

Expression of MUM1/IRF4 correlates with clinical outcome in patients with B-cell chronic lymphocytic leukemia

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In this study, we evaluated the prognostic significance of multiple myeloma-1/interferon regulatory factor-4 (MUM1/IRF4) expression in B-cell chronic lymphocytic leukemia (B-CLL). Our results demonstrated that the absence of MUM1/IRF4 expression showed the highest relative risk among the factors analyzed in determining the probability for death in patients with B-CLL using univariate and multivariate Cox regression analysis. Pa-

tients without MUM1/IRF4 expression had significantly worse overall survival than did those with MUM1/IRF4 expression (52% cumulative survival, 63 months vs not reached, Kaplan-Meier survival analysis; $P < .03$, log-rank test). Patients with MUM1/IRF4 expression were more likely to have disease at low Rai stage and interstitial/nodular marrow involvement. Furthermore, only 1 of 11 patients with MUM1/IRF4 expression and interstitial/

nodular marrow involvement died during a 100-month follow-up. Our results suggest that B-CLL with expression of MUM1/IRF4, indicative of postgerminal center origin, has a more favorable clinical course and that MUM1/IRF4 is an important prognostic marker in B-CLL. (Blood. 2002;100:4671-4675)

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Introduction

B-cell chronic lymphocytic leukemia (B-CLL) is the most common form of leukemia in the Western world,¹ and it follows a heterogeneous clinical course.² Available staging systems and prognostic markers, including lymphocyte doubling time, bone marrow histology, and cytogenetic abnormalities, have limited usefulness in predicting outcomes in individual patients.³⁻⁷

Recently, the histogenesis of B-CLL has been shown to be a promising prognostic indicator for patients.⁸⁻¹⁰ These studies have revealed that B-CLL cells are derived from lymphocytes at 2 different stages of differentiation, one with mutated immunoglobulin V (*IgV*) genes corresponding to postgerminal center (post-GC) origin, and the other with unmutated *IgV* genes, corresponding to pregerminal center (pre-GC) origin. Patients with unmutated *IgV* genes have worse prognoses than do those with mutated *IgV* genes.

Recent studies have demonstrated that the late-stage-GC B cells and post-GC B cells express multiple myeloma-1/interferon regulatory factor-4 (MUM1/IRF4) cells but not pre-GC B cells.¹¹⁻¹⁵ By immunohistochemistry, MUM1/IRF4 is expressed in a morphologic spectrum of B-lymphocytes ranging from centrocytes to plasmablasts/plasma cells.^{11,14,16} Polymerase chain reaction analysis of MUM1/IRF4+ cells has shown that they contain rearranged immunoglobulin heavy-chain (*IgH*) genes with a varying number of V_H somatic mutations.¹⁴ Functionally, MUM1/IRF4 gene expression has been shown to be essential for the function and homeostasis of mature B cells and cytotoxic T lymphocytes.¹⁷

The prognostic significance of MUM1/IRF4 expression has not been systematically studied in B-CLL, although its expression may serve as an important prognostic marker because of the linkage between MUM1/IRF4 expression and histogenesis of B lympho-

cytes as described above. The goal of the current study was to examine whether MUM1/IRF4 expression correlated with clinical outcome in B-CLL patients.

Study design

We retrospectively studied 30 patients with B-CLL (18 men, 12 women; age, 62.4 ± 12.7 years) diagnosed from January 1990 to December 1999. These patients were selected on the basis of the availability of marrow core biopsy samples and complete clinical data. All patients had the typical morphology and classic immunophenotypic pattern for B-CLL.

Pertinent clinical information for these patients was obtained by reviewing the records from the tumor registry, from patient charts, or both (Table 1). Rai stages were defined according to the parameters at the time of initial diagnosis. Median length of follow-up was 62.5 months (range, 1-107 months). All surviving patients were followed up for at least 25 months. The end point of follow-up was either the date of the last visit or the date of death until September 2001.

Dual immunohistochemical stains for MUM1/IRF4 (ISCAT/M-17; Santa Cruz Biotechnology, CA) and CD20 (L-26; DAKO, Carpinteria, CA) were performed on Bouin or B5-fixed, paraffin-embedded, decalcified marrow core biopsy samples. Dual staining was performed to confirm that the expression of MUM-1/IRF4 was in B cells (CD20⁺) but not in activated T cells or plasma cells¹⁴ (Figure 1A).

We defined positive MUM1/IRF4 expression as consisting of more than 20% CD20⁺ B cells stained positively for MUM1/IRF4 according to the literature—small amounts of residual normal B cells may express MUM1/IRF4.^{11,18} Other morphologic findings, including percentages and patterns of involvement, in the marrow biopsy samples were evaluated. The above

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Table 1. Clinical presentations and outcomes and results of laboratory evaluation of marrow specimens, including MUM1/IRF4

Patient	MUM1	Age at diagnosis	Sex	Rai stage	Chemotherapy	Marrow pattern	Marrow involvement, %	Vital status	Follow-up (mo)	Mutation status
1	Neg	55	M	Low	Yes	D	100	Dead	107	Nonmutated
2	Neg	44	M	Low	Yes	D	100	Dead	69	No clonal sequence*
3	Neg	72	F	Low	Yes	I/N	50	Dead	23	Mutated
4	Neg	67	F	Low	No	I/N	70	Alive	62	Mutated
5	Neg	67	F	Low	Yes	D	100	Dead	103	Nonmutated
6	Neg	66	M	High	Yes	I/N	70	Dead	89	Nonmutated
7	Neg	79	M	High	Yes	I/N	50	Dead	41	No clonal sequence
8	Neg	70	F	High	Yes	D	100	Dead	86	Mutated
9	Neg	77	M	High	No	D	80	Dead	2	No clonal sequence
10	Neg	67	M	Low	Yes	I/N	60	Dead	80	Nonmutated
11	Neg	63	F	Low	No	I/N	70	Dead	87	Mutated
12	Neg	78	M	Low	Yes	I/N	60	Dead	57	Nonmutated
13	Neg	69	M	High	Yes	I/N	50	Alive	40	Mutated
14	Neg	61	F	High	Yes	D	100	Dead	17	Nonmutated
15	Neg	69	M	High	No	D	85	Dead	1	No clonal sequence
16	Neg	79	M	High	No	D	90	Dead	7	Mutated
17	Neg	44	M	High	Yes	D	100	Alive	35	Mutated
18	Neg	63	M	Low	Yes	D	70	Dead	63	Nonmutated
19	Pos	66	M	Low	No	I/N	70	Alive	80	Mutated
20	Pos	69	M	Low	Yes	I/N	25	Alive	100	Mutated
21	Pos	71	M	Low	Yes	I/N	40	Alive	25	Mutated
22	Pos	29	F	Low	Yes	I/N	60	Alive	95	Mutated
23	Pos	47	F	Low	No	I/N	10	Alive	70	Mutated
24	Pos	50	F	Low	Yes	I/N	20	Alive	37	Nonmutated
25	Pos	55	F	Low	No	I/N	50	Alive	28	Mutated
26	Pos	60	F	Low	No	I/N	60	Alive	64	Mutated
27	Pos	60	F	Low	No	I/N	5	Alive	84	No clonal sequence
28	Pos	35	M	Low	Yes	I/N	10	Dead	67	Not done
29	Pos	74	M	Low	Yes	D	80	Dead	40	No clonal sequence
30	Pos	65	M	High	No	I/N	10	Alive	76	No clonal sequence

D indicates diffuse; dx, diagnosis; I/N, interstitial and/or nodular; Neg, negative; and Pos, positive.

*No clonal band was identified for sequencing

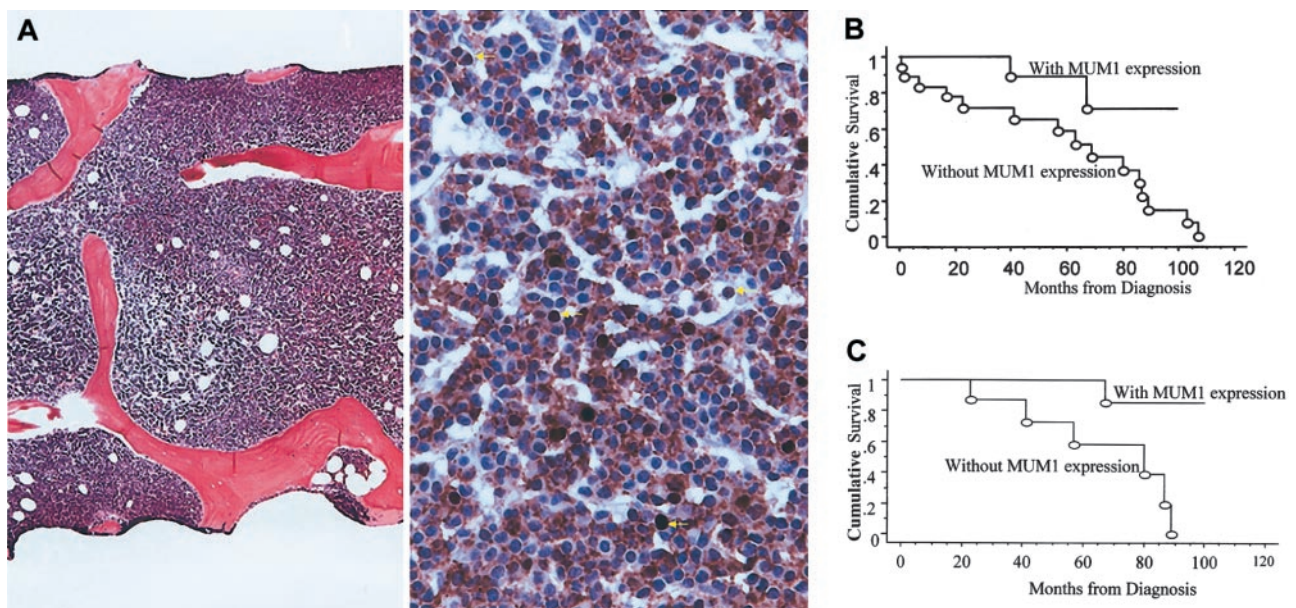


Figure 1. Morphologic evaluation and survival analysis. (A) Example of marrow biopsy sample with diffuse involvement pattern (left) and absence of MUM1/IRF4 expression (right), defined as less than 20% of CD20⁺ B cells stained positively for MUM1/IRF4. Double staining for MUM1/IRF4 (brown; nuclear staining) and CD20 (red; membrane staining) shows that most CD20⁺ B cells do not express MUM1/IRF4. Cells that are positive for MUM1/IRF4 but negative for CD20 represent activated T cells or plasma cells (arrows). Double staining was performed, after heat retrieval, by incubating the marrow biopsy sections with monoclonal antibody against MUM1/IRF4 (1:200 dilution) for 30 minutes, detected by DAB chromogen (DAKO), followed by the second incubation with monoclonal antibody against CD20 (1:800 dilution) for 30 minutes, detected by Vector-Nova Red (Vector, Burlingame, CA). All slides were stained using the DAKO automated immunostainer. In some patients, the monoclonal antibody (MUM1p, a kind gift from Dr B. Falini, University of Perugia, Italy) was used for double staining. Results were comparable between the 2 antibodies. (B) Patients without MUM1/IRF4 expression had significantly worse OS than those with MUM1/IRF4 expression (Kaplan-Meier survival analysis; $P < .03$, log-rank test). (C) Among patients with interstitial/nodular marrow involvement, patients with MUM1/IRF4 expression had significantly better OS than those without (Kaplan-Meier survival analysis; $P < .02$, log-rank test).

Table 2. Results of *IgH* gene mutation status study

Patient	Most similar germline V _H gene	Total mutation (CDR)		Total mutation (FR)		V _H gene difference from germline, %	Mutation status
		R Mut	S Mut	R Mut	S Mut		
1	V _H 1-69	0	0	0	0	0	Nonmutated
2	NA	NA	NA	NA	NA	NA	No clonal sequence
3	V _H 3-21	6	2	0	1	3.29	Mutated
4	V _H 3-7	3	2	10	2	6.22	Mutated
5	V _H 3-15/V _H 4-41	0	0	0	0	0	Nonmutated
6	V _H 3-11	0	0	0	0	0	Nonmutated
7	NA	NA	NA	NA	NA	NA	No clonal sequence
8	V _H 3-23	5	5	9	3	8.05	Mutated
9	NA	NA	NA	NA	NA	NA	No clonal sequence
10	V _H 4-39	0	0	0	0	0	Nonmutated
11	V _H 3-48	4	2	1	6	4.76	Mutated
12	V _H 1-69	0	0	0	0	0	Nonmutated
13	V _H 3-49	5	5	7	8	8.96	Mutated
14	V _H 1-69	0	0	0	0	0	Nonmutated
15	NA	NA	NA	NA	NA	NA	No clonal sequence
16	V _H 3-33	10	6	7	5	10.25	Mutated
17	V _H 3-7	2	1	7	6	5.86	Mutated
18	V _H 1-8	0	0	0	0	0	Nonmutated
19	V _H 1-69	5	3	7	8	8.42	Mutated
20	V _H 3-23	3	1	8	5	6.22	Mutated
21	V _H 3-48	3	2	0	3	2.93	Mutated
22	V _H 5-51	2	1	4	1	3.06	Mutated
23	V _H 1-18	3	1	5	2	4.02	Mutated
24	V _H 1-69	0	0	0	0	0	Nonmutated
25	V _H 3-23	10	1	8	3	8.05	Mutated
26	V _H 4-59	4	2	6	6	6.59	Mutated
27	NA	NA	NA	NA	NA	NA	No clonal sequence
28	NA	NA	NA	NA	NA	NA	Not done
29	NA	NA	NA	NA	NA	NA	No clonal sequence
30	NA	NA	NA	NA	NA	NA	No clonal sequence

In this study, DNA was extracted from the Giemsa-stained bone marrow aspirate smear of each patient using the protocol modified from Court et al.¹⁹ Bone marrow material was scraped from the slide, using a sterile scalpel blade, directly to an Eppendorf centrifuge tube containing the digestion buffer, as described,¹⁹ and was incubated at 55°C overnight. After overnight digestion, DNA extraction was performed using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. The amount of DNA was quantified spectrometrically (Lambda 3A; Perkin-Elmer, Norwalk, CT). As a positive control for the quality of the extracted DNA, all DNA samples were tested by an in-house real-time quantitative polymerase chain reaction (PCR) assay targeting on β-globin. This ensured that the extracted DNA was suitable for further PCR analysis of *IgH* mutation status. PCR amplification of V_H genes included preamplification and amplification steps. For preamplification, approximately 25 to 250 ng DNA, 2 pmol each V_H leader primer, and the J_H primer were used. The reaction was carried out in a 25-μL volume with Advantage 2 PCR enzyme system (Clontech, Palo Alto, CA). The amplification program consisted of 30 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 60 seconds at 72°C, followed by a final incubation step at 72°C for 5 minutes. For the second amplification, the 25-μL reaction was carried out, using 2 μL first-round reaction mixture, 2 pmol each V_H family-specific forward primers, and the J_H* primer. The amplification program consisted of 25 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 60 seconds at 72°C, followed by a final incubation step at 72°C for 5 minutes. Then 4 μL PCR product was analyzed by electrophoresis in 2% agarose gel and ethidium bromide staining. The PCR products were directly sequenced after purification with MultiScreen Filtration System (catalog no. MAFBN0B10; Millipore, Danvers, MA) using an automated ABI PRISM 310 Genetic Analyzer. Nucleotide sequences were aligned to EMBL/GenBank and V-BASE sequence directory. A V_H gene with somatic mutations was defined as previously reported.²⁰ V_H leader primers: V_H1 and V_H7, CACCATGG ACTG(G/C)ACCTGGA; V_H2, CAC(A/G)CTC CTGCTGCTGACCA; V_H3, CACCATGGAGTTTGGGCTGA; V_H3*, GGCTGAGCTGG(C/G)T TTT(C/T)CT(C/T)GT; V_H4, T(C/G)CTGGTGGC(A/G)GCTCCCAGA; V_H5, TCCTCCTGGCT GTTCTCCAA; V_H6, ACAATGTCTGTCTCCTCCTCAT. V_H family-specific forward primers: V_H1/V_H7, CAGGT(C/G)CAGCTGGT(A/G)CAGTCTGG; V_HF2, CAG(A/G)TCACCTTGA AGGAGTCTGG; V_HF3, AGGTGCAGCTGGTGGAGTCTGG; V_HF4, CAGGTGCAGCTGCA GGAGTCTG; V_HF5, GA(A/G)GTGCAGCTGGTGCAGTCTG; V_HF6, CAGGTACAGCTGCA GCAGTCAGG. J-chain primers: J_H primer, ACCTGAGGAGACGGTGACC; J_H* primer, TGAGGAGACGGTGACC(A/G)(T/G)(T/G)GT.

NA indicates not applicable; CDR, complementarity-determining regions; FR, framework region; S Mut, silence mutation; and R mut, replacement mutation.

Table 3. Results of univariate Cox regression analysis

Variable	No. patients	P	RR	95% CI	
				Lower bound	Upper bound
Age (as a continuous variable)	NA	.0464	1.070	1.001	1.144
MUM1 negative (vs positive)	18 of 30	.0394	4.808	1.080	21.28
Diffuse marrow pattern (vs nondiffuse)	11 of 30	.0840	2.457	0.886	6.812
Marrow involvement as a continuous variable, %	NA	.0782	1.018	0.998	1.039
Rai stage high (vs low)	10 of 30	.0412	2.904	1.043	8.038
Male (vs female)	18 of 30	.2830	1.789	0.619	5.171
Chemotherapy no (vs yes)	11 of 30	.7922	0.854	0.264	2.760

Chemotherapy consisted of chlorambucil, fludarabine, or both. NA indicates not applicable.

evaluation was performed without knowledge of the patient's clinical information.

DNA was extracted from the Giemsa-stained bone marrow aspirate smear of each patient¹⁹ (further details are outlined in Table 2). An *IgH* gene mutation study was then performed, as outlined in Table 2. A *V_H* gene with somatic mutations was defined as reported by Fais et al.²⁰

Results and discussion

In the current study, the frequency of MUM1/IRF4 expression was 40% (12 of 30 patients). Our study is the first to evaluate the expression of MUM1/IRF4 in marrow core biopsy samples of B-CLL patients. In agreement with our results, but with much smaller sample sizes, Tsuboi et al,¹¹ Natkunam et al,¹⁸ and Falini et al¹⁴ observed that MUM1/IRF4 expression was seen in 3 (43%) of 7, 3 (43%) of 7, and 10 (66%) of 15 small lymphocytic lymphoma (B-CLL/SLL) lymph node biopsy samples, respectively.

There are clear differences between patients with and without MUM1/IRF4 expression. MUM1/IRF4 expression was more frequent in patients with disease at low Rai stage (stages 0, 1, and 2) than at high Rai stage (stages 3 and 4) (11 of 20 vs 1 of 10; $P < .02$, χ^2 analysis). Additionally, MUM1/IRF4 expression occurred more frequently in patients with interstitial or nodular marrow involvement than in patients with diffuse marrow involvement (11 of 19 vs 1 of 11; $P < .009$, χ^2 analysis). Patients whose neoplastic cells expressed MUM1/IRF4 were slightly younger than those without MUM1/IRF4 expression (56.8 ± 14.2 years vs 66.1 ± 10.3 years; $P = .045$, Student *t* test). However, no difference in sex was noted between patients with and without MUM1/IRF4 expression.

Our results showed that absence of MUM1/IRF4 expression was associated with the highest relative risk (RR) among the factors analyzed in determining the probability for death in patients with B-CLL using univariate Cox proportional hazards regression analysis (Table 3). Most important, when controlling for MUM1/IRF4 expression status using multivariate Cox proportional hazards regression analysis, none of the other factors demonstrated a significant impact on overall survival (OS). Patients without MUM1/IRF4 expression had significantly worse OS than those with MUM1/IRF4 expression (52% cumulative survival, 63 months vs not reached, Kaplan-Meier survival analysis; $P < .03$, log-rank test; Figure 1B).

Furthermore, our results demonstrate that MUM1/IRF4 expression with concurrent interstitial or nodular marrow involvement in B-CLL indicates a more favorable prognosis. Among patients with interstitial or nodular marrow involvement, those with MUM1/IRF4 expression had a significantly better OS than those without (58% cumulative survival, not reached vs 57 months, Kaplan-Meier survival analysis; $P < .02$, log-rank test; Figure 1C). Only 1

Table 4. Correlation between MUM1/IRF4 expression and *IgH* gene mutation status

<i>IgH</i> gene	MUM1/IRF4 positive	MUM1/IRF4 negative
Nonmutated	1	7
Mutated	7	7

of 11 patients with MUM1/IRF4 expression and interstitial/nodular marrow involvement died during the follow-up of this study. The maximal length of follow-up in these 11 patients was 100 months.

MUM1/IRF4 expression is highly predictive of post-GC origin of B-CLL. Most patients (7 of 8, or 88%; Table 4) have mutated *IgH* genes. In addition, the absence of MUM1/IRF4 expression was observed in most patients (7 of 8, or 88%) with nonmutated *IgH* genes. These findings agree with those of previous study showing that MUM1/IRF4 expression is present in lymphocytes containing the mutated *IgH* gene in normal lymphoid tissue.¹⁴ However, the absence of MUM1/IRF4 expression was observed in 7 (50%) of 14 of the patients with mutated *IgH* genes. We hypothesize that these patients may lose or down-regulate the expression of MUM1/IRF4 through gene mutation/deletion or through unknown regulatory mechanisms during disease progression. This is partially supported by our finding that the absence of MUM1/IRF4 expression in patients with mutated *IgH* genes is associated with advanced disease at a high Rai stage (4 of 7 vs 0 of 7 patients with mutated *IgH* gene and MUM1/IRF4 expression; $P < .07$, Fisher exact test) at presentation. Further follow-up of our patients to examine whether any of the MUM1/IRF4-positive patients may lose MUM1/IRF4 expression during their disease course will be important for understanding the role of MUM1/IRF4 expression in B-CLL progression.

In summary, our results suggest that B-CLL with MUM1/IRF4 expression, indicative of post-GC origin, has a more favorable clinical course and that patients are more likely to have disease at low Rai stage and nodular or interstitial marrow involvement. Additional studies are suggested to confirm this observation and to investigate the mechanisms of MUM1/IRF4 expression in B-CLL progression.

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References

- Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *CA Cancer J Clin*. 1999;49:8-31.
- Zwiebel JA, Cheson BD. Chronic lymphocytic leukemia: staging and prognostic factors. *Semin Oncol*. 1998;25:42-59.
- Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*. 1981;48:198-206.
- Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med*. 1990;323:720-724.
- Montserrat E, Sanchez-Bisono J, Vinolas N, Rozman C. Lymphocyte doubling time in chronic lymphocytic leukaemia: analysis of its prognostic significance. *Br J Haematol*. 1986;62:567-575.
- Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. *Blood*. 1975;46:219-234.
- Rozman C, Montserrat E, Rodriguez-Fernandez JM, et al. Bone marrow histologic pattern—the best single prognostic parameter in chronic lymphocytic leukemia: a multivariate survival analysis of 329 cases. *Blood*. 1984;64:642-648.
- Damle RN, Wasil T, Fais F, et al. *Ig V* gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94:1840-1847.
- Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated *Ig V(H)* genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94:1848-1854.
- Naylor M, Capra JD. Mutational status of *Ig V(H)* genes provides clinically valuable information in B-cell chronic lymphocytic leukemia. *Blood*. 1999;94:1837-1839.
- Tsuboi K, Iida S, Inagaki H, et al. MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. *Leukemia*. 2000;14:449-456.

12. Carbone A, Ghoghini A, Larocca LM, et al. Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. *Blood*. 2001;97:744-751.
13. Gaidano G, Carbone A. MUM1: a step ahead toward the understanding of lymphoma histogenesis. *Leukemia*. 2000;14:563-566.
14. Falini B, Fizzotti M, Pucciarini A, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. *Blood*. 2000;95:2084-2092.
15. Iida S, Rao PH, Butler M, et al. Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. *Nat Genet*. 1997;17:226-230.
16. Carbone A, Ghoghini A, Cozzi MR, et al. Expression of MUM1/IRF4 selectively clusters with primary effusion lymphoma among lymphomatous effusions: implications for disease histogenesis and pathogenesis. *Br J Haematol*. 2000;111:247-257.
17. Mittrucker HW, Matsuyama T, Grossman A, et al. Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science*. 1997;275:540-543.
18. Natkunam Y, Warnke RA, Montgomery K, Falini B, van De Rijn M. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. *Mod Pathol*. 2001;14:686-694.
19. Court EL, Davidson K, Smith MA, et al. C-kit mutation screening in patients with acute myeloid leukaemia: adaptation of a Giemsa-stained bone-marrow smear DNA extraction technique. *Br J Biomed Sci*. 2001;58:76-84.
20. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest*. 1998;102:1515-1525.