

ORIGINAL ARTICLE

Early Experience With Pembrolizumab in Bacillus Calmette-Guérin Unresponsive Non-Muscle-Invasive Bladder Cancer

Chung Un Lee¹, Wan Song¹, Minyong Kang¹, Hyun Hwan Sung¹, Hwang Gyun Jeon¹,
Seong Il Seo¹, Seong Soo Jeon¹, Se Hoon Park², Byong Chang Jeong¹

¹Department of Urology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

²Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Received November 8, 2023
Revised November 15, 2023
Accepted November 15, 2023

Corresponding author:

Byong Chang Jeong
Department of Urology, Samsung
Medical Center, Sungkyunkwan
University School of Medicine,
81 Irwon-ro, Gangnam-gu, Seoul
06351, Korea

Email: bc2.jung@samsung.com
<https://orcid.org/0000-0002-5399-2184>

Purpose: Radical cystectomy (RC) is recommended for patients with non-muscle-invasive bladder cancer (NMIBC) who are unresponsive to intravesical bacillus Calmette-Guérin (BCG) instillation. However, RC is a very risky treatment, and some patients cannot undergo RC due to old age, patient preference, and comorbidities. In this study, we investigated the efficacy of pembrolizumab, a programmed cell death protein 1 inhibitor, in patients with NMIBC unresponsive to intravesical BCG instillation.

Materials and Methods: Between December 2016 and February 2023, 24 patients who experienced recurrence after BCG treatment and subsequently received pembrolizumab were enrolled. We evaluated the patients' response to pembrolizumab therapy using urine cytology, cystoscopic examination (with/without biopsy), and/or computed tomography imaging. The primary endpoint was the complete response (CR) rate 3 months after the first dose of pembrolizumab. Patients were followed up every 3 months for the first 2 years and every 6 months thereafter. Kaplan-Meier survival analysis was used to illustrate CR and the individual treatment course was demonstrated.

Results: The median follow-up period was 16 months (range, 2–68 months) and the median number of pembrolizumab administrations was 5 times (range, 3–39 times). Thirteen of the 18 patients (54.2%) with BCG-unresponsive NMIBC achieved CR at 3 months. The median duration of CR maintenance was 15 months (range, 5–47 months). Five patients (20.8%) showed no recurrence for 12 months after pembrolizumab administration. Seven patients underwent RC, and pathological reports showed T2 stage in 3 patients. To date, 1 patient (4.2%) has died.

Conclusions: Our early experience with pembrolizumab treatment for BCG-unresponsive NMIBC showed better results than those of the KEYNOTE-057 trial, which reported a CR rate of 40% at 3 months. However, long-term data and more cases are required to establish pembrolizumab therapy in patients with BCG-unresponsive NMIBC in a real-world setting.

Key Words: BCG vaccine, Non-muscle invasive bladder neoplasms, Pembrolizumab

INTRODUCTION

Bladder cancer (BC) is the tenth most prevalent cancer globally, with an annual incidence of approximately 573,000

cases, and 213,000 deaths [1]. The most common subtype is urothelial BC. Approximately 75% of patients initially present with non-muscle-invasive bladder cancer (NMIBC) confined to the bladder mucosa and submucosa [2,3].

Treatment of NMIBC primarily consists of localized treatment and surveillance. The high prevalence of this stage is due to its nonaggressive characteristics. In cases of NMIBC with a high risk of cancer recurrence and progression, adjuvant intravesical instillation of bacillus Calmette-Guérin (BCG) immunotherapy is considered [2,3].

Intravesical BCG instillation after transurethral resection of bladder tumor (TURBT) has been demonstrated to decrease recurrence significantly [4]. However, recurrence is identified within 5 years of starting BCG treatment in approximately half of high-risk cases, and the prognosis for patients with BCG treatment failure is poor [5-7]. Several guidelines recommend radical cystectomy (RC) for patients who develop carcinoma *in situ* (CIS) and/or high-grade tumor recurrence despite sufficient BCG therapy due to the significant risk of disease progression [2,3].

However, as RC can degrade the quality of life [8], many patients opt for bladder preservation. Furthermore, many patients suffer from concurrent illnesses such as cardiovascular or pulmonary diseases due to tobacco exposure, which makes them unsuitable candidates for RC. Hence, there is a clinical need for alternative bladder-preserving treatments for patients with NMIBC who do not respond to BCG.

Pembrolizumab, a U.S. Food and Drug Administration (FDA)-approved immune checkpoint inhibitor (ICI), is an IgG4 anti-programmed cell death protein 1 (PD-1) humanized antibody. It functions by attaching to PD-1, thereby blocking the binding between PD-1 and its ligands, programmed death-ligand 1 (PD-L1) and programmed death-ligand 2. FDA approval was granted based on a randomized, phase 3 trial known as KEYNOTE-045, which was an open-label study that assigned 542 randomly selected patients who had recurrence or progression following platinum therapy [9]. Pembrolizumab also gained approval as a first-line therapy for cisplatin-ineligible patients with metastatic urothelial carcinoma based on early data from the phase 2 KEYNOTE-052 study [10,11].

The KEYNOTE-057 study demonstrated that pembrolizumab monotherapy was well tolerated and showed promising results in patients with BCG-unresponsive NMIBC who were unsuitable for or refused RC [12]. Consequently, it should be considered as a clinically active nonsurgical

treatment alternative for this challenging patient population. In this study, we investigated the efficacy of pembrolizumab, a PD-1 inhibitor, in patients with NMIBC that was unresponsive to intravesical BCG administration in a real-world setting.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the Samsung Medical Center (IRB No. 2023-07-146-001), which waived the requirement for informed consent because of the retrospective nature of this study. All the study protocols were performed in accordance with the principles of the Declaration of Helsinki.

We retrospectively reviewed the records of patients who experienced recurrence after BCG treatment and subsequently received pembrolizumab treatment between December 2016 and February 2023. Patients who experienced recurrence after intravesical BCG instillation were included in this study. They were pathologically diagnosed with urothelial carcinoma by TURBT before intravesical BCG instillation, and NMIBC was confirmed by TURBT before pembrolizumab administration. Among the included patients, 1 patient was diagnosed with muscle-invasive BC at the first TURBT; however, the patient wanted bladder preservation. Intravesical BCG instillation was performed as part of the treatment, and then NMIBC was confirmed by TURBT before pembrolizumab administration.

When intermediate-to-high NMIBC was confirmed after TURBT, intravesical BCG induction was initiated and performed 6 times weekly. After intravesical BCG induction, the response was evaluated through a cystoscopic examination with or without biopsy after 3 months. Depending on the response, a second intravesical BCG induction or intravesical BCG maintenance was performed 3 times weekly, and after 3 months, post-BCG cystoscopic examination with/without biopsy was used to evaluate the response. In the absence of recurrence, intravesical BCG maintenance was continued every 6 months.

BCG-unresponsive NMIBC was defined as persistent CIS, high-grade Ta tumors, or high-grade T1 tumors at 6 months after receiving adequate BCG therapy. Adequate BCG therapy referred to the administration of at least 5 of

6 induction doses and 2 of three maintenance treatments of BCG, or at least 2 of 6 instillations of a second induction course when maintenance BCG was not provided.

The definition of BCG-unresponsive NMIBC also included patients who experienced recurrences of high-grade Ta or T1 NMIBC within 6 months, or CIS within 12 months following a disease-free state after BCG treatment; patients who continued to exhibit persistent high-grade Ta or CIS, or showed progression to T1 disease after BCG therapy, were also considered to have BCG-unresponsive NMIBC.

Patients with BCG-unresponsive NMIBC received intravenous pembrolizumab 200 mg every 3 weeks. We evaluated the patient's response to pembrolizumab therapy via urine cytology, cystoscopic examination (with/without biopsy), and/or CT imaging after 4 administrations. Patients were followed up every 3 months for the first 2 years and every 6 months thereafter. The primary endpoint was the complete response (CR) rate 3 months after the first dose of pembrolizumab. Secondary outcomes included duration of response, progression-free survival, complications, and recurrence after pembrolizumab treatment.

Descriptive statistics included frequencies and proportions of categorical variables. Continuous variables are presented as median (range). Kaplan-Meier survival analysis was used to illustrate CR and demonstrate the individual treatment course. All statistical analyses were performed using IBM SPSS Statistics ver. 27.0 (IBM Co., Armonk, NY, USA).

RESULTS

1. Baseline Characteristic

In total, 24 patients who experienced recurrence after BCG treatment and subsequently underwent pembrolizumab treatment were analyzed. As shown in Table 1, the median age was 70.5 years (range, 47–85 years), and 5 patients had a history of surgery for upper urinary tract carcinoma (3 and 2 patients had underwent radical nephroureterectomy and distal ureterectomy, respectively). Eight patients (33.3%) had CIS on the initial TURBT, and 15 patients (62.5%) had CIS on TURBT just before the administration of pembrolizumab. The median number of pembrolizumab administration was 5 times (range, 3–39 times) and the duration was 3 months

(1–64 months). The total follow-up period was 16 months (2–68 months).

2. Response to Pembrolizumab

The primary outcome, CR at the first assessment (usually 3 months after pembrolizumab administration), was observed in 54.2% (13 patients) in this study. CR was maintained for 15 months (range, 5–47 months) in these patients. Eleven patients (45.8%) showed CR at the second assessment (usually 6 months after pembrolizumab administration). Five patients (20.8%) maintained a recurrence-free status 1 year after pembrolizumab administration (Table 2).

The median duration of CR at onset was 22 months (95% confidence interval, 9.1–34.9), and 5 of 13 patients (38.5%) had CR for 12 months or longer (Fig. 1). The detailed responses and clinical courses of the individual patients are shown in Fig 2.

Eight patients (33.3%) experienced adverse events following pembrolizumab administration. Fatigue was the most

Table 1. Baseline characteristics of patients with BCG-unresponsive NMIBC (N=24)

Characteristic	Value
Age (yr)	70.5 (47–85)
Sex	
Male	21 (87.5)
Female	3 (12.5)
Body mass index (kg/m ²)	25.2 (20.4–28.9)
Hypertension	16 (66.7)
Diabetes mellitus	8 (33.3)
History of upper tract urothelial carcinoma	5 (20.8)
Pathology at 1st TURB	
CIS only	4 (16.7)
Ta	2 (8.3)
T1	13 (5.2)
T1 + CIS	4 (16.7)
T2	1 (4.2)
Pathology before pembrolizumab	
CIS only	6 (25.0)
Ta	2 (8.3)
Ta + CIS	1 (4.2)
T1	7 (29.2)
No. of pembrolizumab administration	5 (3–39)
Duration of pembrolizumab administration (mo)	3 (1–64)
Total follow-up (mo)	16 (2–68)
T1 + CIS	8 (33.3)

Values are presented as median (range) or number (%).

BCG, bacillus Calmette-Guérin; NMIBC, non-muscle-invasive bladder cancer; TURB, transurethral resection of bladder tumor; CIS, carcinoma *in situ*.

common symptom, followed by pruritus, and features of hyperthyroidism (Table 3).

Table 2. Complete response and recurrence-free survival in patients with BCG-unresponsive NMIBC (N=24)

Variable	Value
Patients with complete response at 3 months	13 (54.2)
Duration of complete response (mo)	15 (5–47)
Patients who were free from recurrence	
6 Months	10 (41.7)
9 Months	8 (33.3)
12 Months	5 (20.8)

Values are presented as number (%) or median (range).

BCG, bacillus Calmette-Guérin; NMIBC, non-muscle-invasive bladder cancer.

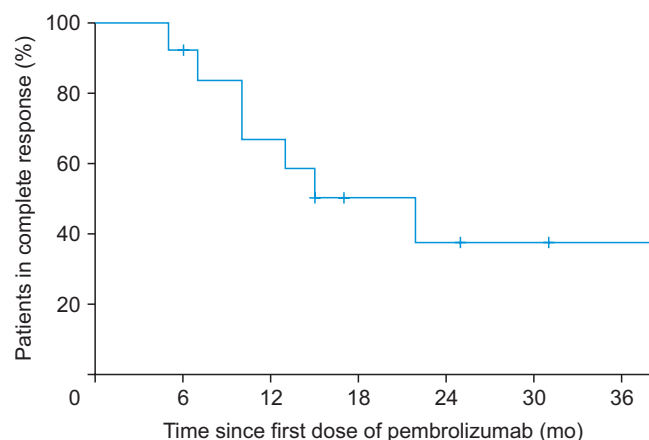


Fig. 1. Kaplan-Meier survival analysis depicting the complete response rate after the first dose of pembrolizumab.

3. Treatment for Patients With Recurrence After Pembrolizumab

No CR was observed in 11 patients during the first assessment. Eight patients (72.7%) either underwent RC or planned RC (Fig. 2). Three patients either planned or underwent other intravesical therapies, including gemcitabine and mitomycin.

In total, 7 patients (29.2%) underwent RC because of recurrence after pembrolizumab administration. There was no aggravation of pathological results after TURBT when comparing the pathology before RC with that before pembrolizumab administration. When comparing the pathology of RC with that before pembrolizumab administration, aggravation of pathology was observed in 3 patients (Table 4).

Table 3. Treatment-related adverse events (N=24)

Adverse events	No. (%)
Any	8 (33.3)
Fatigue	4 (16.7)
Pruritis	2 (8.3)
Hyperthyroidism	2 (8.3)
Hypothyroidism	1 (4.2)
Rash	1 (4.2)
Dizziness	1 (4.2)
Diaphoresis	1 (4.2)
Arthralgia	1 (4.2)
Constipation	1 (4.2)

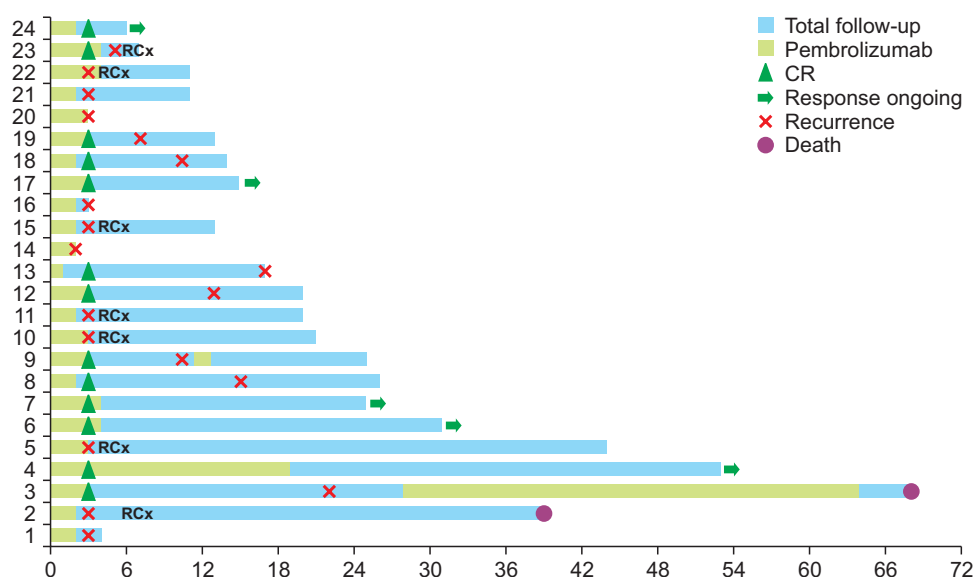


Fig. 2. Detailed response and clinical course for individual patients following the first dose of pembrolizumab. RCx, radical cystectomy; CR, complete response.

Table 4. Pathological stage at the time of radical cystectomy in patients who discontinued pembrolizumab

Pathology before pembrolizumab	Pathology before RCx.	Interval between initial dose of pembrolizumab and RCx. (mo)	Interval between last dose of pembrolizumab and RCx. (mo)	No. of pembrolizumab doses	Pathology after RCx.
T1 + CIS	T1	7	4	5	T1
T1	T1	5	2	6	T2 + CIS
T1 + CIS	CIS	6	2	6	T2
T1 + CIS	T1	4	2	4	TX
T1 + CIS	T1G3 + CIS	4	2	4	T1
T1 + CIS	Ta + CIS	7	2	4	T1 + CIS
T1	-	6	2	7	T2

RCx., radical cystectomy; CIS, carcinoma *in situ*.

DISCUSSION

Although platinum-based combination chemotherapy continues to be the primary treatment for advanced BC, the surge in the understanding of the biological intricacies of BC has spurred a growing interest in ICIs and molecularly targeted treatments [13]. BC is characterized by frequent mutations [14], and its high tumor mutational burden makes it susceptible to ICIs targeting PD-1 and its ligand, PD-L1 [15,16]. Consequently, ICIs that inhibit PD-1 or PD-L1 have gained FDA approval for the first- and second-line treatment of metastatic BC.

Additionally, a role for the PD-1–PD-L1 pathway in fostering resistance to BCG in NMIBC has been proposed. Tumors that have recurred or progressed following BCG treatment have been found to exhibit a noticeable increase in PD-L1 expression compared to tumors that have never been treated with BCG. Furthermore, increased PD-L1 expression has been associated with recurrence and progression [17].

Despite RC being recommended as a treatment option for BCG-unresponsive NMIBC by several guidelines [2,3], the demand for alternative treatments has surged because of the high complication rate associated with RC and patients' preference for preserving the bladder. Based on the findings of the PD-L1 study, pembrolizumab is expected to be a viable treatment option for BCG-unresponsive NMIBC.

This study aimed to investigate the efficacy of pembrolizumab in patients with NMIBC who did not respond to intravesical BCG instillation through our initial experience. The tolerability and antitumor activity of pembrolizumab in patients with BCG-unresponsive NMIBC were reported in the KEYNOTE-057 study [12]. However, there have been no studies on pembrolizumab in patients with NMIBC

in a clinical setting. In real clinical situations, a patient's disease state is not constant, and there are various factors to be considered. Our study demonstrated the efficacy of pembrolizumab in patients with BCG-unresponsive NMIBC.

In our study, the primary outcome, CR at the first assessment (usually at 3 months after pembrolizumab administration), was observed in 54.2% of patients; among them, 38.5% maintained a recurrence-free status at 1 year after pembrolizumab administration. The KEYNOTE-057 study showed a 41% CR rate at 3 months after the administration of pembrolizumab for patients with BCG-unresponsive CIS of the bladder with or without papillary tumors, and 46% of responders remained in CR for 12 months or longer [12]. In terms of complications, 33.3% of patients had treatment-related adverse events in our study, and 66% of patients had treatment-related adverse events in KEYNOTE-057. Our study showed better results than the KEYNOTE-057 study.

Additionally, we analyzed the pathological results of patients who underwent RC after the failure of pembrolizumab treatment. Seven patients underwent RC; among them, 3 patients showed upgrading of T stage to T2, and 1 patient showed downgrading of T stage to TX. Although the patients experienced aggravation of the disease stage, they were able to receive appropriate treatment by undergoing RC. Therefore, the period before surgical treatment can be extended by administering pembrolizumab. Although pembrolizumab treatment results in failure of disease control, it can be properly managed by RC, and we can confirm that pembrolizumab could affect pathological complete remission. Therefore, clinicians should consider pembrolizumab administration to patients with BCG-unresponsive NMIBC.

Previous studies suggest a variance in the 1- to 2-year recurrence-free survival rates, ranging from 18% to 43%, following diverse salvage therapies in patients experiencing BCG treatment failure [18-23]. Consequently, the International Bladder Cancer Group posits a benchmark for clinical significance, advocating for an initial CR rate of 50% at the 6-month interval, coupled with sustained response rates of 30% at 12 months and 25% at 18 months in patients with BCG-unresponsive CIS. In instances of BCG-unresponsive papillary disease, recurrence-free benchmarks of 30% at 12 months and 25% at 18 months are deemed clinically significant [5]. Our study, albeit lacking a control cohort of NMIBC patients not treated with pembrolizumab, demonstrates that pembrolizumab surpasses the efficacy of extant salvage therapies, aligning with the recommendations of the International Bladder Cancer Group.

Some studies have attempted to demonstrate the efficacy of novel treatment options in patients with BCG-unresponsive NMIBC. One concerns hyperthermic intravesical chemotherapy for BCG-unresponsive NMIBC. The study reported that the 3-month, 6-month, 9-month, and 1-year cumulative incidence rates of disease recurrence/progression were 25%, 35%, 44%, and 53%, respectively [24]. Another option is intravesical gemcitabine treatment. There have been some reports on intravesical gemcitabine use in patients with NMIBC with recurrence after intravesical BCG instillation. Hurle et al. [25] reported a disease-free survival rate of 68.8% after induction (once a week for 6 consecutive weeks) and 44.4% disease-free survival after 12 months of treatment. Skinner et al. [19] reported 47% and 28% CR rates at 3 and 12 months, respectively. Another study reported on intravesical nadofaragene firadenovec gene therapy for BCG-unresponsive NMIBC. Boorjian et al. [26] reported 53.4% and 45.5% CR rates at 3 and 12 months, respectively, after initial administration. Direct comparison between these treatments is difficult because each study has a different group of patients and research methods; however, based on the results of our study, it can be suggested that pembrolizumab can also be considered as a treatment option in patients with BCG-unresponsive NMIBC.

Despite the strengths of this study, it has several limitations. First, the retrospective design may have resulted in a significant selection bias. Second, it is limited to establishing

the effect of pembrolizumab, and additional analysis of factors such as treatment success was not possible due to the relatively small cohort. Third, the patients were not uniform in terms of tumor characteristics or previous therapy. Additionally, the absence of PD-L1 testing in this study represents a missed opportunity for more detailed understanding of pembrolizumab's clinical implications. Further, well-designed studies are required to determine the clinical significance and efficacy of pembrolizumab therapy in patients with BCG-unresponsive NMIBC.

CONCLUSIONS

Our early experience with pembrolizumab treatment for BCG-unresponsive NMIBC showed better results than those of the KEYNOTE-057 trial. Pembrolizumab treatment can be considered in patients with BCG-unresponsive NMIBC who decline or are not eligible for RC. However, long-term data and more cases are needed to establish pembrolizumab treatment for patients with BCG-unresponsive NMIBC in real-world settings.

NOTES

- **Conflicts of Interest:** MK and HHS, members of the Editorial Board of *Journal of Urologic Oncology*, is the co-first authors of this article. However, they played no role whatsoever in the editorial evaluation of this article or the decision to publish it. The other authors have nothing to disclose.
- **Funding/Support:** This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
- **Author Contribution:** Conceptualization: CUL; Data curation: CUL, WS, MK, HHS, HGJ, SIS, SSJ, SHP; Formal analysis: CUL, WS; Methodology: WS, MK, HHS; Project administration: BCJ; Visualization: CUL; Writing - original draft: CUL; Writing - review & editing: BCJ.
- **ORCID**
Chung Un Lee: <https://orcid.org/0000-0002-3030-0405>
Wan Song: <https://orcid.org/0000-0003-0971-1805>
Minyong Kang: <https://orcid.org/0000-0002-6966-8813>
Hyun Hwan Sung: <https://orcid.org/0000-0001-9845-3742>

Hwang Gyun Jeon: <https://orcid.org/0000-0002-5613-8389>

Seong Il Seo: <https://orcid.org/0000-0002-9792-7798>

Seong Soo Jeon: <https://orcid.org/0000-0002-3265-6261>

Se Hoon Park: <https://orcid.org/0000-0001-5084-9326>

Byong Chang Jeong: <https://orcid.org/0000-0002-5399-2184>

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209-49.
2. Chang SS, Bochner BH, Chou R, Dreicer R, Kamat AM, Lerner SP, et al. Treatment of non-metastatic muscle-invasive bladder cancer: AUA/ASCO/ASTRO/SUO guideline. *J Urol* 2017;198:552-9.
3. Babjuk M, Burger M, Capoun O, Cohen D, Comperat EM, Dominguez Escrig JL, et al. European Association of Urology guidelines on non-muscle-invasive bladder cancer (Ta, T1, and carcinoma in situ). *Eur Urol* 2022;81:75-94.
4. Han RF, Pan JG. Can intravesical bacillus Calmette-Guerin reduce recurrence in patients with superficial bladder cancer? A meta-analysis of randomized trials. *Urology* 2006;67:1216-23.
5. Kamat AM, Sylvester RJ, Bohle A, Palou J, Lamm DL, Brausi M, et al. Definitions, end points, and clinical trial designs for non-muscle-invasive bladder cancer: recommendations from the International Bladder Cancer Group. *J Clin Oncol* 2016;34:1935-44.
6. Oddens J, Brausi M, Sylvester R, Bono A, van de Beek C, van Andel G, et al. Final results of an EORTC-GU cancers group randomized study of maintenance bacillus Calmette-Guerin in intermediate- and high-risk Ta, T1 papillary carcinoma of the urinary bladder: one-third dose versus full dose and 1 year versus 3 years of maintenance. *Eur Urol* 2013;63:462-72.
7. Li R, Tabayoyong WB, Guo CC, Gonzalez GMN, Navai N, Grossman HB, et al. Prognostic implication of the United States Food and Drug Administration-defined BCG-unresponsive disease. *Eur Urol* 2019;75:8-10.
8. Yang LS, Shan BL, Shan LL, Chin P, Murray S, Ahmadi N, et al. A systematic review and meta-analysis of quality of life outcomes after radical cystectomy for bladder cancer. *Surg Oncol* 2016;25:281-97.
9. Fradet Y, Bellmunt J, Vaughn DJ, Lee JL, Fong L, Vogelzang NJ, et al. Randomized phase III KEYNOTE-045 trial of pembrolizumab versus paclitaxel, docetaxel, or vinflunine in recurrent advanced urothelial cancer: results of >2 years of follow-up. *Ann Oncol* 2019;30:970-6.
10. Balar AV, Castellano D, O'Donnell PH, Grivas P, Vuky J, Powles T, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *Lancet Oncol* 2017;18:1483-92.
11. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N Engl J Med* 2017;376:1015-26.
12. Balar AV, Kamat AM, Kulkarni GS, Uchio EM, Boormans JL, Roumiguie M, et al. Pembrolizumab monotherapy for the treatment of high-risk non-muscle-invasive bladder cancer unresponsive to BCG (KEYNOTE-057): an open-label, single-arm, multicentre, phase 2 study. *Lancet Oncol* 2021;22:919-30.
13. Patel VG, Oh WK, Galsky MD. Treatment of muscle-invasive and advanced bladder cancer in 2020. *CA Cancer J Clin* 2020;70:404-23.
14. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013;499:214-8.
15. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature* 2013;500:415-21.
16. Aggen DH, Drake CG. Biomarkers for immunotherapy in bladder cancer: a moving target. *J Immunother Cancer* 2017;5:94.
17. Fukumoto K, Kikuchi E, Mikami S, Hayakawa N, Matsumoto K, Niwa N, et al. Clinical role of programmed cell death-1 expression in patients with non-muscle-invasive bladder cancer recurring after initial bacillus Calmette-Guerin therapy. *Ann Surg Oncol* 2018;25:2484-91.
18. Dalbagni G, Russo P, Bochner B, Ben-Porat L, Sheinfeld J, Sogani P, et al. Phase II trial of intravesical gemcitabine in bacille Calmette-Guerin-refractory transitional cell carcinoma of the bladder. *J Clin Oncol* 2006;24:2729-34.
19. Skinner EC, Goldman B, Sakr WA, Petrylak DP, Lenz HJ, Lee CT, et al. SWOG S0353: Phase II trial of intravesical gemcitabine in patients with nonmuscle invasive bladder cancer and recurrence after 2 prior courses of intravesical bacillus Calmette-Guerin. *J Urol* 2013;190:1200-4.
20. Morales A, Herr H, Steinberg G, Given R, Cohen Z, Amrhein J, et al. Efficacy and safety of MCNA in patients with nonmuscle invasive bladder cancer at high risk for recurrence and progression after failed treatment with bacillus Calmette-Guerin. *J Urol* 2015;193:1135-43.
21. Rosevear HM, Lightfoot AJ, Birusingh KK, Maymi JL, Nepple KG, O'Donnell MA, et al. Factors affecting response to bacillus Calmette-Guerin plus interferon for urothelial carcinoma in situ. *J Urol* 2011;186:817-23.

22. Steinberg G, Bahnson R, Brosman S, Middleton R, Wajzman Z, Wehle M. Efficacy and safety of valrubicin for the treatment of Bacillus Calmette-Guerin refractory carcinoma in situ of the bladder. The Valrubicin Study Group. *J Urol* 2000;163:761-7.
23. Gallagher BL, Joudi FN, Maymi JL, O'Donnell MA. Impact of previous bacille Calmette-Guerin failure pattern on subsequent response to bacille Calmette-Guerin plus interferon intravesical therapy. *Urology* 2008;71:297-301.
24. de Jong JJ, Hendricksen K, Rosier M, Mostafid H, Boormans JL. Hyperthermic intravesical chemotherapy for BCG unresponsive non-muscle invasive bladder cancer patients. *Bladder Cancer* 2018;4:395-401.
25. Hurlle R, Casale P, Morengi E, Saita A, Buffi N, Lughezani G, et al. Intravesical gemcitabine as bladder-preserving treatment for BCG unresponsive non-muscle-invasive bladder cancer. Results from a single-arm, open-label study. *BJUI Compass* 2020;1:126-32.
26. Boorjian SA, Alemozaffar M, Konety BR, Shore ND, Gommella LG, Kamat AM, et al. Intravesical nadofaragene firadenovec gene therapy for BCG-unresponsive non-muscle-invasive bladder cancer: a single-arm, open-label, repeat-dose clinical trial. *Lancet Oncol* 2021;22:107-17.