Prognostic Factors in Survival of Patients With Stage Ta and T1 Bladder Urothelial Tumors

The Role of G_1-S Modulators (p53, p21Waf1, p27Kip1, Cyclin D1, and Cyclin D3), Proliferation Index, and Clinicopathologic Parameters

Antonio Lopez-Beltran, MD, PhD,[1,2] Rafael J. Luque, MD, PhD,[3] Jose Alvarez-Kindelan, MD,[4] Ana Quintero, PhD,[5] Felix Merlo, MD,[5] Maria J. Requena, MD,[3] and Rodolfo Montironi, MD, FRCPath[6]

Key Words: Bladder cancer; Survival; Cyclin D3; Cyclin D1; Tumor size; Ki-67; Grade; p21Waf1; p27Kip1; p53

DOI: 10.1309/LTFU3UUMBY095HUM

Abstract

*We studied 159 cases of superficial (stage Ta or T1) bladder tumors to determine the significance on survival of a subset of regulators of transition from G_1 to S phase of the cell cycle (p53, p21Waf1, p27Kip1, cyclin D1, cyclin D3) and tumor proliferation (Ki-67 [MIB-1]). Clinical findings (patient age, sex, tumor size, grade, stage [Ta or T1]) were included in the analysis. Univariate analysis revealed association of tumor size (*P = .0353*), grade in stage Ta tumors (*P = .0074*), cyclin D1 expression (*P = .0182*), and Ki-67 index (*P = .0033*) with disease-free survival and of tumor size (*P = .0005*), stage (*P = .0494*), cyclin D3 expression (*P = .0105*), and Ki-67 index (*P = .0272*) with overall survival. Cox multivariate analysis revealed cyclin D1 expression and high proliferation index (disease-free) and tumor size, cyclin D3 expression, and high proliferation index (overall survival) as independent predictors. Results suggest that alterations of the progression from the G_1 to S phase of the cell cycle are common in papillary urothelial bladder tumors. High tumor proliferation, expression of cyclins D1 and D3, and tumor size at diagnosis might be relevant predictors of survival in patients with stage Ta and T1 bladder urothelial tumors.*

At diagnosis, most bladder tumors are superficial (stage Ta or T1); 70% of them are papillary noninvasive (stage Ta), and the remaining 30% manifest with early stromal invasion (stage T1).[1,2] Following initial transurethral resection of the bladder tumor, patients at risk for recurrence or progression can be identified by a variety of prognostic factors, including tumor grade, the depth of bladder wall invasion, the presence of vascular or lymphatic invasion and early recurrence, the number and size of tumors, and the finding of concomitant dysplasia or carcinoma in situ.[1,3-6] However, it has been recognized that these prognostic factors are not sufficiently accurate to predict true biologic behavior[1]; therefore, more reliable indicators of biologic aggressiveness are needed in stage Ta and T1 bladder tumors.[7]

In these tumors, although studies on prognostic biologic markers are limited, a number of cell cycle aberrations have been studied in an attempt to identify tumors prone to progress, but most reports show variable and somewhat contradictory results.[7-10] Tumor proliferation is considered a powerful prognostic indicator of tumor recurrence, and p53 nuclear accumulation is viewed as a marker of progression in superficial bladder tumors[11-15]; but some studies failed to demonstrate any independent prognostic significance in patients with superficial bladder tumors.[16-21] Data concerning other G_1-S modulators such as p21Waf1, p27Kip1, and cyclin D1 are limited,[8,9,18,21-28] and alterations of cyclin D3, another upstream regulator of the cell cycle, have not been reported in bladder cancer.[29,30] Nevertheless, it has been suggested that alterations of cell cycle regulatory proteins involved in the progression from G_1 to S phase are among the most promising markers,[29] and their role in the prognosis of superficial bladder tumors needs to be established.

The purpose of our study was to determine the predictive value of the expression of the G_1-S modulators p53, p21Waf1, p27Kip1, cyclin D1, and cyclin D3 and of cell proliferation as measured by the Ki-67 (MIB-1) labeling index in the survival of patients with primary Ta and T1 bladder cancer. Patient age and sex, tumor size, pathologic stage, and grade also entered the analysis as clinical and pathologic variables.

Materials and Methods

Patients and Clinical Follow-up

A sequential cohort series of 159 patients with primary bladder tumors who underwent complete transurethral resection of bladder and random bladder biopsies was the study group. Resection of primary tumors was performed by 6 staff urologists, and the patients were seen at Reina Sofia University Hospital, Cordoba, Spain, between January 1990 and December 1995. Patients diagnosed as having high-grade bladder carcinoma additionally received intravesical instillation of bacille Calmette-Guérin (Organon, West Orange, NJ; Tice strain, 2-5 × 10^8 colony-forming units weekly for 6 consecutive weeks, starting 10-15 days after transurethral resection of bladder) with no maintenance as the current protocol in clinical practice.

Patient follow-up, calculated as the number of months from the date of the diagnostic surgical procedure to the date of the most recent cystoscopy (or to the date of the last visit or death), was 5 to 12 years (mean ± SD, 74.8 ± 28.1 months; range, 6-120 months).

Tumor recurrence was defined as reappearance of tumor after the initial treatment, with at least 1 tumor-free cystoscopy in the interval. *Survival time* was defined as the period between the time of diagnosis and the time of death. *Cancer-related death* was defined as that caused by bladder carcinoma. The end point of the study was disease-free and overall cancer-specific survival. Preoperative excretory urography was performed to exclude cases with evidence of upper urinary tract disease, and *tumor size* was defined as the largest tumor measured with the resection loop that is 1 cm long; thus, patients were stratified as having tumors of less than 3 cm, 3 to 5 cm, and more than 5 cm.

All available H&E-stained slides, including primary tumors and recurrences, were reevaluated by 3 dedicated pathologists (A.L.B., R.J.L., and A.Q.) without knowledge of the clinical status and were graded in accordance with the 2004 World Health Organization (WHO) classification (former WHO/International Society of Urological Pathology, 1998).[5,6] Clinical and pathologic staging was determined in accordance with the 2002 TNM revision.[31]

Immunohistochemical Analysis

A representative paraffin-embedded specimen was selected from each tumor based on the amount of neoplastic tissue present. Then, blocks were cut serially at 4 µm thick, deparaffinized in xylene, rehydrated in graded ethanol, and washed for 5 minutes with phosphate-buffered saline. For antigen retrieval, the sections were boiled immersed in a 10-mmol/L concentration of citrate buffer (pH 6.0). Endogenous peroxidase was blocked by incubation of the slides for 30 minutes with 3% hydrogen peroxide in methanol. Sections then were incubated with primary mouse monoclonal antibodies at room temperature ▮Table 1▮. Immunohistochemical stains were performed using a highly sensitive, polymer-based detection system (EnVision, DAKO, Glostrup, Denmark) for 30 minutes at room temperature. Diaminobenzidine substrate solution (0.6 mg/mL in tris(hydroxymethyl)aminomethane-buffered saline, pH 7.6 with 12 mL of 30% hydrogen peroxide) was used as a chromogen. Sections were counterstained with Mayer hematoxylin, dehydrated, and mounted using a standard procedure. Appropriate controls, positive and negative (replacing the primary antibody with distilled water), were included (Table 1).

Evaluation

The quantitative and qualitative assessment of the immunohistochemical study was conducted using a Nikon Labophot optical microscope (Tokyo, Japan). Three dedicated pathologists (A.L.B., R.J.L., and A.Q.) independently evaluated all immunohistochemical slides in a blinded manner without knowledge of clinical information. The same area on each slide

▮Table 1▮
Characteristics of Immunohistochemical Markers

Marker	Clone*	Source	Working Dilution	Positive Control Sample	Incubation Time (min)
Cyclin D1	DCS-6	DAKO, Glostrup, Denmark	1:25	Mantle cell lymphoma	60
Cyclin D3	DCS-22	DAKO	1:25	Breast carcinoma	60
Ki-67	MIB-1	Conepta Biosystems, Barcelona, Spain	Prediluted	Tonsil	60
p21WAF1/Cip1	SX118	DAKO	1:25	Breast carcinoma	45
p27Kip1	SX53G8	DAKO	1:25	Colon adenocarcinoma	45
p53	PAb1801	Novocastra, Newcastle upon Tyne, England	1:40	Breast carcinoma	60

* All were mouse monoclonal antibodies.

DOI: 10.1309/LTFU3UUMBY095HUM

was examined. All markers were quantitated by using random fields measuring 62,500 μm^2 delineated by using a 1-cm^2 graded ocular grid attached to the eyepiece of the microscope. The regions were chosen inside areas of tumor with high immunoreactivity and were examined under high-power (×400) view, counting a mean of 1,000 cells per case. A labeling index expressed as the percentage of positive nuclei was established for each marker, and the cases were stratified according to a low vs high expression scheme (cyclins D1 and D3, <15% vs ≥15%; p21Waf1, <10% vs ≥10%; p27Kip1, <30% vs ≥30%; Ki-67, < 13% vs ≥13%; p53, <6% vs ≥6%) **Table 2**.

Statistical Analysis

Univariate survival analysis was conducted using the Kaplan-Meier method, and differences among groups were tested for significance using the log-rank test. In addition, significant variables in the univariate analysis were entered in a multivariate analysis of probable prognostic factors for survival using Cox proportional hazards regression analysis, and the relative risk with 95% confidence interval was calculated. All statistical analyses were performed using SPSS for Windows (SPSS, Chicago, IL). A *P* value of .05 or less was considered indicative of a statistically significant difference. All tests were 2-sided.

Results

Demographic and clinical data for the 159 patients (18 women) are shown in Table 2. The mean age was 61

Table 2
Univariate Analysis of Survival in 159 Patients* With Stage Ta and T1 Bladder Urothelial Tumors According to the Kaplan-Meier Method and the Log-Rank Test†

	Survival						
Variable	**Overall**	**Disease-Free**	**Log-Rank Test**	***P***	**Alive**	**Log-Rank Test**	***P***
Age (y)			0.95	.3305		2.09	.1483
<65	66 (41.5)	28 (42)			59 (89)		
≥65	93 (58.5)	50 (54)			77 (83)		
Sex			1.05	.3058		2.84	.0921
F	18 (11.3)	8 (44)			18 (100)		
M	141 (88.7)	70 (49.6)			118 (83.7)		
Tumor size (cm)			6.69	.0353		15.13	.0005
<3	104 (65.4)	55 (52.9)			95 (91.3)		
3-5	44 (27.7)	20 (45)			34 (77)		
>5	11 (6.9)	3 (27)			7 (64)		
Stage			1.82	.1768		3.86	.0494
Ta	75 (47.2)	41 (55)			68 (91)		
T1	84 (52.8)	37 (44)			68 (81)		
Ta/grade (n = 75)			9.80	.0074		2.79	.0950
PUNLMP	26 (35)	14 (54)			24 (92)		
Low	34 (45)	23 (68)			31 (91)		
High	15 (20)	4 (27)			11 (73)		
T1/grade (n = 84)			0.15	.7031		1.35	.3748
Low	58 (69)	24 (41)			49 (84)		
High	26 (31)	13 (50)			21 (81)		
Cyclin D3 (%)			0.22	.6368		6.55	.0105
<15	138 (86.8)	65 (47.1)			121 (87.7)		
≥15	21 (13.2)	13 (62)			15 (71)		
Cyclin D1 (%)			5.58	.0182		1.99	.1581
<15	106 (66.7)	60 (56.6)			94 (88.7)		
≥15	53 (33.3)	18 (34)			42 (79)		
p21Waf1 (%)			0.53	.4662		0.01	.9216
<10	72 (45.3)	38 (53)			62 (86)		
≥10	87 (54.7)	40 (46)			74 (85)		
p27Kip1 (%)			0.01	.9081		2.61	.1065
<30	86 (54.1)	42 (49)			77 (90)		
≥30	73 (45.9)	36 (49)			59 (81)		
Ki-67 (MIB-1) (%)			8.64	.0033		4.88	.0272
<13	142 (89.3)	74 (52.1)			124 (87.3)		
≥13	17 (10.7)	4 (24)			12 (71)		
p53 (%)			0.42	.5178		1.50	.2211
<6	105 (66.0)	49 (47)			92 (87.6)		
≥6	54 (34.0)	29 (54)			44 (81)		

PUNLMP, papillary urothelial neoplasia of low malignant potential.
* Unless otherwise indicated.
† Marker categories are percentages of expression. Data are given as number (percentage). Percentages for disease-free survival and alive are based on the number of patients listed in the "Overall Survival" column.

DOI: 10.1309/LTFU3UUMBY095HUM

years (range, 29-93 years). The mean ± SD age for the group of patients older than 65 years was 75.2 ± 27.4 years. Most patients (65.4%) had tumors smaller than 3 cm. Almost half of the patients had stage Ta noninvasive tumors; of those 75 tumors, 26 (35%), 34 (45%), and 15 (20%) were classified as papillary urothelial neoplasia of low malignant potential (PUNLMP), low-grade carcinoma, and high-grade carcinoma, respectively. Most stage T1 tumors (58/84 [69%]) were classified as low-grade carcinoma. High nuclear expression (≥15%) of cyclins D3 and D1 was observed in 13.2% and 33.3% of cases, respectively. Low expression of p21Waf1 (<10%) and p27Kip1 (<30%) was present in 45.3% and 54.1% of tumors. Tumor proliferation of 13% or more was present in 10.7% and high p53 accumulation (≥6%) in 34.0% of tumors **Image 1**.

Disease-Free Survival

During the follow-up period, 78 patients (49.1%) patients remained free of disease. Univariate analysis showed tumor size, grade in stage Ta tumors (according to the 2004 WHO classification), cyclin D1 overexpression, and proliferation index to be associated with survival; age, sex, stage, grade in stage T1, and expression of cyclin D3, p21Waf1, p27Kip1, and p53 were not related to disease-free survival (Table 2). The tumor proliferation index and cyclin D1 expression were the only independent predictors of disease-free survival **Table 3** and **Figure 1**.

Overall Cancer-Specific Survival

At last follow-up, 136 patients (85.5%) remained alive. Tumor size and stage, cyclin D3 expression, and proliferation index were associated with overall survival in univariate analysis; age, sex, tumor grade in stage Ta and T1 tumors, and cyclin D1, p21Waf1, p27Kip1, and p53 nuclear accumulation were not related to survival (Table 2). Cyclin D3 expression, proliferation index, and tumor size were independent predictors of overall cancer-specific survival (Table 3) **Figure 2**.

Table 3
Cox Multivariate Analysis Showing Significant Variables Independently Related to Disease-Free and Cancer-Specific Overall Survival in 159 Patients With Stage Ta and T1 Bladder Urothelial Tumors

Variable	Relative Risk	95% Confidence Interval	*P*
Disease-free survival			
Cyclin D1 (%), <15 vs ≥15	3.2623	1.4192-7.4988	.0054
Ki-67 (%), <13 vs ≥13	5.0793	1.8009-14.3258	.0021
Overall survival			
Cyclin D3 (%), <15 vs ≥15	3.8154	1.2404-11.7360	.0195
Ki-67 (%), <13 vs ≥13	3.6645	1.0744-12.4986	.0380
Tumor size (cm), <3, 3-5, >5	2.7948	1.0245-7.6242	.0447

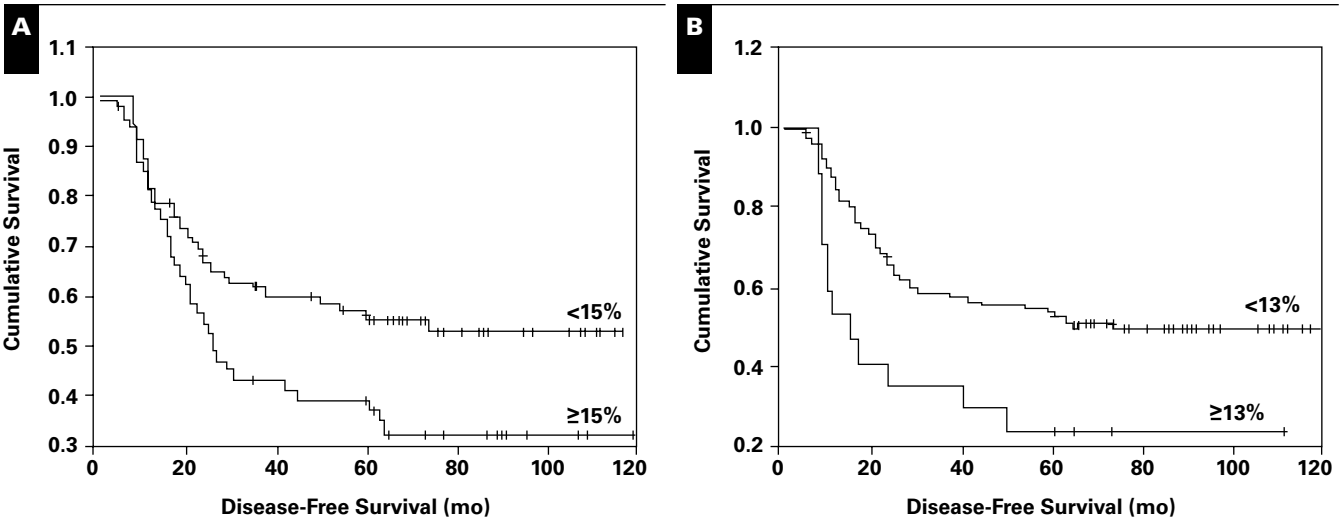

Figure 1 Kaplan-Meier plots illustrating the association between cyclin D1 (**A**) and Ki-67 (MIB-1) proliferative index (**B**) and disease-free survival. These markers were selected as independent predictors in the Cox multivariate analysis (see text). **A**, *P* = .0182. **B**, *P* = .0033.

DOI: 10.1309/LTFU3UUMBY095HUM

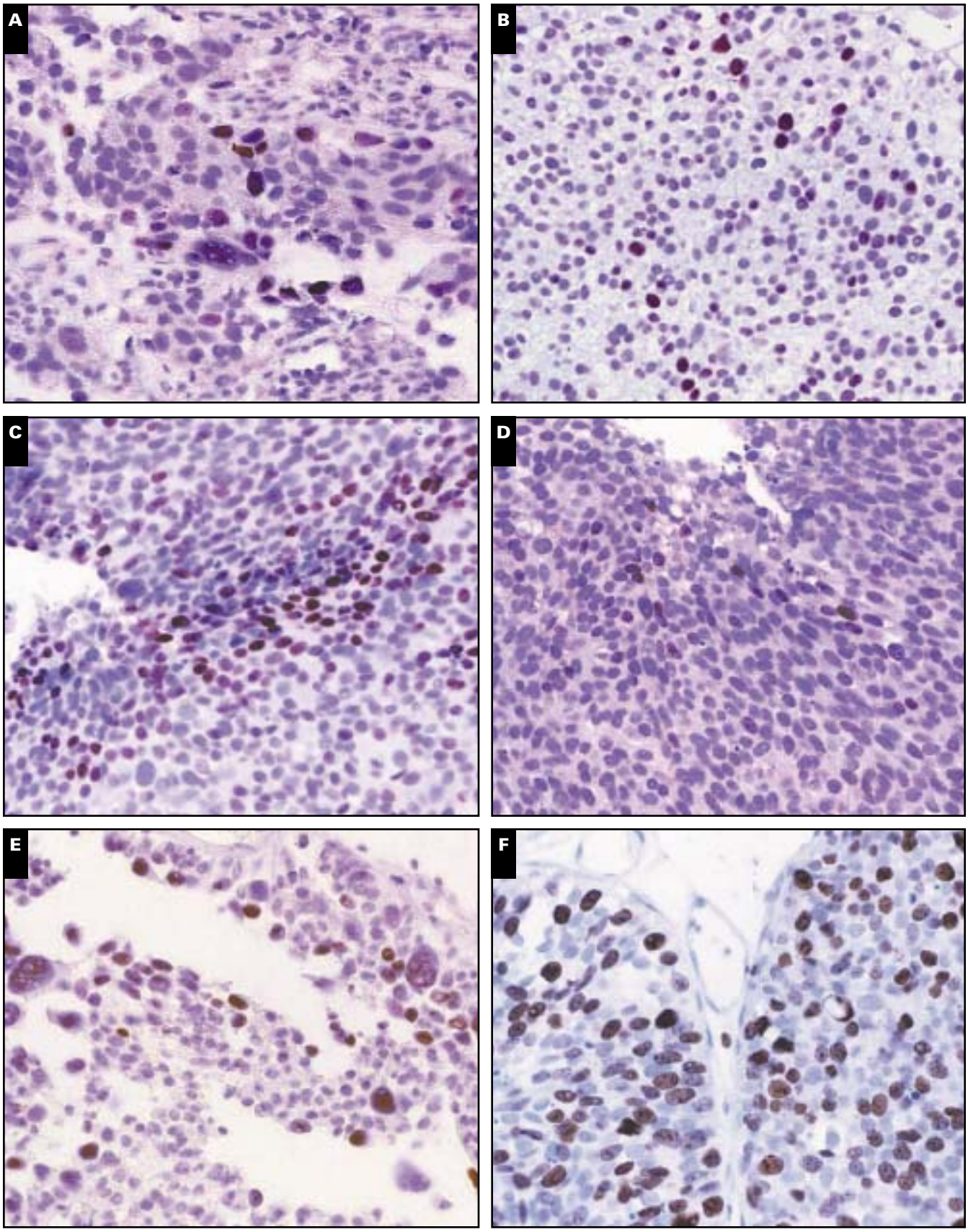

Image 1 Immunohistochemical nuclear expression of cell cycle–related proteins and tumor proliferative activity in stage Ta and T1 bladder urothelial tumors. **A**, p53 (×250). **B**, p21Waf1 (×250). **C**, p27Kip1 (×250). **D**, Cyclin D1 (×250). **E**, Cyclin D3 (×250). **F**, Ki-67 (MIB-1) (×250).

DOI: 10.1309/LTFU3UUMBY095HUM

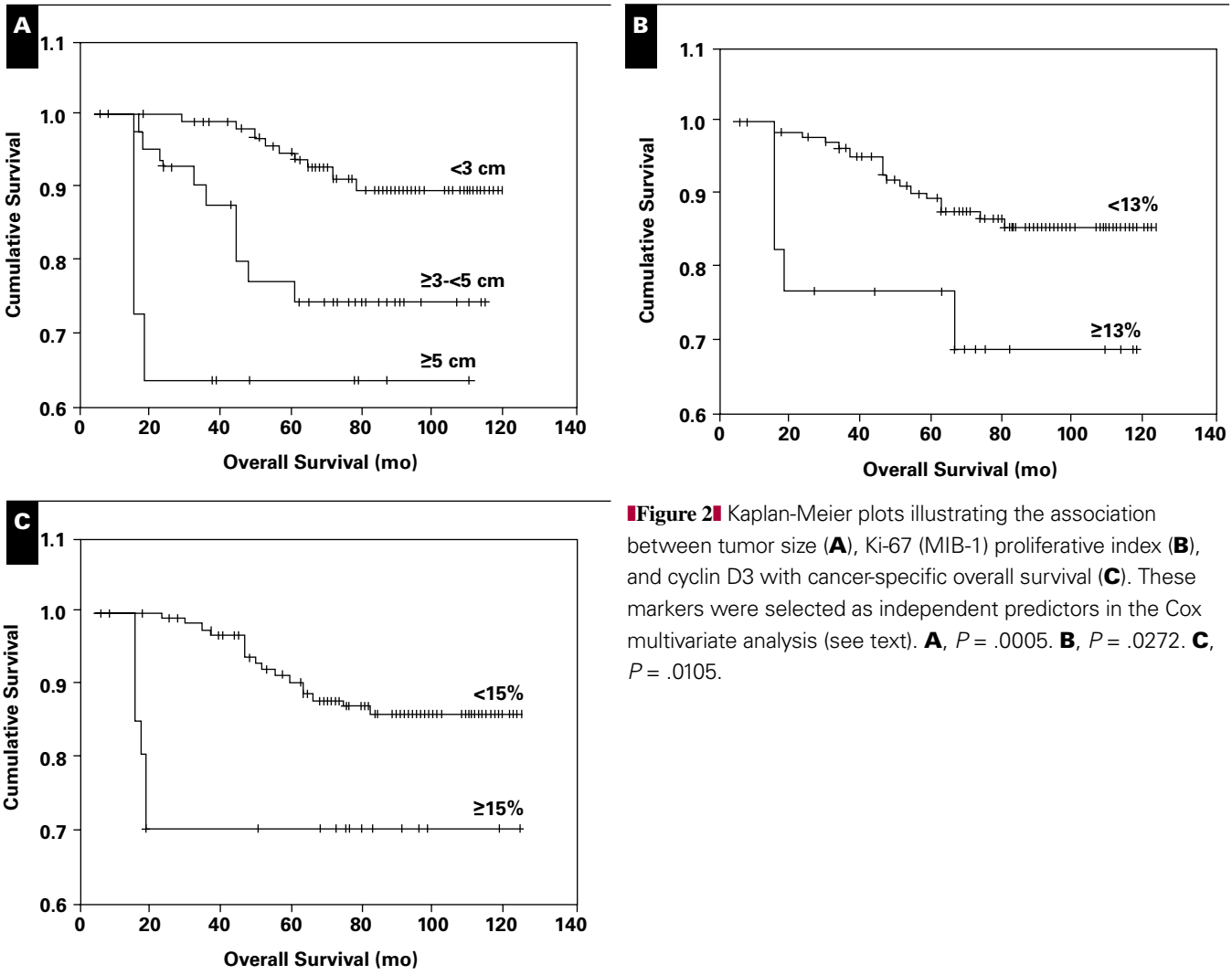

Figure 2 Kaplan-Meier plots illustrating the association between tumor size (**A**), Ki-67 (MIB-1) proliferative index (**B**), and cyclin D3 with cancer-specific overall survival (**C**). These markers were selected as independent predictors in the Cox multivariate analysis (see text). **A**, $P = .0005$. **B**, $P = .0272$. **C**, $P = .0105$.

Discussion

Following the introduction of the WHO/International Society of Urological Pathology grading scheme for bladder urothelial tumors (recently recognized as the 2004 WHO grading system of noninvasive urothelial tumors),[5,6] a limited number of studies have addressed the issue of immunohistochemical markers in predicting outcome of superficial (stages Ta and T1) bladder tumors.[13,14,20] Most available studies included a small number of cases with variable stage selection.[8-12,16-24,32,33] Thus, we decided to restrict our study to stage Ta and T1 urothelial tumors, a common group of tumors in which prognostic parameters are particularly needed, incorporating a series of 159 patients with long-term follow-up.

Most of the more recent studies have addressed mainly the use of p53 and the Ki-67 proliferation index as prognostic tools, suggesting that they might be related to tumor progression or recurrence, respectively.[10,12-14] Limited and somewhat controversial prognostic information regarding G_1-S modulators is available,[1,8,9,18,22,23,25-28,33] making our study design of particular relevance. Indeed, it seems like aberrations of cell cycle regulators in different pathways are common in bladder tumors classified according to former grading systems.[11] Markers such as the cyclin-dependent kinase (CDK) inhibitors, p21Waf1 and p27Kip1, well-known downstream regulators; cyclins D1 and D3, known upstream regulators; p53, a protein with a central role in cell cycle regulation; and tumor proliferation frequently are altered in bladder cancer,[9,18,25,28] and, therefore, the prognostic role of these aberrations in the prognosis of superficial bladder tumors needs to be substantiated. Besides, a study of cell cycle regulatory proteins involved in the progression from G_1 to S phase using an expanded panel of markers, including tumor proliferation and conventional clinicopathologic parameters in a multivariate analysis, as performed in the present study, would provide superior clinically useful information for future prospective studies.

DOI: 10.1309/LTFU3UUMBY095HUM

In our study, tumor size, grading of stage Ta tumors according to the WHO 2004 classification system, cyclin D1 expression, and tumor proliferation index were predictors of disease-free survival in univariate analysis, but only cyclin D1 (relative risk [RR], 3.2623) and the proliferative index (RR, 5.0793) were independent predictors of disease-free survival in Cox multivariate analysis. This result agrees with findings of Sgambato et al,[22] who, in a similar study including stage Ta and T1 tumors, reported a significant correlation between shorter disease-free survival and cyclin D1 expression and high tumor proliferation by Ki-67 labeling index, with both acting as independent predictors. In addition, Pich et al[13] found the Ki-67 labeling index to be the best predictor of recurrence in noninvasive, stage Ta bladder tumors (PUNLMP and low-grade carcinoma), although these authors included a remarkably small number of cases in their study. Yan et al[12] found that Ki-67 can be used to identify patients at high risk for a first recurrence. But this is not an unexpected finding because it well known that the tumor proliferation index increases with grade in bladder tumors.[14,34,35] Our study suggests additional prognostic value for tumor size and the 2004 WHO grading scheme for noninvasive tumors: patients with PUNLMP or low-grade bladder carcinoma and tumors smaller than 3 cm have longer disease-free survival, a previously unreported finding in superficial bladder tumors classified according to the 2004 WHO grading scheme.

Other cell cycle–related proteins such as p21Waf1, p27Kip1, p53, and cyclin D3 did not have significant association with disease-free survival, as reported by others who found that low p27Kip1 and p21Waf1 expression are more common in poorly differentiated, muscle-invasive bladder carcinomas with low cancer-specific overall survival but are not independent predictors.[8,9,15,23-28] Similarly, Zlotta et al[16] did not indicate independent prognostic significance of p21Waf1, p53, and tumor proliferation in a cohort of patients with superficial bladder tumors treated with bacille Calmette-Guérin.

Our study also tested the role of G_1-S modulators, tumor proliferation, and clinicopathologic parameters as markers of cancer-specific overall survival. We found that increased tumor size, stages Ta and T1, cyclin D3 overexpression, and increased tumor proliferation were markers of reduced cancer-specific survival in univariate analysis; cyclin D3 overexpression, high tumor proliferation index, and increased tumor size were independent predictors of overall cancer-specific survival in our study group of 159 patients with superficial bladder tumors. This is an important and original finding because, to our knowledge, cyclin D3 expression has not been reported in Ta and T1 tumors as a prognostic marker.

The cyclin D family, mainly cyclins D1 and D3, form complexes with CDKs 4 and 6, which promote phosphorylation and inactivation of the retinoblastoma protein, thus releasing the promoter factor E2F-1.[7,29,30] This, in turn activates genes involved in DNA synthesis, thereby mediating the progression of cells from G_1 to the S phase of the cell cycle.[29] Expression of cyclin D3, a 34-kd protein that shares 53% sequence homology with cyclin D1 but is induced later in the G_1 phase of the cell cycle, was the main independent predictor of overall cancer-specific survival in our study (RR, 3.8154) of superficial bladder tumors, a finding that, to our knowledge, has not been reported. This is a potentially relevant finding because the use of cell cycle regulatory proteins as therapeutic targets of their modulators represents an active field of research.[29,30,36]

In addition, overexpression of cyclin D3, as a specific G_1-S aberration, was associated with increased proliferation and low expression of p27Kip1 in renal cell carcinoma, suggesting that different aberrations of G_1-S regulatory proteins are linked.[37,38] Similarly, our study showed that Ta and T1 bladder urothelial tumors have important alterations of the cell cycle regulatory pathways of transition from G_1 to S phase of the cell cycle (the so-called pRb pathway), mainly characterized by overexpression of the upstream regulators cyclin D3 and cyclin D1, high tumor cell proliferation, and down-regulation of the CDK inhibitors p21Waf1 and p27Kip1. This, together with tumor size, might represent a finding of potential clinical relevance because these markers could be used for stratifying patients into risk categories.

The alteration of cell cycle regulatory proteins found in Ta and T1 bladder tumors warrants further prospective study on the prognostic significance of these markers and the development of modulators of these regulatory proteins as therapeutic agents. Available modulators of expression of the cyclin D family such as interferon-α and flavopiridol,[36] which can induce cell cycle arrest by directly inhibiting CDK inhibitors and by down-regulation of cyclin D1 and cyclin D3, might represent an alternative to conventional therapeutic approaches in a subset of patients with Ta and T1 bladder tumors at higher risk of aggressive behavior.

From the [1]Department of Pathology, [4]Urology Service, and [5]Biomedical Research Unit, Reina Sofia University Hospital and [2]Cordoba University Medical School, Cordoba, Spain; [3]Department of Pathology, Jaen University Hospital, Jaen, Spain; and [6]Institute of Pathological Anatomy and Histopathology, Polytechnic University of the Marche Region, Ancona, Italy.

Presented in part at the US and Canadian Academy of Pathology meeting, Vancouver, Canada, March 2004.

Supported by grants 00/898 and 03/952 from the Health Research Fund, Madrid, Spain.

Address reprint requests to Dr Lopez-Beltran: Faculty of Medicine, Avda Menendez Pidal S/N, 14004 Cordoba, Spain.

DOI: 10.1309/LTFU3UUMBY095HUM

References

1. Lee R, Droller MJ. The natural history of bladder cancer: implications for therapy. *Urol Clin North Am*. 2000;27:1-13, vii.
2. Montironi R, Lopez-Beltran A, Mazzucchelli R, et al. Classification and grading of the non-invasive urothelial neoplasms: recent advances and controversies. *J Clin Pathol*. 2003;56:91-95.
3. Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, et al. Multivariate analysis of the prognostic factors of primary superficial bladder cancer. *J Urol*. 2000;163:73-78.
4. Solsona E, Iborra I, Dumont R, et al. The 3-month clinical response to intravesical therapy as a predictive factor for progression in patients with high risk superficial bladder cancer. *J Urol*. 2000;164:685-689.
5. Epstein JI, Amin MB, Reuter VR, et al, for the Bladder Consensus Conference Committee. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am J Surg Pathol*. 1998;22:1435-1448.
6. Sauter G, Algaba F, Amin MB, et al. Non-invasive urothelial tumours. In: Eble JN, Sauter G, Epstein JI, et al, eds. *Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs*. Lyon, France: IARC Press; 2004:110. *World Health Organization Classification of Tumours*.
7. Fradet Y, Lacombe L. Can biological markers predict recurrence and progression of superficial bladder cancer? *Curr Opin Urol*. 2000;10:441-445.
8. Lipponen P, Aaltomaa S, Eskelinen M, et al. Expression of p21(waf1/cip1) protein in transitional cell bladder tumours and its prognostic value. *Eur Urol*. 1998;34:237-243.
9. Liukkonen T, Lipponen P, Raitanen M, et al, for the Finnbladder Group. Evaluation of p21WAF1/CIP1 and cyclin D1 expression in the progression of superficial bladder cancer. *Urol Res*. 2000;28:285-292.
10. Liukkonen T, Rajala P, Raitanen M, et al, for the Finnbladder Group. Prognostic value of MIB-1 score, p53, EGFr, mitotic index and papillary status in primary superficial (stage pTa/T1) bladder cancer: a prospective comparative study. *Eur Urol*. 1999;36:393-400.
11. Pfister C, Larue H, Moore L, et al. Tumorigenic pathways in low-stage bladder cancer based on p53, MDM2 and p21 phenotypes. *Int J Cancer*. 2000;89:100-104.
12. Yan Y, Andriole GL, Humphrey PA, et al. Patterns of multiple recurrences of superficial (Ta/T1) transitional cell carcinoma of bladder and effects of clinicopathologic and biochemical factors. *Cancer*. 2002;95:1239-1246.
13. Pich A, Chiusa L, Formiconi A, et al. Proliferative activity is the most significant predictor of recurrence in noninvasive papillary urothelial neoplasms of low malignant potential and grade 1 papillary carcinomas of the bladder. *Cancer*. 2002;95:784-790.
14. Cina SJ, Lancaster-Weiss KJ, Lecksell K, et al. Correlation of Ki-67 and p53 with the new World Health Organization/International Society of Urological Pathology Classification System for Urothelial Neoplasia. *Arch Pathol Lab Med*. 2001;125:646-651.
15. Dybowski B, Kupryjanczyk J, Rembiszewska A, et al. p27(Kip1) and Ki-67 expression analysis in transitional cell carcinoma of the bladder. *Urol Res*. 2003;31:397-401.
16. Zlotta AR, Noel JC, Fayt I, et al. Correlation and prognostic significance of p53, p21WAF1/CIP1 and Ki-67 expression in patients with superficial bladder tumors treated with bacillus Calmette-Guérin intravesical therapy. *J Urol*. 1999;161:792-798.
17. Takagi Y, Takashi M, Koshikawa T, et al. Immunohistochemical demonstration of cyclin D1 in bladder cancers as an inverse indicator of invasiveness but not an independent prognostic factor. *Int J Urol*. 2000;7:366-372.
18. Tut VM, Braithwaite KL, Angus B, et al. Cyclin D1 expression in transitional cell carcinoma of the bladder: correlation with p53, waf1, pRb and Ki67. *Br J Cancer*. 2001;84:270-275.
19. Van Keymeulen A, Bartek J, Dumont JE, et al. Cyclin D3 accumulation and activity integrate and rank the comitogenic pathways of thyrotropin and insulin in thyrocytes in primary culture. *Oncogene*. 1999;18:7351-7359.
20. van Rhijn BW, Vis AN, van der Kwast TH, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. *J Clin Oncol*. 2003;21:1912-1921.
21. Wagner U, Suess K, Luginbuhl T, et al. Cyclin D1 overexpression lacks prognostic significance in superficial urinary bladder cancer. *J Pathol*. 1999;188:44-50.
22. Sgambato A, Migaldi M, Faraglia B, et al. Cyclin D1 expression in papillary superficial bladder cancer: its association with other cell cycle–associated proteins, cell proliferation and clinical outcome. *Int J Cancer*. 2002;97:671-678.
23. Sgambato A, Migaldi M, Faraglia B, et al. Loss of p27Kip1 expression correlates with tumor grade and with reduced disease-free survival in primary superficial bladder cancers. *Cancer Res*. 1999;59:3245-3250.
24. Migaldi M, Sgambato A, Garagnani L, et al. Loss of p21Waf1 expression is a strong predictor of reduced survival in primary superficial bladder cancers. *Clin Cancer Res*. 2000;6:3131-3138.
25. Kamai T, Takagi K, Asami H, et al. Decreasing of p27(Kip1) and cyclin E protein levels is associated with progression from superficial into invasive bladder cancer. *Br J Cancer*. 2001;84:1242-1251.
26. Korkolopoulou P, Christodoulou P, Konstantinidou AE, et al. Cell cycle regulators in bladder cancer: a multivariate survival study with emphasis on p27Kip1. *Hum Pathol*. 2000;31:751-760.
27. Korkolopoulou P, Konstantinidou AE, Thomas-Tsagli E, et al. WAF1/p21 protein expression is an independent prognostic indicator in superficial and invasive bladder cancer. *Appl Immunohistochem Mol Morphol*. 2000;8:285-292.
28. Lacoste-Collin L, Gomez-Brouchet A, Escourrou G, et al. Expression of p27(Kip1) in bladder cancers: immunohistochemical study and prognostic value in a series of 95 cases. *Cancer Lett*. 2002;186:115-120.
29. Bartek J, Lukas J, Bartkova J. Perspective: defects in cell cycle control and cancer. *J Pathol*. 1999;187:95-99.
30. Bartkova J, Zemanova M, Bartek J. Abundance and subcellular localisation of cyclin D3 in human tumours. *Int J Cancer*. 1996;65:323-327.
31. Greene FL, Page DL, Fleming ID, et al. *AJCC Cancer Staging Manual*. New York, NY: Springer-Verlag; 2002.

DOI: 10.1309/LTFU3UUMBY095HUM

32. Wolf HK, Stober C, Hohenfellner R, et al. Prognostic value of p53, p21/WAF1, bcl-2, Bax, Bak and Ki-67 immunoreactivity in pT1 G3 urothelial bladder carcinomas. *Tumour Biol.* 2001;22:328-336.
33. Schmitz-Drager BJ, Goebell PJ, Ebert T, et al. p53 immunohistochemistry as a prognostic marker in bladder cancer: playground for urology scientists? *Eur Urol.* 2000;38:691-699.
34. Bol MG, Baak JP, de Bruin PC, et al. Improved objectivity of grading of T(A,1) transitional cell carcinomas of the urinary bladder by quantitative nuclear and proliferation related features. *J Clin Pathol.* 2001;54:854-859.
35. Bol MG, Baak JP, van Diermen B, et al. Proliferation markers and DNA content analysis in urinary bladder TaT1 urothelial cell carcinomas: identification of subgroups with low and high stage progression risks. *J Clin Pathol.* 2003;56:447-452.
36. Filipits M, Jaeger U, Pohl G, et al. Cyclin D3 is a predictive and prognostic factor in diffuse large B-cell lymphoma. *Clin Cancer Res.* 2002;8:729-733.
37. Hedberg Y, Davoodi E, Ljungberg B, et al. Cyclin E and p27 protein content in human renal cell carcinoma: clinical outcome and associations with cyclin D. *Int J Cancer.* 2002;102:601-607.
38. Hedberg Y, Roos G, Ljungberg B, et al. Cyclin D3 protein content in human renal cell carcinoma in relation to cyclin D1 and clinico-pathological parameters. *Acta Oncol.* 2002;41:175-181.

DOI: 10.1309/LTFU3UUMBY095HUM