky K: Clonal selection and learning in the antibody system. Nature 381:751-758, 1996

23. Stamatopoulos K, Belessi C, Papadaki T, et al: Somatic hypermutation patterns in germinal center B cell malignancies. Hematology 8:319-328, 2003

24. Kuppers R: Identifying the precursors of Hodgkin and Reed-Sternberg cells in Hodgkin's disease: Role of the germinal center in B-cell lymphomagenesis. J Acquir Immune Defic Syndr 21: S74–S79, 1999 (suppl 1)

25. Warren AJ, Colledge WH, Carlton MB, et al: The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. Cell 78:45-57, 1994

26. Yamada Y, Pannell R, Forster A, et al: The oncogenic LIM-only transcription factor Lmo2 regulates angiogenesis but not vasculogenesis in mice. Proc Natl Acad Sci U S A 97:320-324, 2000

27. Look AT: Oncogenic transcription factors in the human acute leukemias. Science 278:1059-1064, 1997

28. Nam CH, Rabbitts TH: The role of LMO2 in development and in T cell leukemia after chromosomal translocation or retroviral insertion. Mol Ther 13:15-25, 2006

29. Yamada Y, Warren AJ, Dobson C, et al: The T cell leukemia LIM protein Lmo2 is necessary for adult mouse hematopoiesis. Proc Natl Acad Sci U S A 95:3890-3895, 1998

30. Boehm T, Foroni L, Kaneko Y, et al: The rhombotin family of cysteine-rich LIM-domain oncogenes: Distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc Natl Acad Sci U S A 88:4367-4371, 1991

31. Royer-Pokora B, Loos U, Ludwig WD: TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11;14)(p13;q11). Oncogene 6:1887-1893, 1991

32. McCormack MP, Rabbitts TH: Activation of the T-cell oncogene LMO2 after gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 350:913-922, 2004

33. Dong WF, Billia F, Atkins HL, et al: Expression of rhombotin 2 in normal and leukaemic haemopoietic cells. Br J Haematol 93:280-286, 1996

34. Barrans SL, Carter I, Owen RG, et al: Germinal center phenotype and bcl-2 expression combined with the International Prognostic Index improves patient risk stratification in diffuse large B-cell lymphoma. Blood 99:1136-1143, 2002

35. Saez AI, Saez AJ, Artiga MJ, et al: Building an outcome predictor model for diffuse large B-cell lymphoma. Am J Pathol 164:613-622, 2004