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A territory-wide study investigating the dose and efficacy of different BCG strains in patients with intermediate- and high-risk non-muscle-invasive bladder cancer

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Suggested Reviewers:	
Opposed Reviewers:	

Response to Reviewer's Comments

Reviewer #1: The authors did a retrospective multi institutional database search on pts with NMIBC receiving BCG (n=2600)- 1600 w/ adequate BCG) between 2001 and 2020. Thy report cancer outcomes (OS, CSS, RFS, PFS) on long-term use of different BCG strains. Median FU is 11 yr on the 19 yr inclusion. interesting report

Intro- update references (ie.PMID: 32446864)
Response: Updated as the 4th reference. Thank you!

Methods- add ethics approval; add more detail on data and variables extraction.
Response: Ethics approval added (Methods- paragraph 1). The details on data and variables extraction were described in the Methods-Data Collection part. Thank you very much!

Results:
Why did you choose HTN and DLP as covariates to highlight?
Response: Thank you for your question. Based on the CDARS, we can retrieve diagnosis data for assessing the baseline healthy status of target population. We assessed the patient's comorbidity index according to the public webpage (https://www.mdcalc.com/calc/3917/charlson-comorbidity-index-cci). Although the Charlson Comorbidity Index already includes 16 types of diseases, the hypertension and hyperlipidemia were excluded. Considering that these two diseases are also very common and patients would take related drugs for them, we think it is worth to add in.

It seems like the population included belongs to a very sick subgroup of patients (50% CCI 3-4, 28% CCI 5)- please provide a rational-
Response: Thank you for the question. CDARS is a very comprehensive electronic database so it can capture most if not all of the morbidity information of our patients. The median age of intermediate- and high-risk non-muscle-invasive bladder cancer patients in Hong Kong was over 70 years old (Table 1) and it is common for them to develop other medical conditions at this age, so this may also explain the relatively high CCI in our cohort.

one would expect those subgroups to receive less adequate BCG and DOC to be primary. You might have to provide more detail on the results section on that sense.
Response: Thank you for the question. The additional details were provided in supplementary Table 2.

please provide more detail on number of events per group.
Response: Thank you for the question. The additional details were provided in supplementary Table 3.

Discussion:

please add PMID: 28286068; PMID: 37046598 and discuss NIMBUS trial for dosing purpose (PMID: 32446864)
Response: We have revised the discussion section accordingly, thank you very much!

please discuss applicability of your findings on daily clinical practice.
Response: We have revised the discussion section accordingly, thank you very much!

Minor:
% on abstract don't add up;
Response: Updated the Tokyo strain group percentage as 39.0%. Thank you!

please make sure to keep up with terminology- either BCSM or CSS on methods;
Response: Unified as CSS. Thank you!

since median FU is 11 yr might be worth updating the figure for 10-15 years max for visual purpose
Response: Figures were updated, thank you.

Study title:

A territory-wide study investigating the dose and efficacy of different BCG strains in patients with intermediate- and high-risk non-muscle-invasive bladder cancer

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Running head: BCG strains in NMIBC

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Ethics approval

Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee

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Abstract

Background

Current EAU guidelines support Adjuvant intravesical BCG treatment after Tran Urethral Resection of Bladder Tumour (TURBT) on intermediate- or high-risk Non-Muscle-Invasive Bladder Cancer (NMIBC) patients, aiming to reduces the risk of tumor recurrence. The quality of data, however, does not allow definitive conclusions on whether different strains and dosages of BCG have different efficacies on long-term survival outcomes.

Objective

To evaluate long-term survival outcomes of different strains and dosages of BCG in patients with NMIBC.

Design, setting, and participants

All NMIBC patients treated with intravesical BCG therapy from 2001 to 2020 were identified using a territory-wide database in Hong Kong.

Exposures

BCG strains and dosages (Connaught strain 81mg, Connaught strain 27mg, Tokyo strain 80mg, Danish strain 30mg) were retrieved from medical records.

Main outcomes and measures

Overall Survival (OS), Cancer-Specific Survival (CSS), Recurrence-Free Survival (RFS), and Progression-Free Survival (PFS) were analyzed using Kaplan-Meier method. Multivariable Cox regression analysis was used to adjust potential confounding factors, and to estimate Hazard Ratio (HR) and 95% Confidence Interval (CI) of different BCG strains. Further subgroup analysis on adequate versus inadequate BCG treatment was performed.

Results

A total of 2,602 NMIBC patients treated with intravesical BCG were identified. Among them, 1291 (49.6%) received Connaught strain 81mg, 199 (7.6%) received Connaught strain 27mg, 1014 (39.0%) received Tokyo strain, and 98 (3.8%) received Danish strain. The median follow-up was 11.0 years. No statistical significant differences in OS, CSS, RFS and PFS were detected among the different groups. At multivariable analysis, the Connaught strain 27mg group was inferior to the Connaught strain 81mg group in OS (HR: 1.26, 95% CI: 1.05-1.51), CSS (HR: 1.69, 95% CI: 1.08-2.66) and PFS (HR: 1.86, 95% CI: 1.20-2.88). Adequate BCG treatment was associated with improved OS (HR: 0.82, 95% CI: 0.73-0.92), CSS (HR: 0.64, 95% CI: 0.47-0.86), RFS (HR: 0.80, 95% CI: 0.70-0.92), and PFS (HR: 0.52, 95% CI: 0.39-0.68). Among patients treated with adequate BCG, at multivariable analysis the Connaught strain 27mg group showed worst results as compared to the Connaught strain 81mg group in CSS (HR: 1.93, 95% CI: 1.07-3.51). Compared to the Connaught strain 81mg group, both Tokyo and Danish strains had similar survival outcomes in the whole cohort and the adequate BCG treatment subgroup.

Conclusions

In this cohort study, adequate BCG remains the most important factor in optimizing survival outcomes in patients with intermediate- and high-risk NMIBC. No significant differences in survival outcomes were observed between full-dose Connaught, Tokyo, and Danish strains. Reduced-dose Connaught strain was associated with the worst survival outcomes.

Patient Summary

We evaluated the efficacy of different strains and dosages of BCG in patients with intermedia- or high-risk NMIBC in the past two decades in Hong Kong. We conclude no significant differences in long-term survival outcomes in terms of full-dose Connaught, Tokyo, and Danish strains, while the reduced-dose Connaught strain was inferior to the full-dose group. Adequate BCG treatment benefits long-term survival.

1. Introduction

Bladder cancer is the tenth most common malignancy worldwide, while it ranks sixth when only the male gender is considered. The estimated number of new bladder cancer cases in 2020 was 573,278, and the deaths reached 212,536 [1]. Its mortality rate ranks 13th in developed countries [2] with a huge burden to health care system [3].

The standard first-line treatment of intermediate- and high-risk NMIBC is Transurethral Resection of Bladder Tumour (TURBT) followed by adjuvant intravesical Bacillus Calmette–Guérin (BCG) therapy [4]. Currently, several strains of BCG are available for clinical usage; However, whether there is any difference between different strains and dosages of BCG in terms of long-term survival outcomes remains controversial and the available evidences does not allow definitive conclusions: Rentsch et al. reported that treatment with BCG Connaught conferred significantly greater 5-yr recurrence-free survival than with BCG Tice [5]; Sengiku et al. compared the complete response, recurrence-free survival and adverse event rates of the Tokyo and Connaught strains, and concluded that there were no significant differences between the two strains [6]. The aim of our study was to assess the efficacy of different BCG strains by using a territory-wide database in Hong Kong.

2. Method

Study Design and Data Source

Our BCG cohort was identified using the Clinical Data Analysis and Reporting System (CDARS) under the management of the Hospital Authority, Hong Kong. CDARS is an electronic healthcare database that covers the patients' demographics, death, diagnoses, procedures, drug prescription and dispensing history, and laboratory results from all public hospitals and clinics in Hong Kong. It represents inpatient and outpatient data of approximately 80% of the 7.4 million population in Hong Kong. Patients are de-identified in CDARS to ensure confidentiality. Different territory-wide studies were previously conducted using CDARS. The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) coding was used in CDARS [7-9]. The study protocol was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee. The ethics approval reference number is CRE-2021.599. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consented was waived given the retrospective nature of this study.

Cohort definition

All consecutive adult patients with NMIBC who were treated with BCG intravesically between January 2001 and December 2020 in Hong Kong were identified. In Hong Kong, the treatment indications for BCG followed the clinical practice guideline of the European Association of Urology (EAU) [10]. Surveillance cystoscopy was also performed according to the EAU guidelines [10]. There were two main exclusion criteria: (1) Patients who received more than one strain of BCG, and (2) patients who had known history of or concomitant Upper Tract Urothelial Carcinoma (UTUC).

Data Collection

Baseline data was retrieved at the date of initiation of intravesical BCG treatment. Demographic data, including date of birth, sex, date of registered death, and main death

cause were captured. At baseline, data on 16 comorbidities (myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular accident or transient ischemic attack, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, moderate to severe chronic kidney disease, solid tumour other than bladder cancer, leukemia, lymphoma, AIDS) based on the Charlson Comorbidity Index (CCI) and two other diseases (hypertension and hyperlipidemia) were retrieved using the ICD-9-CM diagnosis codes (Supplementary Table 1). Briefly, the unique reference key of patients, the exact diagnosis date, and the diagnosis description of comorbidities were extracted until the last follow-up date.

Outcomes

The primary outcome was Overall Survival (OS), the secondary outcomes were Cancer-Specific Survival (CSS), Recurrence-Free Survival (RFS) and Progression-Free Survival (PFS). The INDEX date was the starting date of BCG.

OS was identified as the period from INDEX to date of registered death or last follow-up. The CSS was determined by the registered cause of death. Recurrence date was defined as the date of first TURBT after INDEX. Progression date was defined as the date of radical cystectomy or the date of first radiotherapy/ chemotherapy/ PD-L1 or PD-1 drugs administration after INDEX.

The adequate BCG therapy was defined as at least five of six induction instillations and at least one maintenance (two of three instillations) in a 6-month period [11].

Statistical Analysis

Data was analyzed using SPSS version 25.0 (SPSS, Inc., Chicago, Illinois) and R software (4.2.0; R Foundation for Statistical Computing, Vienna, Austria). Analysis was first performed for the whole BCG cohort. Further subgroup analysis was performed for patients who received adequate BCG treatment. Continuous variables were expressed in mean ± Standard Deviation (SD) or median (Interquartile Range [IQR]), as appropriate, whereas categorical variables were presented as numbers

(percentage). Qualitative and quantitative differences between subgroups were analyzed by the χ^2 test for categorical parameters and Mann-Whitney test for continuous parameters, as appropriate. Kaplan-Meier analysis was performed, and significance was determined by a log-rank test. Multivariate Cox regression analyses were performed to adjust for potential confounding factors. A p-value of <0.05 was considered statistically significant.

3. Result

Baseline Demographic Characteristics

A total of 3334 NMIBC patients who received intravesical BCG were identified from CDARS. Among them, 732 patients were excluded: 507 patients received two or more strains of BCG, and 225 patients had history of or concomitant UTUC. Finally, 2602 NMIBC patients who received one stain of intravesical BCG therapy were included; 1291 received 81mg Connaught strain, 199 received 27mg Connaught, 1014 received Tokyo strain, and 98 received Danish strain (Figure 1).

Among the 2602 patients, 1351 (51.9%) received adequate BCG treatment (Table 1). The detail of adequate BCG treatment distribution was provided in supplementary Table 2. The median age of the whole cohort was 73 years (IQR 65-79), 78.7% were males. Regarding CCI, 26.9% had a score of 0-2, 44.9% had a score of 3-4, and 28.3% had a score of □5. In addition, 52.3% of the patients had hypertension, and 27.6% had hyperlipidemia. The median follow-up was 11.0 years. The number of events and median follow-up of each group were depicted in supplementary Table 3.

Comparison between Different BCG Groups

There were significant differences in the rates of adequate treatment rate ($p < 0.01$), age ($p = 0.01$), and the proportion of patients with hypertension ($p < 0.01$) and hyperlipidemia ($p < 0.01$), between different BCG groups (Table 1). On the other hand, there was no difference in gender ($p = 0.73$) and the CCI score ($p = 0.07$) between the BCG groups.

Survival Outcomes

Upon Kaplan-Meier analysis, no statistical significant differences were found in OS ($p = 0.06$), CSS ($p = 0.09$), RFS ($p = 0.13$), and PFS ($p = 0.10$) among the different BCG strains groups (Figure 2). At Multivariable Cox regression analysis, adequate BCG treatment was an independent protective factor for OS (HR: 0.82, 95% CI: 0.73-0.92), CSS (HR: 0.64, 95% CI: 0.47-0.86), RFS (HR: 0.80, 95% CI: 0.70-0.92), and PFS (HR: 0.52, 95% CI: 0.39-0.68). Compared to the 81mg Connaught group, the 27mg (reduced)

Connaught group was associated with worse OS (HR: 1.26, 95%CI:1.05-1.51), CSS (HR: 1.69, 95%CI:1.08-2.66), and PFS (HR: 1.86, 95%CI:1.20-2.88). Both the Tokyo and Danish strain had comparable survival outcomes with the 81mg Connaught group. Higher age was an independent risk factor for OS (HR: 1.06, 95% CI: 1.05-1.07), CSS (HR: 1.05, 95% CI: 1.02-1.07), and RFS (HR: 1.01, 95% CI: 1.00-1.02), while male gender was an independent risk factor for OS (HR: 1.49, 95% CI: 1.29-1.73), RFS (HR: 1.26, 95% CI: 1.06-1.49), and PFS (HR: 1.84, 95% CI: 1.25-2.73). A CCI score of ≥5 was associated with worse OS (HR: 3.02 95%CI:1.17-7.78). On the other hand, hyperlipidemia was associated with better OS (HR: 0.82, 95%CI:0.70-0.97) (Table 2).

Subgroup Analysis for adequate BCG treatment

We performed further subgroup analysis in patients receiving adequate BCG usage. There were no significant differences in terms of age ($p = 0.36$), gender ($p = 0.57$) and CCI score ($p = 0.18$) between the BCG groups. There were significant differences in the proportion of patients with hypertension ($p<0.01$) and hyperlipidemia ($p<0.01$) between the BCG groups (Table 3).

On Kaplan-Meier analysis, there were no significant differences between groups in OS ($p = 0.33$), CSS ($p = 0.07$), RFS ($p = 0.14$), and PFS ($p = 0.54$) (Figure 3). Multivariable Cox regression showed that the 27mg (reduced) Connaught dose was inferior to the 81mg (full) Connaught dose in CSS (HR: 1.93, 95%CI: 1.07-3.51). Both Tokyo and Danish strains had comparable survival outcomes with the 81mg (full) Connaught group. Older patients showed worse OS (HR: 1.07, 95%CI: 1.05-1.09), CSS (HR: 1.07, 95%CI: 1.03-1.11) and RFS (HR: 1.02, 95%CI: 1.00-1.03), while male gender was associated with worse OS (HR: 1.52, 95%CI: 1.23-1.89) and RFS (HR: 1.39, 95%CI: 1.08-1.80). CCI score of ≥5 was no longer associated with worse OS, but hyperlipidemia remained a significant factor associated with better OS (HR: 0.75, 95% CI: 0.59-0.94) (Table 4).

4. Discussion

To our knowledge, the is one of the largest BCG cohorts[12] and we utilized it to investigate the efficacies of different strains and dosages of intravesical BCG in patients with NMIBC. Our cohort also had a long median follow-up of 11.0 years, which provided important information regarding the long-term survival outcomes follow intravesical BCG therapy. Our study results highlighted three key messages: 1) Adequate BCG treatment is extremely important to optimize survival outcomes, 2) Full-dose Connaught strain, Tokyo strain and Danish strain had no difference in long-term survival outcomes, and 3) Reduced-dose Connaught strain had worse survival outcomes than full-dose Connaught strain. Therefore, in patients with intermediate- and high-risk NMIBC, we should always aim for adequate full-dose BCG treatment, regardless the type of BCG strain.

So far, at least 16 different strains of BCG have been used in clinical practice worldwide[13]. The first BCG was developed when mycobacterium bovis underwent the process of attenuation from 1908 to 1921. It is actually quite common for BCG strains to lost one of the key regions, RD1[14,15]. A series of genomic alterations can occur over time, which branches them off from the original BCG strain [16]. Due to the genetic differences between BCG vaccine strains, phenotypic properties such as reactogenicity and immunogenicity might be influenced, and whether such differences could affect their clinical efficacies in treating bladder cancer remained controversial [17].

Thyavihally et al. reported no significant differences between Danish 1331 and Moscow-I strain in RFS and PFS, but a significantly higher incidence of moderate to severe adverse events in BCG Moscow-I strain[18]. Another study comparing adjuvant intravesical BCG Tice strain with RIVM strain for high-risk NMIBC demonstrated that the Tice strain was superior to RIVM for RFS, but no significant differences were detected for PFS and CSS [19]. For the Tice strain vs. Connaught strain, Chen et al. also reported no difference in 3-year RFS and PFS[20]. A network meta-analysis focus on the impact of different BCG strains on the RFS demonstrated that Tokyo, Pasteur, and TICE

strains were both superior to chemotherapy, but there wasn't a clear superiority of one strain [17]. Recently, a systematic review highlights potential enhanced benefits from the genetically different BCG RIVM, Tice, and Tokyo 172 strains[21]. However, no clinically significant lower recurrence rate was identified. Our study showed no difference between full-dose Connaught, Tokyo and Danish strains in terms of OS, CSS, RFS and PFS. Unfortunately, BCG Tice strain was not used in Hong Kong and we are able to investigate its efficacy in this study. However, we provide Real word-evidence of worst results in terms of CSS for patient receiving reduced-dose Connaught strain, regardless of whether adequate treatment is administered or not. Grimm et al. reported reduced BCG instillation frequency from 15 to 9 led to higher recurrence rates (HR: 0.39, upper part of the one-sided 97.5% CI being 0.66)[4]. The current evidence suggested that both reducing the dosage of BCG and frequency of instillation will lead to suboptimal therapeutic efficacy.

Several factors were associated with survival outcomes according to our findings. First, older patients had worse survival outcomes: with increasing age, the natural immune system aging [22] could led to a down-regulated of the immune response towards BCG treatment. Second, male gender was associated with worse survival outcomes. In Hong Kong, the rate of tobacco use was also much higher in the male population [23], and this might explain our findings [24]. However, data on smoking was not retrievable through CDARS, and we were not able to further confirm our hypothesis. Third, we found that hyperlipidemia was associated with better OS: this counterintuitive phenomenon might be explained by the potential benefit of statins usage. Ferro et al. reported results from 1510 patients with T1 high grade NMIBC and they found statin use was independently associated with a lower risk of recurrence (HR:0.80, 95%CI: 0.67-0.95; P=0.009). The median recurrence-free survival was 47 (95%CI 40-49) months in those classified as non-statin users vs. 53 (95%CI 48-68) months in those classified as statin users [25]. Further investigations are needed to assess the potential benefits of statins usage in NMIBC patients.

In conclusion, our findings suggest that different full-dose adequate BCG treatment had similar long-term survival benefits, which encourage clinical practice follow the current guideline. It is possible to postulate potential benefits from drugs related to chronic diseases (i.e.: hyperlipidemia), that however needs further investigations.

Several limitations need to be reported in our study. First, our BCG patients and their medical conditions were retrieved primarily by diagnostic codes. Although the same electronic system was used in all public hospitals in Hong Kong, there could be missing data and the data accuracy can be affected. Second, only three strains (Connaught, Tokyo, and Danish strain) were available and used in Hong Kong for the past two decades. Therefore, we were not able to extend our analysis to all of the currently available BCG strains worldwide. Third, some data such as the histological reports and imaging results could not be retrieved through the electronic database. As urologists in Hong Kong follow the EAU guidelines closely, we assumed that intravesical BCG was only use for patients with intermediate- and high-risk NMIBC. Recurrence was defined by the need of TURBT, and progression event was defined by the need of cystectomy, radiotherapy or initiation of systemic treatment. However, these assumptions are prone to error, and the data accuracy might be affected. Despite these limitations, this is by far the largest BCG cohort around the world with long-term survival data, and we do believe our study provide important information regarding the efficacy of BCG in the long-run.

5. Conclusion

Adequate BCG remains the most important factor to optimize survival outcomes in patients with intermediate- and high-risk NMIBC. We did not observe any significant differences in survival outcomes were between full-dose Connaught, Tokyo, and Danish strains. Patients who received reduced-dose Connaught strain had worst survival outcomes.

Figure and Table

PRIOR PRESENTATION

Presented in part at the European Association of Urology annual meeting, Milan, Italy, March 10-13, 2023.

DATA SHARING STATEMENT

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

All authors declare they have no conflicts of interest to disclose.

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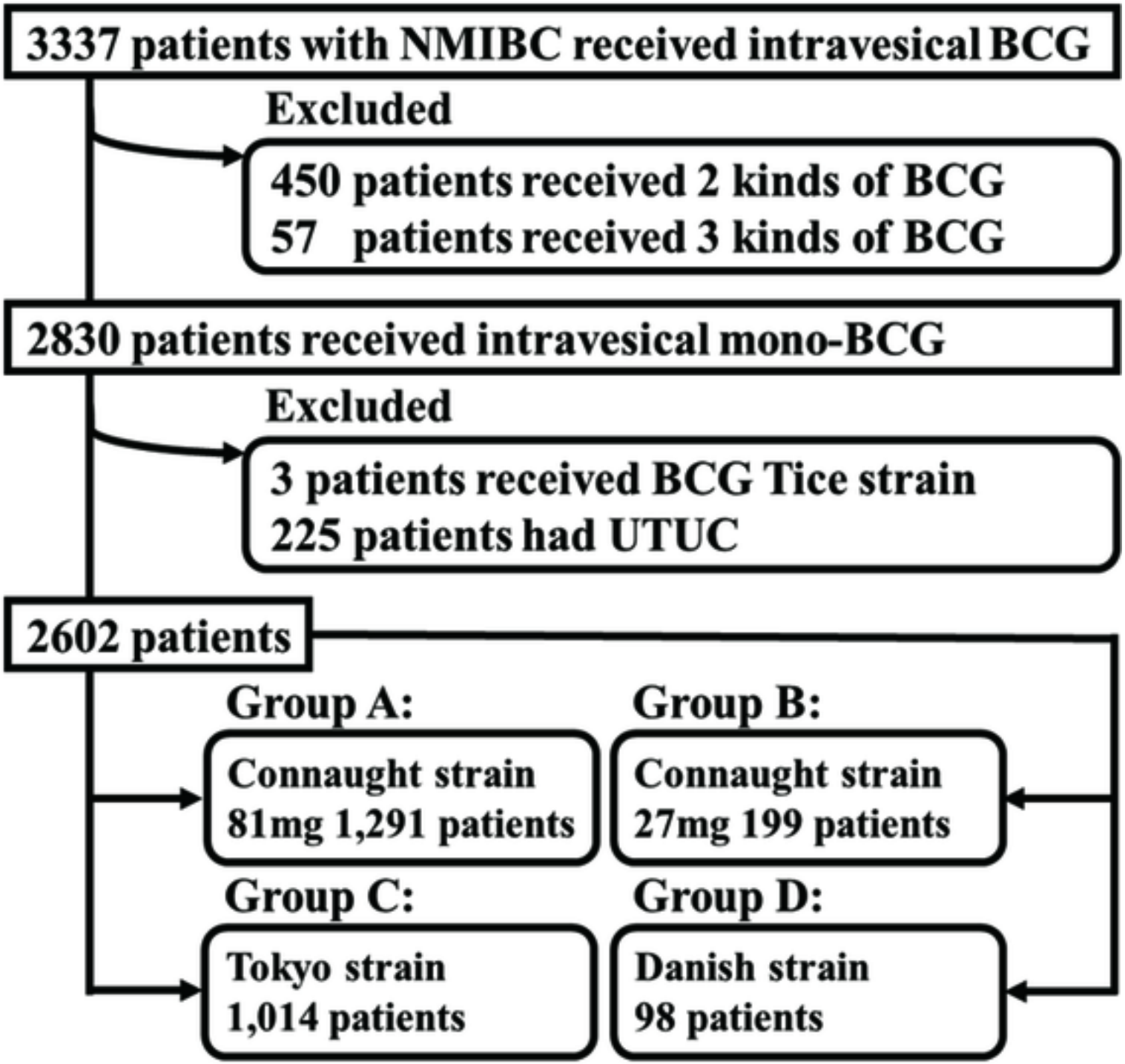
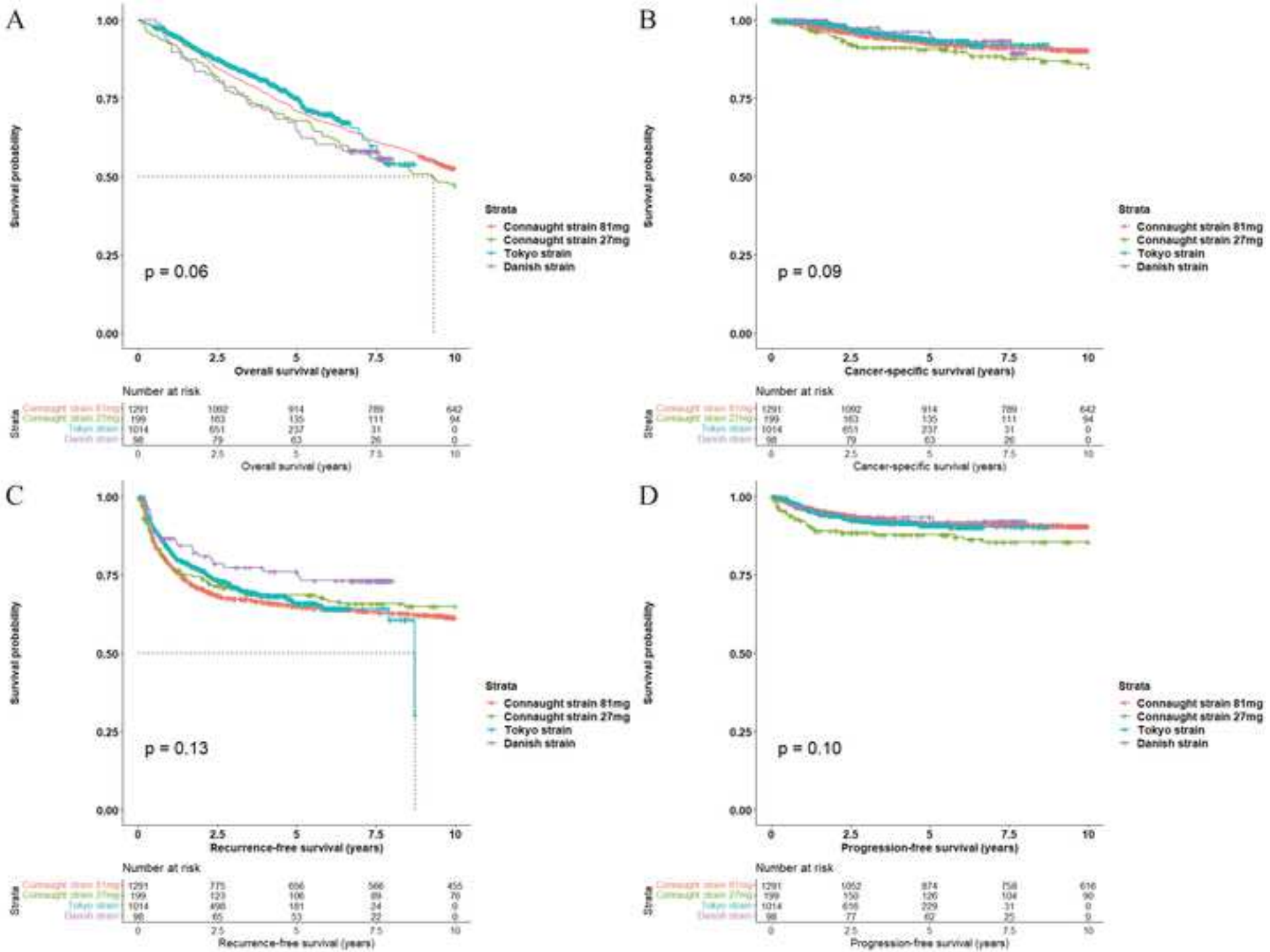
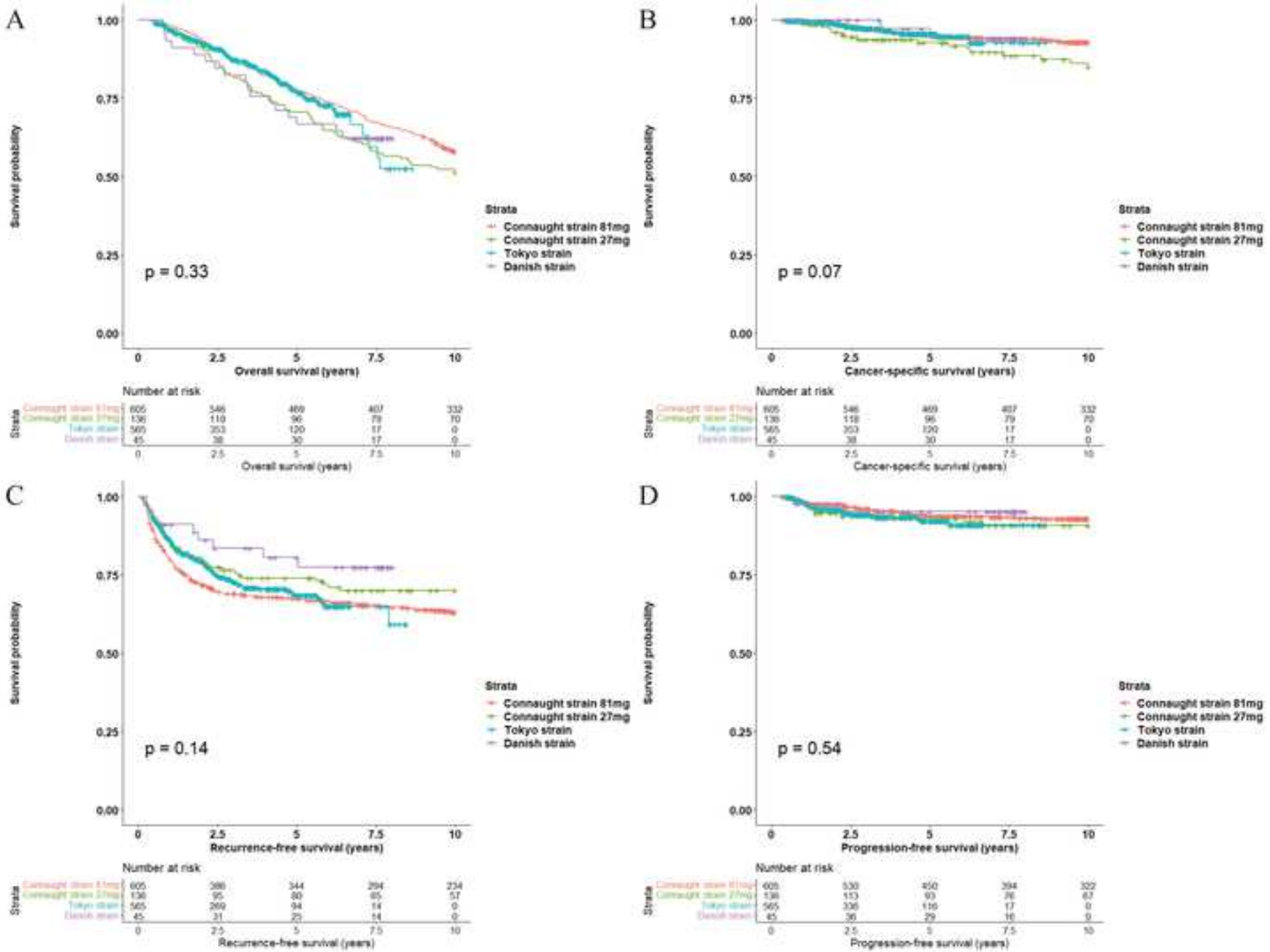
Take home message

In this large BCG cohort study, we demonstrated that no significant differences in survival outcomes were observed between full-dose Connaught, Tokyo, and Danish strains. Reduced-dose Connaught strain was associated with the worst survival outcomes. Adequate BCG remains the most important factor in optimizing survival outcomes in patients with intermediate- and high-risk NMIBC.







Characteristics	Connaught Strain 81mg N = 1291	Connaught Strain 27mg N = 199	Tokyo Strain N = 1014	Danish Strain N = 98	Total N = 2602	*P value*
Adequate BCG						
No	686 (53.1)	63 (31.7)	449 (44.3)	53 (54.1)	1251 (48.1)	<0.01
Yes	605 (49.6)	136 (68.3)	565 (55.7)	45 (45.9)	1351 (51.9)	
Age, median [IQR]	74.0 [66.0-80.0]	74.0 [66.0-79.0]	71.0 [65.0-79.0]	74.0 [64.8-82.0]	73.0 [65.0-79.0]	0.01
Male Gender, n (%)	1010 (78.2)	160 (80.4)	804 (79.3)	74 (75.5)	2048 (78.7)	0.73
Charlson Comorbidity Index, n (%)						
0	37 (2.9)	4 (2.0)	17 (1.7)	6 (6.1)	64 (2.5)	0.07
1-2	293 (22.7)	51 (25.6)	271 (26.7)	20 (20.4)	635 (24.4)	
3-4	603 (46.7)	93 (46.7)	428 (42.2)	43 (43.9)	1167 (44.9)	
≥5	358 (27.7)	51 (25.6)	298 (29.4)	29 (29.6)	736 (28.3)	
Other Comorbidities, n (%)						
Hypertension	636 (49.3)	55 (27.6)	612 (60.4)	58 (59.2)	1361 (52.3)	<0.01
Hyperlipidemia	206 (16.0)	13 (6.5)	462 (45.6)	37 (37.8)	718 (27.6)	<0.01

Variables	Overall survival	Cancer-specific survival	Recurrence-free survival	Progression-free survival
Adequate BCG				
No	Ref	Ref	Ref	Ref
Yes	**0.82 (0.73-0.92)**	**0.64 (0.47-0.86)**	**0.80 (0.70-0.92)**	**0.52 (0.39-0.68)**
BCG Strain				
Connaught strain 81mg	Ref	Ref	Ref	Ref
Connaught strain 27mg	**1.26 (1.05-1.51)**	**1.69 (1.08-2.66)**	0.97 (0.74-1.26)	**1.86 (1.20-2.88)**
Tokyo strain	0.95 (0.80-1.13)	0.87 (0.59-1.29)	0.91 (0.78-1.07)	1.17 (0.85-1.60)
Danish strain	1.20 (0.87-1.65)	0.88 (0.38-2.02)	0.67 (0.44-1.01)	0.97 (0.45-2.10)
Age	**1.06 (1.05-1.07)**	**1.05 (1.02-1.07)**	**1.01 (1.00-1.02)**	0.99 (0.97-1.01)
Gender				
Female	Ref	Ref	Ref	Ref
Male	**1.49 (1.29-1.73)**	1.13 (0.79-1.61)	**1.26 (1.06-1.49)**	**1.84 (1.25-2.73)**
Charlson Comorbidity Index				
0	Ref	Ref	Ref	Ref
1-2	1.30 (0.53-3.24)	>100 (<0.01->100)	0.92 (0.55-1.52)	4.13 (0.97-17.60)
3-4	1.96 (0.77-4.95)	>100 (<0.01->100)	0.83 (0.47-1.47)	1.02 (0.87-18.64)
≥5	**3.02 (1.17-7.78)**	>100 (<0.01->100)	0.93 (0.51-1.71)	4.32 (0.88-21.22)
Hypertension				
No	Ref	Ref	Ref	Ref
Yes	1.11 (0.97-1.26)	1.19 (0.85-1.66)	1.15 (0.98-1.34)	0.98 (0.72-1.34)
Hyperlipidemia				
No	Ref	Ref	Ref	Ref
Yes	**0.82 (0.70-0.97)**	0.83 (0.56-1.23)	0.91 (0.76-1.08)	0.74 (0.50-1.09)

Characteristics	Connaught Strain 81mg N = 605	Connaught Strain 27mg N = 136	Tokyo Strain N = 565	Danish Strain N = 45	Total N = 1351	*P value*
Age, median [IQR]	73.0 [64.0-79.0]	73.0 [66.0-78.0]	71.0 [64.0-78.0]	72.0 [61.5-81.5]	72.0 [64.0-79.0]	0.36
Male Gender, n (%)	469 (77.5)	109 (80.1)	455 (80.5)	34 (75.6)	1067 (79.0)	0.57
Charlson Comorbidity Index, n (%)						
0	18 (3.0)	4 (2.9)	13 (2.3)	4 (8.9)	39 (2.9)	0.18
1-2	159 (26.3)	39 (28.7)	149 (26.4)	10 (22.2)	357 (26.4)	
3-4	284 (46.9)	66 (48.5)	238 (42.1)	18 (40.0)	606 (44.9)	
≥5	144 (23.8)	27 (19.9)	165 (29.2)	13 (28.9)	349 (25.8)	
Comorbidities, n (%)						
Hypertension	291 (48.1)	40 (29.4)	355 (62.8)	26 (57.8)	712 (52.7)	**<0.01**
Hyperlipidemia	96 (15.9)	11 (8.1)	278 (49.2)	18 (40.0)	403 (29.8)	**<0.01**

Variables	Overall survival	Cancer-specific survival	Recurrence-free survival	Progression-free survival
BCG Strain				
Connaught strain 81mg	Ref	Ref	Ref	Ref
Connaught strain 27mg	1.22 (0.96-1.55)	**1.93 (1.07-3.51)**	0.76 (0.53-1.07)	1.26 (0.64-2.48)
Tokyo strain	1.03 (0.79-1.34)	1.07 (0.57-2.00)	0.87 (0.69-1.09)	1.32 (0.79-2.20)
Danish strain	1.46 (0.88-2.40)	1.01 (0.24-4.27)	0.57 (0.29-1.12)	0.83 (0.20-3.45)
Age	**1.07 (1.05-1.09)**	**1.07 (1.03-1.11)**	**1.02 (1.00-1.03)**	0.99 (0.96-1.03)
Gender				
Female	Ref	Ref	Ref	Ref
Male	**1.52 (1.23-1.89)**	1.13 (0.66-1.96)	**1.39 (1.08-1.80)**	1.77 (0.94-3.35)
Charlson Comorbidity Index				
0	Ref	Ref	Ref	Ref
1-2	0.73 (0.26-2.07)	>100 (<0.01->100)	1.08 (0.50-2.33)	3.54 (0.44-28.45)
3-4	1.03 (0.35-3.05)	>100 (<0.01->100)	1.11 (0.47-2.63)	3.57 (0.38-33.93)
≥5	1.85 (0.60-5.70)	>100 (<0.01->100)	1.18 (0.47-2.98)	3.08 (0.28-33.43)
Hypertension				
No	Ref	Ref	Ref	Ref
Yes	1.03 (0.85-1.25)	1.09 (0.64-1.85)	1.12 (0.90-1.40)	0.81 (0.49-1.33)
Hyperlipidemia				
No	Ref	Ref	Ref	Ref
Yes	**0.75 (0.59-0.94)**	0.80 (0.43-1.51)	0.92 (0.71-1.19)	1.17 (0.66-2.06)

Revision notes

Study title:

A territory-wide study investigating the dose and efficacy of different BCG strains in patients with intermediate- and high-risk non-muscle-invasive bladder cancer

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Running head: BCG strains in NMIBC

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Ethics approval

Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee

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Abstract

Background

Current EAU guidelines support ~~There~~ Adjuvant intravesical BCG treatment after Tran Urethral Resection of Bladder Tumour (TURBT) on intermediate- or high-risk Non-Muscle-Invasive Bladder Cancer (NMIBC) patients, aiming to reduces the risk of tumor recurrence. The quality of data, however, does not allow definitive conclusions ~~remains controversy~~ on whether different strains and dosages of BCG have different efficacies on long-term survival outcomes.~~s of intermedia- or high-risk non-muscle-invasive bladder cancer (NMIBC) patients.~~

Objective

To evaluate long-term survival outcomes of different strains and dosages of BCG in patients with NMIBC.~~.~~

Design, setting, and participants

All NMIBC patients ~~who received~~treated with intravesical BCG therapy from 2001 to 2020 were identified using a territory-wide database in Hong Kong.

Exposures

BCG strains and dosages (Connaught strain 81mg, Connaught strain 27mg, Tokyo strain 80mg, Danish strain 30mg) were ~~abstracted~~ retrieved from medical records.

Main outcomes and measures

Overall ~~su~~Survival (OS), C~~e~~ancer-S~~s~~pecific S~~s~~urvival (CSS), R~~r~~ecurrence-F~~f~~ree S~~s~~urvival (RFS), and P~~p~~rogression-F~~f~~ree S~~s~~urvival (PFS) were analyzed using Kaplan-Meier method. Multivariable Cox regression analysis was used to adjust potential confounding factors, and to estimate H~~h~~azard R~~r~~atio (HR) and 95% C~~e~~onfidence I~~i~~nterval (CI) of different BCG strains. Further subgroup analysis on adequate versus inadequate BCG treatment was performed.

Results

A total of 2,602 NMIBC patients ~~who received~~ treated with intravesical BCG were identified. Among them, 1291 (49.6%) received Connaught strain 81mg, 199 (7.6%) received Connaught strain 27mg, 1014 (39.0%) received Tokyo strain, and 98 (3.8%) received Danish strain. The median follow-up was 11.0 years. No statistical ~~On Kaplan-Meier analysis, no~~ significant differences in OS, CSS, RFS and PFS were detected ~~between~~ among the different ~~strains and dosages of BCG~~groups. ~~However,~~ At~~upon~~ multivariable analysis, the Connaught strain 27mg group was inferior to the Connaught strain 81mg group in OS (HR: 1.26, 95% CI: 1.05-1.51), CSS (HR: 1.69, 95% CI: 1.08-2.66) and PFS (HR: 1.86, 95% CI: 1.20-2.88). ~~Of note, adequate~~Adequate BCG treatment was associated with improved OS (HR: 0.82, 95% CI: 0.73-0.92), CSS (HR: 0.64, 95% CI: 0.47-0.86), RFS (HR: 0.80, 95% CI: 0.70-0.92), and PFS (HR: 0.52, 95% CI: 0.39-0.68). ~~Focusing~~ Among ~~on~~ patients ~~who received~~treated with adequate BCG, at multivariable analysis ~~also showed that~~ the Connaught strain 27mg group showed worst results ~~was inferior~~as compared to the Connaught strain 81mg group in CSS (HR: 1.93, 95% CI: 1.07-3.51). Compared to the Connaught strain 81mg group, both Tokyo and Danish strains had similar survival outcomes in the whole cohort and the adequate BCG treatment subgroup.

Conclusions

In this cohort study, adequate BCG remains the most important factor in optimizing survival outcomes in patients with intermediate- and high-risk NMIBC. No significant differences in survival outcomes were observed between full-dose Connaught, Tokyo, and Danish strains. Reduced-dose Connaught strain was associated with the worst survival outcomes.

Patient Summary

We evaluated the efficacy of different strains and dosages of BCG in patients with intermedia- or high-risk NMIBC in the past two decades in Hong Kong. We conclude no significant differences in long-term survival outcomes in terms of full-dose

Connaught, Tokyo, and Danish strains, while the reduced-dose Connaught strain was inferior to the full-dose group. Adequate BCG treatment benefits long-term survival.

1. Introduction

Bladder cancer is the tenth most common malignancy worldwide, while it ranks sixth when only the male gender is considered. The estimated number of new bladder cancer cases in 2020 was 573,278, and the deaths reached 212,536 [1]. Its mortality rate ranks 13th in developed countries [2]. Bladder cancer is a urological malignancy that imposeswith a huge burden to our health care system [3].

The standard first-line treatment of intermediate- and high-risk NMIBC is Ttransurethral Rresection of Bbladder Ttumour (TURBT) followed by adjuvant intravesical Bacillus Calmette–Guérin (BCG) therapy [4]. Currently, several strains of BCG are available for clinical usage; However, wWhether there is any difference between different strains and dosages of BCG in terms of long-term survival outcomes remains controversial and the available evidences does not allow definitive conclusions: Rentsch et al. reported that treatment with BCG Connaught conferred significantly greater 5-yr recurrence-free survival than with BCG Tice [5]; Sengiku et al. compared the complete response, recurrence-free survival and adverse event rates of the Tokyo and Connaught strains, and concluded that there were no significant differences between the two strains [6]. However, no definite conclusion could be drawn from these limited evidences. Therefore, we conductedThe aim of this our study was to assess the efficacy of different BCG strains by using a territory-wide database in Hong Kong.

2. Method

Study Design and Data Source

Our BCG cohort was identified using the Clinical Data Analysis and Reporting System (CDARS) under the management of the Hospital Authority, Hong Kong. CDARS is an electronic healthcare database that covers the patients' demographics, death, diagnoses, procedures, drug prescription and dispensing history, and laboratory results from all public hospitals and clinics in Hong Kong. It represents inpatient and outpatient data of approximately 80% of the 7.4 million population in Hong Kong. Patients are de-identified in CDARS to ensure confidentiality. Different territory-wide studies were previously conducted using CDARS. The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) coding was used in CDARS [7-9]. The study protocol was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee. The ethics approval reference number is CRE-2021.599. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consented was waived given the retrospective nature of this study.

~~*Subjects*~~*Cohort definition*

All consecutive adult patients with NMIBC who were treated with BCG intravesically between January 2001 and December 2020 in Hong Kong were identified. In Hong Kong, the treatment indications for BCG followed the clinical practice guideline of the European Association of Urology (EAU) [10]. Surveillance cystoscopy was also performed according to the EAU guidelines [10]. There were two main exclusion criteria: (1) Patients who received more than one strain of BCG, and (2) patients who had known history of or concomitant U~~u~~pper T~~t~~ract U~~u~~rothelial C~~c~~arcinoma (UTUC).

Data Collection

Baseline data was retrieved at the date of initiation of intravesical BCG treatment. Demographic data, including date of birth, sex, date of registered death, and main death

cause were captured. At baseline, data on 16 comorbidities (myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular accident or transient ischemic attack, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, moderate to severe chronic kidney disease, solid tumour other than bladder cancer, leukemia, lymphoma, AIDS) based on the Charlson Comorbidity Index (CCI) and two other diseases (hypertension and hyperlipidemia) were retrieved using the ICD-9-CM diagnosis codes (Supplementary Table 1). Briefly, the unique reference key of patients, the exact diagnosis date, and the diagnosis description of comorbidities were extracted until the last follow-up date.

~~*Definition*~~*Outcome~~s~~*

The primary outcome~~s~~ w~~as ere~~ O~~o~~verall S~~s~~urvival (OS)~~,~~, ~~and~~ the secondary outcomes were C~~c~~ancer-S~~s~~pecific S~~s~~urvival (CSS), R~~r~~ecurrence-F~~f~~ree S~~s~~urvival (RFS) and P~~p~~rogression-F~~f~~ree S~~s~~urvival (PFS). The INDEX date was the starting date of BCG.

_OS was identified as the period ~~between the~~from INDEX to ~~initiation date of BCG and the~~ date of registered death or ~~the~~ last follow-up ~~date~~. ~~Bladder cancer specific mortality~~The CSS was determined by the registered cause of death. Recurrence date was defined as the date of first TURBT after ~~BCG treatment~~INDEX. Progression date was defined as the date of radical cystectomy or the date of first radiotherapy/ chemotherapy/ PD-L1 or PD-1 drugs administration after ~~BCG treatment~~INDEX.

The adequate BCG therapy was defined as at least five of six induction instillations and at least one maintenance (two of three instillations) in a 6-month period [11].

Statistical Analysis

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Data was analyzed using SPSS version 25.0 (SPSS, Inc., Chicago, Illinois) and R software (4.2.0; R Foundation for Statistical Computing, Vienna, Austria). Analysis was first performed for the whole BCG cohort. Further subgroup analysis was performed for patients who received adequate BCG treatment. Continuous variables

were expressed in mmean ± Sstandard Ddeviation (SD) or median (Iinterquartile Rrange [IQR]), as appropriate, whereas categorical variables were presented as numbers (percentage). Qualitative and quantitative differences between subgroups were analyzed by the χ^2 test for categorical parameters and Mann-Whitney test for continuous parameters, as appropriate. Kaplan-Meier analysis was performed, and significance was determined by a log-rank test. Multivariate Cox regression analyses were performed to adjust for potential confounding factors. A p-value of <0.05 was considered statistically significant.

3. Result

Baseline Demographic Characteristics

A total of 3334 NMIBC patients who received intravesical BCG were identified from CDARS. Among them, 732 patients were excluded: ~~;~~ 507 patients received two or more strains of BCG, and 225 patients had history of or concomitant ~~upper tract urothelial carcinoma~~UTUC. Finally, 2602 NMIBC patients who received one stain of intravesical BCG therapy were included; 1291 received 81mg Connaught strain, 199 received 27mg Connaught, 1014 received Tokyo strain, and 98 received Danish strain (Figure 1).

Among the 2602 patients, 1351 (51.9%) received adequate BCG treatment (Table 1). The detail of adequate BCG treatment distribution was provided in supplementary Table 2. The median age of the whole cohort was 73 years (IQR 65-79 ~~years~~), ~~and~~ 78.7% were ~~of~~ males ~~gender~~. ~~For~~ Regarding CCI, 26.9% had a score of 0-2, 44.9% had a score of 3-4, and 28.3% had a score of ≥5. In addition, 52.3% of the patients had hypertension, and 27.6% had hyperlipidemia. The median follow-up was 11.0 years. The number of events and median follow-up of each group were depicted in supplementary Table 3.

Comparison between Different BCG Groups

There were significant differences in the rates of adequate treatment rate ($p < 0.01$), age ($p = 0.01$), and the proportion of patients with hypertension ($p < 0.01$) and hyperlipidemia ($p < 0.01$), between different BCG groups (Table 1). On the other hand, there was no difference in gender ($p = 0.73$) and the CCI score ($p = 0.07$) between the BCG groups.

Survival Outcomes

Upon Kaplan-Meier analysis, ~~there were~~ no statistical significant differences were found in OS ($p = 0.06$), CSS ($p = 0.09$), RFS ($p = 0.13$), and PFS ($p = 0.10$) among the different BCG strains groups (Figure 2). At Multivariable Cox regression analysis, ~~indicated that~~ adequate BCG treatment was an independent protective factor for OS (HR: 0.82, 95% CI: 0.73-0.92), CSS (HR: 0.64, 95% CI: 0.47-0.86), RFS (HR: 0.80, 95% CI: 0.70-0.92), and PFS (HR: 0.52, 95% CI: 0.39-0.68). Compared to the 81mg

Connaught group, the 27mg (reduced) Connaught group was associated with worse OS (HR: 1.26, 95%CI:1.05-1.51), CSS (HR: 1.69, 95%CI:1.08-2.66), and PFS (HR: 1.86, 95%CI:1.20-2.88). Both the Tokyo and Danish strain had comparable survival outcomes with the 81mg Connaught group. Higher age was an independent risk factor ~~in~~ for OS (HR: 1.06, 95% CI: 1.05-1.07), CSS (HR: 1.05, 95% CI: 1.02-1.07), and RFS (HR: 1.01, 95% CI: 1.00-1.02), while male gender was an independent risk factor ~~in~~ for OS (HR: 1.49, 95% CI: 1.29-1.73), RFS (HR: 1.26, 95% CI: 1.06-1.49), and PFS (HR: 1.84, 95% CI: 1.25-2.73). A CCI score of ≥5 was associated with worse ~~n~~ OS (HR: 3.02 95%CI:1.17-7.78). On the other hand, hyperlipidemia was associated with better OS (HR: 0.82, 95%CI:0.70-0.97) (Table 2).

Subgroup Analysis for ~~patients received~~ adequate BCG treatment

We performed further subgroup analysis in patients receiving adequate BCG usage. There were no significant differences in terms of age (p =0.36), gender (p =0.57) and CCI score (p =0.18) between the BCG groups. There were significant differences in the proportion of patients with hypertension (p<0.01) and hyperlipidemia (p<0.01) between the BCG groups (Table 3).

On Kaplan-Meier analysis, there were no significant differences between groups in OS (p =0.33), CSS (p =0.07), RFS (p =0.14), and PFS (p =0.54) (Figure 3). Multivariable Cox regression showed that the 27mg (reduced) Connaught dose was inferior to the 81mg (full) Connaught dose in CSS (HR: 1.93, 95%CI: 1.07-3.51). Both Tokyo and Danish strains had comparable survival outcomes with the 81mg (full) Connaught group. Older patients showed worse OS (HR: 1.07, 95%CI: 1.05-1.09), CSS (HR: 1.07, 95%CI: 1.03-1.11) and RFS (HR: 1.02, 95%CI: 1.00-1.03), while male gender was associated with worse OS (HR: 1.52, 95%CI: 1.23-1.89) and RFS (HR: 1.39, 95%CI: 1.08-1.80). CCI score of ≥5 was no longer associated with worse OS, but hyperlipidemia remained a significant factor associated with better OS (HR: 0.75, 95% CI: 0.59-0.94) (Table 4).

4. Discussion

To our knowledge, the is one of the largest BCG cohorts[12] and we utilized it to investigate the efficacies of different strains and dosages of intravesical BCG in patients with NMIBC. Our cohort also had a long median follow-up of 11.0 years, which provided important information regarding the long-term survival outcomes follow intravesical BCG therapy. Ours study results highlighted three key messages: 1) Adequate BCG treatment is extremely important to optimize survival outcomes, 2) Full-dose Connaught strain, Tokyo strain and Danish strain had no difference in long-term survival outcomes, and 3) Reduced-dose Connaught strain had worse survival outcomes than full-dose Connaught strain. Therefore, in patients with intermediate- and high-risk NMIBC, we should always aim for adequate full-dose BCG treatment, regardless the type of BCG strain.

So far, at least 16 different strains of BCG have been used in clinical practice worldwide[13]. The first BCG was developed when mycobacterium bovis underwent the process of attenuation from 1908 to 1921. It is actually quite common for BCG strains to lost one of the key regions, RD1[14,15]. A series of genomic alterations can occur over time, which branches them off from the original BCG strain [16]. Due to the genetic differences between BCG vaccine strains, phenotypic properties such as reactogenicity and immunogenicity might be influenced, and whether such differences could affect their clinical efficacies in treating bladder cancer remained controversial [17].

Thyavihally et al. reported no significant differences between Danish 1331 and Moscow-I strain in RFS and PFS, but a significantly higher incidence of moderate to severe adverse events in BCG Moscow-I strain[18]. Another study comparing adjuvant intravesical BCG Tice strain with RIVM strain for high-risk NMIBC demonstrated that the Tice strain was superior to RIVM for RFS, but no significant differences were detected for PFS and CSS [19]. For the Tice strain vs. Connaught strain, Chen et al. also reported no difference in 3-year RFS and PFS[20]. A network meta-analysis focus on the impact of different BCG strains on the RFS demonstrated that Tokyo, Pasteur, and TICE

strains were both superior to chemotherapy, but there wasn't a clear superiority of one strain [17]. Recently, a systematic review highlights potential enhanced benefits from the genetically different BCG RIVM, Tice, and Tokyo 172 strains[21]. However, no clinically significant lower recurrence rate was identified. Our study showed no difference between full-dose Connaught, Tokyo and Danish strains in terms of OS, CSS, RFS and PFS. Unfortunately, BCG Tice strain was not used in Hong Kong and we are able to investigate its efficacy in this study. However, we provide Real word-evidence of worst results in terms of CSS for patient receiving reduced-dose Connaught strain, regardless of whether adequate treatment is administered or not. Grimm et al. reported reduced BCG instillation frequency from 15 to 9 led to higher recurrence rates (HR: 0.39, upper part of the one-sided 97.5% CI being 0.66)[4]. The current evidence suggested that both reducing the dosage of BCG and frequency of instillation will lead to suboptimal therapeutic efficacy.

~~In our study, we also found~~ S~~s~~everal factors ~~which~~ were associated with survival outcomes according to our findings. First~~ly~~, ~~our results showed that old~~older patients ~~age was associated with~~had worse survival outcomes: ~~,~~ w~~W~~ith increasing age, the natural immune system ~~could change~~ aging [22], ~~,~~ could led to a down-regulated of ~~and~~ the immune response towards BCG treatment ~~might be down regulated. Therefore, we should be cautious that the efficacy of BCG could be reduced in elderly patients~~. Second~~ly~~, ~~we found that~~ male gender was associated with worse survival outcomes. In Hong Kong, the rate of tobacco use was also much higher in the male population [23], and this might explain ~~the observed worse survival outcomes in male patients~~our findings [24]. However, data on smoking was not retrievable through CDARS, and we were not able to further ~~analyze~~ confirm our hypothesis. ~~this factor further.~~ Third~~ly~~, we found that higher BMI was associated with better OS: ~~,~~ t~~T~~his counterintuitive phenomenon might be explained by the potential benefit of statins usage. Ferro et al. reported results from 1510 patients with T1 high grade NMIBC and they found statin use was independently associated with a lower risk of recurrence (HR:0.80, 95%CI: 0.67-0.95; P=0.009). The median recurrence-free survival was 47 (95%CI 40-49)

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months in those classified as non-statin users vs. 53 (95%CI 48-68) months in those classified as statin users [25]. Further ~~research~~ investigations ~~is warranted~~are needed to ~~investigate~~ assess the potential benefits of statins usage in NMIBC patients.

In ~~short~~conclusion, our findings ~~highlights~~suggest that different full-dose adequate BCG treatment had similar long-term survival benefits, which encourage clinical practice follow the current guideline. It is possible to postulate ~~And~~potential ~~some potential enhanced~~ benefits from drugs related to chronic diseases (i.e.: hyperlipidemia)~~-~~, that however needs further investigations~~should also be investigate~~.

~~There are several limitations in our study~~Several limitations need to be reported in our study. First, our BCG patients and their medical conditions were retrieved primarily by diagnostic codes. Although the same electronic system was used in all public hospitals in Hong Kong, there could be missing data and the data accuracy can be affected. Second, only three strains (Connaught, Tokyo, and Danish strain) were available and used in Hong Kong for the past two decades. Therefore, we were not able to extend our analysis to ~~other~~ all of the currently available BCG strains worldwide. Third, some data such as the histological reports and imaging results could not be retrieved through the electronic database. As urologists in Hong Kong follow the EAU guidelines closely, we assumed that intravesical BCG was only use for patients with intermediate- and high-risk NMIBC. Recurrence was defined by the need of TURBT, and progression event was defined by the need of cystectomy, radiotherapy or initiation of systemic treatment. However, these assumptions are prone to error, and the data accuracy might be affected. Despite these limitations, this is by far the largest BCG cohort around the world with long-term survival data, and we do believe our study provide important information regarding the efficacy of BCG in the long-run.

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5. Conclusion

~~In conclusion, adequate~~Adequate BCG remains the most important factor to optimize survival outcomes in patients with intermediate- and high-risk NMIBC. We did not observe any significant differences in survival outcomes were between full-dose Connaught, Tokyo, and Danish strains. Patients who received reduced-dose Connaught strain ~~was associated with~~had ~~the~~ worst survival outcomes.

Figure and Table

PRIOR PRESENTATION

Presented in part at the European Association of Urology annual meeting, Milan, Italy, March 10-13, 2023.

DATA SHARING STATEMENT

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

All authors declare they have no conflicts of interest to disclose.

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