INTRODUCTION

Approximately 75–85% of all patients with bladder cancer are initially diagnosed with non-muscle-invasive disease (i.e. stage Ta, Tis or T1) [1]. Adjuvant intravesical BCG therapy is the most effective regimen for non-muscle-invasive bladder cancer (NMIBC) among common intravesical agents; however, some patients with NMIBC experience tumour recurrence after BCG therapy, and this has been termed 'BCG failure'.

Owing to the lack of a standard classification system for BCG failure, previous studies have noted erratic rates of stage progression after BCG therapy [2–8]. Some studies classified patients as having BCG failure after a single induction course of BCG [9,10], while others used failure after two courses as their definition [5,6,11]. In addition, patients with persistent disease and patients with recurrent disease after an initial response have been combined in some studies [9,10]. A few reports combined all patients who could not complete the BCG therapy because of toxicity [11,12]. Meanwhile, in the most general sense, any recurrence of disease after BCG therapy can be referred to as 'BCG failure'. Most studies did not indicate the disease-free interval after the last BCG induction. These inconsistencies have led to comparisons of outcome in a very heterogeneous population, and this has resulted in confusion regarding treatment decisions in patients classified as having BCG failure.

To avoid unnecessary salvage therapy and to accurately evaluate the results of salvage therapy, Herr and Dalbagni [13] noted the need for a standard definition of BCG failure. Nieder *et al.* [14] stratified BCG-failure patterns into four major groups, BCG-refractory, BCG-resistant, BCG-relapsing, and BCG-intolerant, which were based mainly on the responsiveness to BCG therapy and duration until tumour recurrence. They recommended that the classification of BCG failure into specific types of BCG failure should be used whenever possible to provide more uniformity in reporting.

In the present study, using Nieder *et al.*'s classification, we divided patients with NMIBC who had undergone induction BCG therapy that had resulted in failure into the four major BCG-failure groups. We then investigated whether the defined classification of BCG failure could successfully identify patients with a higher malignant potential.

PATIENTS AND METHODS

Data were retrospectively reviewed from the medical records of patients who had received induction BCG therapy that had resulted in BCG failure for NMIBC, excluding *carcinoma in situ* (CIS), between 1987 and 2009 at Keio University Hospital and Saiseikai Central Hospital. During this period, >1800 patients (>1400 patients at Keio University Hospital and 400 patients at Saiseikai Central Hospital) were diagnosed with NMIBC and treated using transurethral resection of bladder tumour (TURBT). After excluding patients with CIS, 521 patients who underwent induction BCG therapy were included in the study population. Some patients had undergone TURBT and intravesical chemotherapy one or more times before the initial BCG therapy. Of these 521 patients, 173 patients with BCG failure (142 men and 31 women), who matched the BCG-failure criteria, were selected for the study. Nieder *et al.* [14] defined the groups as follows: BCG-refractory as the presence of the disease at 6-month follow-up after BCG therapy, or any progression in stage, grade, or disease extent at 3-month follow-up, BCG-resistant as the disappearance of disease at 6-month follow-up, despite presence of disease that was of a lesser degree, stage, or grade 3 months after induction BCG therapy, BCG-relapsing as recurrence after disease-free status at 6-month follow-up, accompanied by a complete response to induction BCG therapy, and BCG-intolerant as recurrence after administering inadequate BCG therapy because of BCG toxicity. Using this classification system, we divided the 173 patients into their corresponding groups.

The patients were treated with Tokyo 172 strain or Connaught strain induction BCG therapy scheduled for weekly administration for 6–8 weeks at a full dose of BCG in 40 mL of saline with retention for 1–2 h. Patients were assessed at follow-up using urine cytology and cystoscopy at 3-month intervals during the initial 2 years, every 6 months for the next 3 years and yearly thereafter. I.v. urography, ultrasonography, or CT was used to evaluate distant metastasis and the upper urinary tract every year for at least 5 years. Stage progression was defined as confirmed histological muscle invasion or distant metastases.

In the analysis of stage progression-free survival, the starting point was defined as the date of BCG failure, and the endpoint as the date of stage progression. In the analysis of disease-specific survival, the starting point was defined as the date of BCG failure, and the endpoint as the date of disease-specific death. Stage progression-free survival and disease-specific survival rate curves were constructed using the Kaplan–Meier method and were compared using the log-rank test. A P value of <0.05 was considered to indicate statistical signficance. Univariate and multivariate analyses of data were performed using the Cox proportional hazards model with stepwise forward selection. The variables used were age, sex, pathological grade, stage, tumour multiplicity at the time of both initial induction BCG therapy and BCG failure, and BCG-failure classification. These analyses were performed using a JMP version 8.02 statistical software package (SAS Institute, Cary, NC, USA).

RESULTS

Table 1 shows the clinicopathological profiles of the 173 patients divided by the four BCG-failure groups. The mean (range) age of the patients was 65.5 (35–89) years. Of these 173 patients, 42 (24.3%) were assigned to the BCG-refractory group, three (1.7%) to the BCG-resistant group, 106 (61.3%) into the BCG-relapsing group and 22 (12.7%) into the BCG-intolerant group. The median follow-up period from TURBT just before initial induction BCG therapy was 6.7 years. The median (range) follow-up duration from initial BCG failure was 4.7 (1.0–13.3) years. Of 173 patients, five (2.9%) underwent total cystectomy before stage progression during the follow-up period owing to recurrence. Of these five patients, three were in the BCG-refractory group, one was in the BCG-relapsing group and one was in the BCG-intolerant group. Twenty-four patients (13.9%) experienced stage progression during follow-up. The mean (range) length of time from initial BCG failure to stage progression was 2.4

TABLE 1 Clinicopathological profiles of 173 patients divided into the four BCG-failure groups

	Study population	BCG-failure groups			
		Refractory	Resistant	Relapsing	Intolerant
No. of patients	173	42	3	106	22
Mean (range) age, years	65.5 (34–89)	65.6 (34–88)	72.9 (68–79)	65.1 (34–89)	65.8 (44–87)
Sex, *n* (%)					
Male	142 (82)	30 (71)	1	92 (87)	19 (86)
Female	31 (8)	12 (29)	2	14 (13)	3 (14)
Findings before initial BCG therapy					
Grade, *n* (%)					
G1–2	111 (64)	20 (48)	1	75 (71)	15 (68)
G3	62 (36)	22 (52)	2	31 (29)	7 (32)
Stage, *n* (%)					
Ta	100 (58)	19 (45)	0	67 (63)	14 (64)
T1	73 (42)	23 (55)	3	39 (37)	8 (36)
Concurrent CIS, *n* (%)					
Yes	21 (12)	10 (24)	0	9 (8)	2 (9)
No	152 (88)	32 (76)	3	97 (92)	20 (91)
Tumour multiplicity, *n* (%)					
Solitary	39 (23)	7 (17)	0	25 (24)	7 (32)
Multiple	134 (77)	35 (83)	3	81 (76)	15 (68)
Pathological category, *n* (%)					
TaG1–2	68 (39)	12 (29)	0	44 (42)	12 (55)
T1G1–2	33 (19)	7 (17)	0	23 (22)	3 (14)
TaG3	42 (24)	8 (19)	1	31 (29)	2 (9)
T1G3	30 (17)	15 (36)	2	8 (8)	5 (23)
Findings at BCG failure					
Grade, *n* (%)					
G1–2	126 (73)	27 (64)	2	82 (77)	15 (68)
G3	47 (27)	15 (36)	1	24 (23)	7 (32)
Stage, *n* (%)					
Ta	96 (55)	16 (38)	2	62 (58)	16 (73)
T1	72 (42)	25 (60)	1	41 (39)	5 (23)
Tis	5 (3)	1 (2)	0	3 (3)	1 (5)
Concurrent CIS, *n* (%)					
Yes	10 (6)	5 (12)	1	3 (3)	1 (4)
No	163 (94)	37 (88)	2	103 (97)	21 (95)
Tumour multiplicity, *n* (%)					
Solitary	83 (48)	17 (40)	2	53 (50)	11 (50)
Multiple	90 (52)	25 (60)	1	53 (50)	11 (50)
Pathological category, *n* (%)					
TaG1–2	76 (44)	11 (26)	2	50 (47)	13 (59)
T1G1–2	50 (29)	16 (38)	0	32 (30)	2 (9)
TaG3	20 (12)	5 (12)	0	12 (11)	3 (14)
T1G3	22 (13)	9 (21)	1	9 (8)	3 (14)

(0.1–10.5) years. Of the 24 patients who experienced stage progression, 14 (58.3%) were in the BCG-refractory group, eight (33.3%) were in the BCG-relapsing group, one (4.2%) was in the BCG-resistant group, and one (4.2%) was in the BCG-intolerant group at the time of the first BCG failure. Metastases appeared in 12 patients (50.0%) and muscle invasion was observed in 12 patients (50.0%). The clinicopathological characteristics of the patients were compared between the BCG-refractory group and the BCG-relapsing group. The pathological results at the time of both TURBT just before initial induction BCG therapy and BCG failure showed that the BCG-refractory group contained significantly more T1 or concurrent CIS components than the BCG-relapsing group. In addition, the pathological results at the time of TURBT just before initial induction BCG therapy showed that the BCG-refractory group contained more G3 components than the BCG-relapsing group.

TABLE 2 Univariate and multivariate analysis for stage progression

	Univariate	Multivariate		
	P	*P*	Hazard ratio	95% CI
Age (<65 vs ≥65)	0.058			
Sex (Male vs Female)	0.634			
Finding at initial BCG therapy				
Grade (G1-2 vs G3)	0.029			
Stage (pTa vs pT1)	0.307			
Concurrent CIS (Yes vs No)	0.122			
Tumour multiplicity (Solitary vs Multiple)	0.975			
Findings at BCG-failure				
Grade (G1-2 vs G3)	0.003	0.014	2.84	1.24–6.52
Stage (pTa vs pT1 and pTis)	0.252			
Concurrent CIS (Yes vs No)	0.053			
Tumour multiplicity (Solitary vs Multiple)	0.113			
BCG failure group (BCG-refractory vs Others)	<0.001	<0.001	4.68	2.07–10.97

FIG. 1. Stage progression survival curves for each BCG-failure group.

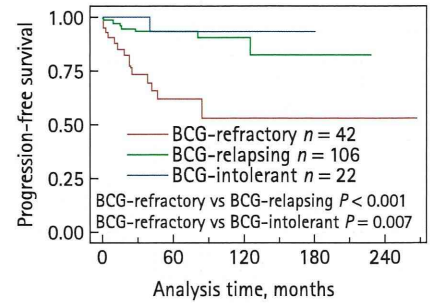

FIG. 2. Disease-specific survival rate curves for each BCG-failure group.

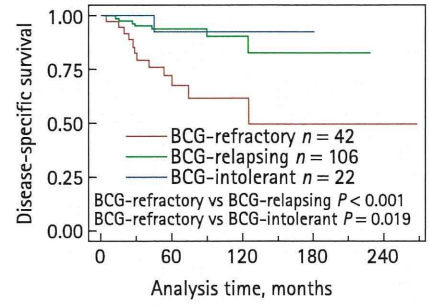

Table 2 shows the results of univariate and multivariate analysis of stage progression. Multivariate analysis showed that pathological grade G3 at BCG failure ($P = 0.014$; risk ratio 2.84; 95% CI 1.24–6.52) and BCG-refractory group ($P < 0.001$; risk ratio 4.68; 95% CI 2.07–10.97) were independent predictors for stage progression. The 10-year progression-free survival rates in the BCG-refractory group, BCG-relapsing group and BCG-intolerant group were 53.2%, 91.1%, and 93.8%, respectively (Fig. 1). Kaplan–Meier curve analysis showed that the BCG-refractory group progressed more than the BCG-relapsing group ($P < 0.001$) and BCG-intolerant group ($P = 0.007$).

A total of 20 patients (11.6%) died from their disease during follow-up. At the time of initial BCG failure, 12 (60.0%) were in the BCG-refractory group, seven (35.0%) in the BCG-relapsing group, and one (5.0%) in the BCG-intolerant group. The 10-year disease-specific survival rates in the BCG-refractory group, BCG-relapsing group and BCG-intolerant group were 61.8%, 90.6% and 92.9%, respectively (Fig. 2). Kaplan–Meier curve analysis showed that patients in the BCG-refractory group were significantly more likely to die from their disease than patients in the BCG-relapsing group ($P < 0.001$) and the BCG-intolerant group ($P = 0.019$).

DISCUSSION

In the present study, we have shown that patients in the BCG-refractory group were at higher risk for subsequent stage progression and disease-specific death over a long duration compared with patients in the other BCG-failure groups. Our results indicated that a recurrent pattern after induction BCG therapy according to Nieder's classification had an association with subsequent clinical course and we could identify patients who had a markedly worse outcome after initial induction BCG therapy.

A close association between responsiveness to the initial induction BCG therapy and subsequent stage progression has been shown in previous studies. Lerner *et al.* [15] reported that failure to achieve a complete response during induction BCG before maintenance BCG therapy for CIS, Ta or T1 bladder cancer was associated with a significant risk for disease-specific death. The 5-year probability of survival for patients who achieved a complete response to induction BCG was 77% compared with 62% for the patients who did not. Herr *et al.* [16] showed that evaluation of patients with superficial bladder carcinoma at 6 months after BCG therapy best defined the probability of stage progression. Andius and Holmäng [17] concluded that the disappearance of disease at the time of the first cystoscopy after BCG therapy was an independent risk factor for stage progression. Although the patient profiles and protocols of BCG therapy were different among the studies, all of the studies noted that failure to achieve a complete response by about 6 months, which was defined as BCG-refractory in our population, could be a risk factor for subsequent stage progression.

Our results indicate that the term 'BCG failure' involves heterogeneous characteristics and behaviour with respect to stage progression and disease-specific survival. Current guidelines have their unique definitions of BCG failure and recommendations for its treatment. European Association of Urology guidelines state that the following is regarded as BCG failure: the detection of muscle-invasive disease, the presence of high grade, non-muscle-invasive tumour at both 3 and 6 months, or any worsening of the disease under BCG treatment in spite of an initial response [1]. National Comprehensive Cancer Network guidelines do not clearly define BCG failure and state that if high-risk NMIBC is managed conservatively and does not respond to BCG, a cystectomy should be performed. For patients with recurrent persistent NMIBC that responds to induction intravesical therapy, a second course of BCG or mitomycin C induction therapy is recommended. Meanwhile, treatment

decision criteria for BCG-relapsing cases are not mentioned in any current guidelines. A clear and consistent definition of BCG failure should be used, such as BCG-refractory and BCG-relapsing, in future studies.

In the present study, we showed that definitive classification of BCG failure could estimate the different malignant potentials for subsequent stage progression and disease-specific survival. We found that more patients experienced stage progression (14/42 patients: 33.3%) and more patients died from their disease (12/42 patients: 28.6%) in the BCG-refractory group than in the other BCG-failure groups. We propose that patients in the BCG-refractory group should be advised of these potential worse outcomes during the follow-up. These patients might then be recommended to have more aggressive treatment, e.g. total cystectomy, at an early time because of this higher risk of stage progression and disease-specific death compared with the other BCG-failure groups. By contrast, total cystectomy does not appear to be directly indicated for patients in the BCG-relapsing group. Future studies concerning an appropriate treatment strategy for patients in the BCG-relapsing group are warranted.

The present study has several limitations. It was performed in a retrospective manner and included a small number of patients. BCG instillation with the maintenance schedule and second-look transurethral resection were not commonly practised at our institution during this period, and if they had been, they may have improved the results. Nevertheless, complete TURBTs until the muscle layer could be observed were performed in principle, except for tiny papillary tumours or CIS lesions. Moreover, all patients with a reddish or mossy area apart from the main tumour and oedema of the mucosa underwent biopsy of suspicious-appearing urothelium including biopsies from prostatic fossa. Disparities in treatments after BCG failure may have introduced bias into the results. Most of the patients had undergone an additional course of common intravesical agents, such as mitomycin C, epirubicin, pirarubicin or a second course of BCG.

In conclusion, patients in the BCG-refractory group had a potentially higher risk for subsequent stage progression and disease-specific death over a long duration compared with patients in the other BCG-failure groups. As the reporting definitions of 'BCG-failure' have been decidedly heterogeneous to date, standardized treatment decisions, protocols and recommendations should be established according to each individual BCG-failure pattern.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **NMIBC**, non-muscle-invasive bladder cancer; **TURBT**, transurethral resection of bladder tumour.

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A 2-week Maintenance Regimen of Intravesical Instillation of Bacillus Calmette–Guérin is Safe, Adherent and Effective in Patients with Non-muscle-invasive Bladder Cancer: A Prospective, Multicenter Phase II Clinical Trial

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Objective: To investigate the safety and efficacy of a maintenance regimen of bacillus Calmette–Guérin therapy including 6-week induction and 2-week maintenance instillation for patients with recurrent or multiple Ta, T1 tumors or carcinoma *in situ* of the urinary bladder.
Methods: This study was performed as single-arm multi-institutional study. The enrolled patients had been diagnosed with urothelial carcinoma of the bladder, including the presence of at least two bladder tumors, single tumors recurring within 12 months of follow-up, any Grade 3 Stage Ta or T1 tumor, and primary or recurrent biopsy proven carcinoma *in situ*. Patients received 81 mg intravesical bacillus Calmette–Guérin (Connaught strain). The instillation was repeated once a week for another 5 weeks, followed by once a week for 2 weeks at months 3, 6, 12, 18, 24, 30 and 36, for a total of 20 instillations in 3 years.
Results: From 28 hospitals, 202 patients were registered. A total of 186 patients matched the inclusion criteria: 139 patients in the Ta/T1group and 47 patients in the carcinoma *in situ* group. At the 4-year median point of follow-up, recurrence-free survival rates in the Ta/T1 group and the carcinoma *in situ* group were 76.7 and 77.7%, respectively. Completion rates for maintenance therapy in both groups at months 3, 6, 12, 24 and 36 were 81.7, 68.9, 58.1, 42.5 and 35.0%, respectively. Common toxicities were pain on urination, urinary frequency and gross hematuria. There was no treatment-related death.
Conclusions: This regimen may be feasible in patients with Ta/T1 tumor or carcinoma *in situ*; however, future Phase III randomized study is needed to determine whether this regimen would be truly safe and effective compared with 3-week maintenance regimen.

Key words: bladder cancer – bacillus Calmette–Guérin – recurrence – urothelial carcinoma – transurethral resection

INTRODUCTION

Intravesical bacillus Calmette–Guérin (BCG) instillation is a standard treatment for intermediate- and high-risk Ta, T1 lesions and carcinoma *in situ* (CIS), as well as for the prophylaxis of recurrence after transurethral resection of the bladder tumor (TURBT) (1–3). Recent meta-analyses have shown that intravesical BCG reduces the risk of both recurrence and progression in patients with non-muscle-invasive cancer (NMIC) of the urinary bladder (4,5).

The Southwest Oncology Group (SWOG) 8507 study by Lamm et al. (6) showed that 3-year intravesical BCG maintenance therapy remarkably prevents recurrence and disease

progression in comparison with the conventional 6-week induction therapy. In addition, BCG maintenance therapy is recommended to prolong survival in patients with high-risk NMIC (1,2).

Intravesical BCG provokes more side effects than intravesical chemotherapy (4,7–10), and some urologists are reluctant to use it. However, in the SWOG randomized study, maintenance therapy had to be discontinued in many patients because of exacerbation of drug-related adverse events; only 16% of the patients were able to complete the full treatment, lasting 36 months (6). There are a number of reasons why patients may not complete the entire BCG maintenance regimen, including the 3-week schedule, which seems to be regarded as the standard for instillation. It is more likely that patients will develop severe sensitivity when instillations are given over a long period of time (6).

While maintenance instillations are necessary to reduce the risk of recurrence and progression, it is important to know whether or not adverse effects increase with time during maintenance. Efforts should be made to develop alternate BCG maintenance schedules. We carried out a prospective study to investigate the safety and efficacy of a BCG maintenance schedule consisting of 6 weeks of induction and 2-week cycles of maintenance instillations for patients with recurrent or multiple Ta or T1 tumors or CIS of the urinary bladder.

PATIENTS AND METHODS

This clinical trial is a prospective, multicenter, open-label study. The protocol was planned by Kanagawa Urological Research Group (KURG) and was approved by the institutional review board of each participating institution. Patients gave informed consent in accordance with the Declaration of Helsinki or existing national and local regulations.

This trial included patients who had been diagnosed with non-muscle-invasive urothelial carcinoma based on histological examination. Ta and T1 tumors were completely resected, and cancers were categorized as one of the following: (i) the presence of at least two bladder tumors, irrespective of primary or recurrent lesion; (ii) a single tumor recurring within 12 months of the previous TURBT for NMIC; or (iii) any Grade 3 tumor. In cases of CIS, all primary or recurrent biopsy-proven tumors were included, irrespective of concomitant Ta or T1 tumors, which were completely resected.

Other inclusion criteria were (i) World Health Organization (WHO) performance status 0 or 1; (ii) <80 years old; (iii) no history of intravesical BCG instillation; (iv) no history of intravesical instillation of any anti-cancer drug prior to BCG instillation; and (v) functioning main organs (i.e. heart, lung, liver, kidney and bone marrow).

Exclusion criteria were (i) Stage T2 or higher muscle-invasive bladder cancer; (ii) a concurrent tumor of the upper primary urinary tract or urethra; (iii) a history of bladder-sparing treatment for invasive bladder cancer; (iv) the presence of any lymph node metastases or distant metastases; (v) active double cancers; (vi) cured or active tuberculosis or strong tuberculin reaction; (vii) pregnancy or lactation; (viii) serious bacterial infection of the urinary tract; (ix) any other immunodeficiency disease or serious medical complication; and (x) patients whom the attending clinician considered to be inappropriate for inclusion in this study.

Patients received 81 mg intravesical BCG, Connaught strain (ImmuCyst; manufactured by Sanofi Pasteur Ltd, Toronto, Canada) in 40 ml saline. BCG solution was instilled into the bladder using a urethral catheter. Patients tried to retain the solution for 2 h and then voided. The instillation was started at least 7 days after TURBT and no later than 1 month. The instillation was repeated once a week for another 5 weeks as induction therapy, then once a week for 2 weeks at months 3, 6, 12, 18, 24, 30 and 36, for a total of 20 instillations in 3 years.

Efficacy of the treatment was assessed on the basis of cystoscopy and urinary cytology findings. Cystoscopy and urinary cytology were performed every 3 months during the first 2 years and every 6 months thereafter. Patients stopped protocol treatment upon recurrence after TURBT, progression to muscle-invasive disease, appearance of CIS, positive urinary cytology results, development of carcinoma in the upper urinary tract or prostatic urethra, or distant metastasis. Further treatment was then administered at the discretion of the local investigator.

Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Pain on urination, urinary frequency, hematuria etc., related to local bladder reactions, were categorized as local adverse events. Fever, general fatigue, nausea, vomiting etc., including Reiter syndrome, were categorized as systemic adverse events. The completion rate was defined as the number of patients with completed instillations in each period (i.e. months 3, 6, 12, 24 and 36) divided by the total number of eligible patients in each group.

The primary endpoint was time to first recurrence. Secondary endpoints were progression rate, overall survival and patient adherence. Variables analyzed were recurrence rate, number of recurrences, progression rate, cancer-specific survival rate, overall survival rate, disease-free interval, time to progression, cancer-specific survival time, overall survival time and adverse events. Recurrence was defined as biopsy confirmed NMIC or malignancy detected by cytology. Progression was defined as the development of a muscle-invasive tumor or more advanced disease. Progression was also regarded as recurrence if it was the first event. We calculated the overall median follow-up time based on either time of last treatment for surviving patients or time of death.

The starting point was the date of induction therapy. Overall survival and recurrence- free survival were determined by the Kaplan–Meier analysis. Patients without an event were censored at the date of the last follow-up unless

they died from other causes. All analyses were performed with StatView, version 5.0 (SAS Institute, Cary, NC, USA). We considered a P value of <0.05 to be statistically significant.

RESULTS

From December 2003 to March 2007, 202 patients from 28 hospitals were registered (see Appendix). Of these 202 patients, 152 were in the Ta/T1 group. Fifty were in the CIS group. The median follow-up period was 46.6 months (mean 43.5, range: 2–78) for the Ta/T1 group and 50.6 months (mean 48.4, range: 11–75) for the CIS group. Sixteen patients were excluded from the study because of the presence of upper urinary tract urothelial carcinoma and a single primary tumor. A total of 186 (92.1%) patients matched the inclusion criteria: 139 (91.5%) patients in the Ta/T1group and 47 (94.0%) patients in the CIS group (Table 1).

Table 1. Patient characteristics

Characteristic	Ta/T1 group, *n* (%)	CIS group, *n* (%)
Patients	139 (100)	47 (100)
Sex		
Male	123 (88.5)	37 (78.7)
Female	16 (11.5)	10 (21.3)
Age (years)		
<65	54 (38.8)	18 (38.3)
≥65	85 (61.2)	29 (61.7)
Stage		
Ta	77 (55.4)	—
T1	62 (44.6)	—
Grade		
1	23 (16.5)	3 (6.4)
2	76 (54.7)	21 (44.7)
3	37 (26.6)	18 (38.3)
Unknown	3 (2.2)	5 (10.6)
History		
Primary	93 (66.9)	37 (78.7)
Recurrence	44 (31.7)	10 (21.3)
Unknown	2 (1.4)	
Multiplicity		
Solitary	21 (15.1)	—
Multiple	118 (84.9)	—
Concomitant Ta/T1		
Yes	—	21 (44.7)
No	—	26 (55.3)

n, number of patients; CIS, carcinoma *in situ*.

The recurrence rates and the number of recurrences in the Ta/T1 group are shown in Figure 1A. Univariate analysis of data from the Ta/T1 group revealed no statistically significant differences in disease-free interval for Grade 1–2 versus Grade 3 tumors, different numbers of tumors or primary versus recurrent lesions. The recurrence rate and the number of recurrences in the CIS group are shown in Fig. 1B. Univariate analysis of data from the CIS group revealed no statistically significant differences in disease-free interval for primary versus recurrent lesions, with or without concomitant Ta or T1 lesions.

The overall survival rates and the number of survivors in both groups are reported in Fig. 2. There were six cases of progression in the Ta/T1 group and two in the CIS group.

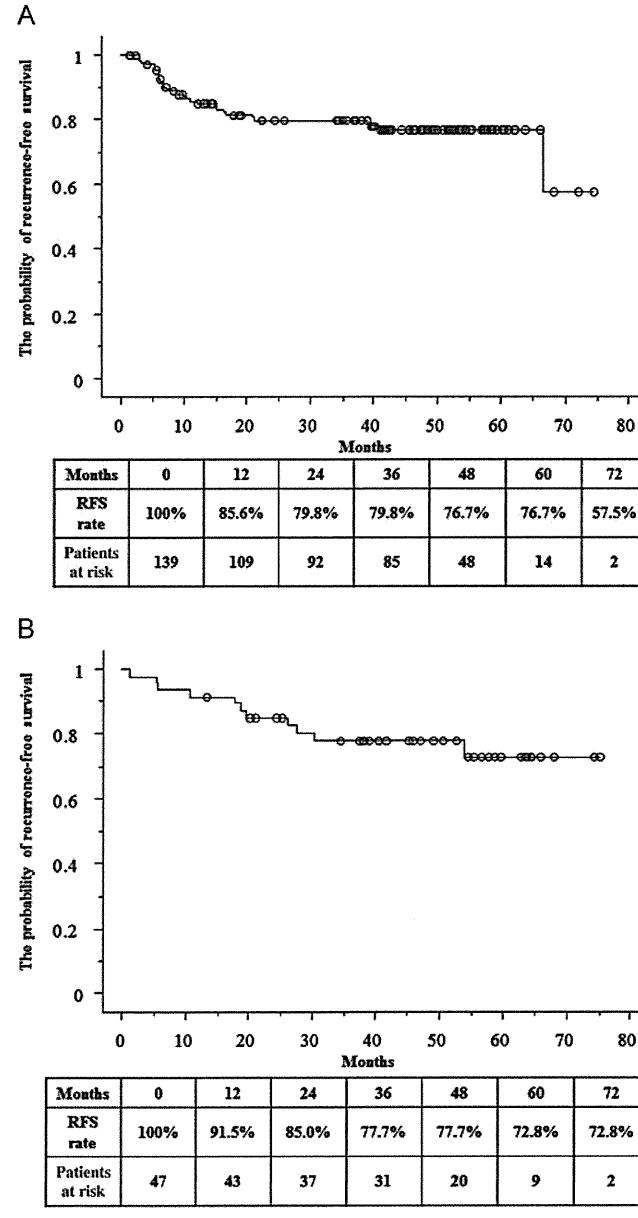
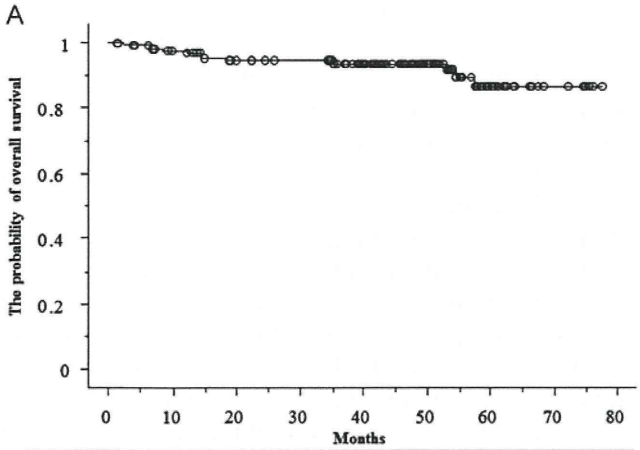
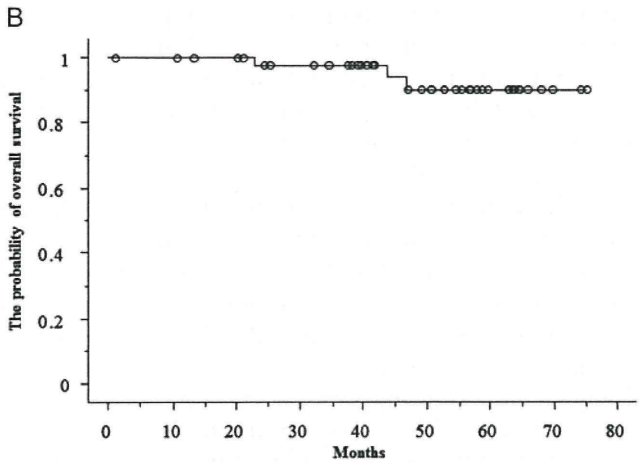


Figure 1. Recurrence-free survival for the Ta/T1 group (A) and for the carcinoma *in situ* (CIS) group (B). RFS, recurrence-free survival.



Months	0	12	24	36	48	60	72
OS rate	100%	97.7%	94.6%	93.7%	93.7%	86.6%	86.6%
Patients at risk	139	126	112	102	64	20	6



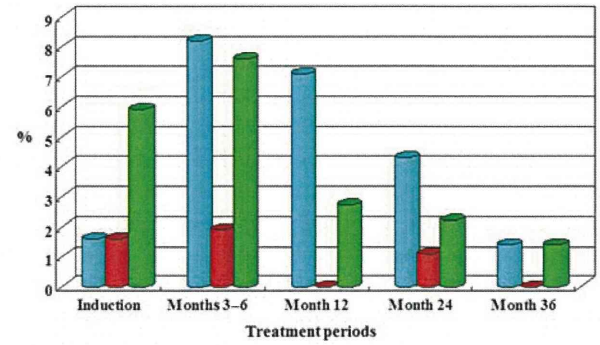
Months	0	12	24	36	48	60	72
OS rate	100%	100%	97.6%	97.6%	90.4%	90.4%	90.4%
Patients at risk	47	45	41	37	24	12	2

Figure 2. Survival for the Ta/T1 group (A) and for the CIS group (B). OS, overall survival.

Table 2. Treatment-related adverse events

Adverse event ($n = 186$)	Patients (n)	Incidence (%)	
		Grade 2	Grade 3
Pain on urination	64	28.0	6.5
Urinary frequency	63	28.5	5.4
Gross hematuria	40	20.4	1.1
Difficulty urinating	10	4.8	0.5
Malaise	6	2.7	0.5
Fever	5	2.7	—
Anorexia	4	1.6	0.5
Arthralgia	2	0.5	0.5
Ophthalmia	2	0.5	0.5
Urgency	1	0.5	—
Mouth ulcer	1	0.5	—
Nausea	1	0.5	—
Residual urine	1	0.5	—

n, number of patients.



Patients at risk (%)	186	158	112	93	74
Local AE	3 (1.6)	13 (8.2)	8 (7.1)	4 (4.3)	1 (1.4)
Systemic AE	3 (1.6)	3 (1.9)	0 (0)	1 (1.1)	0 (0)
Recurrence	11 (5.9)	12 (7.6)	3 (2.7)	2 (2.2)	1 (1.4)

Figure 3. Bacillus Calmette–Guérin therapy was discontinued in varying treatment periods, upon local (blue) or systemic (red) adverse events or recurrence (green). AE, adverse events.

Three patients in the Ta/T1 group and one in the CIS group died from bladder cancer. Eight more in the Ta/T1 group died: two from lung cancers, two from acute pneumonias, one from rhabdomyosarcoma, one from acute myocardial infarction, one from cerebral bleeding and one of suicide. One patient in the CIS group died of gastric cancer.

Common adverse events were pain on urination, urinary frequency and gross hematuria (Table 2). Grade 3 pain on urination occurred in 12 patients (6.5%). Grade 3 urinary frequency occurred in 10 patients (5.4%). One patient had a contracted bladder. There was no treatment-related death.

To identify toxicity (local or systemic) and recurrence during the maintenance period, we divided the total treatment period into five intervals and identified the number of patients who discontinued treatment during each interval (Fig. 3). Local adverse events were most frequent during months 3–6 but gradually decreased in frequency after that. The percentage of patients with systemic adverse events ranged from 0.0 to 1.9 and did not differ much during the five treatment periods. Recurrence was the most common reason for discontinuing BCG treatment after the induction period and during months 3–6. After the first 6 months of BCG therapy, the frequency of recurrence declined.

The number of patients in both groups continuing therapy was 152 (81.7%) at month 3, 128 (68.9%) at month 6, 108 (58.1%) at month 12, 79 (42.5%) at month 24 and 65 (35.0%) at month 36. Completion rates for each group are reported in Table 3.

Table 3. Completion rates

	Ta/T1 group ($n = 139$)		CIS group ($n = 47$)	
Month	n	(%)	n	(%)
3	112	80.6	40	85.1
6	92	66.2	36	76.6
12	80	57.6	28	59.6
24	59	42.4	20	42.6
36	48	34.5	17	36.2

n, number of patients.

DISCUSSION

Intravesical BCG has been used for more than 25 years. The present study demonstrates that a BCG intravesical instillation schedule consisting of 6 weeks of induction and 2 weeks of maintenance is a safe, adherent and effective modality for patients with biologically aggressive NMIC. Various trials have suggested that maintenance therapy can improve the outcome of BCG treatment, but the optimal maintenance scheme is yet to be defined (5). According to the results of the SWOG randomized clinical trial (6), a 3-week maintenance schedule seems to be regarded as the standard. Andius and Holmang (3) showed that multiple instillation cycles improve recurrence-free survival, compared with a single 6-week induction, in 236 Ta/T1 bladder cancer patients. However, they suggested that maintenance therapy may not be necessary for pTa and lower-grade tumors. van der Meijden et al. (11) concluded that maintenance therapy should mainly be applied to intermediate- and high-risk tumors. Hinotsu et al. (12) demonstrated that BCG maintenance therapy consisting of one instillation per week for 6 weeks followed by three once-weekly instillations at 3, 6, 12 and 18 months significantly prolongs time to recurrence compared with BCG induction therapy alone or epirubicin intravesical therapy. Decobert et al. (13) retrospectively studied the relationship between recurrence and the number of cycles of 3-week maintenance instillations and suggested that a minimum of three cycles of BCG (nine instillations) is required to significantly improve the recurrence-free survival rate. Han and Pan (14), in a meta-analysis of 25 trials (4767 patients), concluded that intravesical BCG with maintenance treatment should be offered as the treatment of choice to patients with papillary carcinomas. On the basis of other reports, the guidelines for the treatment of intermediate- or high-risk NMIC recommended that BCG maintenance therapy be continued for at least 1 year (15).

On the other hand, Herr (16) recently argued that there is insufficient evidence indicating that BCG maintenance therapy is effective in suppressing disease progression. However, most progression events occur repeatedly before they are detected, particularly in NMIC; therefore, preventing recurrence over a long period of time by conducting maintenance treatment may also reduce the risk of cancer progression. BCG maintenance therapy using a 2-week maintenance modality results in good cancer control, including recurrence and progression, irrespective of biologically aggressive Ta/T1 tumors or CIS.

Saint et al. (17) similarly demonstrated that the maintenance therapy reported on by Lamm et al. (6) for NMIC yielded a significant reduction in recurrence, but adherence was hindered by adverse reactions: 81% of the patients discontinued the treatment. In Koga et al. (18), the number of maintenance instillations was set at four. Maintenance consisting of just four instillations decreased the recurrence rate, compared with induction alone, although this study failed to show significant superiority of the four-instillation maintenance schedule without serious adverse events. It is difficult for clinicians to determine what number of maintenance instillations will lead to better outcomes, without severe adverse events. Because the present KURG study reduced the number of maintenance instillations from three to two, patients tolerated BCG treatment well and the completion rates were high during 3 years and kept the cancer control rates comparable to other reports (6,12,17).

Although severe adverse events may not be predictable, many believe that mild adverse events during BCG therapy are favorable, suggesting an immune reaction which should result in a better clinical response. For a number of years, there has been an assumption that adverse events increase further into the maintenance schedule. The completion rate of maintenance therapy in the SWOG trial was 16%, whereas another study reported that only one of 111 patients completed seven cycles of maintenance therapy (13). However, Sylvester et al. (19) reported that local side effects of BCG do not increase during maintenance therapy and that systemic side effects are more frequent during the first 6 months of treatment, after which time they decrease. In a European Organisation for Research and Treatment of Cancer (EORTC) prospective study, the majority of discontinuations resulting from adverse events occurred during induction therapy and the first 6 months of maintenance therapy, suggesting that BCG maintenance does not necessarily increase adverse events (11). Consistent with other reports, the present KURG study demonstrated the same reasons for discontinuation during induction and maintenance. In addition, recurrence was also the reason of discontinuation of maintenance instillation particularly after the induction period and during months 3–6. In addition, 60% of the patients who stopped BCG because of toxicity did so within the first 6 months of treatment. Most patients who adhere to BCG treatment and do not experience recurrence beyond months 3–6 can possibly continue maintenance therapy safely.

A possible explanation for the efficacy of a 2-week maintenance schedule is that the secondary immune response occurs more rapidly and is more vigorous than the primary

immune response. Immune stimulation, as measured by urinary cytokine excretion, in patients receiving the induction of intravesical BCG generally peaks at 6 weeks (20,21). Immune stimulation and protection from recurrence following BCG instillation appears to be long-term, but lymphocytic infiltration and immunoproliferative responses persist for as little as 6 months (22). Saint et al. (23) found that a high urinary leukocyte count correlates with both a reduction in recurrence and a greater severity and duration of adverse events. In addition, they showed that the level of urinary leukocytes is higher at the time of second instillation compared with other instillations during the maintenance period. Based on these results, we used a 2-week maintenance schedule and demonstrated that our protocol was not less effective than the 3-week maintenance schedule in terms of cancer control. Although we did not show any immunological data in this study, further studies were warranted to reveal the association of immunological changes and clinical outcomes during or after 2-week maintenance therapy.

A limitation of this clinical trial is that there was only one arm, which included BCG induction and maintenance therapy consisting of 2-week instillation cycles. This regimen may be feasible in patients with Ta/T1 tumor or CIS; however, future Phase III randomized study is needed to determine whether this regimen would be truly safe and effective compared with 3-week maintenance regimen. Another limitation is that we evaluated only clinical outcome. It would be useful to evaluate treatment from the patient's point of view by assessing the quality of life. However, the reduction in maintenance from 3 to 2 weeks may improve patient adherence. Another treatment option is possibly utilized low-dose of BCG in the maintenance period. Low-dose of BCG has been shown the clinical effectiveness in the induction period (24,25). Further prospective study is warranted to investigate the appropriate maintenance period, number of intravesical instillations and BCG dosage.

In conclusion, this prospective, multicenter clinical trial showed that a 2-week maintenance regimen of BCG intravesical instillation is safe, adherent and effective in preventing cancer recurrence in patients with primary or recurrent multiple tumors, single tumors recurring within 12 months of follow-up or any Grade 3 Stage Ta or T1 tumors; and primary or recurrent CIS. In addition, this maintenance regimen resulted in better adherence and completion rates than the SWOG 8507 regimen. The results of this clinical trial provide a valuable modality for preventing recurrence and encouraging adherence in patients with non-muscle-invasive bladder cancer.

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Conflict of interest statement

None declared.

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Appendix

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UROLOGIC ONCOLOGY

Original article

Expression profile of E-cadherin and N-cadherin in non-muscle-invasive bladder cancer as a novel predictor of intravesical recurrence following transurethral resection

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Abstract

The objective of this study was to investigate the impact of the expression profile of E-cadherin and N-cadherin in newly diagnosed non-muscle-invasive bladder cancer (NMIBC) on the probability of intravesical recurrence in patients undergoing transurethral resection (TUR). This study included 115 consecutive patients diagnosed as having NMIBC following TUR. Expression levels of E-cadherin and N-cadherin in TUR specimens from these patients were measured by immunohistochemical staining. In this series, intravesical recurrence occurred in 35 of 115 patients (30.4%). Immunohistochemical study showed that positive expression of E-cadherin and N-cadherin were noted in 62 (53.9%) and 48 (41.7%) specimens, respectively. Intravesical recurrence was detected in only 7 of 62 patients (11.3%) with positive E-cadherin expression, while 33 of 48 patients (68.8%) with positive N-cadherin expression developed intravesical recurrence. When patients were divided into 4 groups according to the positivities of E-cadherin and N-cadherin expression, intravesical recurrence was detected in 27 of 30 patients (90.0%) with negative E-cadherin as well as positive N-cadherin expression, and the intravesical recurrence-free survival of this group was significantly poorer than those of the remaining 3 groups. Furthermore, negative E-cadherin as well as positive N-cadherin expression was identified as the most powerful independent predictor for intravesical recurrence following TUR on multivariate analysis. These findings suggest that the loss of E-cadherin and gain of N-cadherin expression in on NMIBC appeared to be significantly associated with postoperative recurrence; therefore, the switch from E-cadherin to N-cadherin expression might be involved in the mechanism underlying intravesical recurrence of on NMIBC.

Keywords: Non-muscle-invasive bladder cancer; Intravesical recurrence; E-cadherin; N-cadherin

1. Introduction

Approximately 80% of newly diagnosed bladder cancers are classified into non-muscle-invasive tumors that are limited to the urothelium or infiltrate no deeper than the lamina propria. The current standard treatment of patients with non-muscle-invasive bladder cancer (NMIBC) is complete removal of the visible tumor burden by transurethral resection (TUR), and the prognosis of such patients is generally favorable, achieving 5-year survival rates greater than 80% [1]. However, the high probability of intravesical recurrence in patients undergoing TUR has been reported to range between 30% and 80% in patients with NMIBC [1,2]. Intensive efforts, therefore, have been made to identify factors precisely predicting the clinical course of NMIBC following TUR, which are of great utility in planning both postoperative adjuvant therapy and follow-up schedule in an individual patient.

Cadherins are the transmembrane glycoproteins that mediate cell–cell adhesion through the extracellular domains and connect to the actin cytoskeleton by cooperating with catenins through the cytosolic domains [3]. In normal tissues, epithelial and mesenchymal cells mainly express E-cadherin and N-cadherin, respectively; however, down-regulation of E-cadherin and/or up-regulation of N-cadherin, which are observed in various kinds of malignant tumors, have been shown to be associated with the acquisition of

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aggressive phenotypes [4–11]. Furthermore, several recent studies have demonstrated the important role of a switch from E-cadherin to N-cadherin in cancer cells, which is regarded as one aspect of the epithelial-to-mesenchymal transition (EMT), in the malignant progression through their enhanced motility and invasive potential [12,13].

In bladder cancer, the significance of cadherins in invasive disease has been well characterized [6,7,10,11]; however, it remains largely unknown whether the expression profile of cadherins, particularly N-cadherin, is correlated to intravesical recurrence of NMIBC. In the present study, therefore, we evaluated the expression levels of E-cadherin and N-cadherin in newly diagnosed NMIBC specimens from 115 patients undergoing TUR, and then investigated the impact of these findings on the probability of postoperative intravesical recurrence.

2. Patients and methods

Of consecutive patients who were treated with TUR of newly diagnosed primary bladder cancer between April 2000 and December 2007, this study included a total of 115 patients fulfilling the following criteria: (1) superficial pure urothelial carcinoma of the bladder (i.e., pTa or pT1) was pathologically confirmed; (2) concomitant carcinoma in situ (CIS) was not detected; (3) intravesical instillation therapy was not performed postoperatively. In this series, complete resection of all visible tumors was carried out, and several deep muscular samples were taken using the resection instrument. Irrespective of the findings of preoperative urinary cytology, random bladder biopsy specimens, targeting the trigone, posterior wall, bilateral lateral walls, dome, anterior wall, prostatic urethra in men, and/or suspicious regions, were obtained before TUR in all patients. Histopathologic examinations were performed by a single pathologist according to the 2002 American Joint Committee on Cancer TNM classification system, and tumor grade was assigned based on the 1973 World Health Organization (WHO) grading system.

Follow-up of patients after TUR was conducted as follows: cystoscopy and urinary cytologic examination were performed every 3 months for 3 years after TUR, then every 6 months from 3 to 5 years, and then annually after 5 years, and intravenous pyelography was performed every 6 months until 3 years after TUR and then annually until 5 years after TUR. On detection of tumors or hyperemic mucosa by cystoscopy and/or positive findings on urinary cytology, transurethral biopsy of the abnormal region and/or TUR of the tumor were performed. Informed consent for performing this study was obtained from each of these patients, and the study design was approved by the Research Ethics Committee of our institution.

Immunohistochemical staining of TUR specimens was performed as previously described [14]. Briefly, sections from formaldehyde-fixed, paraffin-embedded tissue from 115 specimens were deparaffinized by xylene and rehydrated in decreasing concentrations of ethanol. After blocking endogenous peroxidase with 3% hydrogen peroxidase in methanol, sections were boiled in 0.01 M citrate buffer for 10 min and incubated with 5% normal blocking serum in Tris-buffered saline for 20 min. The sections were then incubated with anti-human E-cadherin mouse monoclonal antibody and N-cadherin mouse monoclonal antibody (DAKO, Carpinteria, CA) for 60 minutes, followed by incubation with biotinylated rabbit anti-mouse IgG (Vector Laboratories, Burlingame, CA) for 30 minutes. After incubation in an avidin-biotin peroxidase complex for 30 minutes, the sections were exposed to diaminobenzidine tetrahydrochloride solution and counterstained with methyl green.

Staining results were interpreted by 2 independent observers who were blinded to the clinicopathologic data. Immunoreactivities of E-cadherin and N-cadherin in tissue sections were scored as previously reported [6,10]; that is, positive staining of E-cadherin was defined as the proportion of tumor cells with membranous staining $> 90\%$, while the definition of positive staining for N-cadherin was the presence of tumor cells showing immunoreactivity with membranous staining irrespective of the proportion of positively stained tumor cells due to the lack of expression of N-cadherin in normal bladder.

All statistical analyses were performed using Statview 5.0 software (Abacus Concepts, Berkeley, CA), and P values < 0.05 were considered significant. Fisher's exact test was used to analyze associations between intravesical recurrence and several parameters. The intravesical recurrence-free survival rates were calculated by the Kaplan-Meier method, and differences were determined by log-rank test. The prognostic significance of certain factors was assessed by the Cox proportional hazards regression model.

3. Results

The median age of 115 patients (male, 95; female 20) included in this study was 69 years (range, 43–84 years). Distribution of T stage and tumor grade in TUR specimens from these 115 patients were Ta in 59 (51.3%), T1 in 56 (48.7%), G1 in 36 (31.3%), G2 in 66 (57.4%), and G3 in 13 (11.3%). In addition, multiple tumors were detected in 59 patients (51.7%). During the observation period of this study (median, 34 months; range, 6–94 months), intravesical recurrence occurred in 35 patients (30.4%).

As shown in Table 1, positive staining of E-cadherin and N-cadherin were detected in 62 (53.9%) and 48 (41.7%) patients, respectively. Representative findings of immunohistochemical study for detecting E-cadherin and N-cadherin expression are presented in Fig. 1. The incidence of intravesical recurrence in patients with negative E-cadherin expression was significantly greater than that in those with positive expression, whereas patients with positive N-cad-

Table 1
Incidence of intravesical recurrence according to expression of E-cadherin and N-cadherin

	No. pts (%)	No. pts with intreavesical recurrence (%)	*P* value
E-cadherin expression			0.0004
Negative	53 (46.1)	28 (52.8)	
Positive	62 (53.9)	7 (11.3)	
N-cadherin expression			<0.0001
Negative	67 (58.3)	2 (3.0)	
Positive	48 (41.7)	33 (68.8)	

herin expression were significantly more likely to develop intravesical recurrence than those with negative expression (Table 1). Furthermore, there were significant differences in intravesical recurrence-free survival based on positivities of both E-cadherin and N-cadherin in TUR specimens (Fig. 2).

We then divided the 115 patients into 4 groups according to the expression pattern of E-cadherin and N-cadherin as follows: group A, E-cadherin positive and N-cadherin positive; group B, E-cadherin positive and N-cadherin negative; group C, E-cadherin negative and N-cadherin positive; and group D, E-cadherin negative and N-cadherin negative. Intravesical recurrence was most likely to occur in group C, and there were significant differences in the incidence of intravesical recurrence between group C and the remaining 3 groups (Table 2). In addition, intravesical recurrence-free survival in group C was significantly poorer than those in the remaining 3 groups (Fig. 3).

As shown in Table 3, univariate and multivariate analyses were performed to evaluate the significance of several parameters, including the expression patterns of E-cadherin and N-cadherin, as predictors of intravesical recurrence. Multiple tumors, negative E-cadherin expression, positive N-cadherin expression, and classification into group C were identified by univariate analysis as factors significantly associated with intravesical recurrence. Multivariate analysis of these 4 factors identified as significant on univariate analysis showed that all 4 factors were independently associated with intravesical recurrence; however, classification into group C, which was characterized by negative E-cadherin as well as positive N-cadherin expression, appeared to be the most powerful independent predictor of intravesical recurrence following TUR.

4. Discussion

Cadherins are a family of calcium-dependent, cell–cell adhesion molecules implicated in the progression of malignant tumors [3]. E-cadherin, the most intensively investi-

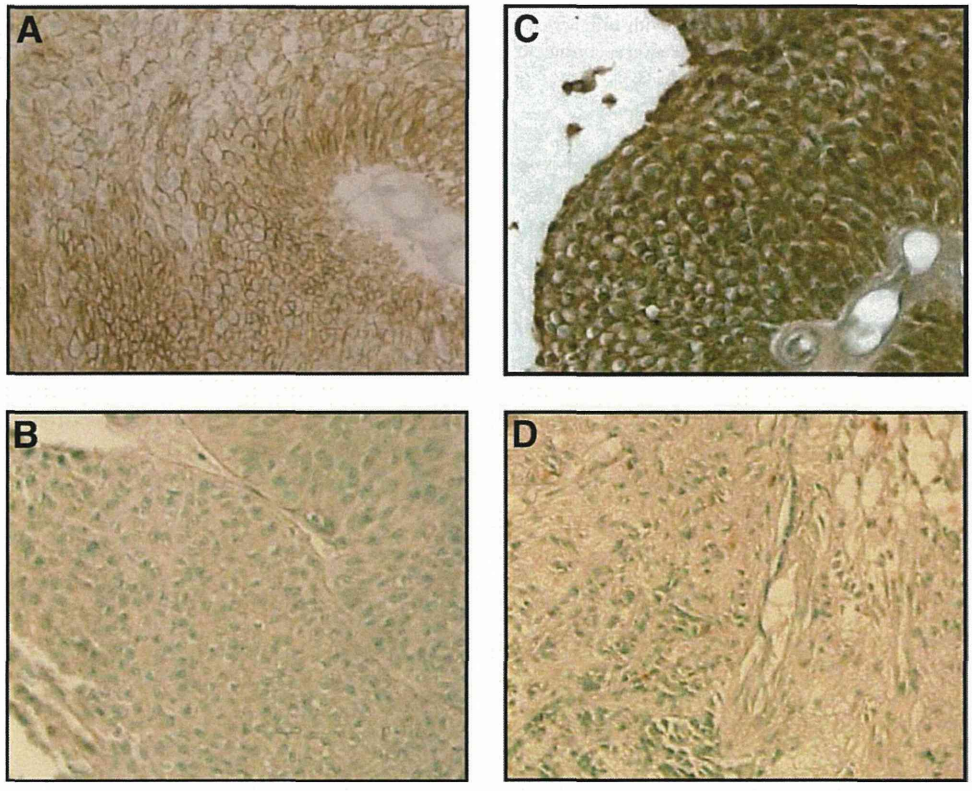

Fig. 1. Typical outcomes of immunohistochemical staining of primary superficial bladder cancer with E-cadherin or N-cadherin antibody. (A) Positive expression of E-cadherin. (B) Negative expression of E-cadherin. (C) Positive expression of N-cadherin. (D) Negative expression of N-cadherin. (Color version of figure is available online.)

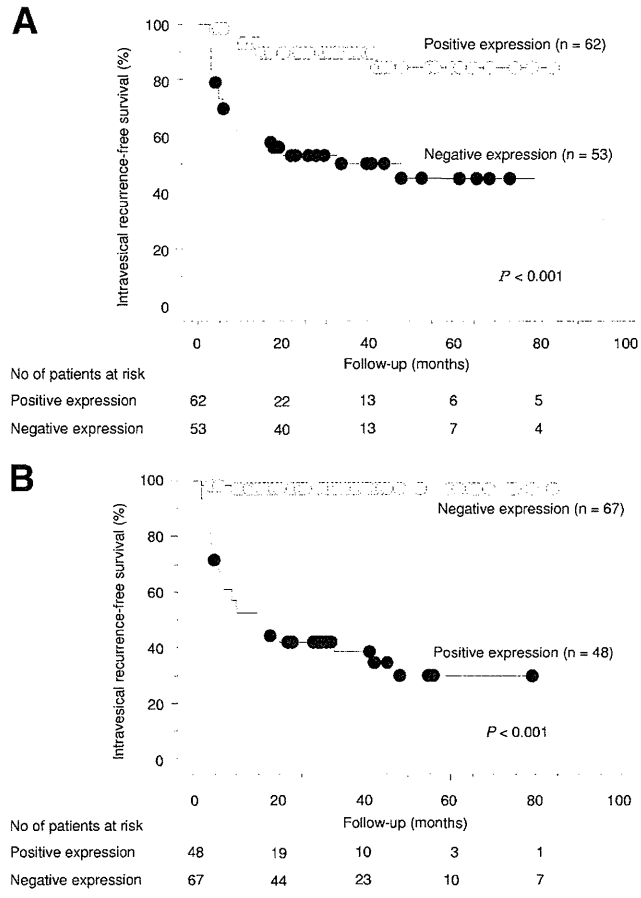

Fig. 2. Intravesical recurrence-free survival of patients with primary superficial bladder cancer who underwent transurethral resection according to the expression profiles of E-cadherin (A) and N-cadherin (B).

gated member of the cadherin family, plays a potential role in the suppression of tumor cell invasion, and loss or decreased expression of E-cadherin was observed in various kinds of cancer specimens [4,5]. In recent years, the importance of EMT, a process in which epithelial cells lose their characteristic polarity, disassemble cell–cell junctions, and become more migratory, has been recognized in several pathophysiologic processes [12]. During EMT, cadherin switching, characterized by the down-regulation of E-cadherin as well as up-regulation of N-cadherin, has been shown, resulting in the extensive reorganization of cell–cell junctions; therefore, the significance of N-cadherin has also been intensively studied, and it has been well documented that aberrant expression of N-cadherin in cancer cells helps to promote their aggressiveness through enhanced invasive potential [8–11]. In bladder cancer, the involvement of E-cadherin and N-cadherin in the progression of invasive disease has been described [6–11]; however, it remains largely unknown whether the expression profile of E-cadherin and N-cadherin is associated with the probability of intravesical recurrence in NMIBC. Considering these findings, we assessed the impact of E-cadherin and N-cadherin expression in TUR specimens obtained from 115 patients with NMIBC on their postoperative intravesical recurrence.

In this series, 53.9% and 41.7% of NMIBC specimens were judged positive for E-cadherin and N-cadherin expression, respectively. Furthermore, the incidence of intravesical recurrence was significantly associated with both negative expression of E-cadherin and positive expression of N-cadherin, and there were significant differences in intravesical recurrence-free survival following TUR according to the positivities of these 2 adhesion molecules. To date, despite limited data concerning the prognostic significance of E-cadherin and N-cadherin in NMIBC, the present study clearly showed a significant association of these 2 molecules with intravesical recurrence of NMIBC.

To more precisely predict the probability of intravesical recurrence of NMIBC based on the expression of either E-cadherin or N-cadherin, it would be of interest to consider the expression patterns of these 2 adhesion molecules simultaneously. Accordingly, we divided the included patients into 4 groups as described above, and compared the incidence of intravesical recurrence according to this classification. As expected, the incidence of intravesical recurrence in group C, characterized by negative E-cadherin and positive N-cadherin expression, was extremely high, and this proportion was significantly greater than those in the other 3 groups. Intravesical recurrence-free survival in group C was also significantly unfavorable compared with those in the other 3 groups. These findings suggest that it would be particularly useful to perform a combined assessment of E-cadherin and N-cadherin expression in TUR specimens for predicting the prognosis of patients with NMIBC.

Table 2
Incidence of intravesical recurrence according to classification based on expression patterns of E-cadherin and N-cadherin

Classification*	No pts (%)	No pts with intreavesical recurrence (%)	*P* value			
			Group A vs. others	Group B vs. others	Group C vs. others	Group D vs. others
Group A	19 (16.5)	6 (31.6)	—	0.0022	<0.0001	0.036
Group B	44 (38.3)	1 (2.3)	<0.0022	—	<0.0001	0.99
Group C	30 (26.1)	27 (90.0)	<0.0001	<0.0001	—	<0.0001
Group D	22 (19.1)	1 (4.5)	0.0036	0.99	<0.0001	—

* Group A, E-cadherin positive and N-cadherin positive; Group B, E-cadherin positive and N-cadherin negative; Group C, E-cadherin negative and N-cadherin positive; Group D, E-cadherin negative and N-cadherin negative.

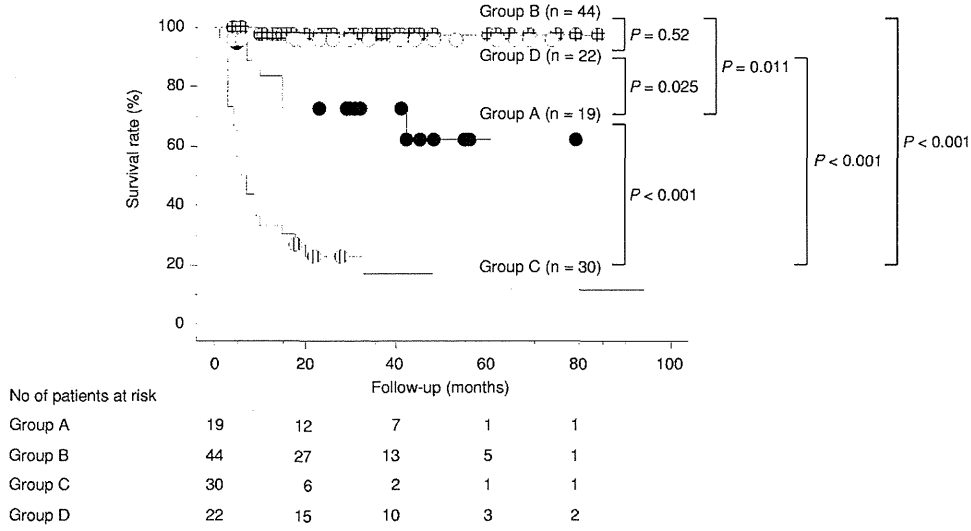

Fig. 3. Intravesical recurrence-free survival of patients with primary superficial bladder cancer who underwent transurethral resection according to the classification based on expression patterns of E-cadherin and N-cadherin. Group A, E-cadherin positive and N-cadherin positive; group B, E-cadherin positive and N-cadherin negative; group C, E-cadherin negative and N-cadherin positive; and group D, E-cadherin negative and N-cadherin negative.

It would be potentially useful to develop a prediction system for intravesical recurrence following TUR of NMIBC for determining postoperative follow-up as well as treatment schedules; hence, the impact of conventional prognostic parameters and the expression profile of E-cadherin and N-cadherin on intravesical recurrence-free survival following TUR were compared. Of several factors examined in this series, multiple tumor, negative E-cadherin expression, positive N-cadherin expression, and classification into group C appeared to be significantly associated with intravesical recurrence on univariate analysis. Furthermore, these 4 factors were also identified as independent predictors of intravesical recurrence following TUR on multivariate analysis. To date, a number of studies have reported several predictive factors for intravesical recurrence, of which tumor multiplicity is regarded as one of the most useful indicators of clinical outcome in patients with NMIBC undergoing TUR [15–17]. However, it may not be sufficient to analyze clinicopathologic parameters alone for stratifying patients with superficial bladder cancer into risk groups with respect to intravesical recurrence following TUR, considering the diverse genetic as well as biological features of NMIBC [18]. Collectively, these findings suggest that consideration of expression profile of both E-cadherin and N-cadherin in addition to conventional prognostic parameters, such as tumor multiplicity, may allow more accurate individualization of risk for postoperative intravesical recurrence in patients with NMIBC.

Here, we would like to emphasize the limitations of this study. A series of 115 patients with a disease as common as NMIBC treated with TUR is not a sufficient sample size. Furthermore, this study excluded patients with concomitant CIS and/or those receiving adjuvant intravesical instillation therapy, in order to avoid simultaneous assessment of pa-

Table 3
Univariate and multivariate analyses of association between various parameters and intravesical recurrence-free survival in 115 patients with non-muscle-invasive bladder cancer who underwent transurethral resection

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (years) (<70 vs. 70 or older)	1.02	0.54–2.11	0.57	—	—	—
Gender (male vs. female)	1.21	0.47–3.99	0.42	—	—	—
Multiplicity (single vs. multiple)	2.20	1.73–5.65	0.024	1.91	1.14–4.11	0.042
T stage (Ta versus T1)	1.42	0.31–2.54	0.070	—	—	—
Grade (G1 or 2 vs. G3)	1.33	0.37–4.74	0.65	—	—	—
E-cadherin (negative vs. positive)	4.97	1.12–10.82	0.036	4.15	1.22–10.43	0.020
N-cadherin (negative vs. positive)	5.35	1.86–12.90	0.022	5.88	2.63–18.96	0.018
Classification* (Group C vs. Group A, B or D)	8.81	1.34–19.68	0.010	9.61	1.31–21.33	0.0010

CI = confidence interval.

* Group A, E-cadherin positive and N-cadherin positive; Group B, E-cadherin positive and N-cadherin negative; Group C, E-cadherin negative and N-cadherin positive; and Group D, E-cadherin negative and N-cadherin negative.

tients with varied characteristics; therefore, it would be difficult to apply the findings obtained from this study to an entire cohort of NMIBC, particularly those showing high grade disease. In addition, the outcomes presented in this study strongly suggest the involvement of a switch from E-cadherin to N-cadherin in the molecular mechanism mediating intravesical recurrence; however, it would apparently be warranted to perform experimental studies using human NMIBC model systems to address the functional role of cadherin switching in intravesical recurrence following TUR.

In conclusion, we analyzed the expression profiles of E-cadherin and N-cadherin in NMIBC as predictors of intravesical recurrence following TUR, and demonstrated that patients with NMIBC characterized by negative E-cadherin as well as positive N-cadherin expression are significantly more likely to develop postoperative intravesical recurrence. These findings suggest that switching from E-cadherin to N-cadherin expression might be involved in the mechanism underlying intravesical recurrence of NMIBC.

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Watchful Waiting Versus Intravesical BCG Therapy for High-grade pT1 Bladder Cancer with pT0 Histology After Second Transurethral Resection: Japan Clinical Oncology Group Study JCOG1019

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A Phase III clinical trial has been started in Japan to determine the optimal treatment strategy for patients with high-grade pT1 bladder cancer who have pT0 histology after second transurethral resection. The aim of this trial is to demonstrate the non-inferiority of relapse-free survival (excluding Tis or Ta intravesical recurrence) for watchful waiting compared with intravesical bacillus Calmette–Guérin therapy for pT0 after second transurethral resection. Patients with high-grade pT1 bladder cancer at the first registration and pT0 after second transurethral resection at the second registration are randomized to either a watchful waiting arm or an intravesical bacillus Calmette–Guérin therapy arm. A total of 575 patients at the first registration and 260 patients at the second registration will be accrued for this study from 38 institutions over 5 years. The primary endpoint is relapse-free survival (excluding Tis or Ta intravesical recurrence), and the secondary endpoints are overall survival, metastasis-free survival with bladder preserved, annual proportion of intravesical relapse-free survival, annual proportion of T2 or deeper relapse-free survival, adverse events and serious adverse events.

Key words: bladder cancer – second transurethral resection – BCG – watchful waiting – Phase III clinical trial

INTRODUCTION

Bladder cancer is a common disease in urologic oncology. Non-muscle invasive bladder cancer (NMIBC) comprises about 70% of all bladder cancers. NMIBC consists of Ta, Tis and T1 bladder cancers. The main problems with treatment for NMIBC are recurrence and progression after transurethral resection of bladder tumor (TURBT). Above all, high-grade pT1 bladder cancer has a high risk for progression. Sylvester et al. (1) published risk tables for predicting recurrence and progression in Stage Ta and T1 bladder cancers and showed that T1 category and high-grade disease were the predominant risk factors for progression. In fact, some researchers have demonstrated that the 3-year relapse-free survival (RFS) rate of watchful waiting after initial

TURBT is approximately 40%, whereas that of intravesical bacillus Calmette–Guérin (BCG) therapy is approximately 70% for high-grade pT1 bladder cancer (2–5). The European Association of Urology (EAU) guidelines, therefore, advocate intravesical BCG therapy or total cystectomy as the standard treatments for bladder cancer in high-risk progression groups (6). Meanwhile, cystectomy is an invasive intervention and is generally considered to be a treatment option only for high-risk patients or poor BCG responders (6). Thus, intravesical BCG therapy is considered the first choice after TURBT for high-grade pT1 bladder cancer in clinical practice.

Jakse et al. (7) reported that residual tumors were observed in 27–62% of cases following second TUR after initial TURBT for high-grade Ta or T1 bladder cancer. It was recognized that one-time TURBT is insufficient for complete resection of bladder cancer and leads to an underdiagnosis of muscle invasive cancer. Based on this background, the practice of performing second TUR spreads widely. Actually, second TUR is the recommended therapy for high-grade Ta and T1 bladder cancers in the EAU guidelines (6). In addition, the National Comprehensive Cancer Network (NCCN) guidelines recommend repeat resection for any pT1 bladder cancers if the first TURBT does not allow adequate staging or if no muscle is observed in biopsy (8). Second TUR is currently recognized as the standard therapy for high-grade pT1 bladder cancer.

The diagnostic significance of second TUR is that it avoids the underdiagnosis of the first TURBT, but the treatment significance of second TUR is unknown. Before the concept of second TUR was proposed, the standard treatment for high-grade pT1 bladder cancer following TURBT was intravesical BCG therapy. A meta-analysis demonstrated the efficacy of intravesical BCG therapy in preventing recurrence and progression without second TUR (2). The recurrence rate of high-grade pT1 bladder cancer is 50–80% and the progression rate of high-grade pT1 bladder cancer is 30–60% when watchful waiting is selected after TURBT, but the recurrence rate of high-grade pT1 bladder cancer is 30–50% and the progression rate is 15–20% when intravesical BCG therapy is selected after TURBT (2–5,9–11). However, there is no evidence showing whether or not intravesical BCG therapy is necessary for patients with high-grade pT1 bladder cancer who have pT0 histology after second TUR. The current standard treatment for patients with high-grade pT1 bladder cancer who have pT0 histology after second TUR is intravesical BCG therapy. NCCN guidelines recommend intravesical BCG or mitomycin therapy when there is no residual tumor after second TUR. On the other hand, another opinion holds that pT0 status after second TUR carries minimal risk for recurrence or progression and that intravesical BCG therapy is overtreatment for these patients. It takes about 2 months to complete intravesical BCG therapy, and adverse events such as pollakisuria, macrohematuria and dysuria occur in almost all patients.

Based on this background, we began a multi-institutional Phase III trial (JCOG1019) to evaluate the non-inferiority in terms of RFS (excluding Tis or Ta intravesical recurrence) of a watchful waiting arm compared with an intravesical BCG therapy arm for patients with high-grade pT1 bladder cancer who have pT0 histology after second TUR.

The study protocol was designed by the Urologic Oncology Study Group (UOSG) of the Japan Clinical Oncology Group (JCOG), approved by the Protocol Review Committee of JCOG on September 2008 and activated on July 2011. This trial was registered at the UMIN Clinical Trials Registry as UMIN000006930.

PROTOCOL DIGEST OF THE JCOG 1019

Purpose

The aim of this study is to demonstrate the non-inferiority in terms of RFS (excluding Tis or Ta intravesical recurrence) of watchful waiting compared with intravesical BCG therapy for pT0 after second TUR after TURBT for high-grade pT1 bladder cancer.

Study Setting

This study is a multi-institutional open-label randomized Phase III trial.

Resources

This study is supported by a Health and Labour Sciences Research Grant for Clinical Cancer Research (H22-67) from the Ministry of Health, Labour and Welfare, Japan, and National Cancer Center Research and Development Funds (23-A-16 and 23-A-20).

Endpoints

The primary endpoint is RFS (excluding Tis or Ta intravesical recurrence), which is defined as days from randomization to first evidence of either intravesical recurrence of pT1 or deeper, distant metastasis, cystectomy or death from any cause, and censored at the latest day without events. Tis and Ta intravesical recurrence were excluded from the primary endpoint because Ta intravesical recurrence can be treated by TURBT and these recurrences are not critical. We considered adopting 'overall survival' (OS) or 'metastasis-free survival with bladder preserved' as the primary endpoint, but the prognosis of the study subjects is too good to evaluate by OS and the adaptation of cystectomy depends on a patient's preference or the general condition. Therefore, we selected 'RFS (excluding Tis or Ta intravesical recurrence)' as the primary endpoint because it is more objective and harder endpoint than 'metastasis-free survival with bladder preserved'.