Cyclin D3 Is a Predictive and Prognostic Factor in Diffuse Large B-cell Lymphoma[1]

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ABSTRACT

Cyclin D3 is an important regulator for transition from G_1 to the S phase of the cell cycle. Cyclin D3 expression is associated with cell proliferation in lymphoid tissues, but its impact on clinical outcome in non-Hodgkin's lymphomas has not been studied. Therefore, we determined the clinical relevance of cyclin D3 expression in patients with diffuse large B-cell lymphoma. We examined the relation between cyclin D3 expression at diagnosis and response to conventional polychemotherapy and overall survival in 81 previously untreated patients with diffuse large B-cell lymphoma. Cyclin D3 expression was assessed by immunohistochemistry. Cyclin D3 immunostaining ranged from 0–100% (median, 30%) of the lymphoma cells. Patients with high (≥50% cyclin D3-positive lymphoma cells) cyclin D3 expression had a more advanced clinical stage ($P = 0.003$) and more often had extranodal disease in more than one site ($P = 0.007$) than patients with low cyclin D3 expression. Patients with high cyclin D3 expression had a significantly lower complete response rate (17% *versus* 74%; $P < 0.001$) and a shorter overall survival (3-year survival rate, 18% *versus* 74%; $P < 0.001$) than those with low cyclin D3 expression. Multivariate analyses that included cyclin D3 and the International Prognostic Index demonstrated that cyclin D3 expression had independent effects on the complete response rates and overall survival of the patients. In conclusion, high cyclin D3 expression is an independent predictive and prognostic factor associated with poor clinical outcome in patients with diffuse large B-cell lymphoma.

INTRODUCTION

Diffuse large B-cell lymphomas can be treated effectively with conventional polychemotherapy regimens with or without radiotherapy (1, 2). High-risk patients may benefit from either initial high-dose induction chemotherapy with stem cell support (3) or high-dose consolidation treatment with hematopoietic stem cell support after having achieved complete response from initial chemotherapy (4). However, many patients are not cured by chemotherapy because of drug-resistant disease.

Different cellular mechanisms including drug transport, decreased activation or increased inactivation of anticancer drugs, drug target alteration, enhanced DNA repair, and altered apoptosis can contribute to drug resistance. A high tumor proliferation rate may also cause drug resistance. In patients with aggressive lymphoma, Ki-67 expression as a marker for increased cell proliferation predicted poor response to chemotherapy and shorter survival of the patients (5).

Cell proliferation is controlled by a complex network of cell cycle regulators including cyclin D3 (6). Cyclin D3 is a member of the cyclin D family, which also includes cyclin D1 and D2. The cyclin D family forms complexes with cyclin-dependent kinases 4 and 6, which promote phosphorylation and inactivation of the retinoblastoma protein, thereby mediating the progression of cells from G_1 to the S phase of the cell cycle (6). Cyclin D3 has a dual role: it can promote cell proliferation as seen in lymphoid tissue, and it can induce and/or maintain terminal differentiation in other tissues (7). Because of its potential association with proliferation in lymphoma cells (7, 8), cyclin D3 expression may affect clinical outcome in non-Hodgkin's lymphomas. Therefore, we have investigated the relationship between cyclin D3 expression and both response to chemotherapy and overall survival in previously untreated patients with diffuse large B-cell lymphoma.

PATIENTS AND METHODS

Patients and Chemotherapy. Eighty-one previously untreated patients with diffuse large B-cell lymphoma who had been treated at our institution between 1991 and 2000 and from whom paraffin blocks were available were included in this study. All biopsy samples were diagnosed and reviewed at the Institute of Clinical Pathology by experienced hematopathologists. Between 1991 and 1994, diagnosis was based on the criteria of the Kiel classification (9). Later on, lymphomas were typed according to the criteria provided in the Revised European-American Lymphoma classification (10–12), and the former cases were reviewed and also classified according to Revised European-American Lymphoma criteria. Lymphomas were subtyped using standard histological and immunohistological methods. Cases with antecedent low-grade B-cell lymphoma were not included in this study.

The clinical characteristics of the patients are summarized in Table 1 and are similar to those of a large clinical trial of

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Table 1 Cyclin D3 and characteristics of patients with diffuse large B-cell lymphoma

		Low cyclin D3		High cyclin D3		
	Total	*n*	%	*n*	%	*P*
No. of patients	81	50	62	31	38	
Age (yrs)						
≤60	46	31	67	15	33	0.2
>60	35	19	54	16	46	
Sex						
Female	37	27	73	10	27	0.06
Male	44	23	52	21	48	
ECOG performance status*						
≤1	66	44	67	22	33	0.06
>1	15	6	40	9	60	
Stage						
I or II	43	33	77	10	23	0.003
III or IV	38	17	45	21	55	
Extranodal sites						
≤1 site	56	40	71	16	29	0.007
>1 site	25	10	40	15	60	
Lactate dehydrogenase						
≤240 units/liter	32	23	72	9	28	0.1
>240 units/liter	49	27	55	22	45	
International Prognostic Index						
Low risk	36	26	72	10	28	
Low-intermediate risk	15	11	73	4	27	0.007
High-intermediate risk	16	10	63	6	37	
High risk	14	3	21	11	79	
MIB1 (n = 76)						
<80%	42	29	69	13	31	0.2
≥80%	34	19	56	15	44	
Chemotherapy						
CHOP	58	33	57	25	43	
ProMACE-CytaBOM	19	14	77	5	23	
CEP	1	1	100	0	0	0.4
CHEOP/IMVP-16	1	1	100	0	0	
IMVP-16	1	1	100	0	0	
No chemotherapy	1	0	0	1	100	

patients with diffuse large B-cell lymphoma (4). Eighty patients received polychemotherapy with established protocols. One patient died before chemotherapy. Fifty-eight patients were treated with CHOP,[3] and 19 patients were treated with ProMACE-CytaBOM. One patient received CEP, one patient received IMVP-16, and one patient received CHEOP/IMVP-16. Patients with stage I disease received three to four cycles of chemotherapy plus consecutive radiotherapy (1). Patients with stage II-IV disease received six cycles of chemotherapy. Eight of them with bulky disease who achieved a complete response were treated with additional radiotherapy after six cycles of chemotherapy. All 80 patients were evaluable for response. Response to chemotherapy was assessed according to standard criteria (13). Complete response was defined as the absence of clinical and radiological evidence of disease for a minimum of at least 2 months. Nineteen patients received either additional autologous bone marrow transplantation or autologous stem cell transplantation. In these patients, response assessment was performed after chemotherapy but before transplantation.

Age, tumor stage, lactate dehydrogenase, ECOG performance status, and the number of extranodal sites of the disease were used to determine the International Prognostic Index (14). For statistical analysis, patients were classified as low risk, low-intermediate risk, high-intermediate risk, and high risk.

Immunohistochemical Detection of Cyclin D3. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded lymphoma specimens. Paraffin sections were mounted on poly-L-lysine-coated glass microslides. Sections were deparaffinized and rehydrated by consecutive submersions in xylene (two changes, 10 min each), absolute ethanol (two changes, 5 min each), 70% ethanol (two changes, 5 min each), and distilled water (3 min). Endogenous peroxidase activity was blocked by incubation in 0.06% hydrogen peroxide for 10 min at room temperature, and slides were washed in PBS (pH 7.4). Sections were boiled for 10 min in 10 mM citrate buffer (pH 6.0) in a standard pressure cooker for antigen retrieval. After cooling down for 15 min and washing in PBS (pH 7.4), the tissues were preincubated for 20 min in normal serum (1:50; Dako, Glostrup, Denmark) before a 60-min incubation with the cyclin D3 monoclonal antibody (clone DCS-22; antibody used at 4 μg/ml; Neomarkers, Fremont, CA). Antibody binding was detected by the avidin-biotin-peroxidase method. Bound peroxidase was developed with 3,3′-diaminobenzidine (Dako). The slides were counterstained with Mayer's Hämalaun and mounted with Aquatex (Merck, Darmstadt, Germany). All of the washes were performed in PBS (pH 7.4).

Expression of cyclin D3 in normal epithelial cells, endothelial cells of small blood vessels, or blast cells of germinal centers was used as an internal positive control of immunostaining (7, 8). Small lymphocytes that usually do not express cyclin D3 served as an internal negative control (8). In addition, negative controls without the primary antibody were performed as described above. Staining of lymphoma cells was examined by two investigators (M. F. and I. S.) without prior knowledge of the clinical outcome of the patients, and the concordance of their evaluation was high. The rare discrepant cases were reassessed together by both investigators, and a consensus was reached. Specimens were scored for the percentage of stained nuclei of lymphoma cells. Only nuclear staining was scored as positive.

Statistical Analysis. Associations of cyclin D3 expression with clinical as well as laboratory parameters and with response to chemotherapy were assessed by the χ^2 test. The values of cyclin D3 expression were compared by Mann-Whitney U tests. Survival probabilities were calculated with the product limit method according to Kaplan-Meier (15). Overall survival time was defined as the period between the time of diagnosis and the time of death. Survival times of patients still alive or of patients who underwent transplantation were censored with the date of the last follow-up or transplantation, respectively. Differences between survival curves were analyzed by means of the log-rank test. To describe the

[3] The abbreviations used are: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; ProMACE-CytaBOM, prednisone, doxorubicin, cyclophosphamide, etoposide, cytarabine, bleomycin, vincristine, methotrexate, and leucovorin; CEP, lomustine, etoposide, and prednimustine; IMVP-16, ifosfamide, methotrexate, and etoposide; CHEOP/IMVP-16, cyclophosphamide, doxorubicin, vincristine, prednisone, and etoposide and ifosfamide, methotrexate, and etoposide; ECOG, Eastern Cooperative Oncology Group; CI, confidence interval.

unadjusted effects of covariates on response rates and on survival, univariate logistic and Cox proportional hazards regression models were used. Multiple logistic regression models and Cox proportional hazards regression models were used to assess the independent effects of cyclin D3 expression and the International Prognostic Index on response rates and survival (16). All *P*s are the results of two-sided tests. The SAS statistical software system (SAS Institute Inc., Cary, NC) was used for calculations.

Table 2 Logistic regression analysis for no complete response to chemotherapy

	Univariate			Multivariate		
	Odds ratio	95% CI*	*P*	Odds ratio	95% CI	*P*
High cyclin D3	14.2	4.5–44.9	<0.001	18.8	4.3–83.3	<0.001
International Prognostic Index	3.6	2.1–6.3	<0.001	4.4	2.1–9.2	<0.001

RESULTS

Cyclin D3 Expression in Diffuse Large B-cell Lymphomas at Diagnosis. Cyclin D3 expression was immunohistochemically determined in 81 previously untreated patients with diffuse large B-cell lymphoma. Cyclin D3 immunostaining was nuclear and ranged from 0–100% of the lymphoma cells. The median value of cyclin D3 expression of the series was 30%. Comparisons of cyclin D3 expression with clinical parameters including response to chemotherapy and survival were performed with cyclin D3 expression as a continuous variable and as a dichotomized variable classified as high (≥50% cyclin D3-positive lymphoma cells) or low (<50% cyclin D3-positive lymphoma cells). This classification as high and low has the advantage of being more easily reproducible. High cyclin D3 expression was observed in 31 (38%) patients.

Correlation of Cyclin D3 with Clinical and Laboratory Parameters. The major clinical and laboratory findings of the patients grouped according to high or low cyclin D3 expression are summarized in Table 1. Patients with high cyclin D3 expression in their tumors had a more advanced clinical stage ($P = 0.003$) and more often had extranodal disease in more than one site ($P = 0.007$) than patients with low cyclin D3 expression. There was also a significant difference in the proportion of patients with high cyclin D3 expression between the group with either low risk, low-intermediate risk, or high-intermediate risk and the group with high risk according to the International Prognostic Index ($P = 0.007$). Patients with high or low cyclin D3 expression did not differ significantly in other clinical or laboratory parameters including age and sex of the patients, ECOG performance status, lactate dehydrogenase, and treatment. In addition, we observed no significant association between cyclin D3 expression and the proliferation marker MIB1 (Table 1).

Comparable results were obtained if cyclin D3 expression as a continuous variable was compared with the characteristics of the patients (data not shown).

Cyclin D3 and Response to Chemotherapy. Eighty patients were treated with conventional polychemotherapy. The treatment protocols were equally distributed among patients with high cyclin D3 expression and those with low cyclin D3 expression (Table 1). All 80 patients were evaluable for response to chemotherapy. The complete response rate of the total study population was 53%. Partial responses and no responses were seen in 17% and 30% of the patients, respectively. The complete response rate was 17% for patients with high cyclin D3 expression and 74% for those with low cyclin D3 expression ($P < 0.001$). A similar difference (12% *versus* 79%; $P < 0.001$) was observed when only patients receiving CHOP chemotherapy ($n = 58$) were analyzed.

Cyclin D3 evaluated as a continuous variable was also associated with response to chemotherapy. Patients with no complete response had significantly higher percentages of cyclin D3-expressing tumor cells than patients who achieved a complete response (median, 55% *versus* 10%; $P < 0.001$).

Advanced tumor stage ($P < 0.001$), extranodal disease in more than one site ($P < 0.001$), poor ECOG performance status ($P = 0.002$), elevated lactate dehydrogenase ($P = 0.001$), and the International Prognostic Index ($P < 0.001$) were also significantly associated with poor outcome of chemotherapy.

We also evaluated the importance of high cyclin D3 expression in subgroups of patients classified according to the International Prognostic Index. There was a significant difference in complete response rates between patients with high cyclin D3 expression and those with low cyclin D3 expression in the group with low risk or low-intermediate risk (36% *versus* 87%; $P < 0.001$) and in the group with high-intermediate risk or high risk (0% *versus* 39%; $P = 0.006$).

The impact of cyclin D3 on outcome of chemotherapy was also observed in patients with stage I or II disease and in those with stage III or IV disease. In patients with stage I or II disease, the complete response rate was 30% (3 of 10 patients) for patients with high cyclin D3 expression and 85% (28 of 33 patients) for patients with low cyclin D3 expression ($P = 0.001$). In patients with stage III or IV disease, the complete response rate was 10% (2 of 20 patients) for patients with high cyclin D3 expression and 53% (9 of 17 patients) for patients with low cyclin D3 expression ($P = 0.004$).

We performed logistic regression analyses that included cyclin D3 and the International Prognostic Index. In the univariate analysis, the odds ratios of failure to achieve a complete response were 14.2 for high cyclin D3 expression (95% CI, 4.5–44.9; $P < 0.001$) and 3.6 for the International Prognostic Index (95% CI, 2.1–6.3; $P < 0.001$; Table 2). In the multivariate analysis, these odds ratios were 18.8 for high cyclin D3 expression (95% CI, 4.3–83.3; $P < 0.001$) and 4.4 for the International Prognostic Index (95% CI, 2.1–9.2; $P < 0.001$; Table 2). Comparable results were obtained when only patients receiving CHOP chemotherapy were analyzed or when cyclin D3 was included as a continuous variable in the model (data not shown). Thus, cyclin D3 expression had an independent effect on the rate of complete response.

Cyclin D3 and Survival. The median follow-up of the total study population was 2.5 years, and the maximum follow-up was 8.5 years. Thirty patients died (18 patients with high cyclin D3 expression and 12 patients with low cyclin D3 expression). The estimated 3-year overall survival of all 81 patients was 54%. Patients with high cyclin D3 expression had a

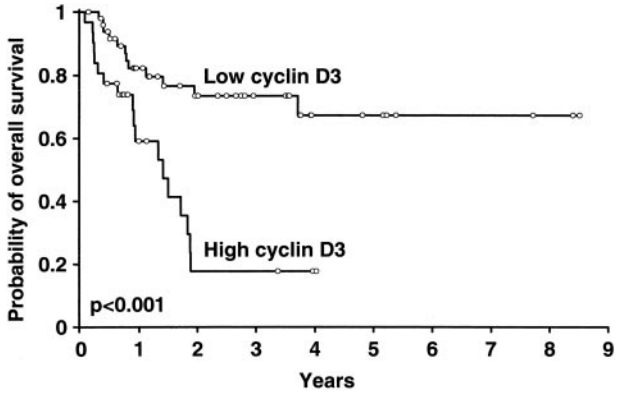

Fig. 1 Cyclin D3 expression and overall survival of patients with diffuse large B-cell lymphoma. Cyclin D3 expression in lymphoma cells was determined by immunohistochemistry, and overall survival was estimated according to the method of Kaplan-Meier in 81 patients. Survival data based on cyclin D3 expression are shown. The 31 patients with high cyclin D3 expression had a significantly shorter overall survival than the 50 patients with low cyclin D3 expression. Statistical comparison between survival curves was done by the log-rank test.

shorter overall survival than those with low cyclin D3 expression (Fig. 1). Kaplan-Meier estimate of survival at 3 years was 18% for patients with high cyclin D3 expression and 74% for patients with low cyclin D3 expression ($P < 0.001$). A difference in overall survival was also seen when only patients treated with CHOP were analyzed (3-year survival rate, 15% *versus* 71%; $P < 0.001$).

In the group with low risk or low-intermediate risk, patients with high cyclin D3 expression had a 3-year overall survival of 38%, whereas patients with low cyclin D3 expression had a 3-year overall survival of 80% ($P = 0.02$). However, in the group with high-intermediate or high risk, survival was not significantly different between patients with high cyclin D3 expression (3-year survival rate, 0%) and those with low cyclin D3 expression (3-year survival rate, 56%; $P = 0.07$).

In patients with stage III-IV disease, overall survival remained significantly shorter in patients with high cyclin D3 expression than in those with low cyclin D3 expression (3-year survival rate, 11% *versus* 65%; $P = 0.04$).

In the univariate Cox regression analysis, the relative risk for death was 3.9 for high cyclin D3 expression (95% CI, 1.9–8.3; $P < 0.001$) and 2.1 for the International Prognostic Index (95% CI, 1.5–2.9; $P < 0.001$; Table 3). Age > 60 years (relative risk, 2.1; $P = 0.04$), advanced clinical stage (relative risk, 3.1; $P = 0.003$), extranodal disease in more than one site (relative risk, 2.5; $P = 0.01$), and poor ECOG performance status (relative risk, 3.2; $P = 0.006$) were also significantly associated with shorter overall survival. In the multivariate Cox regression analysis that included cyclin D3 and the International Prognostic Index, the relative risk for death was 2.6 for high cyclin D3 expression (95% CI, 1.2–5.8; $P = 0.02$) and 1.8 for the International Prognostic Index (95% CI, 1.2–2.5; $P = 0.002$; Table 3). Comparable results were obtained when only patients receiving CHOP chemotherapy were analyzed or when cyclin D3 was included as a continuous variable in the model (data not shown). Thus, cyclin D3 expression was an independent prognostic factor.

Table 3 Cox regression analysis for overall survival of patients with diffuse large B-cell lymphoma

	Univariate			Multivariate		
	Relative risk	95% CI*	*P*	Relative risk	95% CI	*P*
High cyclin D3	3.9	1.9–8.3	<0.001	2.6	1.2–5.8	0.02
International Prognostic Index	2.1	1.5–2.9	<0.001	1.8	1.2–2.5	0.002

DISCUSSION

In the present study, high cyclin D3 expression in the tumor cells of patients with diffuse large B-cell lymphoma predicted poor response to chemotherapy and shorter overall survival. Although high cyclin D3 expression was associated with other predictors of poor outcome in diffuse large B-cell lymphomas including advanced tumor stage and extranodal disease in more than one site, multivariate analyses demonstrated that the effect of cyclin D3 expression was independent of the International Prognostic Index. Thus, these data suggest an important role of cyclin D3 in the clinical outcome of non-Hodgkin's lymphomas.

Our study is the first to address the impact of cyclin D3 on clinical outcome in diffuse large B-cell lymphoma. The degree of cyclin D3 expression in our study is similar to the ones in two other studies (17, 18). We did not observe a correlation between cyclin D3 expression and proliferation as measured by MIB1 in our study. Møller *et al.* (19) studied cyclin D3 in B-cell lymphomas and indolent lymphomas and found a higher mean proliferation rate in cyclin D3-positive lymphomas than in cyclin D3-negative lymphomas but did not find a difference in proliferation rate in the subgroup of diffuse large B-cell lymphoma, which is consistent with our study. In another study (17), cyclin D3 expression correlated with proliferation rate, but these findings were based on only 12 patients with diffuse large B-cell lymphoma.

The importance of the cell cycle for clinical outcome requires the development of modulators of cell cycle regulators. Several agents that interfere with cell cycle-regulatory proteins have already entered preclinical and clinical trials (20). Cyclin D3 may be an interesting target for new therapeutic approaches in refractory diffuse large B-cell lymphomas. Possible modulators of cyclin D3 expression are IFN-α and flavopiridol. In Daudi Burkitt's lymphoma cells, IFN-α inhibits growth, and this inhibition is associated with a rapid decrease of cyclin D3 expression (21). A subset of patients with relapsed or refractory diffuse large-cell lymphoma may respond to IFN-α therapy (22), but no data on cyclin D3 expression are available in these patients. IFN-α has also been studied as maintenance therapy in stage III and IV diffuse large-cell lymphoma patients in complete remission after chemotherapy (23). Patients who received IFN-α had a significantly longer disease-free survival and overall survival than patients without maintenance therapy (23). Flavopiridol, a semisynthetic flavonoid, can induce cell cycle arrest by directly inhibiting cyclin-dependent kinase inhibitors

and by down-regulation of cyclin D1 and cyclin D3 (20). In a clinical Phase I trial, flavopiridol resulted in minor responses in refractory cancers including non-Hodgkin's lymphoma (24).

In conclusion, high cyclin D3 expression is an independent predictive and prognostic factor associated with poor clinical outcome in patients with diffuse large B-cell lymphoma. After confirmation of our results by other investigators and on other patient populations, cyclin D3 might become a clinically useful marker for the selection of patients for specific treatments, and modulation of cyclin D3 expression may be a potential therapeutic strategy to improve clinical outcome in patients with diffuse large B-cell lymphoma in the future.

REFERENCES

1. Miller, T. P., Dahlberg, S., Cassady, J. R., Adelstein, D. J., Spier, C. M., Grogan, T. M., LeBlanc, M., Carlin, S., Chase, E., and Fisher, R. I. Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. N. Engl. J. Med., *339:* 21–26, 1998.

2. Fisher, R. I., Gaynor, E. R., Dahlberg, S., Oken, M. M., Grogan, T. M., Mize, E. M., Glick, J. H., Coltman, C. A., and Miller, T. P. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N. Engl. J. Med., *328:* 1002–1006, 1993.

3. Gianni, A. M., Bregni, M., Siena, S., Brambilla, C., Di Nicola, M., Lombardi, F., Gandola, L., Tarella, C., Pileri, A., Ravagnani, F., Valagussa, P., and Bonadonna, G. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N. Engl. J. Med., *336:* 1290–1297, 1997.

4. Haioun, C., Lepage, E., Gisselbrecht, C., Bastion, Y., Coiffier, B., Brice, P., Bosly, A., Dupriez, B., Nouvel, C., Tilly, H., Lederlin, P., Biron, P., Brière, J., Gaulard, P., and Reyes, F. Benefit of autologous bone marrow transplantation over sequential chemotherapy in poor-risk aggressive non-Hodgkin's lymphoma: updated results of the prospective study LNH87–2, the Groupe d'Etude des Lymphomes de l'Adulte. J. Clin. Oncol., *15:* 1131–1137, 1997.

5. Miller, T. P., Grogan, T. M., Dahlberg, S., Spier, C. M., Braziel, R. M., Banks, P. M., Foucar, K., Kjeldsberg, C. R., Levy, N., Nathwani, B. N., Schnitzer, B., Tubbs, R. R., Gaynor, E. R., and Fisher, R. I. Prognostic significance of the Ki-67-associated proliferative antigen in aggressive non-Hodgkin's lymphomas: a prospective Southwest Oncology Group trial. Blood, *83:* 1460–1466, 1994.

6. Sherr, C. J. Cancer cell cycles. Science (Wash. DC), *274:* 1672–1677, 1996.

7. Bartkova, J., Lukas, J., Strauss, M., and Bartek, J. Cyclin D3: requirement for G_1/S transition and high abundance in quiescent tissues suggest a dual role in proliferation and differentiation. Oncogene, *17:* 1027–1037, 1998.

8. Doglioni, C., Chiarelli, C., Macri, E., Dei Tos, A. P., Meggiolaro, E., Dalla Palma, P., and Barbareschi, M. Cyclin D3 expression in normal, reactive, and neoplastic tissues. J. Pathol., *185:* 159–166, 1998.

9. National Cancer Institute-sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer (Phila.), *49:* 2112–2135, 1982.

10. Harris, N. L., Jaffe, E. S., Stein, H., Banks, P. M., Chan, J. K. C., Cleary, M. L., Delsol, G., De Wolf-Peeters, C., Falini, B., Gatter, K. C., Grogan, T. M., Isaacson, P. G., Knowles, D. M., Mason, D. Y., Muller-Hermelink, H-K., Pileri, S. A., Piris, M. A., Ralfkiaer, E., and Warnke, R. A. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood, *84:* 1361–1392, 1994.

11. Armitage, J. O., and Weisenburger, D. D., for the Non-Hodgkin's Lymphoma Classification Project. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. J. Clin. Oncol., *16:* 2780–2795, 1998.

12. Harris, N. L., Jaffe, E. S., Diebold, J., Flandrin, G., Muller-Hermelink, H. K., Vardiman, J., Lister, T. A., and Bloomfield, C. D. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the clinical advisory committee meeting, Airlie House, Virginia, November 1997. J. Clin. Oncol., *17:* 3835–3849, 1999.

13. Cheson, B. D., Horning, S. J., Coiffier, B., Shipp, M. A., Fisher, R. I., Connors, J. M., Lister, T. A., Vose, J., Grillo-López, A., Hagenbeek, A., Cabanillas, F., Klippensten, D., Hiddemann, W., Castellino, R., Harris, N. L., Armitage, J. O., Carter, W., Hoppe, R., and Canellos, G. P. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J. Clin. Oncol., *17:* 1244–1253, 1999.

14. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N. Engl. J. Med., *329:* 987–994, 1993.

15. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc., *53:* 457–481, 1958.

16. Cox, D. R. Regression models and life tables. J. R. Stat. Soc., *34:* 187–220, 1972.

17. Ott, M. M., Bartkova, J., Bartek, J., Dürr, A., Fischer, L., Ott, G., Müller-Hermelink, H. K., and Kreipe, H. Cyclin D1 expression in mantle cell lymphoma is accompanied by down-regulation of cyclin D3 and is not related to the proliferative activity. Blood, *90:* 3154–3159, 1997.

18. Sánchez-Beato, M., Camacho, F. I., Martinez-Montero, J. C., Sáez, A. I., Villuendas, R., Sánchez-Verde, L., Garcia, J. F., and Piris, M. A. Anomalous high p27/KIP1 expression in a subset of aggressive B-cell lymphomas is associated with cyclin D3 overexpression. p27/KIP1-cyclin D3 colocalization in tumor cells. Blood, *94:* 765–772, 1999.

19. Møller, M. B., Nielsen, O., and Pedersen, N. T. Cyclin D3 expression in non-Hodgkin lymphoma. Correlation with other cell cycle regulators and clinical features. Am. J. Clin. Pathol., *115:* 404–412, 2001.

20. Senderowicz, A. M., and Sausville, E. A. Preclinical and clinical development of cyclin-dependent kinase modulators. J. Natl. Cancer Inst. (Bethesda), *92:* 376–387, 2000.

21. Tiefenbrun, N., Melamed, D., Levy, N., Resnitzky, D., Hoffman, I., Reed, S. I., and Kimchi, A. α Interferon suppresses the *cyclin D3* and *cdc25A* genes, leading to a reversible G_0-like arrest. Mol. Cell. Biol., *16:* 3934–3944, 1996.

22. Armitage, J. O., and Coiffier, B. Activity of interferon-α in relapsed patients with diffuse large B-cell and peripheral T-cell non-Hodgkin's lymphoma. Ann. Oncol., *11:* 359–361, 2000.

23. Aviles, A., Diaz-Maqueo, J. C., Garcia, E. L., Talavera, A., and Guzman, R. Maintenance therapy with interferon-α2b in patients with diffuse large-cell lymphoma. Invest. New Drugs, *10:* 351–355, 1992.

24. Senderowicz, A. M., Headlee, D., Stinson, S. F., Lush, R. M., Kalil, N., Villalba, L., Hill, K., Steinberg, S. M., Figg, W. D., Tompkins, A., Arbuck, S. G., and Sausville, E. A. Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. J. Clin. Oncol., *16:* 2986–2999, 1998.