significant efficacy in suppressing disease progression. However, most events of progression are detected during the course of repeated recurrence in the bladder, and it can therefore be thought that achieving RFS over a long period of time by performing maintenance therapy will also reduce the risk of progression. Actually, in our study no progression was observed in the maintenance group. The efficacy results for BCG maintenance therapy that we have demonstrated in our present randomized trial not only support the findings reported by Lamm *et al.* [1] but are also able to partially counter the recent discussion regarding BCG maintenance therapy [11,32–34].

For the tolerability evaluation of 81 mg of BCG dose, our present protocol stipulated that the dose of BCG could not be reduced, because one objective of the study was to confirm the tolerability of BCG when administered in a dose of 81 mg. Treatment-related AEs were clearly more severe during the maintenance therapy. It was reported that treatment-related AEs could be reduced, without sacrificing efficacy, by lowering the BCG dose [35,36] or by administering a quinolone antibiotic 8 h after BCG instillation [21]. We think that future studies are warranted to investigate the possibility of further increasing the duration of long-term prevention of bladder cancer recurrence by improving treatment compliance through better management of treatment-related AEs during the course of BCG maintenance therapy.

In conclusion, this randomized, comparative clinical trial demonstrated that BCG intravesical instillation maintenance therapy is able to significantly prolong post-TURBT RFS in patients with recurrent or multiple, stage Ta or T1, bladder cancer that is at moderate-to-high risk of recurrence. The results of this clinical trial represent valuable new evidence in support of the efficacy of BCG maintenance therapy in preventing bladder cancer recurrence.

FIG. 3. Recurrence-free survival. Plot of Kaplan-Meier estimates for recurrence-free survival (FAS population) for the combined BCG group and EPI group (upper), and for the maintenance group and non-maintenance group (lower).

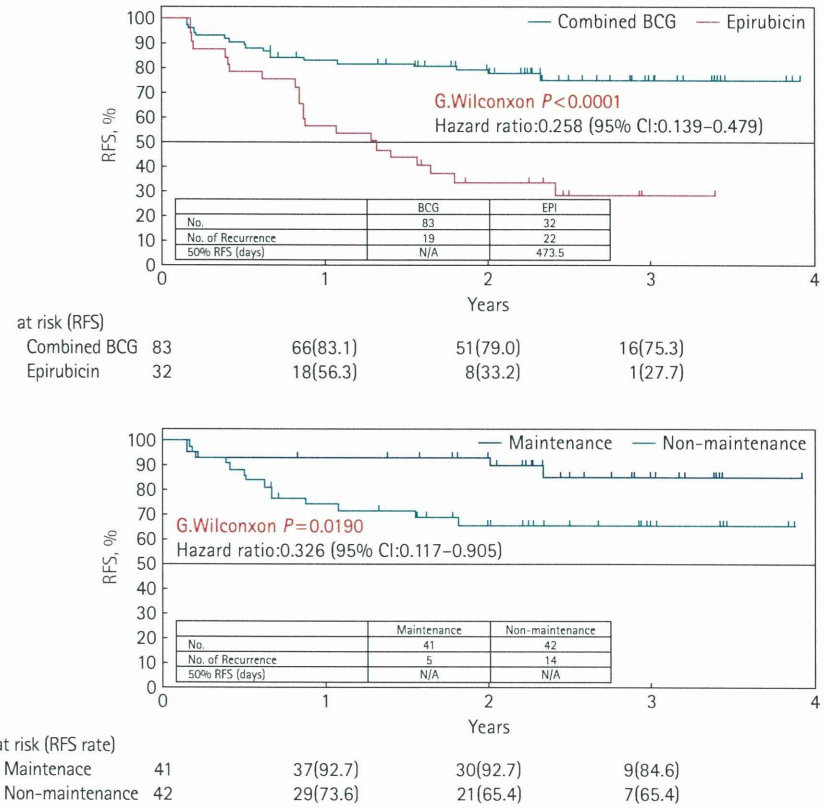
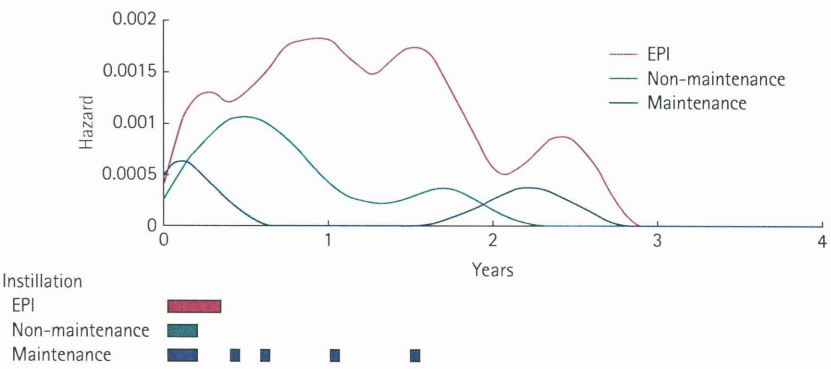


FIG. 4. Smoothed hazards analysis of recurrence.



ACKNOWLEDGEMENTS

We appreciate the cooperation afforded by all of the patients and institutions involved in this study. In Japan, neither the usage of BCG adjuvant treatment after TURBT nor a schedule of maintenance therapy for NMIBC has yet been approved. Accordingly, the present clinical study was carried out with the objective of supporting an approval application for BCG maintenance therapy for preventing the recurrence of NMIBC in Japan. The trial is supported by Nippon Kayaku Co. Ltd (Tokyo, Japan), which is the current Japanese licence holder for the BCG Connaught strain.

CONFLICT OF INTEREST

None declared.

TABLE 3 The major treatment-related adverse events (AEs) in the maintenance group and the non-maintenance group

	Maintenance arm ($N = 42$)*			Non-maintenance arm ($N = 42$)*		
Event	Pts *n*	Incidence (%)	≥Grade 3 (%)	Pts *n*	Incidence (%)	≥Grade 3 (%)
Urinary frequency	39	92.9	40.5	30	71.4	19.0
Pain on urination	39	92.9	9.5	29	69.0	2.4
Difficulty in urination	21	50.0	4.8	12	28.6	
Gross haematuria	39	92.9	19.0	30	71.4	11.9
Residual urine	8	19.0		2	4.8	
Bladder pain	3	7.1	2.4	0		
Lower abdominal pain	4	9.5		2	4.8	2.4
Epididymitis	1	2.4	2.4	0		
Bladder tamponade	1	2.4	2.4	0		
Pyrexia (≥38 °C)	18	42.9		11	26.2	
Malaise	21	50.0		18	42.9	
Anorexia	13	31.0		4	9.5	
Arthralgia	7	16.7		4	9.5	4.8
Headache	5	11.9		3	7.1	
Hypertension	1	2.4	2.4	0		
Leukocytosis†	6	14.3		3	7.1	
Urinary protein positive	20	47.6		10	23.8	
Microscopic haematuria†	31	73.8		25	59.5	
Urinary red blood cell increase†	29	69.0		24	57.1	
Urinary white blood cell increase†	36	85.7		31	73.8	

*The severity of AEs was judged in accordance with the criteria stipulated in the Japanese version of the Japan Clinical Oncology Group 2nd edition of the National Cancer Institute – Common Toxicity Criteria version 2.0. *N represents the number of patients for whom safety was evaluable; †not graded.*
Pts, Patients.

TABLE 4 Completion rates and performance rates for the maintenance therapy cycles in the patients advanced to the maintenance therapy

		Performance rate		Completed rate	
Cycle	*N*	*n*	%	*n*	%
At 3 months	36	36	100.0	32	88.9
At 6 months	36	35	97.2	23	63.9
At 12 months	36	30	83.3	19	52.8
At 18 months	36	25	69.4	15	41.7

The completion rate (i.e. all three of the planned instillations in the cycle were administered) and performance rate (i.e. at least one of the three planned instillations in the cycle was administered) are shown for each of the instillation cycles at the 3-, 6-, 12- and 18-month points of the maintenance therapy for the 36 patients who were advanced to that therapy.

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Correspondence: Hideyuki Akaza, Department of Strategic Investigation on Comprehensive Cancer Network, Research Centre for Advanced Science and Technology, The University of Tokyo 4-6-1, Komaba, Meguro-ku, Tokyo 153-8904, Japan.
e-mail: akazah@med.rcast.u-tokyo.ac.jp

Abbreviations: **NMIBC**, non-muscle-invasive bladder cancer; **RFS**, recurrence-free survival; **SWOG**, Southwest Oncology Group; **TURBT**, transurethral resection of bladder tumour; **AE**, adverse event; **EPI**, epirubicin.

APPENDIX

The authors wish to thank the investigators for performing this study. Investigators and institutions: T. Tsukamoto, Sapporo Medical University Hospital; T. Ohyama, Hirosaki University School of Medicine and Hospital; A. Ito, Tohoku University Hospital; N. Tsuchiya, Akita University Hospital; N. Miyanaga, Tsukuba University Hospital; K. Tanabe, Tokyo Women's Medical University Hospital; E. Kikuchi, Keio University Hospital; I. Fukui, The Cancer Institute Hospital of JFCR; K. Tomita, The University of Tokyo Hospital; S. Egawa, The Jikei University Hospital; S. Komatsubara, Niigata Cancer Centre Hospital; S. Mugiya, Hamamatsu University School of Medicine, University Hospital; Y. Hirano, Fujieda Municipal General Hospital; H. Kobayashi, Nagoya Daini Red Cross Hospital; K. Kohri, Nagoya City University Hospital; K. Miki, University Hospital, Kyoto Prefectural University of Medicine; H. Nishiyama, Kyoto University Hospital; M. Usami, Osaka Medical Centre for Cancer and Cardiovascular Disease; N. Nonomura, Osaka University Hospital; K. Fujimoto, Nara Medical University Hospital; T. Inagaki, Wakayama Medical University Hospital; M. Takahashi, Tokushima University Hospital; M. Inui, Faculty of Medicine, Kagawa University; A. Yokomizo, Kyushu University Hospital; M. Nakagawa, Kagoshima University Medical and Dental Hospital. The Protocol and Evaluation Committee: Y. Kubota, Yokohama City University Hospital; T. Habuchi, Akita University Hospital; H. Kanetake, Nagasaki University Hospital. Coordinating Committee: T. Tsukamoto, Sapporo Medical University Hospital; Y. Hirao, Nara Medical University Hospital.

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Original Article: Clinical Investigation

Overexpression of Eg5 predicts unfavorable prognosis in non-muscle invasive bladder urothelial carcinoma

Sentai Ding,[1,3] Naidong Xing,[1] Jiaju Lu,[1] Hui Zhang,[1] Koji Nishizawa,[3] Shuai Liu,[1] Xiaodong Yuan,[1] Yejun Qin,[2] Ying Liu,[2] Osuma Ogawa[3] and Hiroyuki Nishiyama[3]

Departments of [1]Urology and [2]Pathology, Provincial Hospital affiliated to Shandong University, Jinan, Shandong, China; and [3]Department of Urology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Objective: To investigate the relationship between Eg5 expression and prognosis of patients with non-muscle invasive bladder urothelial carcinoma.

Methods: Eg5 expression was examined by immunohistochemistry in non-muscle invasive urothelial carcinoma specimens (grade: G1, 32 cases; G2, 92 cases; and G3, 39 cases. Stage: pTa, 49 cases and pT1, 114 cases). The correlation between clinicopathological characteristics and Eg5 expression was evaluated. The prognostic significance of Eg5 immunoreactivity was analyzed through survival analysis in 163 non-muscle invasive cases that were treated with transurethral resection and adjuvant intravesical instillations.

Results: The expression of Eg5 was significantly associated with tumor grade ($P = 0.006$), with a trend towards significant association with stage ($P = 0.057$). The 163 patients with non-muscle invasive tumors were regularly followed with the mean of 32.52 (from 6 to 72) months. Univariate analysis showed Eg5 overexpression exhibited a significant unfavorable influence on intravesical recurrence ($P = 0.012$) while having only a marginal correlation with disease progression ($P = 0.070$). Subsequent Cox hazard multivariate analysis showed that both grade ($P = 0.045$) and Eg5 expression ($P = 0.029$) were independent predictors for early intravesical recurrence.

Conclusions: Overexpression of Eg5 correlates with poor differentiation of bladder cancer, and it represents an independent prognostic factor in predicting early intravesical recurrence in non-muscle invasive bladder carcinoma patients.

Key words: bladder, Eg5, prognosis, recurrence, urothelial carcinoma.

Introduction

Bladder urothelial carcinoma (UC) is the fifth most common malignancy worldwide, and the large majority of patients (~75–85%) present with non-muscle invasive tumors, which are mainly confined to the mucosa (Ta) or subepithelial layer (T1).[1] Despite receiving standard transurethral resection (TUR) and adjuvant intravesical chemotherapeutic instillations, approximately two-thirds of patients have the malignancy recur, and 15–30% will progress to a higher stage and/or grade with greater mortality.[2]

Besides the clinical parameters (e.g. grade, stage), a number of markers were expected to predict the prognosis of non-muscle invasive UC, but few of them have been recognized as reliable methods until now.[3,4] Thus, there is a considerable need for investigating effective markers that can accurately identify non-muscle invasive cases with a high risk of recurrence or progression. This could contribute not only to the improvement of the prognosis by treating such individual patients more aggressively, but also to the development of novel anticancer targets.

Eg5 (also known as kinesin spindle protein, KSP), a member of the kinesin-5 family of molecular motors (previously referred to as the BimC family), plays a critical role for proper bipolar spindle formation and maintenance during mitosis in proliferating cells.[5] Eg5 has a catalytic motor domain at the N-terminus, an α-helical coiled coil stalk domain in the middle, and a tail domain at the C-terminus, and forms a homotetrameric structure with motor domains located at each end of a central stalk.[6] This relatively compact domain is responsible for adenosine triphosphate hydrolysis and generation of motile force along the microtubule, thus Eg5 is thought to crosslink and slide microtubules relative to each other, pushing apart interpolar microtubules and resulting in bipolarity, and might also play a role in bundling microtubules together.[5]

Eg5 expression is most abundant in proliferating human tissues, including thymus, tonsils, testis, esophageal epithelium and bone marrow, and is absent from postmitotic

Correspondence: Jiaju Lu M.D., Ph.D., Department of Urology, Provincial Hospital affiliated to Shandong University, Jinan, Shandong 250021, China. Email: kyoto2310@hotmail.com
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human cells (e.g. central nervous system neurons). These data suggest that KSP would be an attractive target for the discovery of novel and specific antimitotic cancer therapies.[7]

Therefore, Eg5 has become an attractive anticancer therapeutic target, and induction of Eg5 dysfunction through specific small molecular inhibitors[7,8] or antisense oligonucleotide (ASO)[9] could result in apoptotic cell death after prolonged mitotic arrest. Since the discovery of monastrol by a phenotype-based screening,[10] a large number of KSP inhibitors have shown excellent anticancer efficacy in both *in vitro* and *in vivo* studies, and several KSP inhibitors have been currently undergoing clinical studies.[11]

Consistent with the fact that Eg5 is only expressed in mitotic cells, especially in malignant cells,[7] Eg5 expression levels appeared to be directly proportional to the mitotic population in both cancer cell lines[9] and clinical tumors.[12] Furthermore, Eg5 has been shown to correlate with oncogenesis,[13] proliferative rate and clinical outcomes in cancers.[14] A recent study showed that Eg5 expression levels appeared to be directly proportional to the mitotic population. For example, the mitotic fraction is 20% and 30% in LNCaP and PC-3 cells, respectively, and Eg5 expression is proportionately higher in PC-3 cells consistent with their higher cell growth rates.[9] However, to our knowledge, there is no data on the correlation between Eg5 expression and the prognosis of UC.

In the present study, we analyzed the expression of Eg5 in clinical bladder UC specimens by immunohistochemistry, and evaluated the correlation between Eg5 expression and clinicopathological characteristics, aiming to identify the evidence for its usefulness as a prognostic marker in patients with non-muscle invasive UC.

Methods

We retrospectively reviewed the detailed and comprehensive clinical and pathological information of all the patients who were diagnosed with non-muscle invasive urothelial carcinomas from January 2003 to December 2007. However, the patients diagnosed with pTis were not included in the present study, because such tumors have relatively worse degeneration and only comprise a small proportion of non-muscle invasive cases. A total of 279 cases with non-invasive UC were reviewed in our study initially and the patients were also excluded if they had incomplete medical records (9 cases), inadequate follow up (57 cases), or a history of bladder biopsy before the primary surgery or transurethral resection (TUR) without standard following regular intravesical instillations (50 cases). Thus, 163 patients were included in the present study, including 129 men and 34 women with a mean (SEM, median, range) age of 63.86 (1.022, 65.00, 24–88) years. The staging of tumors and grade evaluation were independently confirmed by two pathologists by the combination of clinical and histological data. There were 49 pTa and 114 pT1 stage tumors according to the TNM classification, and 32 grade 1, 92 grade 2 and 39 grade 3 tumors by using the 1973 World Health Organization (WHO) criteria.[15]

The patients enrolled in the present study were treated with complete transurethral resection (TUR) and received regular adjuvant intravesical instillations of bacillus Calmette-Guérin (BCG) or pirarubicin (tetrahydropyranyladriamycin, THP). During the surgery, after the resection of the tumors, the urothelial mucosa within 2 cm around the tumor was also fulgurized, and any lesions suspicious of carcinoma *in situ* (CIS) were resected and then further confirmed by pathologists. As for the following intravesical instillations, THP (30 mg/kg) was initially given within 24 h after surgery and then given regularly for 1 year as described previously;[16] meanwhile, BCG (40 mg/kg) was given with the first intravesical instillations 2 weeks after the surgery and then given regularly as the protocol described previously.[17] The surveillance included ultrasonography, cystoscopy and urine cytology every 3 months for 2 years after the initial diagnosis or any recurrence, every 6 months for the following 2 years, and yearly thereafter, and patients were followed up until August 2009 with the mean duration (SEM, median, range) of 32.52 (1.44, 30, 6–72) months. By the time the present study was undertaken, 52.8% (86/163) patients had intravesical tumor recurrence and 12.9% (21/163) progressed to a higher tumor stage.

Immunohistochemistry

Immunohistochemistry was carried out to elucidate the Eg5 expression in all 163 UC samples. Briefly, all the tissues were fixed in 10% buffered formalin overnight and then embedded in paraffin wax. Paraffin blocks were cut at 5 um thickness. After deparaffinization, endogenous peroxidase activity was blocked by 0.3% hydrogen peroxidase. The glass slides were washed in PBS (6 times, 5 min each) and mounted with 1% goat normal serum in PBS for 30 min. Subsequently, anti-Eg5 rabbit polyclonal antibody (Cytoskeleton, Denver, CO, USA) was used as the primary antibody at a dilution of 1:300, and the sections were incubated overnight at 4°C. In evaluating immunoreactivity of Eg5 staining, the images were initially analyzed by using the ImageJ software (NIH, Bethesda, MD, USA) for staining intensity. After images were captured (TIFF format) at the same light intensity in the microscope and transformed to 256 grays 8-bit images, the images were analyzed by the "Measure and Label" plugins tool of ImageJ. A total of 18 representative section images containing positive stained tumor cells (6 random sections in each grade, ×400) were evaluated, the intensity of staining was quantifiable between zero (black) and 225 (white). The positive intensity of expression in the tumor cells was standardized by division of the gray level of internal control from that of negative epithelial cells in the

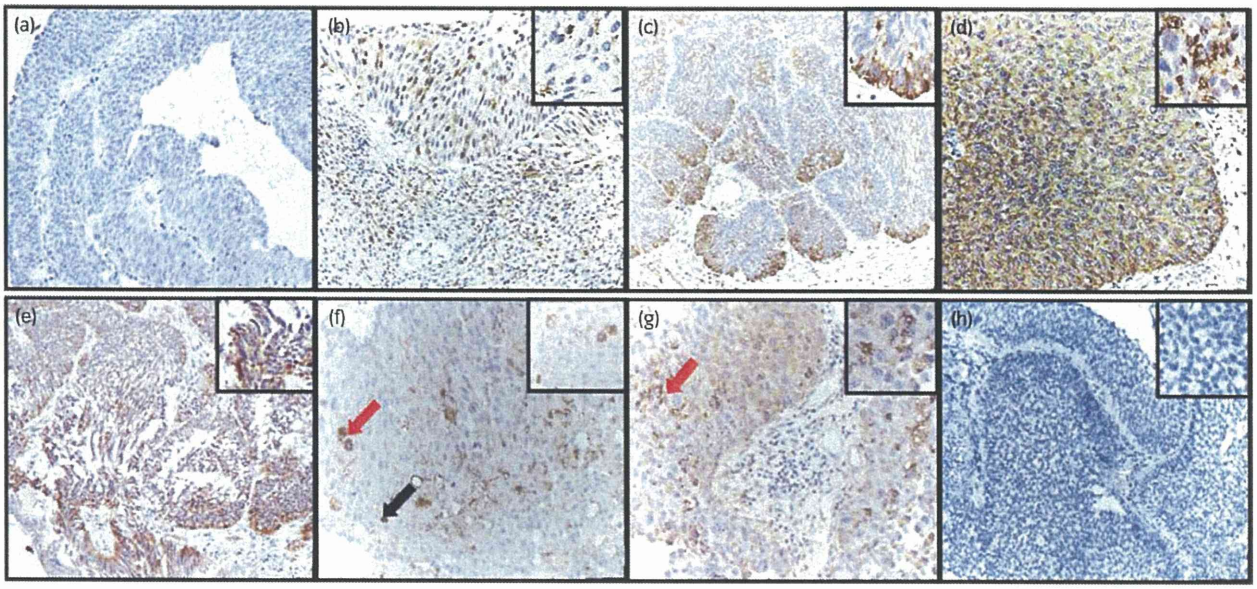

Fig. 1 Immunohistochemistry of Eg5 in bladder urothelial cancer samples. (a) Representative normal urothelium (magnification: ×200) and (b–h) tumor specimens. (b) Grade 1 with low staining reactivity (magnification: ×200). (c) Grade 2 with low (magnification: ×200) and (d) high (magnification: ×400) staining reactivity, and (e) grade 3 with high staining reactivity (magnification: ×200). (f,g) The positive staining signals of Eg5 were mainly detected in cytoplasm as shown by the red arrow (magnification: ×400), (f) with sporadic nuclear staining cell as shown by the black arrow (magnification: ×400). (h) The negative control achieved by omitting the primary antibody during immunohistochemistry procedure, a tumor specimen of grade 2 (magnification: ×200).

same section and was analyzed. These values in each grade were statistically compared by the Students *t*-test by 22 comparisons in the three groups, and no significance was observed (data not shown). Consequently, the positive fractions of Eg5 staining reactivity were accessed by positive cell proportion analysis, so at least 100 tumor cells were counted in three random regions of each section and the mean percentage of positive stained cells were evaluated. The immunoreactivity was classified as follows: no staining reactivity, <10% positive tumor cells; low reactivity, 10–50% positive cells; and high reactivity, >50% positive cells. The immunohistological assessment was carried out by two of the authors (YQ and YL) who were "blinded" to the clinicopathological data.

Statistical analysis

The Pearson χ^2-test was used to analyze the association between the Eg5 expression and clinicopathological parameters. Survival curves were plotted using the Kaplan–Meier method, and the log–rank test was used to access the significance of observed differences. Survival analysis was carried out using recurrence and progression as the endpoints for recurrence-free survival and progression-free survival, respectively. Recurrence and progression were defined as the time from the end of primary TUR surgery until the first evidence of recurrence of disease and the latter was defined as a relapse at more advanced tumor stage and/or a higher pathological grade. The patients who died during the follow-up period were treated as censored. When the log–rank test showed a significant difference between groups, Cox proportional hazards model multivariate analysis was used to calculate the effect of various variables on outcome. All statistical analyses were carried out by using Statistical Package for Social Sciences (SPSS, version 16; SPSS, Chicago, IL, USA) software, and two-sided *P*-values of less than 0.05 were regarded as statistically significant.

Results

Positive signals of Eg5 were detected in all bladder tumors examined to various extents (Fig. 1), with low immunoreactivity in 77 (47.2%) and high in 86 (52.8%) of the 163 tumor samples, respectively. We observed that the frequency of positivity for some cases were roughly more than 90% (Fig. 1d), and Figure 1h shows an Eg5-negative control. As shown in Figure 1, the positive staining signals of Eg5 were primarily detected in the cytoplasm of bladder tumor cells, but some tumors also showed a low number of nuclear staining cells that might be undergoing mitosis (Fig. 1f,g). Although Eg5 expression was observed in several cells in normal urothelium, all nine normal urothelial tissues of the bladder were judged to be negative for Eg5, because the cytoplasm of <10% of the tumor cells showed evidence of staining (Fig. 1a).

Table 1 Correlations of Eg5 reactivity with clinical and pathological characteristics

Variable	No. cases	Eg5 staining		*P*-value
		Low reactivity No. cases (%)	High reactivity No. cases (%)	
Age, years (median, 65)				$P = 0.306$
≤65	82	42 (51.2)	40 (48.8)	
>65	81	35 (43.2)	46 (56.8)	
Sex				$P = 0.682$
Male	129	62 (48.1)	67 (51.8)	
Female	34	15 (44.1)	19 (55.9)	
Past history of bladder tumors				$P = 0.297$
Yes	17	6 (35.3)	11 (64.7)	
No	146	71 (48.6)	75 (51.4)	
Grade				$P = 0.006$*
1	32	22 (68.8)	10 (31.2)	
2	92	43 (45.7)	49 (54.3)	
3	39	12 (30.8)	27 (69.2)	
Stage				$P = 0.057$
Ta	49	27 (55.1)	22 (44.9)	
T1	114	50 (43.9)	64 (56.1)	
Multifocality				$P = 0.061$
Focal	134	69 (55.1)	65 (55.1)	
Multifocal	29	8 (55.1)	21 (55.1)	
Intravesical instillation drugs				$P = 0.666$
Pirarubicin (THP)	142	68	74	
BCG	21	9	12	

*$P < 0.05$. BCG, bacillus Calmette-Guérin; THP, tetrahydropyranyladriamycin.

As for the clinicopathological characteristics, there was a highly significant association between Eg5 staining reactivity and tumor grade ($P = 0.006$), and a marginal significance between Eg5 staining reactivity and stage ($P = 0.057$; Table 1). Additionally, high-staining reactivity for Eg5 was detected in 81.8% (27/33) of pT1G3 tumors, and just 34.8% (8/23) of pTaG1. Multifocal tumors likely showed high Eg5 staining reactivity with a marginal significance ($P = 0.061$). There was no significant association of the Eg5 expression with patients' age ($P = 0.312$), sex ($P = 0.682$) or past history of bladder tumors ($P = 0.297$; Table 1). In addition, 21 cases in the present study chose BCG as the adjuvant intravesical instillations drug for less cost, and there was no significant difference in distribution of intravesical instillations drugs in patients with different Eg5 status ($P = 0.666$; Table 1).

As for the correlations of Eg5 reactivity with recurrence rate, 45.4% (35/77) of patients with low staining reactivity for Eg5 recurred, and 59.3% (51/86) of patients with high staining reactivity for Eg5 recurred. Thus, no significant relationship was found between the recurrence rate and Eg5 status ($P = 0.086$). Nonetheless, we further analyzed the clinical significance of Eg5 staining on recurrence time of non-muscle invasive bladder tumors by Kaplan–Meier analysis, and the result showed that tumors with high reactivity of Eg5 were significantly worse in the recurrence-free survival than those with low reactivity ($P = 0.012$) (Table 2 and Fig. 2a). Subsequently, multivariate analysis by using a Cox proportional hazard model showed that both grade (*P*-value/odds ratio, 0.045/1.411) and Eg5 staining reactivity (*P*-value/odds ratio, 0.029/1.649) were identified as independent predictors for recurrence-free survival with adverse significance in non-muscle invasive UC.

Among the 21 patients who progressed to a higher tumor stage during relapse, the Eg5 expression was low in 33.3% (7/21) of cases and high in 66.7% (14/21), respectively, and it turned out that the high reactivity of Eg5 had no significant impact on the progression rate ($P = 0.580$). However, as for the progression-free survival, tumors with a high expression level of Eg5 tended to be worse in the progression-free survival compared with those with low expression, although statistically not significant ($P = 0.070$). Next, multivariate analysis showed that both stage (*P*-value/odds ratio, 0.047/1.827) and past history of bladder tumors (*P*-value/odds ratio, 0.021/2.28) were independent predictors for progression-free survival with adverse significance, and fur-

Table 2 Univariate analyses (Kaplan–Meier method; log–rank test) of the recurrence-free and progression-free survival in the 163 non-muscle invasive cases

Univariate	*P*-value of Kaplan–Meier analysis	
	Recurrence-free	Progression-free
Grade (1 *vs* 2 *vs* 3)	0.017*	0.093
Stage (Ta *vs* T1)	0.102	0.031*
Multifocality (focal *vs* multifocal)	0.064	0.082
Intravesical instillation drugs (pirarubicin *vs* BCG)	0.209	0.162
Past history of bladder tumors (0 *vs* ≥1)	0.098	0.017*
Eg5 reactivity (low *vs* high)	0.012*	0.070

*$P < 0.05$. BCG, bacillus Calmette-Guérin.

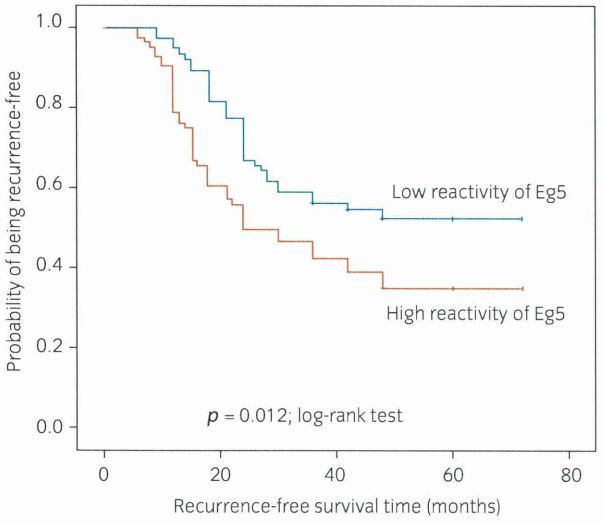

Fig. 2 Kaplan–Meier survival curves of recurrence-free survival for all 163 patients with non-muscle invasive tumors, according to Eg5 staining reactivity.

thermore, Eg5 staining reactivity (*P*-value/odds ratio, 0.105/1.249) failed to show significance again.

Discussion

Non-muscle invasive bladder UC are likely to have a favorable prognosis, but frequent recurrence and progression are the typical adverse outcomes, even after curative resection followed by chemotherapeutic intravesical instillations. In support of this view, approximately half of the patients in the present study relapsed and 12.9% progressed. As reported previously, once progression to invasive cancer (pT2-4) occurs, the median survival rate is just 12–15 months, and this has not been improved over the past two decades, despite the development and use of different chemotherapeutic treatment approaches.[18] Therefore, identification of prognostic clinicopathological factors, especially in relation to tumor recurrence and progression, is crucial to manage UC optimally.[19]

Although tumor grade and stage are the most accurate prognostic factors in the evaluation of bladder UC, they cannot always predict the true tumor biological potential, as non-muscle invasive tumors of the same stage and grade can have completely different clinical courses.[4] Furthermore, non-muscle invasive UC is a heterogeneous spectrum of diseases with different biological and clinical behaviors, determined by distinct molecular alterations.[20] Previous publications have described a number of markers that were identified not only as prognostic factors,[3,4,21] but were also found to contribute to the development of novel anticancer targets in bladder cancer.[22]

Eg5 plays a crucial role in the initial stages of mitosis for formation and maintenance of the bipolar spindle, so Eg5 is only expressed in proliferating tissues, and has especially stronger expression in malignant tissues.[7,14] Thus, its overexpression is a marker of cell proliferation, and inhibition of Eg5 as been identified as a promising novel anticancer therapy in preclinical studies.[9,11] Nevertheless, until recently, there has been no report about the relationship between the Eg5 expression and bladder cancer, and specifically there were no data on the clinical prognosis of non-muscle invasive UC.

Here, we first analyzed the correlation between the Eg5 expression and clinicopathological characteristics and prognosis of bladder UC. The tumor immunohistochemistry (IHC) staining pattern in the current study is consistent with previous findings,[14,23] which was primarily cytoplasmic, except for in the few cells that were undergoing mitotic cell division where staining was strongly nuclear. Previously, when evaluating protein marker expression in bladder cancer, both the percentage of positive stained tumor cells and the staining intensity were commonly analyzed.[24] Here, we assayed staining intensity by using ImageJ software, which has been widely used in biological research,[25,26]

because such image analysis approaches could provide more accurate results for IHC quantitation by measuring the intensity of staining much more precisely.[27] Nonetheless, no significant difference of staining intensity was observed among the positive tumor cells in different tumor grade samples. Thus, only the percentage of positive stained tumor cells was used to score the immunoreactivity.

In the present study, Eg5 expression is a common event in UC samples, and the data suggest that the classical biological indicators (grade and stage) are correlated with Eg5 expression level, showing a role of Eg5 in cell proliferation, differentiation and progression of bladder UC. Consistent with our data, several previous studies have reported that Eg5 is involved in oncogenesis,[13] cell proliferating rate and the progression of human cancers. In an *in vitro* study with several prostate cell lines, Hayashi *et al.*[9] observed that Eg5 expression levels appeared to be directly proportional to the mitotic population, and Eg5 expression is higher in the cells with higher cell growth rates. Additionally, some preliminary data have shown that in breast tumors there was a positive correlation between mitotic index and Eg5 gene expression levels.[12] In another clinical study with analysis for Eg5 expression by IHC, Eg5 expression was detected in all of the cases with squamous cell carcinoma of the head and neck. Furthermore, the Eg5 expression was highest at the basal edge of the tumor nests where the proliferative activity is expected to be highest, showing an agreement with our data.[23]

To our knowledge, there are few data on the prognostic role of Eg5 in human cancers. In the current study, a mean follow up of 32.52 months ensured that most patients in whom recurrence would be detected already had recurred by the time of analysis. By using univariate and subsequent multivariate survival analysis, the increased expression of Eg5 showed a correlation with the intravesical recurrence survival, which was identified as an independent marker in predicting high risk of early recurrence. On one hand, the intravesical recurrence of bladder cancer is usually thought to be a result of the seeding of bladder cancer cells, and Eg5 might help the seeded bladder cancer cells to grow by exerting its proliferative activity. As shown in the present study, this point might be the main reason, which is also in agreement with previous results, that the cells with higher Eg5 expression had higher cell growth rates.[9] On the other hand, a previous report suggested that Eg5 status was likely to be an independent variable related to response to chemotherapy,[14] and here we found that patients with higher Eg5 intensity showed a much poorer recurrence-free survival time compared with those lower Eg5 expression cases, even when considering that intravesical instillation drugs had no significant difference in low and high Eg5 expression groups. Consistent with our data, in a previous clinical study of non-small cell lung cancer, the authors reported that the response rate to chemotherapy of patients with Eg5-positive tumors was 37%, as opposed to 10% for patients with Eg5-negative tumors. Nonetheless, the Eg5-positive cases tended to have a poorer outcome in terms of overall survival than the Eg5-negative cases.[14] As for progression during recurrence of non-muscle invasive tumors, besides a marginal significant association between Eg5 expression and tumor stage, univariate analysis showed that Eg5 might play a role in the early progression of non-muscle invasive UC when cancer relapsed, although the statistical power was low owing to the relatively small sample size. Nevertheless, our results showed that those patients with Eg5 overexpression might have a dismal prognosis with early progression to life-threatening stages, so they should be treated with a more aggressive adjuvant regimen promptly.

As reported previously, tumor multifocality,[28] history of recurrence[29] and CIS[30] were the main prognostic factors for UC of the bladder. In the present study, only the history of recurrence affected tumor progression with significance in univariate analysis, but all three characteristics might lose their significances as a result of a small number of such patients, which was a limitation of this study. However, different studies reported varied results, perhaps originating from different clinical and pathological characteristics. In another study, using multivariate analysis, history of recurrence was found to have independent significance for recurrence, nonetheless, history of recurrence was not an independent and significant predictor for progression.[29] Likewise, several other factors, such as large tumor size, have also been reported to be associated with poor prognosis;[19,30] however, we did not investigate such parameters, because the clinical information was incomplete, which is another limitation of this retrospective study.

In conclusion, Eg5 is generally expressed in bladder UC, and the main finding of the present study was that non-muscle invasive bladder UC patients with Eg5 high level expression had a significantly worse prognosis, especially an earlier recurrence probability, which could assist in identifying a group of patients that possibly require a thorough follow up and a more aggressive adjuvant regimen. Further research in this field will provide more evidence about the function and regulation of Eg5, and the potential role of Eg5 as a novel target for anticancer therapy in UC.

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Urinary pH Is Highly Associated With Tumor Recurrence During Intravesical Mitomycin C Therapy for Nonmuscle Invasive Bladder Tumor

Takahiro Maeda, Eiji Kikuchi,* Kazuhiro Matsumoto, Akira Miyajima and Mototsugu Oya

From the Department of Urology, Keio University School of Medicine, Tokyo, Japan

Abbreviations and Acronyms

BCG = bacillus Calmette-Guérin

CIS = carcinoma in situ

MMC = mitomycin C

NMIBC = nonmuscle invasive bladder cancer

TUR-BT = bladder cancer transurethral resection

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* Correspondence and requests for reprints: Department of Urology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan (telephone: 81-3-5363-3825; FAX: 81-3-3225-1985; e-mail: eiji-k@kb3.so-net.ne.jp).

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Purpose: In recent years some reports have suggested without any significant evidence that mitomycin C instillation would be more effective with urinary alkalinization. We investigated the association between urinary pH and the efficiency of mitomycin C instillation.

Materials and Methods: We identified 130 patients treated with transurethral resection of a bladder tumor and adjuvant intravesical mitomycin C instillation between 1985 and 2008 at Keio University Hospital. Urinary pH was determined in 124 of the 130 patients just before mitomycin C administration during the scheduled instillation period. These 124 patients were assigned to groups according to urinary pH in increments of 0.5 and the association between urinary pH and clinicopathological characteristics was evaluated.

Results: Mean ± SD urinary pH was 5.77 ± 0.05 (range 5.00 to 7.66) during the scheduled instillation period. Urinary pH was 5.00 to 5.49, 5.50 to 5.99, 6.00 to 6.49, 6.50 to 6.99 and 7.00 in 39, 46, 25, 7 and 7 patients, respectively. Patients were further divided into 2 groups by urinary pH using a cutoff of 5.5, including 39 with pH less than 5.5 and 85 with pH 5.5 or more. Age, gender, tumor grade, primary/recurrent disease, pathological stage and the presence or absence of concomitant carcinoma in situ were not significantly difference between the 2 groups. Multivariate analysis revealed that categorical urinary pH was an independent risk factor for tumor recurrence (HR 1.75, $p = 0.032$). Three and 5-year recurrence-free rates were 64.2% and 52.9% in patients with pH 5.5 or greater, and 41.9% and 38.4% in those with pH less than 5.5, respectively ($p = 0.046$). Multivariate analysis showed that the HR of urinary pH for tumor recurrence was 1.84 and 2.54 at the 5.4 and 5.2 cutoffs, respectively.

Conclusions: Results suggest that urinary pH more than 5.5 is associated with a decreased risk of tumor recurrence in patients treated with intravesical mitomycin C for nonmuscle invasive bladder cancer. Monitoring urinary pH during mitomycin C adjuvant treatment and modifying pH for urine alkalization may improve the therapeutic efficacy of mitomycin C instillation.

Key Words: urinary bladder; urothelium; carcinoma; neoplasm recurrence; local; mitomycin

Urothelial carcinoma accounts for more than 90% of all bladder cancer cases.[1] An average of 70% to 75% of bladder urothelial carcinomas present as NMIBC and the remainder present as muscle invasive bladder cancer.[2] Standard initial treatment for patients with NMIBC is complete surgical TUR-

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BT. However, 30% to 80% of these patients have tumor recurrence within 5 years with TUR-BT alone.[3] Intravesical chemotherapy and immunotherapy are widely used as adjuvant therapies after TUR-BT to prevent tumor recurrence. BCG is used as an intravesical immunotherapy agent and thiotepa, MMC, doxorubicin, epirubicin or valrubicin are instilled as chemotherapy agents. Of these methods BCG instillation has become first line treatment for CIS and is effective as prophylaxis to prevent bladder cancer recurrence after TUR-BT, especially in patients at high risk.[4–7] On the other hand, there are some problems in BCG treatment because of the high rate of local side effects.[8] An adverse event at any time during the BCG instillation period is the most common cause of patient dropout, which is a factor affecting treatment success or failure.[9–10]

The current guideline states that an induction course of intravesical therapy with MMC or BCG is recommended in a patient with multifocal or large volume, low grade Ta bladder cancer.[11] For patients who cannot continue BCG instillation due to side effects intravesical instillation of MMC is considered a safe treatment option since there are few problems associated with transurothelial absorption and myelosuppression is rare. However, an optimal therapeutic schedule has not yet been established.

Au et al reported an improved recurrence-free survival rate and prolonged median time to recurrence using methods to enhance MMC activity by improving the MMC concentration and dose, and urine alkalization in a phase III trial.[12] However, to our knowledge an optimal pH during the improved MMC instillation schedule has not yet been proposed. We evaluated whether urinary pH during MMC instillation has an effect on tumor recurrence and determined an appropriate urinary pH for efficient MMC intravesical treatment.

PATIENTS AND METHODS

A review of the medical records of Keio University Hospital between 1985 and 2008 identified more than 1,300 patients diagnosed with pTa or pT1 NMIBT who were treated with TUR-BT, including 130 treated with adjuvant intravesical MMC instillation. After excluding 6 patients for whom urine pH data could not be obtained 93 males and 31 females were included in analysis.

Intravesical instillation of MMC was begun 4 to 5 weeks after TUR-BT and scheduled for weekly administration for 6 to 9 weeks at a dose of 30 mg MMC in 30 ml saline via an 8Fr urethral catheter with retention for at least 1 hour. Patients were followed with urine cytology and cystoscopy at 3-month intervals during the initial 2 years, every 6 months for the next 3 years and yearly thereafter. Excretory urography, ultrasound or computerized tomography was used to evaluate distant metastasis and upper urinary tract tumor recurrence every 1 or 2 years for 5 years after initial treatment.

Recurrence was defined as new tumor in the bladder after initial clearance without stage progression. Side effects observed during MMC instillation were classified as local or systemic. Urinary symptoms were considered local side effects while other side effects, such as dermal rush, itching, epididymitis and general fatigue, were classified as systemic side effects.[13] During each MMC intravesical instillation urine was obtained just before MMC administration. We used the Uriflet 9UB (Menarini Diagnostics, Florence, Italy), a widely used dipstick in Japan, and Aution Max™ AX-4030, an automated urine test-strip analyzer. Urinary pH was measured and calculated as the mean.

The clinicopathological features of each patient, including age, gender, primary/recurrent tumor, multiplicity, tumor grade, pathological stage, concomitant CIS and urinary pH, were analyzed by Cox regression univariate and multivariate analysis. Recurrence-free survival curves were constructed using the Kaplan-Meier method and compared using the log rank test. Differences among groups were considered significant at $p < 0.05$. Univariate and multivariate analysis of data was done using the Cox proportional hazards model with stepwise forward selection using STATA®, version 11.0.

RESULTS

Mean age of the 124 patients was 69.0 years (range 27 to 90) and median followup was 2.5 years (range 1 to 22). TaG1/2, TaG3, T1G2 and T1G3 disease was seen in 62, 22, 15 and 25 patients, respectively, and 17 (13.7%) had a concomitant CIS lesion. Of the cases 79 (63.7%) were recurrent. A total of 85 patients (68.5%) had multiple tumors and histological type was pure urothelial carcinoma in 121. Overall tumor recurrence was observed in 64 cases (51.6%). Three, 5 and 10-year recurrence-free rates were 57.2%, 48.4% and 34.0%, respectively.

Mean urinary pH was 5.77 (range 5.00 to 7.66) during the MMC instillation period. Table 1 shows the number of patients by urinary pH, which was 5.00 to 5.49 in 39 (31.5%). Using a cutoff of pH 5.5 we first divided the patients into 2 groups by urinary pH, including 39 and 85 with pH less than 5.5 (mean 5.2, range 5.0 to 5.4) and 5.5 or greater (mean 6.0, range 5.5 to 7.7), respectively ($p < 0.001$). Mean age in these groups was 70 (range 36 to 90) and 69

Table 1. *Mean urinary pH during MMC instillation in all patients*

Urinary pH	No. Pts (%)
5.00–5.49	39 (31.5)
5.50–5.99	46 (37.1)
6.00–6.49	25 (20.2)
6.50–6.99	7 (5.6)
7.00	7 (5.6)
Total No.	124

years (range 27 to 90), respectively (p = 0.493). Age, gender, primary/recurrent tumor, multiplicity, tumor grade, pathological stage and concurrent CIS were not significantly different between the 2 groups (table 2). Univariate analysis revealed that categorical urinary pH (less than 5.5) was a significant risk factor for tumor recurrence (p = 0.021). Multivariate analysis demonstrated that urinary pH (p = 0.032) and tumor multiplicity (p = 0.033) were independent predictors of tumor recurrence (table 3). Three and 5-year recurrence-free rates were 64.2% and 52.9% in patients with pH 5.5 or more, and 41.9% and 38.4% in those with pH less than 5.5, respectively (p = 0.046, part *A* of figure).

Patients were also divided into 2 groups by urinary pH using cutoffs of 5.4, including less than 5.4 in 32 and 5.4 or greater in 92, and 5.2, including less than 5.2 in 17 and 5.2 or greater in 107. Multivariate analysis revealed that urinary pH and tumor multiplicity were independent predictors of tumor recurrence. The HR of urinary pH for tumor recurrence was 1.84 and 2.54 at the 5.4 and 5.2 cutoffs, respectively. Three and 5-year recurrence-free rates were 63.5% and 53.2% in patients with urinary pH 5.4 or greater, and 39.3% and 33.5% in their counterparts, respectively (p = 0.028). When the cutoff was set at pH 5.2, 3 and 5-year recurrence-free rates were 61.5% and 57.0% vs 30.9% and 23.2%, respectively (p = 0.017, part *B* of figure).

Side effects, which developed in 38 patients (30.6%) during MMC instillation, were local in 31 and systemic in 7. No patient required hospitalization during MMC instillation. We evaluated the difference between nonhematological toxicity and urinary pH at a cutoff of pH 5.5. There were no significant differences in local or systemic toxicity between the 2 groups (table 4).

Table 2. *Patient characteristics and urinary pH at pH 5.5 cutoff*

	Total No.	No. pH Less Than 5.5	No. pH 5.5 or Greater	p Value
Gender:				0.147
M	93	33	60	
F	31	6	25	
Multiplicity:				0.922
Solitary	39	13	26	
Multiple	85	26	59	
Tumor grade:				0.969
G1/G2	76	24	52	
G3	48	15	33	
Pathological stage:				0.973
Ta	84	27	57	
T1	36	12	28	
Concurrent CIS:				
Yes	17	5	12	
No	107	34	73	
Recurrent TUR-BT:				0.951
Yes	79	25	54	
No	45	14	31	

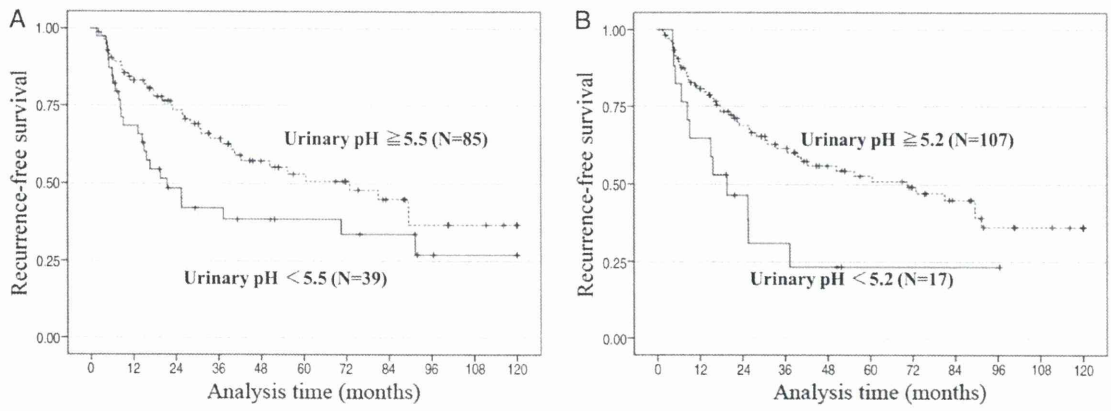
Table 3. *Univariate and multivariate analysis of tumor recurrence in patients with MMC instillation*

	Univariate p Value	Multivariate p Value	Risk Ratio (95% CI)
Age	0.583		
Gender (M vs F)	0.871		
Multiplicity (solitary vs multiple)	0.076	0.033	1.818 (1.047–3.185)
Tumor grade (G1/G2 vs G3)	0.471		
Pathological stage (Ta vs T1)	0.296		
Concurrent CIS (yes vs no)	0.585		
Recurrent TUR-BT (yes or no)	0.366		
Urinary pH (less than 5.5 vs 5.5 or greater)	0.021	0.032	1.752 (1.040–2.927)

DISCUSSION

MMC is an alkylating agent that is only minimally absorbed from the bladder into the systemic circulation due to its high molecular weight. As a result, myelosuppression is uncommon after intravesical use of this agent.[14] Intravesical MMC has been applied with various schedules, including various concentrations, with or without a state of dehydration and at various instillation times during a scheduled period. However, the methods of administering intravesical MMC were constructed empirically. A number of factors, including the dose, effective drug concentration and instillation dwelling time, may determine therapeutic efficacy. Schmittgen et al reported that MMC activity may be enhanced by increasing the area under the drug concentration-time profile in urine and tissue by 1) decreasing urine volume, 2) increasing dwelling time and 3) increasing drug absorption across the urothelium.[15] Based on a phase III trial Au et al advocated increasing the intravesical drug concentration by decreasing the volume in which the drug is instilled (40 mg in 20 cc), decreasing urine volume by dehydration and complete emptying, and alkalinizing urine to stabilize drug improved recurrence-free survival and prolong median time to recurrence.[12] We evaluated whether urinary pH is associated with the efficacy of adjuvant MMC instillation treatment for NMIBC recurrence. Multivariate analysis showed that categorical urinary pH and tumor multiplicity were independent predictors of tumor recurrence. A mean urinary pH cutoff of 5.5 or less (5.2 or 5.4) is thought to be an appropriate level to monitor intravesical MMC instillation therapy.

Our analysis indicates that patients with urinary pH less than 5.5 had the worst tumor recurrence rate. Wientjes et al reported that urine pH may alter the treatment outcome of a chemotherapy agent in 3 ways, that is it may affect MMC absorption, stability



Recurrence-free rates. *A*, at 5.5 urinary pH cutoff. Dotted curve indicates pH 5.5 or greater. Solid curve indicates pH less than 5.5. *B*, at 5.2 urinary pH cutoff. Dotted line indicates pH 5.2 or greater. Solid line indicates pH less than 5.2.

or cytotoxicity.[16] Since MMC is mainly not ionized at the normal urine pH range of 5 to 8, the effect of urine pH on absorption is minor. Urine pH has a significant effect on drug degradation. MMC degradation in human urine was 10-fold greater at pH 5 than at pH 7.[17] Although MMC is more active at acidic pH in monolayer cultures, MMC activity was not affected by pH in a multicellular human solid tumor.[18]

Our results revealed no association between the frequency of adverse events and urinary pH. We are certain that low urinary pH would not affect the frequency of adverse events associated with MMC instillation. Au et al recommended alkalinizing urine with 1.3 gm oral sodium bicarbonate administered the night before, the morning of and 30 minutes before intravesical therapy.[12] Since it was reported that potassium citrate at doses of 30 to 80 mEq/24 hours increased urine pH from 5.3 to 6.2,[19] we surmised that oral potassium citrate would become an alternative to conventional urine alkalinization. The efficacy of MMC instillation for intermediate risk NMIBT should be reevaluated when an improved method such as urine alkalinization is introduced and then be compared to BCG instillation with respect to inhibiting tumor recurrence.

Table 4. *Nonhematological toxicity and urinary pH at pH 5.5 cutoff*

Toxicity	No. 5.5 or Less	No. Greater Than 5.5 (%)	p Value
Local:	10 (25.6%)	21 (24.7%)	0.991
Urinary frequency	7	6	
Hematuria	5	5	
Pain	1	8	
Dysuria	1	5	
Systemic:	2 (5.1%)	5 (5.9%)	0.424
Dermal rash + itching	2	3	
Epididymitis	0	2	

Other tools to improve the efficiency of intravesical MMC therapy have been reported. Di Stasi et al performed a prospective study in patients with high risk NMIBC with the aim of increasing MMC bladder uptake with electromotive administration.[20] Patients were randomly assigned to 1 of 3 groups, including a 40 mg electromotive MMC group, a 40 mg passive MMC group and an 81 mg BCG group. Intravesical electromotive administration increased peak plasma MMC concentration 5-fold compared with passive transport, resulting in an improved response rate. Colombo et al designed a prospective, multicenter, randomized trial to compare the efficacy of intravesical instillation of MMC vs MMC combined with local hyperthermia using a special transurethral catheter with microwaves.[21] Results indicated that combination therapy would be more effective than standard instillation at 24-month followup despite increased but acceptable local toxicity. Heijden et al reported that EO9 (apaziquone), an indolequinone bioreductive prodrug and analog of MMC that requires activation by cellular reductase enzyme to become cytotoxic, was used for adjuvant intravesical instillation and had a good completion rate for NMIBT without an increase in side effects.[22]

Our study has some limitations. It was done in retrospective fashion. No accurate data on post-void residual urine volume and no information on MMC instillation indwelling time could be obtained. This may also have affected the treatment outcome of our study. The MMC dose and concentration (30 mg MMC/30 ml saline) were somewhat lower than previously reported (40 mg/50 ml[23] and 40 mg/20 ml[12]). In the future a randomized, prospective trial is warranted to compare urine alkalinization at defined MMC doses and concentrations.

Variation in repeat urine pH measurements and dipstick test accuracy are also our study limitations. During several weeks of MMC instillation pH

changed but not widely. In a subgroup of patients with urinary pH less than 5.2 the deviation of pH in each individual was not wide (SD 0.00 to 0.24). Similar trends were observed at other pH cutoffs. The gold standard measurement of urinary pH is to use an electrochemical pH meter. However, this device is not generally used in the urological office due to cost and time consumption. The dipstick is rapid, easy, economical and widely used in the clinical setting.

Others reported that long-term intravesical adjuvant MMC decreased the risk of tumor recurrence compared with induction BCG therapy in patients with intermediate and high risk NMIBC. Lamm et al pointed out the usefulness of maintenance BCG therapy with a low 17% rate of completion of BCG maintenance therapy due to BCG related adverse events.[24] An improvement such as modifying urinary pH for MMC instillation therapy may also be applicable to maintenance therapy.

CONCLUSIONS

Results suggest that urinary pH more than 5.5 is associated with a decreased risk of tumor recurrence in patients treated with intravesical MMC for NMIBC. Monitoring urinary pH during MMC adjuvant treatment and modifying urinary pH for urine alkalization may improve the therapeutic efficacy of MMC instillation.

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BJUI
BJU INTERNATIONAL

Risk group stratification to predict recurrence after transurethral resection in Japanese patients with stage Ta and T1 bladder tumours: validation study on the European Association of Urology guidelines

Shigeru Sakano[1], Hideyasu Matsuyama[1], Kimio Takai[2], Satoru Yoshihiro[3], Yoriaki Kamiryo[4], Satoshi Shirataki[5], Yoshitaka Kaneda[6], Osamu Hashimoto[7], Keiji Joko[8], Akinobu Suga[9], Mitsutaka Yamamoto[10], Shigeaki Hayashida[11], Yoshikazu Baba[12], Akihiko Aoki[13] and The Yamaguchi Uro-Oncology Group

[1]Department of Urology, Graduate School of Medicine, Yamaguchi University, [6]Department of Urology, Konan St. Hill Hospital, Ube, [2]Department of Urology, Saiseikai Shimonoseki General Hospital, [3]Department of Urology, Shimonoseki City Central Hospital, [4]Department of Urology, Shimonoseki Municipal Saiseikai Toyoura Hospital, Shimonoseki, [5]Department of Urology, Yamaguchi Rosai Hospital, Sanyo-Onoda, [7]Department of Urology, Ogori Daiichi General Hospital, [8]Department of Urology, Saiseikai Yamaguchi General Hospital, [9]Department of Urology, Yamaguchi Red Cross Hospital, Yamaguchi, [10]Department of Urology, Yamaguchi Grand Medical Center, Hofu, [11]Department of Urology and Nephrology, Tokuyama Central Hospital, Shunan, [12]Department of Urology, Shuto General Hospital, Yanai, Yamaguchi and [13]Department of Urology, Masuda Red Cross Hospital, Masuda, Shimane, Japan

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Study Type – Therapy (case series)
Level of Evidence 4

What's known on the subject? and What does the study add?
EAU guidelines on non-muscle-invasive bladder tumours have been widely used for the prediction of recurrence after TUR. However, there are substantial differences in bladder cancer incidence and mortality rates between European countries and Japan.

This study provides useful factors for predicting recurrence and validation of EAU guidelines on the risk group stratification to predict recurrence in Japanese patients with stage Ta and T1 bladder tumours.

OBJECTIVE

- To validate the European Association of Urology (EAU) guidelines on risk group stratification to predict recurrence in Japanese patients with stage Ta and T1 bladder tumours.

PATIENTS AND METHODS

- A cohort of 592 Japanese patients who were treated with transurethral resection (TUR) and histopathologically diagnosed with Ta and T1 urothelial carcinoma of the bladder were enrolled in this retrospective study.
- The primary endpoint of the present study was recurrence-free survival, and the median follow-up duration was 37 months in recurrence-free survivors.

RESULTS

- Multivariate Cox proportional hazards regression analysis showed that the Eastern Cooperative Oncology Group performance status (ECOG PS), prior recurrence rate, number of tumours and T category were independent predictors of time to recurrence ($P < 0.05$). According to the EAU guidelines for predicting recurrence, the vast majority of Japanese patients were classified into intermediate risk.
- The intermediate-risk patients were further divided into intermediate-low-risk and intermediate-high-risk subgroups based on the European Organization for Research and Treatment of Cancer risk table, and a significant difference in the recurrence-free survival rates was found between these subgroups ($P < 0.001$).
- It was also found that patients with high risk combined with intermediate-high risk had significantly poorer recurrence-free survival rates than those with low risk combined with intermediate-low risk ($P < 0.001$).

CONCLUSIONS

- This is the first report on the ECOG PS as a potentially useful predictor for bladder tumour recurrence.
- The risk group stratification of the EAU guidelines for recurrence might not be applicable to Japanese patients with Ta and T1 bladder tumours, but the subgroup classification of intermediate risk could be appropriate.

KEYWORDS

carcinoma, recurrence, transitional cell, urinary bladder

INTRODUCTION

Urinary bladder cancer is a common malignant disease with 356 000 new cases and 145 000 deaths worldwide in 2002 [1]. Its prevalence is three- to eightfold higher than its incidence, making bladder cancer one of the most prevalent neoplasms, and hence a major burden for healthcare systems [2]. Most bladder cancers are urothelial carcinomas (UCs) and present in two different forms – carcinomas not invading bladder muscle (stages Ta and T1) and carcinoma invading bladder muscle (stages T2–T4) – that often progress to life-threatening disease. Carcinoma *in situ* (CIS) can also be considered not to be invading the bladder muscle. However, CIS tends to behave more aggressively and is often found in association with high-grade carcinomas not invading bladder muscle.

The bladder tumours not invading bladder muscle account for about 70–80% of newly diagnosed cases. These tumours can usually be managed with transurethral resection (TUR), with or without intravesical therapy. However, tumours that are not invading bladder muscle form a heterogeneous group of tumours, for which 1-year recurrence and progression to carcinoma invading bladder muscle are 15–61% and <1–17%, respectively [3]. Despite the absence of progression, the frequent recurrences have a marked effect on patients' quality of life, and frequent follow-up examinations for detecting recurrence, including cystoscopy, are a severe burden on the patients. An accurate estimate of the probability of recurrence in patients with bladder tumours not invading muscle is essential for patient counselling, choosing the appropriate adjuvant treatment, and determining the frequency of follow-up in individual patients.

Recently, European Association of Urology (EAU) guidelines on carcinomas not invading bladder muscle have been widely used for the prediction of recurrence [4–6]. However, there are substantial differences in bladder cancer incidence and mortality rates between European countries and Japan [7,8]. Original guidelines for Japanese patients with bladder tumours not invading muscle might, therefore, be required to predict recurrence. In the present study, we analysed various clinicopathological factors for predicting recurrence after TUR in Japanese patients with stage Ta and T1 bladder tumours, and validated the EAU guidelines on the risk group stratification to predict recurrence.

PATIENTS AND METHODS

In all, 24 hospitals in or around the Yamaguchi prefecture, Japan, participated in this multicentre, retrospective cohort study, and the investigators in each hospital reviewed original medical records for collecting data. Between January 2004 and December 2006, 779 consecutive patients with bladder cancer not invading the muscle were evaluated. They were initially treated with complete TUR and histopathologically diagnosed with Ta and T1 (N0M0) UC of the bladder based on the TNM classification of the International Union Against Cancer (2002) [9]. Patients with non-UC histology, primary CIS or any treatments before TUR were excluded from the present study. Furthermore, patients with systemic chemotherapy and/or radiotherapy or cystectomy after TUR or with no follow-up data were excluded. After exclusion, 592 patients were enrolled in the present study. The median (range) patient age at TUR was 73 (33–95) years, and the median (range) follow-up duration among patients who were recurrence-free was 37 (3–69) months, which was calculated from the date of TUR to the end of observation. The tumour grade was classified according to the World Health Organization system (1973) [10].

The clinicopathological data, including age, sex, Eastern Cooperative Oncology Group performance status (ECOG PS), prior recurrence rate, number of tumours, tumour size, pathological T category, presence of concomitant CIS, histopathology (pure UC or UC with other elements), grade and intravesical therapy, were obtained from each hospital and merged. The patient characteristics are summarized in Table 1. Intravesical chemotherapy or BCG therapy was carried out after TUR in 189 (31.9%) or 92 (15.5%) patients, respectively. None of the patients received maintenance BCG therapy. All patients underwent cystoscopy for the detection of bladder tumour recurrence every 3 months after TUR. The time to recurrence was defined as the time from the date of TUR to the date of the first documented recurrence. Patients who were still alive or who had died before tumour recurrence or progression were censored at the date of the last available follow-up cystoscopy. We defined the recurrence-free survival as a primary endpoint of the present study. Tumour progression was not analysed in the present study because of the shorter follow-up period. Univariate and multivariate Cox proportional hazards regression models were used to assess the impact of various clinicopathological factors on time to first recurrence in 592 patients with bladder cancer not invading the bladder muscle.

To validate the EAU guidelines on the risk group stratification for predicting recurrence in Japanese patients, a total recurrence score for each patient was calculated based on the six clinicopathological factors according to the EAU guidelines scoring system for recurrence, as follows: prior recurrence rate (primary, 0; < one recurrence/year, 2; ≥ one recurrence/year, 4), number of tumours (single, 0; 2–7, 3; ≥8, 6), tumour size (≤3 cm, 0; >3 cm, 3), T category (Ta, 0; T1, 1), concomitant CIS (no, 0; yes, 1) and grade (G1, 0; G2, 1; G3, 2) [4]. Patients were then divided into three risk groups for recurrence based on their total scores according to the EAU guidelines: low risk (score 0), intermediate risk (score 1–9) and high risk (score 10–17) [4]. In the present study, patients with intermediate risk were further divided into two subgroups based on the total scores according to the recurrence risk table of European Organization for Research and Treatment of Cancer (EORTC) (the original article of EAU guidelines) [3]: intermediate-low risk (total recurrence score 1–4) and intermediate-high risk (score 5–9) [3]. The recurrence-free survival curves stratified by risk groups were estimated using the Kaplan–Meier method in 372 patients for whom the data on all six of the earlier-mentioned clinicopathological factors were completely available. The recurrence-free survival probability distributions were compared using the log-rank test. A P value <0.05 was considered to indicate statistical significance and all reported P values were two-sided. All statistical tests were performed using the JMP version 6.0 statistical software package (SAS Institute Inc., Cary, NC, USA).

RESULTS

During the follow-up period, 316 (53.4%) of 592 Japanese patients with Ta and T1 UC of the bladder had at least one tumour recurrence after TUR. Recurrence-free survival rates at 1 and 3 years were 67.7 and

44.3%, respectively, and the median time to first recurrence was 28.8 months.

On univariate Cox proportional hazards regression analysis, age, ECOG PS, prior recurrence rate, number of tumours, T category and grade had a significant influence on time to first recurrence ($P < 0.05$) (Table 2), while sex, tumour size, presence of concomitant CIS, histopathology and intravesical therapy were not significantly associated with time to first recurrence. Multivariate analysis showed that ECOG PS [hazard ratio (HR) = 1.24; 95% CI = 1.06–1.44; $P = 0.008$], prior recurrence rate (HR = 1.12, 95% CI = 0.94–1.33 for primary vs < one recurrence/year; HR = 1.26, 95% CI = 1.04–1.52 for primary vs ≥ one recurrence/year; $P = 0.046$), number of tumours (HR = 1.40, 95% CI = 1.21–1.62 for single vs 2–7; HR = 1.15, 95% CI = 0.76–1.64 for single vs ≥8; $P < 0.001$) and T category (HR = 1.17, 95% CI = 1.00–1.36, $P = 0.044$) were independent predictors for time to first recurrence (Table 2).

Of 372 patients with a complete data set, 12 (3.2%), 344 (92.5%) and 16 (4.3%) were classified into low-, intermediate- and high-risk groups, respectively, according to the EAU guidelines for predicting recurrence (Table 3). Although most Japanese patients with Ta and T1 tumours were intermediate-risk according to the EAU guidelines, these intermediate-risk patients were further divided into intermediate-low-risk ($n = 186$, 50.0%) and intermediate-high-risk ($n = 158$, 42.5%) subgroups based on the EORTC risk table (Table 3). Recurrence-free survival rates were plotted for the risk groups of the EAU guidelines using Kaplan–Meier survival curves ($P = 0.032$, log-rank test; Fig. 1A). Recurrence-free survival curves were also constructed, adding the intermediate-risk subgroup stratification ($P = 0.27$ for low risk vs intermediate-low risk; $P < 0.001$ for intermediate-low risk vs intermediate-high risk; and $P = 0.33$ for intermediate-high risk vs high risk; log-rank test; Fig. 1B). No significant differences in the recurrence-free survival rates were found between low-risk and intermediate-low-risk groups or between intermediate-high-risk and high-risk groups. Therefore, the low-risk and intermediate-low-risk groups were combined into one group (total recurrence score 0–4, $n = 198$) and the intermediate-high-risk and high-risk groups were considered as another group (score 5–17, $n = 174$). Recurrence-free survival curves stratified by this two-tiered risk group were calculated using the Kaplan–Meier method. The median recurrence-free survival times were 58.3 months in patients with score 0–4, and 18.2 months in patients with score 5–17. The patients with score 5–17 had significantly poorer recurrence-free survival rates than those with score 0–4 ($P < 0.001$, log-rank test; Fig. 2).

Characteristic	Number (%) of patients
Age, years	
≤70	218 (36.8)
>70	355 (60.0)
Unknown	19 (3.2)
Sex	
Male	469 (79.2)
Female	120 (20.3)
Unknown	3 (0.5)
ECOG PS	
0	286 (48.3)
1	105 (17.7)
2–4	35 (5.9)
Unknown	166 (28.0)
Prior recurrence rate	
Primary	353 (59.6)
< one recurrence/year	108 (18.2)
≥ one recurrence/year	85 (14.4)
Unknown	46 (7.8)
Number of tumours	
Single	304 (51.4)
2–7	264 (44.6)
≥8	22 (3.7)
Unknown	2 (0.3)
Tumour size	
≤3 cm	562 (94.9)
>3 cm	25 (4.2)
Unknown	5 (0.8)
Pathological T category	
Ta	287 (48.5)
T1	305 (51.5)
Concomitant CIS	
No	360 (60.8)
Yes	53 (9.0)
Unknown	179 (30.2)
Histopathology	
Pure UC	572 (96.6)
UC with other elements	20 (3.4)
Grade	
G1	105 (17.7)
G2	334 (56.4)
G3	145 (24.5)
Unknown	8 (1.4)
Intravesical therapy	
No	311 (52.5)
Chemotherapy	189 (31.9)
BCG	92 (15.5)

TABLE 1
Patient characteristics

DISCUSSION

The recently published EAU guidelines on UC of the bladder not invading bladder

TABLE 2 Univariate and multivariate Cox proportional hazards regression analysis for time to first recurrence

Variable (*n*)	Number of patients	Univariate		Multivariate	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age, years					
≤70	218	1 (reference)	0.003	1 (reference)	0.70
>70	355	1.20 (1.06–1.35)		1.03 (0.88–1.21)	
Sex					
Male	469	1 (reference)	0.68		
Female	120	0.97 (0.84–1.11)			
ECOG PS					
0	286	1 (reference)	0.005	1 (reference)	0.008
1–4	140	1.21 (1.06–1.39)		1.24 (1.06–1.44)	
Prior recurrence rate					
Primary	353	1 (reference)	0.002	1 (reference)	0.046
< one recurrence/year	108	1.12 (0.97–1.29)		1.12 (0.94–1.33)	
≥ one recurrence/year	85	1.31 (1.12–1.52)		1.26 (1.04–1.52)	
Number of tumours					
Single	304	1 (reference)	<0.001	1 (reference)	<0.001
2–7	264	1.33 (1.19–1.49)		1.40 (1.21–1.62)	
≥8	22	1.43 (1.07–1.84)		1.15 (0.76–1.64)	
Tumour size (cm)					
≤3	562	1 (reference)	0.80		
>3	25	1.04 (0.77–1.34)			
Pathological T category					
Ta	287	1 (reference)	0.004	1 (reference)	0.044
T1	305	1.17 (1.05–1.31)		1.17 (1.00–1.36)	
Concomitant CIS					
No	360	1 (reference)	0.56		
Yes	53	0.94 (0.77–1.14)			
Histopathology					
Pure UC	572	1 (reference)	0.92		
UC with other elements	20	0.98 (0.68–1.33)			
Grade					
G1	105	1 (reference)	<0.001	1 (reference)	0.21
G2	334	1.29 (1.09–1.53)		1.22 (0.98–1.54)	
G3	145	1.40 (1.16–1.69)		1.37 (1.01–1.87)	
Intravesical therapy					
No	311	1 (reference)	0.18		
Chemotherapy	189	1.01 (0.89–1.13)			
BCG	92	0.87 (0.73–1.02)			

TABLE 3 Risk group stratification to predict recurrence based on the total recurrence score

Risk group	Total recurrence score	Number of patients (%)
Low risk	0	12 (3.2)
Intermediate risk	1–9	344 (92.5)
Intermediate-low risk	1–4	186 (50.0)
Intermediate-high risk	5–9	158 (42.5)
High risk	10–17	16 (4.3)

muscle seem to offer a useful tool for decision-making in a clinical setting, and, in particular, the critical importance of risk stratification is well illustrated [4,5]. The risk group stratification for predicting recurrence and progression in the guidelines was based on the EORTC database, which provided individual patient data for 2596 patients diagnosed with stage Ta and T1 tumours randomized in seven trials in European countries [3]. However, because there are substantial differences in bladder cancer incidence and mortality rates between European countries and Japan [7,8], prognostic factors for recurrence and progression in Japanese patients might possibly be different from those in Europe. To the best of our knowledge, there have been no validation studies, including in Japan, on the EAU guidelines on UC of the bladder not invading bladder muscle. Thus, we analysed various clinicopathological factors for