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Efficacy Study of Mitomycin C Pre-infusion in Addition to Periodic Bacillus Calmette-Guerin Infusions in the Management of Non-muscle Invasive Bladder Cancers

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What's known on the subject? and What does the study add?

Adjuvant intravesical therapies for the treatment of non-muscle invasive bladder cancers have been consistently recommended, which include intravesical immunotherapy with "bacillus Calmette-Guerin (BCG)" or intravesical chemotherapy with "mitomycin C (MMC), epirubicin, or doxorubicin." BCG holds superiority over MMC, but the additional efficacy of the combination of pre-infusion of MMC in BCG protocol has been seldom studied. Thus, this study aimed to determine the differences between the two different protocols and show that combination therapy (MMC followed by BCG infusion) holds no advantage over BCG alone.

Abstract

Objective: To determine the benefits of the combination of bacillus Calmette-Guerin (BCG) and mitomycin C (MMC) in comparison to BCG alone in the treatment of patients with non-muscle invasive (NMI) bladder cancer.

Materials and Methods: The randomized comparative study was conducted on 54 patients with NMI bladder cancer. Following the transurethral resection, patients were randomly grouped into two: Group A (n=27) included patients who received postoperative MMC at 40 mg diluted in 50 mL of normal saline on postoperative day 1, followed by intravesical BCG at 60 mg per week for 6 weeks and BCG monthly for 1 year, and group B (n=27) included patients who received intravesical BCG at 60 mg postoperatively per week for 6 weeks followed by BCG monthly for 1 year. The outcome measures were time to recurrence, progression of the disease to muscles or other organs, overall survival, and treatment-related side effects.

Results: Compared to BCG alone, perioperative MMC in combination with BCG had comparable disease-free survival (85.18% vs. 66.66%, p=0.202), recurrence of disease (14.81% vs. 33.33%, p=0.202), and progression rate (11.1% vs. 25.9%, p=0.293). Side effects were minor and comparable between the study groups, which included dysuria, bacterial cystitis drug-induced cystitis, macroscopic hematuria prostatitis epididymitis fever, influenza-like symptoms, and fatigue.

Conclusion: Overall, both protocols were found comparable in safety and efficacy in reducing the progression and recurrence of NMI bladder cancers without any significant superiority of MMC in combination with BCG in comparison to BCG alone.

Keywords: BCG, mitomycin C, non-muscle invasive bladder cancer

Introduction

Bladder cancer is one of the rare cancers that ranks ninth in the global list of cancers. In the majority of patients (approximately 80%), the cancer is superficial at presentation. However, despite being superficial [Ta, T1, or carcinoma *in situ* (CIS)], the outcome remains varied (1).

The usual initial management of non-muscle invasive (NMI) bladder cancers includes cystoscopy and transurethral resection (TUR). Despite this, the spread of cancers to the muscle tissue and the recurrence rate are high, which carries a poor prognosis (1).

Given this, the use of adjuvant intravesical therapies has been practically recommended, which include intravesical

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immunotherapy with "bacillus Calmette-Guerin (BCG)" or intravesical chemotherapy with "mitomycin C (MMC), epirubicin, or doxorubicin" (2). They have been proposed to delay the tumor progression and recurrence with minimal side effects (3).

The use of BCG in bladder cancer began after it proved efficacious in melanoma. The effect is based on the mechanism that BCG binds and internalizes in the bladder tumor cells and induces cell death by (1) apoptosis-inducing pathways and (2) stimulation of local inflammation and macrophage-induced destruction. Since an adequate host immunity is required for the BCG immunotherapy to be effective, BCG has been much used in NMI bladder cancers only, that is, with low cancer load and good host immunity (4). The dosage regime of BCG has been decided as per its delayed immunity effects, which begin to show in 3 weeks, and its side effects profile, which subsides within 1 week. Therefore, a weekly regime of 6 weeks has been practically unanimously agreed (5,6). This regime showed to reduce tumor progression and recurrence for up to 10 years (7), after which increased recurrence rates have been reported in a follow-up study of 15 years (8).

MMC, hydrophobic in nature, is an antitumor antibiotic, among others, like epirubicin and doxorubicin, which by local instillation, works as an immediate chemotherapy measure to provide a longer recurrence-free period (9,10). It is minimally absorbed in a dose of 40 mg in saline or water and thus carries minimal side effects (11,12). Unlike BCG multi-dosage regime, MMC shows no superiority in efficacy by multi-dosage in comparison to single immediate instillation (12,13). Thus unanimously, a single dose of 40 mg within 1 h of TUR has been accepted (11).

Among the two, BCG has shown superior efficacy in reducing the recurrence (14-19). With the ongoing medical advancements, combinations of therapies have been tried against monotherapy. However, no consensus was found as to combination therapies are safe and efficacious compared to monotherapy. Some studies showed that BCG in combination with MMC is far better in achieving tumor-free interval and lowering disease progression (20-22), whereas some studies fail to see any significant difference with the combination therapy (23,24).

Therefore, the present study was conducted to determine the benefits of a combination of BCG and MMC compared to BCG alone in the treatment of patients with NMI bladder cancer.

Materials and Methods

The randomized comparative study was conducted in the department of urology of a tertiary care hospital. The study recruitment period was of 1 year with a follow-up of 2 years. The institutional ethical committee approved the study (IEC/SNMC/1210/2010). All patients aged 18 years or older with freshly diagnosed and histologically proven stage pT1 transitional cell carcinoma of the bladder, whether papillary or solid, were included in the study. Any patient with muscle invasion, previous or ongoing treatment with intravesical agents, bladder capacity <2 L, a urethral stricture that would prevent endoscopic procedures and repeated catheterization, diseases of the upper urinary tract, history of tuberculosis, tumor recurrence, cardiac disease, other malignancies, psychiatric or neurological disorder, contraindications to spinal or general anesthesia as required for a TUR, and known hypersensitivity to BCG or MMC were excluded from the study.

Informed written consent was obtained from all patients before enrolling them into the study. The sample size calculation of the study was based on the research of Hurle et al. (25), who observed that in the BCG group, estimated recurrence-free survival was 58.1 months, whereas 34.6 months for the MMC group. Taking these values as a reference and assuming a standard deviation of 30 months, the minimum required sample size with 80% power of study and 5% level of significance is 26 patients in each study group. The total sample size taken is 27 (54 patients per group) to reduce the margin of error.

The enrolled patients underwent an investigative protocol of urine exfoliative cytology, ultrasonography of the upper urinary tract, intravenous urogram, cystoscopy, and biopsy. TUR of bladder tumor (TURBT) for all visible tumors was performed on cystoscopy. The detailed methodology of TURBT has been shown in Supplementary File 1.

Following TURBT, patients were randomly grouped into two using a sealed envelope system. In this, ten sealed opaque envelopes were prepared, assigning A and B in 5 envelopes each, where A is represented by perioperative MMC and BCG and B by BCG alone. Once a patient consented to enter a trial, an envelope was opened, and the patient was then offered the allocated group. In this technique, patients were randomized in a series of blocks of 10. Once 25 patients were allocated to each group, then we used four sealed opaque envelopes, assigning A and B in 2 envelopes each.

Group A (n=27) included patients who received postoperative MMC at 40 mg diluted in 50 mL of NS within 6 h of operation, followed by intravesical BCG at 60 mg per week for 6 weeks followed by monthly BCG for 1 year. BCG therapy was begun 2-4 weeks after the tumor resection to allow time for re-epithelization.

Group B (n=27) included patients who received intravesical BCG at 60 mg postoperatively (after 2-4 weeks) per week for 6 weeks, followed by BCG monthly for 1 year.

The detailed steps of drug instillations are shown in Supplementary File 2, and the procedural flow is shown in Figure 1.

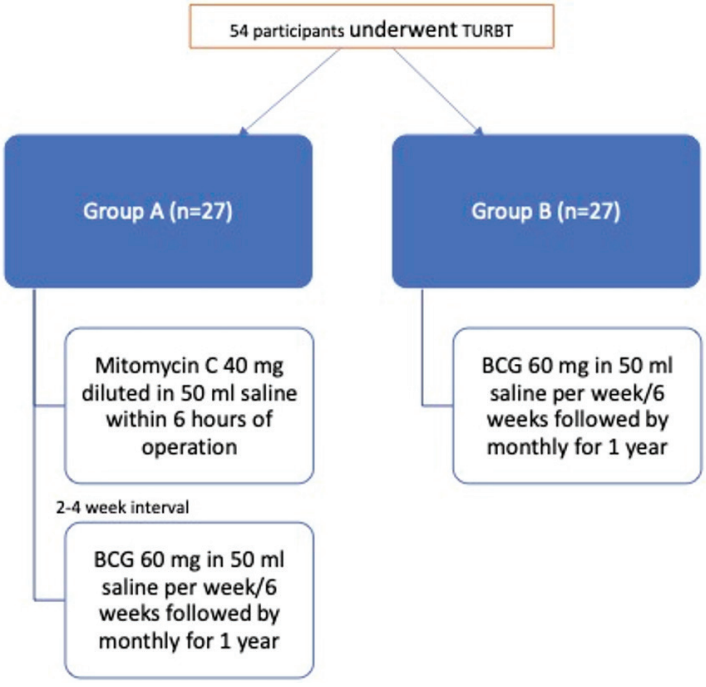

Figure 1. Fifty-four participants underwent TURBT

TURBT: Trans urethral resection of bladder tumour, BCG: Bacillus Calmette-Gu

Follow-up

All patients were properly followed for the next 2 years to fulfill the objectives of the study. Adverse events were recorded according to the World Health Organization toxicity grading (26) after each instillation and a week after the 6th dose. In case of persistent fever for >72 h and sterile urine culture, antitubercular treatment was started depending on the severity of the symptoms, and all records were maintained of the total dose and duration. Follow-up cystoscopy with three urine cytology was done every 3 months for 2 years. Histopathological types and grades were recorded for each recurrence.

The outcome measures were time to recurrence, progression of the disease to muscles or other organs, overall survival, and treatment-related side effects. Recurrence (or persistent disease) was defined as biopsy confirmed CIS or non-invasive papillary carcinoma, or malignant cytology and progression defined as pT1 tumor or more advanced disease. Patients were considered to have a complete response at 1 year if they had no progression during the first 9 months and no recurrent/persistent disease or progression at 12±3 months. The treatment failure was defined as progression or change in therapy resulting from recurrence or side effects during the first year or as recurrence, progression, or change in therapy after the first year.

Statistical Analysis

The categorical variables were presented in the form of numbers and percentages (%). Contrarily, the continuous variables were presented as mean ± standard deviation and median values. Mann-Whitney U test was used to compare quantitative variables, and the chi-square test and Fisher's Exact test were used to compare qualitative variables. The data entry was done in the Microsoft Excel spreadsheet, and the final analysis was done with the use of Statistical Package for Social Sciences software version 21.0. For statistical significance, a p-value of <0.05 was considered significant.

Results

The demographic distribution of the two groups was comparable (Table 1). The median age in the group of perioperative MMC with BCG was 65 years and in BCG alone was 67 years (p=0.361). The gender distribution showed slight male predominance in both groups (p=0.573). All patients were of NMI bladder cancer with T1 stage in both the groups (p=0.967). The majority of tumors were the papillary type of transitional cell carcinoma (88.89% in group A and 92.59% in group B) with others being the solid type of transitional cell carcinoma, with no significant difference (p>0.05). The tumors were majorly low grade (85.19% in group A and 88.89% in group B) with well-differentiated morphology. Among all the cases belonging to T1, 2 cases in group A and 3 cases in group B were CIS.

Compared to BCG alone, perioperative MMC in combination with BCG had comparable disease-free survival (85.18% vs. 66.66%, p=0.202), disease recurrence (14.81% vs. 33.33%, p=0.202), and progression rate (11.1% vs. 25.9%, p=0.293) (Table 2).

The side effects profile of the study participants was comparable in both the study groups (Table 3). The side effects included

Table 1. Demographic characteristics of study groups

Variables	Group A (n=27)	Group B (n=27)	p
Age	65 (58-72) years	67 (64-70) years	0.361
Gender			0.573
Male	16 (59.26%)	18 (66.67%)	
Female	11 (40.74%)	9 (33.33%)	
Histological variant of the tumor			
Papillary	24 (88.89%)	25 (92.59%)	1
Solid	3 (11.11%)	2 (7.41%)	
Carcinoma *in situ*	2 (7.41%)	3 (11.11%)	1
Grade of tumor			
Low grade	23 (85.19%)	24 (88.89%)	1
High grade	4 (14.81%)	3 (11.11%)	
Disease characteristics			0.967
pT1-G2 (multifocal)	15 (55.56%)	16 (59.26%)	
pT1-G3	12 (44.44%)	11 (40.74%)	

dysuria, bacterial cystitis, drug-induced cystitis, macroscopic hematuria, prostatitis, epididymitis, fever, influenza-like symptoms, and fatigue, due to which treatment had to be temporarily stopped in 5 patients in group A and 4 patients in group B. However, overall, they were minor side effects and were effectively managed without any mortality.

Table 2. Comparison of outcomes in the study groups

Variables	Group A (n=27)	Group B (n=27)	p
Disease-free survival	23 (85.18%)	18 (66.66%)	0.202
Recurrence of disease	4 (14.81%)	9 (33.33%)	0.202
Progression rate	3 (11.1%)	7 (25.9%)	0.293
Mortality	0 (0%)	0 (0%)	-

Table 3. Side effects of study groups

Event	Group A (n=27)	Group B (n=27)	p
Dysuria	17 (62.96%)	13 (48.15%)	0.902
Bacterial cystitis	7 (25.93%)	6 (22.22%)	0.869
Drug-induced cystitis	15 (55.56%)	14 (51.85%)	0.984
Macroscopic hematuria	20 (74.07%)	17 (62.96%)	0.743
Prostatitis	0 (0%)	1 (3.70%)	1
Epididymitis	1 (3.70%)	0 (0%)	1
Fever	9 (33.33%)	8 (29.635)	0.914
Influenza-like symptoms	13 (48.15%)	12 (44.44%)	0.984
Fatigue	13 (48.15%)	16 (59.26%)	0.951

Discussion

The present study was a randomized comparative trial on 54 patients (27 patients in each group), where we determined the benefits of a combination of BCG and MMC in comparison to BCG alone in the treatment of patients with NMI bladder cancer by comparing progression-free survival rates in patients.

The randomization ensured that age, gender, and cancer stage were comparable among the two groups and that any difference in outcome is purely due to differential intervention and not due to chance bias (27).

Despite the developments in diagnosis and treatment modalities, a high recurrence rate in bladder tumors is reported. Generally, the progression from non-muscle to muscle-invasive urinary bladder cancer results in metastasis and is considered a bad prognosis. Approximately 70-80% are NMI, which usually recur without aggressive histopathological features, and a subgroup of high-risk lesions that usually progress to invasive forms (MI). Recurrent relapses, disease progression, and chemoresistance lead to urinary bladder cancer that becomes difficult to manage from the diagnosis till death (28).

Our study revealed a comparable progression rate in perioperative MMC in combination with BCG compared to BCG alone (11.1% vs. 25.9%, p=0.293). Among the previous studies that compared the combination therapy with BCG alone, similar findings were reported by Solsona et al. (29), who found no statistically significant difference between MMC + BCG and BCG alone in terms of 5-year PFI (12.3% vs. 12.2%; hazard ratio: 1.05; 95% confidence interval, 0.61-1.83; p=0.852). Even Oosterlinck et al. (30) reported that alternating chemoimmunotherapy schedules with MMC and BCG demonstrated comparable efficacy compared to BCG alone in reducing the rate of progression. In contrast, Di Stasi et al. (23) reported that sequential BCG in combination with electromotive MMC showed lesser progression of the disease than BCG alone [9.3% (3.8-14.8) vs. 21.9% (17.9-25.9); p=0.004].

Disease-free survival is an important landmark in cancer treatment. Our study revealed a comparable disease-free survival in perioperative MMC in combination with BCG compared to BCG alone (85.18% vs. 66.66%, p=0.202), as well as in disease recurrence (14.81% vs. 33.33%, p=0.202). The findings were comparable to Oosterlinck et al. (30), who found that alternating chemoimmunotherapy schedules with MMC and BCG had similar efficacy compared to BCG alone in reducing the rate of recurrence. Contrary to the present study, Di Stasi et al. (23) found a combination of the sequential BCG with electromotive MMC to be superior to BCG alone, as sequential BCG and electromotive MMC had lower recurrence [41.9% (32.7-51.5) vs. 57.9% (48.7-67.5); p=0.0012]. Solsona et al. (29) also reported similar findings as a combination of sequential BCG with MMC significantly reduced the disease relapse at 5 years compared to BCG alone (20.6% vs. 33.9%, p<0.05).

The side effects of both protocols were minor without any mortality. They were mainly macroscopic hematuria, dysuria, and drug-induced cystitis in the BCG + MMC group and macroscopic hematuria, fatigue, and drug-induced cystitis in the BCG alone group (p>0.05). A similar comparable side-effect profile was observed in the study by Di Stasi et al. (23). Kaasinen et al. (31) found significantly more local side effects with BCG monotherapy, which also resulted in premature cessation of instillation treatment compared to the MMC + BCG. However, the difference in serious side effects in both groups was not significantly different. Contrarily, Solsona et al. (29) found that the MMC + BCG had significantly more local toxicity compared to BCG alone (80.4% vs. 69.7%, p<0.05). Even after reducing the dose of MMC to 10 mg, toxicity was still higher compared to BCG alone, specifically in local side effects grade 3 (28.4% vs. 10.9%; p<0.001).

Overall, we found that both protocols were comparable in safety and efficacy in reducing the progression and recurrence of NMI bladder cancers without any significant superiority of MMC in combination with BCG compared to BCG alone. Our

findings were in line with the studies by Solsona et al. (29), who found comparable PFI, and were in contrast to the studies by Oosterlinck et al. (30) and Di Stasi et al. (23), who showed that combination is a better protocol compared to BCG alone in terms of recurrence, progression, and disease-free survival. Among other studies, Kaasinen et al. (31) found that 1-year BCG monotherapy was more effective than the alternating therapy of BCG and MMC for reducing recurrence rates and disease-free survival and similar progression rate.

The difference in the results of various studies could be due to the difference in the schedule of instilling MMC with BCG. In studies by Oosterlinck et al. (30) and Kaasinen et al. (31), MMC plus BCG was administered on a weekly alternating schedule. In a study by Solsona et al. (29), MMC was administered sequentially, 1 d before the BCG instillation. In the study by D i Stasi et al. (23), patients who were assigned to BCG + MMC had electromotive MMC once a month. The comparable reduction in the progression and recurrence of NMI bladder cancers noted in the present study might be because MMC was given only once immediately after the TURBT, whose effect might be overpowered by the continuous instillation of BCG for 1 year given in both the groups.

Thus, combination therapy is proposed to depend specifically on the infusion timing. In contrast to the present study, where MMC was given initially and followed by BCG in due course, better outcomes have been seen with initial treatment with BCG followed by MMC. With the initial treatment with BCG, the immune response is initiated, and the tumor cells get exposed to infiltration of cytokines (some of which exert an antiproliferative action), cytotoxic T-lymphocytes, helper T-lymphocytes, and specifically, non-specific BCG-induced responses. These cascades play an important role for MMC introduction, which by enzymatic reduction, enhances the anticancer effects by crosslinking of DNA to all tissue layers of the bladder wall, which are affected by NMI bladder cancer (such as urothelium and lamina propria). In addition, MMC attacks cancer cells that are resistant to BCG (23).

Study Limitations

One of the limitations of the study was the insufficient long-term maintenance schedule, which is recommended as per the guidelines (32). Another limitation was that this study was conducted at a single center; thus, results cannot be generalized. Lastly, no comparison was made on BCG infusion followed by MMC on the disease-free interval in NMI bladder cancers.

Conclusion

Perioperative MMC in combination with BCG was comparable to BCG alone in the effectiveness in terms of disease-free survival, disease recurrence, and progression rate in patients with NMI bladder cancer. In addition, side effects were also similar in patients who received MMC with BCG and BCG alone, thereby suggesting that the combination therapy (MMC followed by BCG infusion) holds no advantage over BCG alone.

Ethics

Ethics Committee Approval: The institutional ethical committee approved the study (IEC/SNMC/1210/2010).

Informed Consent: Informed written consent was obtained from all patients before enrolling them into the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.K., S.J., D.A., Design: N.K., Data Collection or Processing: N.K., S.J., D.A., Analysis or Interpretation: N.K., S.J., D.A., Literature Search: S.J., D.A., Writing: N.K., S.J.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Lamm D, Herr H, Jakse G, Kuroda M, Mostofi FK, Okajima E, Sakamoto A, Sesterhenn I, da Silva FC. Updated concepts and treatment of carcinoma in situ. Urol Oncol 1998;4:130-138.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
3. Shelley MD, Jones G, Cleves A, Wilt TJ, Mason MD, Kynaston HG. Intravesical gemcitabine therapy for non-muscle invasive bladder cancer (NMIBC): a systematic review. BJU Int 2012;109:496-505.
4. Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J, Rouprêt M; European Association of Urology (EAU). EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update. Eur Urol 2011;59:997-1008.
5. Jiang SJ, Ye LY, Meng FH. Comparison of intravesical bacillus Calmette-Guerin and mitomycin C administration for non-muscle invasive bladder cancer: A meta-analysis and systematic review. Oncol Lett 2016;11:2751-2756.
6. Gandhi NM, Morales A, Lamm DL. Bacillus Calmette-Guérin immunotherapy for genitourinary cancer. BJU Int 2013;112:288-297.
7. Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 1976;116:180-183.
8. Lamm DL, Thor DE, Harris SC, Reyna JA, Stogdill VD, Radwin HM. Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. J Urol 1980;124:38-40.
9. Pinsky CM, Camacho FJ, Kerr D, Geller NL, Klein FA, Herr HA, Whitmore WF Jr, Oettgen HF. Intravesical administration of bacillus Calmette-Guérin in patients with recurrent superficial carcinoma of the urinary bladder: report of a prospective, randomized trial. Cancer Treat Rep 1985;69:47-53.
10. Herr HW. Extravesical tumor relapse in patients with superficial bladder tumors. J Clin Oncol 1998;16:1099-1102.

11. Bouffioux C, Kurth KH, Bono A, Oosterlinck W, Kruger CB, De Pauw M, Sylvester R. Intravesical adjuvant chemotherapy for superficial transitional cell bladder carcinoma: results of 2 European Organization for Research and Treatment of Cancer randomized trials with mitomycin C and doxorubicin comparing early versus delayed instillations and short-term versus long-term treatment. European Organization for Research and Treatment of Cancer Genitourinary Group. J Urol 1995;153:934-941.
12. Krege S, Giani G, Meyer R, Otto T, Rübben H. A randomized multicenter trial of adjuvant therapy in superficial bladder cancer: transurethral resection only versus transurethral resection plus mitomycin C versus transurethral resection plus bacillus Calmette-Guerin. Participating Clinics. J Urol 1996;156:962-966.
13. Tolley DA, Parmar MK, Grigor KM, Lallemand G, Benyon LL, Fellows J, Freedman LS, Grigor KM, Hall RR, Hargreave TB, Munson K, Newling DW, Richards B, Robinson MR, Rose MB, Smith PH, Williams JL, Whelan P. The effect of intravesical mitomycin C on recurrence of newly diagnosed superficial bladder cancer: a further report with 7 years of follow up. J Urol 1996;155:1233-1238.
14. Iqbal, Shergill, Alam, Trivedi,Gujral. Therapies in bladder cancer: intravesical mitomycin-C. Therapy 2007;4:115-117.
15. Shelley MD, Kynaston H, Court J, Wilt TJ, Coles B, Burgon K, Mason MD. A systematic review of intravesical bacillus Calmette-Guérin plus transurethral resection vs transurethral resection alone in Ta and T1 bladder cancer. BJU Int 2001;88:209-216.
16. Han RF, Pan JG. Can intravesical bacillus Calmette-Guérin reduce recurrence in patients with superficial bladder cancer? A meta-analysis of randomized trials. Urology 2006;67:1216-1223.
17. Böhle A, Jocham D, Bock PR. Intravesical bacillus Calmette-Guerin versus mitomycin C for superficial bladder cancer: a formal meta-analysis of comparative studies on recurrence and toxicity. J Urol 2003;169:90-95.
18. Malmström PU. Management of superficial bladder cancer: what is new? Curr Opin Urol 2000;10:447-451.
19. Shelley MD, Wilt TJ, Court J, Coles B, Kynaston H, Mason MD. Intravesical bacillus Calmette-Guérin is superior to mitomycin C in reducing tumour recurrence in high-risk superficial bladder cancer: a meta-analysis of randomized trials. BJU Int 2004;93:485-490.
20. Shelley MD, Mason MD, Kynaston H. Intravesical therapy for superficial bladder cancer: a systematic review of randomised trials and meta-analyses. Cancer Treat Rev 2010;36:195-205.
21. Rintala E, Jauhiainen K, Rajala P, Ruutu M, Kaasinen E, Alfthan O. Alternating mitomycin C and bacillus Calmette-Guerin instillation therapy for carcinoma in situ of the bladder. The Finnbladder Group. J Urol 1995;154:2050-2053.
22. Lamm DL, van der Meijden PM, Morales A, Brosman SA, Catalona WJ, Herr HW, Soloway MS, Steg A, Debruyne FM. Incidence and treatment of complications of bacillus Calmette-Guerin intravesical therapy in superficial bladder cancer. J Urol 1992;147:596-600.
23. Di Stasi SM, Giannantoni A, Giurioli A, Valenti M, Zampa G, Storti L, Attisani F, De Carolis A, Capelli G, Vespasiani G, Stephen RL. Sequential BCG and electromotive mitomycin versus BCG alone for high-risk superficial bladder cancer: a randomised controlled trial. Lancet Oncol 2006;7:43-51.
24. Serretta V, Pavone C, Ingargiola GB, Daricello G, Allegro R, Pavone-Macaluso M. TUR and adjuvant intravesical chemotherapy in T1G3 bladder tumors: recurrence, progression and survival in 137 selected patients followed up to 20 years. Eur Urol 2004;45:730-736.
25. Hurle R, Manzetti A, Losa A, Micheli E, Ranieri A, Chinaglia D, Lembo A. Intravesical instillation of mitomycin-C in 242 patients with superficial bladder cancer at high risk of recurrence: long-term results. Urol Int 1998;61:220-226.
26. Zengina K, Tanika S, Senerb NC, Nalbantc I, Ekicid M, Bozkurte IH, Yigitbasid O, Sertcelikd MN. The effect of postoperative intravesical BCG and mitomycin C therapy on recurrence of non-muscle invasive bladder cancer. World J Nephrol Urol 2013;2:65-69.
27. Roberts C, Torgerson DJ. Understanding controlled trials: baseline imbalance in randomised controlled trials. BMJ 1999;319:185.
28. Afonso JP, Freitas R, Morais FL, Oliveira J, Amaro T, Reis RM, Baltazar FM, Longatto-Filho A, Santos LL. Urothelial bladder cancer progression: lessons learned from the bench. J Cancer Metastasis Treat 2015;1:57-66.
29. Solsona E, Madero R, Chantada V, Fernandez JM, Zabala JA, Portillo JA, Alonso JM, Astobieta A, Unda M, Martinez-Piñeiro L, Rabadan M, Ojea A, Rodriguez-Molina J, Beardo P, Muntañola P, Gomez M, Montesinos M, Martinez Piñeiro JA; Members of Club Urológico Español de Tratamiento Oncológico. Sequential combination of mitomycin C plus bacillus Calmette-Guérin (BCG) is more effective but more toxic than BCG alone in patients with non-muscle-invasive bladder cancer in intermediate- and high-risk patients: final outcome of CUETO 93009, a randomized prospective trial. Eur Urol 2015;67:508-516.
30. Oosterlinck W, Kirkali Z, Sylvester R, da Silva FC, Busch C, Algaba F, Collette S, Bono A. Sequential intravesical chemoimmunotherapy with mitomycin C and bacillus Calmette-Guérin and with bacillus Calmette-Guérin alone in patients with carcinoma in situ of the urinary bladder: results of an EORTC genito-urinary group randomized phase 2 trial (30993). Eur Urol 2011;59:438-446.
31. Kaasinen E, Wijkström H, Malmström PU, Hellsten S, Duchek M, Mestad O, Rintala E; Nordic Urothelial Cancer Group. Alternating mitomycin C and BCG instillations versus BCG alone in treatment of carcinoma in situ of the urinary bladder: a nordic study. Eur Urol 2003;43:637-645.
32. Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Compérat E, Sylvester RJ, Kaasinen E, Böhle A, Palou Redorta J, Rouprêt M; European Association of Urology. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol 2013;64:639-653.

Supplementary File 1

Operative Steps of TURBT:

- A preliminary cystoscopy was performed to assess the shape and size of the tumor.
- An Otis urethrotomy was performed if the urethra was narrow to allow easy passage of a resectoscope.
- A preliminary assessment of whether the tumor could be easily resected completely or not was made. This was possible in those tumors that had not infilterated more deeply than superficial muscle.
- The aim was to resect the tumor level to the rest of the bladder wall.
- With small tumors, it was often best to begin the resection towards the base of tumor, particularly when a papillary growth had a relatively small stalk. On other occasions when the tumor had a wide base, it was necessary to resect from the top or the side of the tumor downwards, towards the base.
- A continuous flow Iglesias system was used, so that the position of the bladder tumor remains static.

- In tumors of the anterior wall, which tend to become inaccessible as the bladder filled, a suprapubic pressure on a half filled bladder was done to bring the tumor into view.
- Diathermy alone was sufficient for small areas of tumor, provided that adequate biopsies had been taken elsewhere.
- During the resection bleeding points were coagulated. Visualizing a small ring of white coagulation confirmed homeostasis and yielded less damage to the bladder than that occurring when the biopsy area was painted with cautery.
- It was important to include muscle in the resection biopsy specimens, so that any invasion could be identified histologically. The resulting bladder defect was inspected carefully for bleeding and for perforation.

Supplementary File 2

- Method of instillation of perioperative chemotherapy.
  1. Intent to administer perioperative chemotherapy (and agent) on actual operative schedule was included.
  2. It was insured that the pharmacy had the medication available. A written prescription was given to all patients.
  3. After resection, absence of clinical perforation was confirmed. Then 3 ways catheter was placed in bladder while patient was still in operating room. Inflow port was attached to saline infusion bag and inflow was clamped.
  4. Mitomycin C 40 mg diluted in 50 mL of NS was administered through catheter outflow port in recovery room within 6 hours of operation and outflow tubing was clamped with hemostat to allow retention.
  5. Outflow tubing was opened for irrigation 1 hour after administration, so that gravity drainage occurs in next 30 to 60 minutes.
  6. Foley catheter was removed and discarded in biohazard container.
  7. Gloves were worn throughout the procedure.
- Method of instillation of BCG.
  1. The vaccine was reconstituted with 50 mL of saline and administered through a urethral catheter under gravity drainage soon thereafter in order to prevent aggregation.
  2. Treatment was begun 2 to 4 weeks after tumor resection, allowing time for reepithelialization to minimize the potential for intravasation of live bacteria.
  3. In the event of a traumatic catheterization, the treatment was delayed for several days.
  4. After instillation, the patient retained the solution for 2 hours.
  5. After 2 hours gravity drainage of the drug was done by opening the catheter opening, followed by removal of catheter.