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Oncolytic adenoviral therapy plus pembrolizumab in BCG-unresponsive non-muscle-invasive bladder cancer: the phase 2 CORE-001 trial

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Cretostimogene grenadenorepvec is a serotype-5 oncolytic adenovirus designed to selectively replicate in cancer cells with retinoblastoma pathway alterations, previously tested as monotherapy in bacillus Calmette–Guérin (BCG)-experienced non-muscle-invasive bladder cancer. In this phase 2 study, we assessed the potential synergistic efficacy between intravesical cretostimogene and systemic pembrolizumab in patients with BCG-unresponsive non-muscle-invasive bladder cancer with carcinoma in situ (CIS). Thirty-five patients were treated with intravesical cretostimogene with systemic pembrolizumab. Induction cretostimogene was administered weekly for 6 weeks followed by three weekly maintenance infusions at months 3, 6, 9, 12 and 18 in patients maintaining complete response (CR). Patients with persistent CIS/high-grade Ta at the 3-month assessment were eligible for re-induction. Pembrolizumab was administered for up to 24 months. The primary endpoint was CR at 12 months as assessed by cystoscopy, urine cytology, cross-sectional imaging and mandatory bladder mapping biopsies. Secondary endpoints included CR at any time, duration of response, progression-free survival and safety. The CR rate in the intention-to-treat population at 12 months was 57.1% (20 out of 35, 95% confidence interval (CI) 40.7–73.5%), meeting the primary endpoint. A total of 29 out of 35 patients (82.9%, 95% CI 70.4–95.3%) derived a CR at 3 months. With a median follow-up of 26.5 months, the median duration of response has not been reached (95% CI 15.7 to not reached). The CR rate at 24 months was 51.4% (18 out of 35) (95% CI 34.9–68.0%). No patient progressed to muscle-invasive bladder cancer in this trial. Adverse events attributed to cretostimogene were low grade, self-limiting and predominantly limited to bladder-related symptoms. A total of 5 out of 35 patients (14.3%) developed grade 3 treatment-related adverse effects. There was no evidence of overlapping or synergistic toxicities. Combination intravesical cretostimogene and systemic pembrolizumab demonstrated enduring efficacy. With a toxicity

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profile similar to its monotherapy components, this combination may shift the benefit-to-risk ratio for patients with BCG-unresponsive CIS. ClinicalTrials.gov Identifier: NCT04387461.

Bladder cancer is the tenth most common cancer worldwide, amounting to 573,000 new cases in 2020, with approximately 75% presenting in the non-muscle-invasive stage (NMIBC)[1]. Carcinoma in situ (CIS) affects 10% of the patients with NMIBC and is an aggressive form associated with increased risks of recurrence and progression[2]. Standard treatment for patients with high-risk NMIBC consists of transurethral resection of bladder tumor (TURBT) followed by intravesical bacillus Calmette–Guérin (BCG). Despite high initial response rates, approximately half of the patients experience relapse. Patients with recurrent high-grade NMIBC following BCG are especially at risk for disease progression, with 20–40% risk of developing muscle-invasive bladder cancer (MIBC)[3,4], of whom over half will eventually succumb to cancer[5]. Traditionally, radical cystectomy has been the definitive standard of care to prevent disease progression and metastasis[6–8]. However, it is fraught with high perioperative morbidity, with many patients unwilling or unable to undergo the complex surgical procedure. Although myriad attempts were made to evaluate the efficacy of bladder-sparing alternatives, drug development was hindered by heterogeneity in patient population, poor definition of disease states and lack of consensus on trial endpoints[9].

To stimulate clinical trial development, a joint panel between the American Urological Association (AUA) and the US Food and Drug Administration (FDA) was convened in 2013. From the discussion, BCG-unresponsive (BCG-UR) NMIBC was defined as persistent or recurrent high-grade NMIBC within 6–12 months despite adequate BCG therapy[10–12]. Additionally, the panel agreed that, as CIS cannot be completely resected at the time of diagnosis, randomized control trials using a placebo arm would be unethical. Without an effective standard bladder-preserving therapy to be used as comparison, a single-arm trial design with complete response (CR) rate and other key efficacy endpoints such as duration of response (DoR) would be acceptable for registrational purposes.

Cretostimogene grenadenorepvec (CG0070), a serotype-5 oncolytic adenovirus, was designed to selectively replicate in cancer cells with retinoblastoma pathway alterations[13]. In addition, cretostimogene also expresses the cytokine granulocyte macrophage colony-stimulating factor, adding to local and systemic cancer control[13]. Cretostimogene was formulated for intravesical infusion and sequentially administered following 0.1% *n*-dodecyl-B-D-maltoside, which was used to enhance adenovirus infection of the urothelium. In a phase 1 dose-finding study in 35 patients with recurrent NMIBC following BCG, viral replication was found to peak at day 2 following primary infusion, with sustained activity out to day 5 post-treatment in the majority of treated patients[14]. Exploratory efficacy rates indicated recommended phase 2 dosing regimen of 1×10^{12} vp infused weekly for 6 weeks. In a subsequent phase 2 study conducted in a more heavily pretreated patient population, 6-month CR was found to be 47% (95% CI 32–62%)[15]. Following the success of the early phase trials, a phase 3, open-label, single-arm study was launched in 2020, accruing 115 patients with BCG-UR CIS (NCT04452591). Given the observed activity and safety of cretostimogene monotherapy, as well as the demonstrated efficacy of pembrolizumab in BCG-UR NMIBC, in this Article, the phase 2 CORE-001 study was designed to assess for superior 12-month CR compared to pembrolizumab alone using the combination of these two agents as well as the safety of this combination strategy.

Results

Patients with BCG-UR CIS with or without concurrent Ta/T1 disease according to the 2018 US FDA guidance[16] were enrolled. All patients were ineligible for or refused radical cystectomy on the basis of informed consent and had not received prior anti-PD-1, anti-PD-L1 inhibitor or adenovirus-based therapy (for example, nadofaragene firadenovec). Before study treatment, all visible tumors were completely resected, with mandatory re-resection performed on patients with T1 disease. Cretostimogene was administered as six weekly intravesical instillations in the induction phase followed by three weekly maintenance instillations at months 3, 6, 9, 12 and 18, or until disease persistence or recurrence. If patients presented with persistent CIS or high-grade Ta NMIBC at month 3, a second induction course of cretostimogene was administered. Pembrolizumab was administered every 6 weeks for approximately 2 years or until disease persistence or recurrence. Disease assessment included quarterly cystoscopies and centrally reviewed urine cytologies (with for-cause biopsy if warranted), as well as a predefined mandatory bladder mapping biopsies performed at the 12-month time point. CR at each time point was defined as negative biopsy, cystoscopy, cytology and axial imaging. The primary endpoint was the proportion of patients with CR at the 12-month assessment. Secondary endpoints included CR at any time point, DoR, defined as time from first CR to documented recurrence, progression or death. Additionally, proportional and time to event analyses evaluating high-grade-recurrence-free survival (as a surrogate measure of DoR), radical cystectomy-free survival, progression-free survival (PFS) and overall survival (OS) were assessed. Safety endpoints included type, incidence, relatedness and severity of adverse events (AEs).

Between 4 November 2020, and 16 August 2022, 44 patients were screened for eligibility, 9 of whom did not meet the study entry criteria (Supplementary Table 1), resulting in an intention-to-treat (ITT) cohort of 35 patients (Fig. 1). All patients enrolled met the FDA BCG-UR definition[16]. No patient with bladder cancer of variant subtype was included. The median age was 72 years (range 57–90), with male predominance (Table 1). A majority of the patients (27 out of 35, 77.1%) had pure CIS, while 4 (11.4%) each had concurrent Ta and T1 high-grade papillary disease. The cohort had been heavily pretreated with a median of 12 (range 9–30) instillations of intravesical BCG before enrollment.

Of 35 patients in the ITT population, 20 (57.1%, 95% CI 40.7–73.5%) had durable CR at 12 months as assessed by mandatory bladder mapping biopsy, rejecting the prespecified null hypothesis of a CR rate ≤19% at $P < 0.0001$. From preplanned analyses of CR at earlier time points, 29 out of 35 (82.9%, 95% CI 70.4–95.3%) derived a CR at 3 months. Two patients received cretostimogene re-induction, neither of whom converted to CR. With a median follow-up of 26.5 months (range 17–37.5 months), median DoR was not reached but exceeded 21 months (95% CI 15.7 to nonresponse (NR); Fig. 2). CR at 6, 18 and 24 months following treatment initiation was 77.1% (95% CI 63.2–91.1%), 54.3% (95% CI 37.8–70.8%) and 51.4% (95% CI 34.9–68.0%) respectively. Excluding 5 patients who dropped out before 12-month evaluation for reasons other than disease recurrence or progression (Supplementary Table 2), 20/30 (66.7%, 95% CI 47.1–82.1%) were complete responders at 12 months.

Overall, 10 of 35 patients developed high-grade non-muscle-invasive recurrences, none of whom suffered progression to MIBC or metastatic bladder cancer, and one (2.8%) died of non-bladder cancer-related cause (with no evidence of recurrence). The median high-grade RFS was not reached (95% CI 6.7 to not reached) (Fig. 2). Radical cystectomy was performed in 7 out of 35 patients (20%) for recurrent high-grade NMIBC, finding pT0 in one patient, pTa LG in one patient, pTa high-grade in two patients and pTis in three patients

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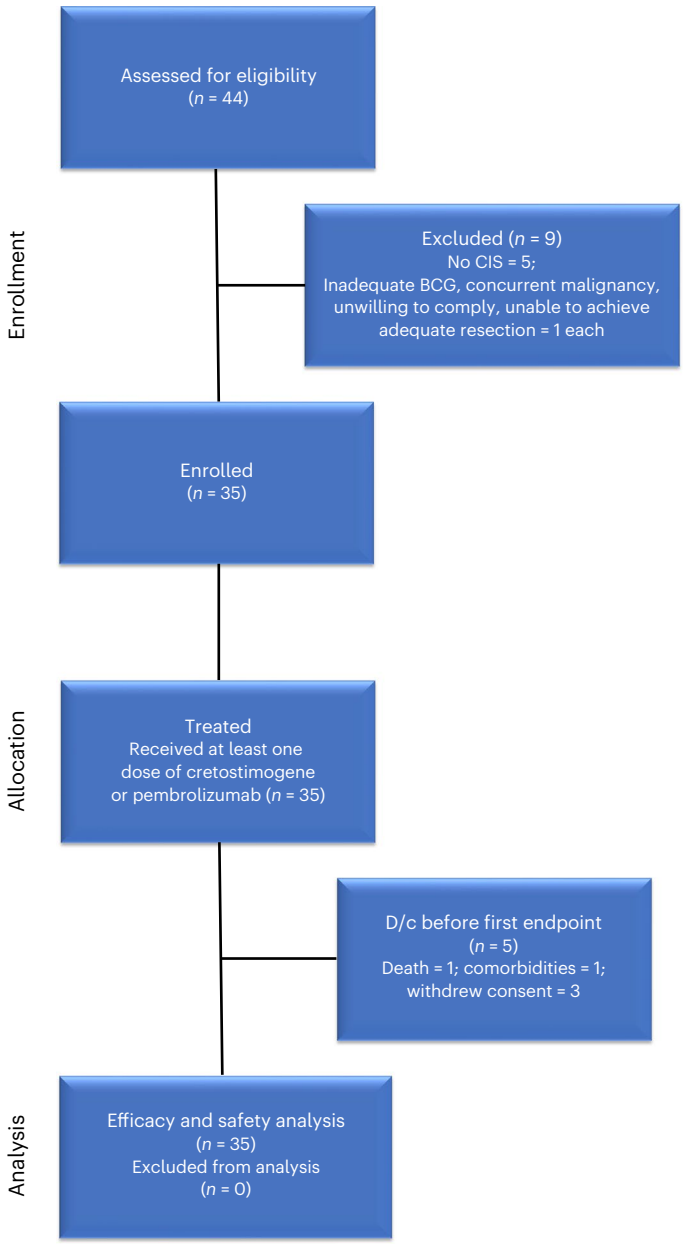

Fig. 1 | CONSORT patient flow diagram. D/c, discontinued.

Table 1 | Baseline characteristics

	n=35
Age	
Median	72
Range	57–90
Sex	
Male	33 (94.3)
Female	2 (5.7)
ECOG	
0	25 (71.4)
1	9 (25.7)
2	1 (2.9)
Country	
USA	30 (85.7)
Korea	5 (14.3)
Race	
White	27 (77.1)
Black/African American	2 (5.7)
Asian	5 (14.3)
Unknown	1 (2.9)
Stage	
CIS	27 (77.1)
CIS+high-grade Ta	4 (11.4)
CIS+high-grade T1	4 (11.4)
Prior BCG courses	
2	18 (51.4)
3	8 (22.9)
4	2 (5.7)
5	4 (11.4)
≥6	3 (8.6)

(Fig. 3a). No occult MIBC was found. Thus, PFS and OS completely overlapped (Fig. 3b).

The median number of doses of cretostimogene was 18.0 (range 16–21). The median number of doses of pembrolizumab was 8.0 (range 2–22). Treatment-related adverse events (TRAEs) attributed to cretostimogene and pembrolizumab are presented in Table 2 and Extended Data Tables 1 and 2. Related adverse effects to cretostimogene are generally low-grade, self-limiting and consistent with typical adverse effects related to intravesical treatment for NMIBC. Related adverse effect to pembrolizumab assessed by the treating physicians are consistent with systemic immunotherapy and demonstrate no unexpected immune-related adverse effects. The most common TRAEs attributed to cretostimogene were limited to bladder symptoms including bladder spasm (45.7%), dysuria (25.7%), pollakiuria (25.7%), micturition urgency (17.1%), hematuria (14.2%) and nocturia (11.4%). The most common TRAEs attributed to pembrolizumab included fatigue (31.4%), diarrhea (14.3%), hypothyroidism (11.4%), ALT (alanine transaminase) increase (8.6%), AST (aspartate aminotransferase) increase (8.6%) and arthralgia (8.6%). No grade 3 TRAE was attributed to cretostimogene. Five patients (14.3%) developed grade 3 TRAEs associated with pembrolizumab (Table 2). There were no grade 4 or 5 events related to either cretostimogene or pembrolizumab, and there was no evidence of overlapping or synergistic toxicities. No patients came off study due to cretostimogene- or pembrolizumab-related toxicity.

Discussion

BCG-UR CIS is an especially aggressive and difficult-to-treat disease. Despite increasing enthusiasm for drug development, only systemic pembrolizumab and intravesical nadofaragene firadenovec have been approved by the US FDA in the last quarter century, with CR rates between 41% and 53% at 3 months, and median DoR of 9.7–16.2 months[17,18]. In our study, the combination of cretostimogene grenadenorepvec and pembrolizumab resulted in a CR rate of 82.9% (95% CI 70.4–95.3%) at 3 months, 57.1% (95% CI 40.7–73.5%) at 12 months and a median DoR for the combination of >21 months with 51.4% (18 out of 35) (95% CI 34.9–68.0) of the ITT population continuing in response at 24 months. Moreover, the toxicity profile of the combination was similar to that described previously in monotherapy trials using cretostimogene[14,15] and pembrolizumab[17], with no synergistic toxicity signal observed. Given the favorable overall benefit-to-risk profile demonstrated in this study, this treatment combination may represent a unique long-term bladder-sparing treatment option for patients with BCG-UR CIS.

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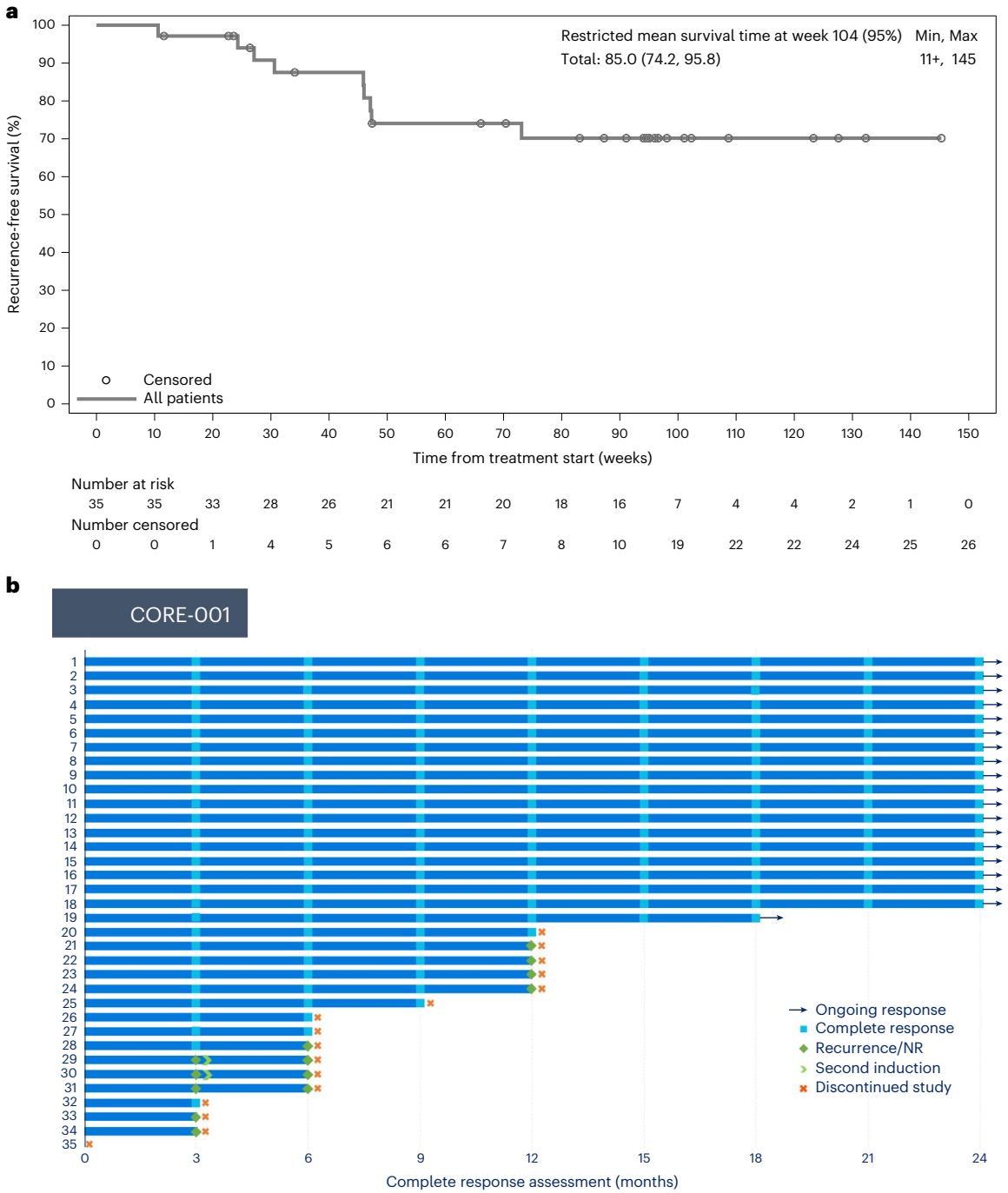

Fig. 2 | High-grade recurrence-free survival in CORE-001. a, Kaplan–Meier plot of high-grade recurrence-free survival. **b**, Swimmer plot of response to treatment from the time of treatment start.

The quest for efficacious, long-term bladder-sparing options for BCG-UR NMIBC was borne out of the need to avoid complications and decrement to quality-of-life associated with radical cystectomy, the current standard-of-care for BCG-UR. Alignment between the FDA and the bladder cancer research community enabled single-armed registrational trials in BCG-UR CIS based on the supposition that in the absence of pharmacologic intervention or cystectomy, this disease will persist and progress, precluding the possibility of placebo-controlled comparator arm. However, the 2018 FDA guidance document for BCG-UR NMIBC drug development did not specify efficacy benchmarks needed for regulatory approval. Instead, it stipulated that clinical response should be considered in the context of DoR, along with associated toxicity, the drug's mechanism of action and other measures of anti-tumor effect[16].

To fill the void, the aspirational goalposts of initial CR rate of 50% at 6 months and durable response rates of 30% at 12 months and 25% at 18 months proposed by the International Bladder Cancer Group (IBCG) have often been used as measuring sticks for single-arm studies. Despite falling short, pembrolizumab (19% CR at 12 months) and nadofaragene (24% CR at 12 months) were both approved by the FDA and have entered into the clinic. However, neither has garnered wide-spread adoption, due to concerns for the unfavorable benefit-to-risk ratio associated with pembrolizumab and manufacturing challenges for nadofaragene. Intravesical nogapendekin alfa inbakicept (NAI), an

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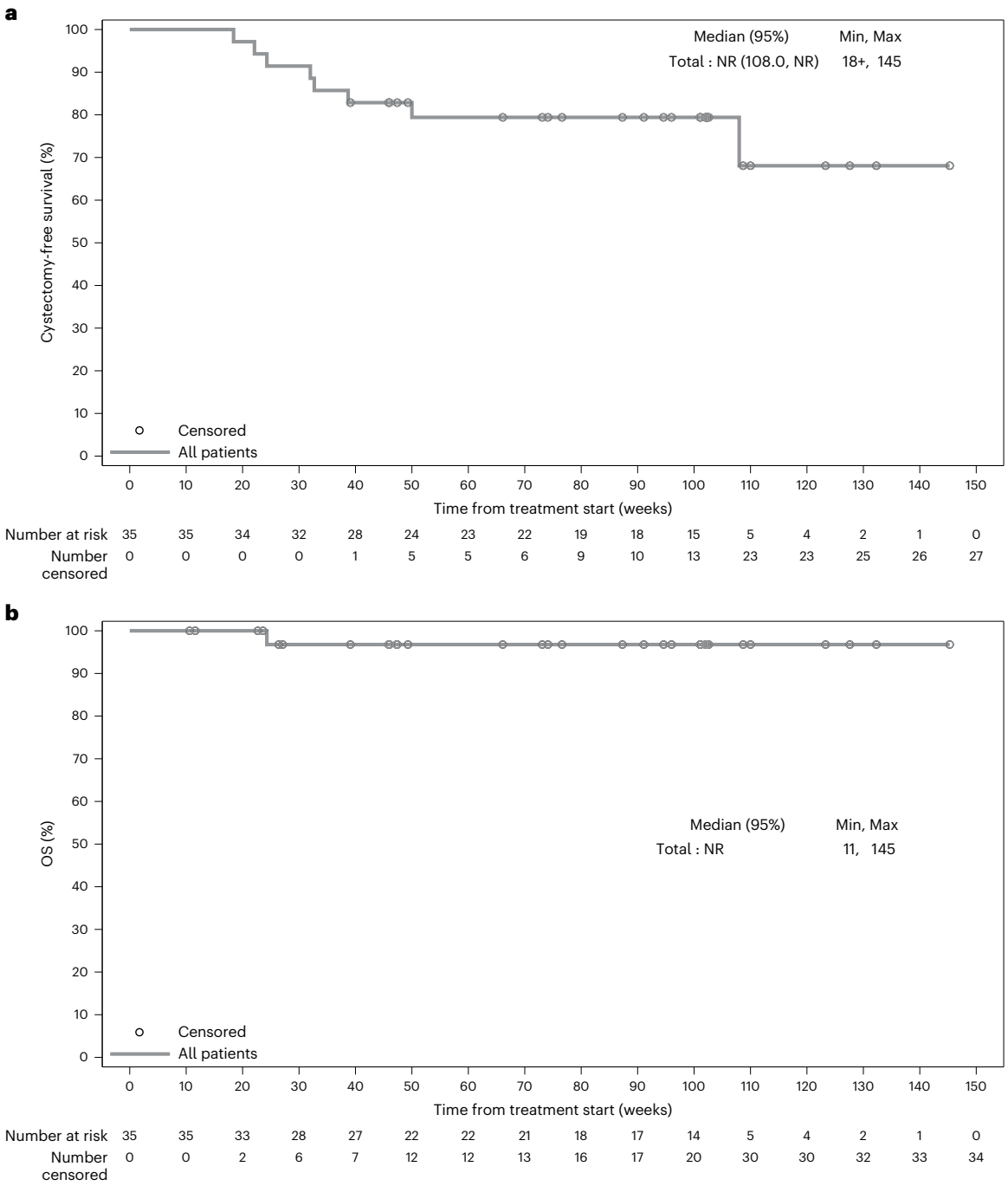

Fig. 3 | Cystectomy-free survival, PFS and OS in CORE-001. a, Kaplan–Meier plot of cystectomy-free survival. **b**, Kaplan–Meier plot of progression free and overall survival. NR, not reached.

interleukin-15 superagonist complex designed to boost the immune response primed by BCG, yielded an impressive initial CR rate of 62% when used in combination with BCG, with 36% of patients maintaining response for ≥12 months. In April 2024, combined intravesical nogapendekin alfa inbakicept and BCG also received approval from the FDA[19].

Beyond these approved agents, various other bladder-sparing strategies have been investigated, with varying degrees of success. Atezolizumab and durvalumab (PD-L1 inhibitors) yielded modest 6-month CRs of 27% (ref. 20) and 12% (ref. 21), respectively. Intravesical oportuzumab monatox, which targets tumor cells expressing Ep-CAM, reported a 3-month CR of 40% (ref. 22) but was denied regulatory approval. TAR-200, an intravesical drug-delivery system providing sustained release of gemcitabine, reported 77% CR in the first 30 patients with BCG-UR CIS[23]. Intravesical combination gemcitabine and docetaxel, the de facto 'standard' bladder-sparing therapy for patients refusing radical cystectomy, has demonstrated 12-month CR rates as high as 56% in retrospective series[24] but has never been evaluated in a prospective, rigorous fashion. Importantly, none of the agents tested so far has yielded satisfactory long-term CR rates to justify shifting the standard of care from radical cystectomy.

Intravesical delivery of oncolytic virus enables easy access to the site of disease while bypassing the systemic antiviral humoral response, thus ensuring sufficient drug delivery[25]. Additionally, as CIS is generally associated with areas of dysplasia and malignant cells that cannot be

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Table 2 | Treatment-related adverse effects

System organ class, *n* (%)/preferred term, *n* (%)	Maximum severity					
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total (*N*=35)
Participants reporting at least one study drug-related treatment-emergent adverse effects	9 (25.7)	18 (51.4)	5 (14.3)	0	0	32 (91.4)
Bladder spasm	13 (37.1)	4 (11.4)	0	0	0	17 (48.6)
Fatigue	11 (31.4)	2 (5.7)	0	0	0	13 (37.1)
Dysuria	8 (22.9)	1 (2.9)	0	0	0	9 (25.7)
Pollakiuria	8 (22.9)	1 (2.9)	0	0	0	9 (25.7)
Hematuria	5 (14.3)	1 (2.9)	0	0	0	6 (17.1)
Micturition urgency	4 (11.4)	2 (5.7)	0	0	0	6 (17.1)
Diarrhea	4 (11.4)	0	1 (2.9)	0	0	5 (14.3)
Nocturia	3 (8.6)	1 (2.9)	0	0	0	4 (11.4)
Hypothyroidism	1 (2.9)	3 (8.6)	0	0	0	4 (11.4)
Urinary tract infection	3 (8.6)	1 (2.9)	0	0	0	4 (11.4)
Blood alkaline phosphatase increased	0	0	1 (2.9)	0	0	1 (2.9)
Ejection fraction decreased	0	0	1 (2.9)	0	0	1 (2.9)
Neutrophil count decreased	0	0	1 (2.9)	0	0	1 (2.9)
Adrenal insufficiency	0	0	1 (2.9)	0	0	1 (2.9)
Immune-mediated hepatitis	0	0	1 (2.9)	0	0	1 (2.9)

Data are *n* (%). The table presents study drug-related adverse effects that occurred in at least 10% or more of all treated patients (*n*=35) and all study drug-related grade 3 events. AEs include all events that occurred or worsened after the first dose of cretostimogene or pembrolizumab. There were no grade 3–5 cretostimogene drug-related adverse effects. There were no grade 4–5 pembrolizumab drug-related adverse effects.

completely resected[26], there is sufficient antigenic burden to enable in situ vaccination following cytolysis. As such, BCG-UR CIS represents the ideal clinical scenario to test the efficacy of intravesical oncolytic virus. Indeed, cretostimogene has previously been demonstrated to be both safe and efficacious, with enhanced anti-tumor activity observed in patients with CIS[14,15]. From the ongoing pivotal BOND-003 trial (NCT04452591), cretostimogene demonstrated a CR rate of 75.2% (95% CI 65.7–82.9%) at any time in 105 patients with BCG-UR CIS (Tyson, AUA 2024).

Although oncolytic virus was originally thought to act predominantly through direct tumor cytolysis, most effective regimens are now believed to engender a long-lasting tumor-specific immune response[27,28]. Infection by oncolytic virus causes endoplasmic reticulum (ER) stress, in turn leading to immunogenic cell death via the translocation of ER-associated calreticulin to the plasma membrane, as well as the release of other damage-associated molecular patterns such as high mobility group protein B1[25]. Additionally, pathogen-associated molecular patterns on oncolytic virus bind pattern recognition receptors on dendritic cells to drive their maturation and expression of co-stimulatory molecules, and promote the release of pro-inflammatory cytokines to create an immunogenic milieu leading to the recruitment of innate lymphoid cells to enhance viral and tumor killing[29]. Finally, type I interferon-mediated effects leads to the emergence of adaptive immune response, completing the elimination of oncolytic virus-treated tumors[25]. These purported mechanisms of action were recently substantiated through a single-cell transcriptomic analysis of serial biopsies obtained from B cell lymphoma lesions treated with talimogene laherparepvec (T-VEC: genetically modified HSV-1), demonstrating rapid eradication of malignant cells, early influx of natural killer cells, monocytes and dendritic cells, followed by enrichment of cytotoxic T cells and a decrease of regulatory T cells in the injected lesions[28].

Leveraging the complementary immunogenic effects of oncolytic virus and immune checkpoint blockade, many trials have attempted to combine the two therapies to achieve synergistic anti-tumor activity[25]. In patients with advanced melanoma, the combination of T-VEC and pembrolizumab showed early promise with an acceptable safety profile and an encouraging CR rate of 43% in a phase 1b study[30]. However, a subsequent randomized, double-blinded, placebo-controlled, phase 3 trial failed to demonstrate improved survival with the addition of T-VEC to pembrolizumab in immunotherapy-naive advanced melanoma patients in the frontline setting[31]. Nevertheless, subgroup analyses showed improved PFS in patients with limited disease burden, as reflected by baseline tumor measurements and lactate dehydrogenase levels. Furthermore, immunogenic effects of oncolytic virus were expectedly strongest within the injected lesions, and attenuated at metastatic sites[28]. We hypothesize that these clinical insights may further support the use of this combinatory strategy in early stage, nonmetastatic cancers as exemplified by BCG-UR CIS. The durability of disease control along with the absence of disease progression observed in our trial may serve as evidence for the long-lasting efficacy of this treatment approach.

To achieve wide adoption for a disease that does not pose imminent lethal risk, careful attention must be paid toward treatment-related toxicity. In our study, the toxicity profile of the combination did not incur any new safety signals beyond those observed from monotherapy trials with cretostimogene or pembrolizumab, with TRAEs predominantly limited to bladder-related symptoms attributed to intravesical infusions. Immune-related adverse effects were most consistent with those previously reported from multiple pembrolizumab monotherapy trials incurring ≥grade 3 TRAEs in 14.3% of patients, in line with what has been previously reported in immune checkpoint blockade monotherapy trials[17,20,21]. No treatment-related death was observed. The incremental increase in severe systemic toxicity is offset by the improved durable response, creating a more favorable benefit-to-risk ratio.

Our study has several limitations. As a single-arm study, results can only be interpreted within the context of other historical controls, such as pembrolizumab and cretostimogene monotherapy trials performed in the same disease space. However, nuanced characteristics of enrolled patients may differ, confounding comparative analysis and generalizability of the results. Notwithstanding, due to the lack of consensus for

bladder-sparing alternatives in the BCG-UR setting, a recently held FDA workshop concluded that randomized controlled trials may not yet be feasible[32]. Second, the lack of randomization makes it impossible to assess the contribution of components and synergy within our combination regimen, in terms of both efficacy and toxicity. Although both drugs were administered in combination and are immunomodulatory agents, individual toxicity attributions were the assessment of site investigators. Absent a randomized controlled trial, they are hypothesis generating. Given the small sample size of 35 patients, none of the reported adverse events are statistically significant. The eventual risk benefit assessment of the pembrolizumab/cretostimogene combination awaits the outcome of a randomized controlled trial. However, this may be somewhat mitigated by the availability of data from the monotherapy trials using both of the therapeutic components. Third, without surgical extirpation, the assessment of response is hampered by the well-documented inaccuracy of clinical staging. This is further obfuscated by the nonstandardized use of enhanced cystoscopy techniques, mandatory versus for-cause biopsies, and operator-dependent interpretation of cystoscopy findings, among other factors. Fourth, our study was limited by its scope. Although the preliminary results are encouraging and hypothesis generating, validation for this combinatory approach must be carried out with additional studies in larger cohorts for confirmation. It was further limited by the predominance of male patients included, although this is reflective of the sex distribution seen in previous BCG-UR trials[17,18,20,33]. Finally, correlative analyses prespecified in the protocol are not yet available; these could provide further insights into the baseline tumor characteristics and mechanisms of action of the treatment combination.

In summary, combination intravesical cretostimogene grenadenorepvec and systemic pembrolizumab achieved a favorable benefit-to-risk profile in patients with BCG-UR CIS, with a 12-month CR of 57.1% and a 24-month CR of 51.4%, with an acceptable toxicity profile. The long-term efficacy in this study may represent progress and a novel mechanism of action for bladder-sparing strategies in the BCG-UR CIS setting. Additional clinical trials may confirm the benefit of oncolytic virus therapy and its potential synergy with immune checkpoint blockade for patients with BCG-UR NMIBC. This treatment regimen may allow patients to preserve their bladders and shift the standard of care in this disease space.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-03025-3.

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Methods

Study design and participants

CORE-001 is a single-arm, open-label, multicenter phase 2 clinical trial performed at 14 clinical sites in the USA and South Korea. The trial adhered to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was provided by all participants on trial. Eligible patients over the age of 18 with BCG-UR CIS with or without concurrent Ta/T1 disease according to the 2018 US FDA Guidance for Industry[16] were enrolled, with persistent CIS at 6 months despite receiving adequate BCG therapy (defined as ≥5/6 induction doses and 2/3 maintenance treatments, or at least 2/6 instillation of a second induction course in which maintenance treatment is not given) or recurrence of CIS within 12 months of disease-free state after BCG. Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 but were ineligible for or refused radical cystectomy on the basis of informed consent and counseling with their treating surgeon. Eligible patients were determined to have adequate organ function, based on liver function tests ≤2.5× the upper limit of normal (ULN), serum bilirubin ≤1.5× ULN, international normalized ratio or prothrombin time ≤1.5× ULN, absolute neutrophil count ≥1,000 cells mm^{-3}, hemoglobin ≥8 g dl^{-1}, platelet count ≥100,000 platelets mm^{-3} and serum chemistries within normal limits. Patients were excluded if they had received a prior anti-PD-1 or PD-L1 agent or other treatment directed at stimulatory or co-inhibitory T cell receptors; had prior treatment with adenovirus-based therapy (for example, nadofaragene firadenovec); had active autoimmune disease that required systemic treatment in the past 2 years; with known infection with human immunodeficiency virus, hepatitis B virus or hepatitis C virus (HCV); had active, noninfectious pneumonitis or a history of interstitial lung disease; current or prior history of T2 or higher-stage bladder cancer; concurrent urethral or upper tract urothelial carcinoma; or concurrent high-dose steroid therapy as well as history of allogenic or solid organ transplant. Patients who received a live vaccine, immunosuppressive therapy, anti-cancer therapy (including investigational agents) or intravesical therapy had to undergo a washout period (30 days before screening for live vaccine, 4 weeks for immunosuppressive and anti-cancer therapy), with the exception of cytotoxic agents administered as a single instillation immediately after TURBT. The full study protocol is included in Supplementary Information.

Inclusion criteria

- Age ≥18 years at the time of consent
- ECOG 0–2
- Have pathologically confirmed CIS unresponsive to prior BCG therapy
  - Relapsed or persistent CIS (with or without Ta high-grade or T1) within 12 months of the completion (last dose) of adequate BCG treatment for high grade urothelial carcinoma (HGUC) (for example, CIS, Ta high-grade, T1 or a combination of these HGUC pathologies).
  - Pathological confirmation of BCG-UR CIS within 10 weeks of study enrollment.
  - CIS specimen must be predominantly urothelial (transitional cell) and have less than 50% variant (for example, sarcomatoid or squamous component) histology.
  - No maximum limit to the amount of BCG administered, but maintenance BCG should be administered on a schedule consistent with the SWOG (Southwest Oncology Group) 8507 regimen
- Have all Ta and/or T1 disease resected and all CIS resected or fulgurated, as feasible, before study treatment (for example, before day 1 treatment)
- Received prior adequate BCG therapy as defined as at least one of the following ('5 + 2' minimum exposure; all seven qualifying doses must be greater than or equal to one-third (1/3) of full-dose BCG):
  - At least five of six doses of an initial induction course (adequate induction) plus at least two of three doses of maintenance therapy, OR
  - At least five of six doses of an initial induction course (adequate induction) plus at least two of six doses of a second induction course
- Ineligible (or medically unfit) to receive radical cystectomy or refusal of radical cystectomy based on investigator assessment
- Adequate organ function
- Willing to use barrier contraception during sexual activity, starting with day 1 for up to 6 weeks after each dose of cretostimogene
- Women of childbearing potential must have negative pregnancy test, not be breast feeding and agree to follow contraception guidance during treatment
- Patients must be willing to comply with study-mandated cystoscopies, urine cytology, urograms, biopsies and other procedures

Exclusion criteria

- Prior therapy with an anti-PD-1, anti-PD-L1 or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T cell receptor (for example, CTLA-4, OX40 and CD137)
- Has current or past history of muscle-invasive (T2 or higher stage) or locally advanced (T3/T4 or any N) or metastatic bladder cancer
- Has current or past history of T2 or higher-stage urothelial carcinoma in the upper genitourinary tract (kidneys, renal collecting systems and ureters) or prostatic urethra OR T1 or CIS urothelial carcinoma in the upper genitourinary tract or prostatic urethra (including CIS of the urethra) within 24 months before enrollment
- Has received systemic anti-cancer therapy, including investigational agents, within 4 weeks of day 1
- Has received prior systemic treatment, radiation therapy or surgery for bladder cancer other than TURBT or bladder biopsies
- Has any of the following within the 6 months before starting study treatment: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, cerebrovascular accident, pulmonary embolus, uncontrolled hypertension or uncontrolled congestive heart failure
- Has used excluded antiviral medication (for example, interferon/peg-interferon, ribavirin and so on) within 14 days of day 1 and that cannot be suspended throughout for at least 14 days before and after each treatment with cretostimogene
- Has had prior treatment with any human adenovirus serotype-5-based therapy (for example, nadofaragene firadenovec)
- Requires use of anti-platelet or anti-coagulant therapy that cannot be safely suspended for per protocol biopsies and other procedures as per standard of care
- Has significant immunodeficiency due to underlying illness (for example, known human immunodeficiency virus/acquired immunodeficiency syndrome)
- Has received systemic immunosuppressive medication including high-dose corticosteroids (for example, systemic corticosteroids >10 mg prednisone or equivalent), within 28 days
- Has had an allogenic tissue/solid organ transplant
- Has a known history of hepatitis B (defined as hepatitis B antigen reactive) or known active HCV (defined as HCV RNA (qualitative) is detected) infection

- Has received prior radiotherapy within 2 weeks of start of study treatment. Patients must have recovered from all radiation-related toxicities, not require corticosteroids and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-central nervous system disease
- Has a known additional malignancy that is progressing or has required active treatment within the past 3 years
- Has an active infection requiring systemic therapy (for example, urinary tract infection or other active infection)
- Has active autoimmune disease that has required systemic treatment in past 2 years (that is, with use of disease modifying agents, corticosteroids or immunosuppressive drugs)
- Has a history of (noninfectious) pneumonitis that required steroids or has current pneumonitis
- Has a history of interstitial lung disease
- Has received a live vaccine within 30 days before the first dose of study drug
- Has severe hypersensitivity (≥grade 3) to pembrolizumab and/or any of its excipients
- Has not recovered (that is, to ≤grade 1 or to baseline status) from adverse effects due to a previously administered agent
- Has an illness, metabolic dysfunction, physical examination finding or clinical laboratory finding that gives reasonable suspicion of a disease or condition that would contraindicate study treatment or that would limit compliance with study requirement
- Is pregnant, currently breastfeeding or intending to breastfeed, within the projected duration of the trial beginning at screening through 150 days after the last study treatment
- Cannot tolerate study-related biopsies, intravesical administration or 1-h bladder hold of cretostimogene
- Intravesical therapy within 8 weeks before beginning study treatment with the exception of cytotoxic agents (for example, mitomycin C, gemcitabine, doxorubicin and epirubicin) when administered as a single instillation immediately following a TURBT procedure, which is permitted up to 14–60 days before beginning study treatment.

Procedures

Before study treatment, all visible Ta/T1 tumors were completely resected and visible areas of CIS fulgurated. All patients received sterile placement of a Foley catheter, with instillations of 0.1% *n*-dodecyl-B-D-maltoside and 1×10^{12} vp of cretostimogene in 100 ml of normal saline. Intravesical dwell time was maintained at 1 h as tolerated. The patient was monitored for an additional hour for safety evaluations and the collection of protocol-specified research samples. Cretostimogene was administered as six weekly intravesical instillations in the induction phase followed by three weekly maintenance instillations at months 3, 6, 9, 12 and 18, or until confirmed disease persistence, recurrence, progression or withdrawal of consent. If patients presented with persistent CIS or high-grade Ta NMIBC at month 3, a second induction course of cretostimogene was administered. No dose modifications of cretostimogene were permitted. In the event of cretostimogene dose holds, dosing was resumed at the next prespecified protocol time point. Cretostimogene doses were held for ≥grade 3 serious adverse events as well as those felt to be clinically significant or likely to impair the efficacy of treatment. In the event of dose delays or discontinuations of cretostimogene, receipt of pembrolizumab was still permitted per protocol.

Pembrolizumab was administered at 400 mg intravenously and continued every 6 weeks for 18 administrations (~2 years) or until confirmed disease persistence, recurrence, progression or withdrawal of consent. On days with simultaneous dosing of cretostimogene and pembrolizumab, dosing was separated by at least 2 h, to facilitate the assessment of infusion and instillation-related adverse effects. Dose modifications of pembrolizumab were precluded per protocol. Dose delays were permitted if occurring within 14 days of the prespecified protocol timeline window. Toxicity management guidelines for immune-related adverse effects were provided in the protocol. In the event of dose delays or discontinuations of pembrolizumab, receipt of cretostimogene was permitted per protocol.

Screening procedures, including urine cytology and biopsy, were done for all patients up to 8 weeks before the start of treatment. All patients with T1 disease were mandated to undergo re-resection. Quarterly, centrally reviewed urine cytologies, along with cystoscopies (with for-cause biopsy if warranted) were performed. At the 12-month time point, patients underwent protocol-defined mandatory bladder mapping biopsies of at least five sites within the bladder, including the bladder dome, trigone, right and left lateral walls, the posterior wall as well as any sites of abnormal appearance or prior sites of disease to ensure rigorous endpoint assessment. Disease assessment at the 12-month time point was based on centrally reviewed mandatory bladder mapping biopsies along with cytology. Upon identification of discordance between local and central pathology interpretation, a third-party expert genitourinary pathologist in the Johns Hopkins University Department of Pathology was consulted for adjudication. Investigators were not required to use enhanced cystoscopy (that is, blue-light cystoscopy), but, if used at baseline, they were required to use the same modality at the 12-month efficacy assessment. Low-grade tumor recurrence or an isolated high-grade or suspicious urine cytology in the absence of pathologic disease confirmation was considered a CR for that time point. In the event of subsequent pathologic confirmation of prior isolated positive or suspicious cytology, not reached was backdated to the original time point.

The evaluation of adverse effects and safety monitoring such as urinalyses, clinical laboratory chemistry and hematology evaluations, electrocardiograms and physical examinations were performed at each clinic visit. All patients were followed for disease status or new anti-cancer therapy initiation until withdrawal of consent, death or upon reaching the 24-month time point. Immune-related and serious adverse effects were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. The assessment of adverse effects was continued at least 30 days beyond the last dose of either study drug.

Outcomes

The primary endpoint was the proportion of patients with CR at the 12-month assessment, incorporating mandatory mapping bladder biopsies. CR was defined as negative biopsy, cystoscopy, cytology and axial imaging. An isolated positive urine cytology, in the absence of abnormal cystoscopy, prompted a repeat urine cytology for confirmation. If the repeated urine cytology remained positive or suspicious, a mandatory mapping biopsy of multiple bladder locations was performed. Secondary endpoints included CR at any time point, DoR, defined as time from first CR to documented recurrence, progression or death. Additionally, proportional and time to event analyses evaluating high-grade-recurrence-free survival (as a measure of DoR), radical cystectomy-free survival, PFS and OS were assessed. Safety endpoints included type, incidence, relatedness and severity of adverse effects, and number of serious adverse effects as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. A data monitoring committee (DMC) oversaw the study conduct to ensure the safety of trial patients.

Statistical analysis

The sample size calculation was based on the previously observed 12-month CR rate of 19% using pembrolizumab in patients with BCG-UR CIS from the KEYNOTE-57 trial[17]. For the initial study design hypothesis, a CR rate at 12 months of 19% (H0) ($0.41 \times 0.46 = 0.19$)[17]

for the experimental regimen was set as the null while a CR rate of 36% (Ha) or higher was considered suitable for further investigation. The study used a single-stage design with standard one-sided normal statistical test of a single binomial proportion with no interim analysis. The primary analysis of the proportion of CR at the 12-month time point tested the null hypothesis H0: $\pi \leq 0.19$ versus Ha: $\pi > 0.19$ using a single-proportion asymptotic normal test statistic, with one-sided α set at 0.10. With the prespecified sample size, there was approximately 81% power to test if the true population proportion achieved 36%. Using these operating characteristics for study design, a total of 30 evaluable patients were estimated to be required. An additional 5 patients were added to account for potential nonevaluable patients (for example, losses to follow-up or withdrawal of consent before the first response assessment) resulting in a total study population of 35 patients. In the ITT assessment, patients who enrolled in the study after completing baseline screening assessment but failed to reach the 12-month evaluation were considered to be in not reached.

All patients enrolled met the US FDA definition of BCG-UR NMIBC with CIS[16]. Efficacy analyses were therefore performed per protocol for all patients. The proportion of patients with CR at the 12-month time point is presented with a two-sided 95% CI by normal approximation. High-grade RFS (as surrogate for DoR), cystectomy-free survival and PFS were evaluated using the Kaplan–Meier method to provide estimates of median duration, along with two-sided 95% CIs for the median. The median DoR was also computed using the Kaplan–Meier method.

Continuous data were summarized using descriptive statistics (number of observations, means, standard deviations, minimum, median and maximum), and categorical variables were summarized using frequency counts and percentages. A DMC oversaw the study according to its charter. Safety analyses were performed on the ITT populations. Statistical analyses utilized SAS version 9.4 (SAS Institute). ClinicalTrials.gov identifier: NCT04387461.

Role of the funding source

The funders contributed to the study design, data analysis and data interpretation, and review of the manuscript in collaboration with the authors; the primary investigator (R.L.) had full access to the data. The funder had no role in data collection. Investigators and site personnel collected data, which were housed on CG Oncology's database. A DMC made recommendations about the overall risk and benefit to trial participants.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Clinical data from this study used to support this publication will be made available upon request from a qualified medical or scientific professional for the specific purpose laid out in that request and may include de-identified individual participant data. Requests for secondary use of this data will require a data use agreement created with CG Oncology and submitting a data access request to K.A.K. at pat.keegan@cgoncology.com. Response to external data requests will be provided within an estimated 4–6-week time frame as much as possible. Source data are provided with this paper.

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Author contributions

R.L.: conceptualization, formal analysis, supervision, investigation, original writing, writing review and editing. P.H.S.: investigation, writing review and editing. T.F.S.: investigation, writing review and editing. J.K.N.: investigation, writing review and editing. T.J.B.: investigation, writing review and editing. D.L.L.: investigation, writing review and editing. E.M.U.: investigation, writing review and editing. D.M.G.: investigation, writing review and editing. J.M.J.: investigation, writing review and editing. J.J.M.: investigation, writing review and editing. R.D.: investigation, writing review and editing. S.M.P.: investigation, writing review and editing. S.H.K.: investigation, writing review and editing. S.I.J.: investigation, writing review and editing. A.M.K.: conceptualization, writing review and editing. J.M.B.: conceptualization, formal analysis, supervision, investigation, writing review and editing. K.A.K.: original writing, writing review and editing. G.D.S.: conceptualization, supervision, original writing, writing review and editing.

Competing interests

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Additional information

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Extended Data Table 1 | Investigator-determined treatment related adverse event attributed to cretostimogene

System Organ Class, n (%)/ Preferred Term, n (%)	Maximum Severity					
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total (N=35)
Participants Reporting at Least One Treatment-Emergent ADR Related to CG0070	18 (51.4)	10 (28.6)	0	0	0	28 (80.0)
Renal And Urinary Disorders	19 (54.3)	7 (20.0)	0	0	0	26 (74.3)
Bladder Spasm	13 (37.1)	3 (8.6)	0	0	0	16 (45.7)
Dysuria	8 (22.9)	1 (2.9)	0	0	0	9 (25.7)
Pollakiuria	8 (22.9)	1 (2.9)	0	0	0	9 (25.7)
Micturition Urgency	4 (11.4)	2 (5.7)	0	0	0	6 (17.1)
Haematuria	4 (11.4)	1 (2.9)	0	0	0	5 (14.3)
Nocturia	3 (8.6)	1 (2.9)	0	0	0	4 (11.4)
Cystitis Noninfective	2 (5.7)	1 (2.9)	0	0	0	3 (8.6)
Polyuria	3 (8.6)	0	0	0	0	3 (8.6)
Bladder Discomfort	2 (5.7)	0	0	0	0	2 (5.7)
Urinary Incontinence	2 (5.7)	0	0	0	0	2 (5.7)
Urinary Tract Pain	2 (5.7)	0	0	0	0	2 (5.7)
Haemorrhage Urinary Tract	1 (2.9)	0	0	0	0	1 (2.9)
Urinary Retention	0	1 (2.9)	0	0	0	1 (2.9)
General Disorders And Administration Site Conditions	6 (17.1)	2 (5.7)	0	0	0	8 (22.9)
Fatigue	5 (14.3)	2 (5.7)	0	0	0	7 (20.0)
Chills	1 (2.9)	0	0	0	0	1 (2.9)
Instillation Site Discomfort	1 (2.9)	0	0	0	0	1 (2.9)
Malaise	1 (2.9)	0	0	0	0	1 (2.9)
Suprapubic Pain	1 (2.9)	0	0	0	0	1 (2.9)
Gastrointestinal Disorders	5 (14.3)	0	0	0	0	5 (14.3)
Abdominal Pain	3 (8.6)	0	0	0	0	3 (8.6)
Abdominal Discomfort	2 (5.7)	0	0	0	0	2 (5.7)
Abdominal Distension	1 (2.9)	0	0	0	0	1 (2.9)
Infections And Infestations	3 (8.6)	1 (2.9)	0	0	0	4 (11.4)
Urinary Tract Infection	3 (8.6)	1 (2.9)	0	0	0	4 (11.4)
Musculoskeletal And Connective Tissue Disorders	1 (2.9)	1 (2.9)	0	0	0	2 (5.7)
Arthralgia	0	1 (2.9)	0	0	0	1 (2.9)
Muscle Spasms	1 (2.9)	0	0	0	0	1 (2.9)
Not Coded	0	1 (2.9)	0	0	0	1 (2.9)
Not Coded	0	1 (2.9)	0	0	0	1 (2.9)
Reproductive System And Breast Disorders	1 (2.9)	0	0	0	0	1 (2.9)
Penile Haemorrhage	1 (2.9)	0	0	0	0	1 (2.9)

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Extended Data Table 2 | Investigator-determined treatment related adverse events attributed to pembrolizumab

System Organ Class, n (%)/ Preferred Term, n (%)	Maximum Severity					
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total (N=35)
Participants Reporting at Least One Treatment-Emergent ADR Related to Pembrolizumab	8 (22.9)	14 (40.0)	5 (14.3)	0	0	27 (77.1)
General Disorders And Administration Site Conditions	13 (37.1)	3 (8.6)	0	0	0	16 (45.7)
Fatigue	9 (25.7)	2 (5.7)	0	0	0	11 (31.4)
Asthenia	1 (2.9)	1 (2.9)	0	0	0	2 (5.7)
Chills	2 (5.7)	0	0	0	0	2 (5.7)
Influenza Like Illness	1 (2.9)	0	0	0	0	1 (2.9)
Malaise	1 (2.9)	0	0	0	0	1 (2.9)
Pyrexia	1 (2.9)	0	0	0	0	1 (2.9)
Investigations	5 (14.3)	1 (2.9)	3 (8.6)	0	0	9 (25.7)
Alanine Aminotransferase Increased	3 (8.6)	0	0	0	0	3 (8.6)
Aspartate Aminotransferase Increased	2 (5.7)	1 (2.9)	0	0	0	3 (8.6)
Blood Glucose Increased	2 (5.7)	0	0	0	0	2 (5.7)
Activated Partial Thromboplastin Time Prolonged	1 (2.9)	0	0	0	0	1 (2.9)
Blood Alkaline Phosphatase Increased	0	0	1 (2.9)	0	0	1 (2.9)
Blood Testosterone Decreased	1 (2.9)	0	0	0	0	1 (2.9)
Blood Thyroid Stimulating Hormone Increased	1 (2.9)	0	0	0	0	1 (2.9)
Ejection Fraction Decreased	0	0	1 (2.9)	0	0	1 (2.9)
Neutrophil Count Decreased	0	0	1 (2.9)	0	0	1 (2.9)
Skin And Subcutaneous Tissue Disorders	3 (8.6)	6 (17.1)	0	0	0	9 (25.7)
Pruritus	1 (2.9)	1 (2.9)	0	0	0	2 (5.7)
Rash	1 (2.9)	1 (2.9)	0	0	0	2 (5.7)
Dermatitis	0	1 (2.9)	0	0	0	1 (2.9)
Eczema	0	1 (2.9)	0	0	0	1 (2.9)
Erythema	0	1 (2.9)	0	0	0	1 (2.9)
Night Sweats	1 (2.9)	0	0	0	0	1 (2.9)
Psoriasis	0	1 (2.9)	0	0	0	1 (2.9)
Skin Hypopigmentation	1 (2.9)	0	0	0	0	1 (2.9)
Gastrointestinal Disorders	6 (17.1)	1 (2.9)	1 (2.9)	0	0	8 (22.9)
Diarrhoea	4 (11.4)	0	1 (2.9)	0	0	5 (14.3)
Abdominal Discomfort	1 (2.9)	0	0	0	0	1 (2.9)
Dental Caries	0	1 (2.9)	0	0	0	1 (2.9)
Dry Mouth	0	1 (2.9)	0	0	0	1 (2.9)
Gingival Pain	1 (2.9)	0	0	0	0	1 (2.9)
Nausea	1 (2.9)	0	0	0	0	1 (2.9)
Stomatitis	1 (2.9)	0	0	0	0	1 (2.9)
Tongue Discolouration	1 (2.9)	0	0	0	0	1 (2.9)
Toothache	1 (2.9)	0	0	0	0	1 (2.9)
Vomiting	1 (2.9)	0	0	0	0	1 (2.9)
Musculoskeletal And Connective Tissue Disorders	5 (14.3)	2 (5.7)	0	0	0	7 (20.0)
Arthralgia	2 (5.7)	1 (2.9)	0	0	0	3 (8.6)
Joint Stiffness	2 (5.7)	0	0	0	0	2 (5.7)
Myalgia	2 (5.7)	0	0	0	0	2 (5.7)
Joint Swelling	0	1 (2.9)	0	0	0	1 (2.9)
Musculoskeletal Stiffness	1 (2.9)	0	0	0	0	1 (2.9)
Endocrine Disorders	1 (2.9)	3 (8.6)	1 (2.9)	0	0	5 (14.3)
Hypothyroidism	1 (2.9)	3 (8.6)	0	0	0	4 (11.4)
Adrenal Insufficiency	0	0	1 (2.9)	0	0	1 (2.9)
Autoimmune Thyroiditis	1 (2.9)	0	0	0	0	1 (2.9)
Hyperthyroidism	1 (2.9)	0	0	0	0	1 (2.9)
Nervous System Disorders	4 (11.4)	0	0	0	0	4 (11.4)
Headache	3 (8.6)	0	0	0	0	3 (8.6)
Lethargy	1 (2.9)	0	0	0	0	1 (2.9)
Renal And Urinary Disorders	2 (5.7)	0	0	0	0	2 (5.7)
Bladder Spasm	1 (2.9)	0	0	0	0	1 (2.9)
Haematuria	1 (2.9)	0	0	0	0	1 (2.9)
Respiratory, Thoracic And Mediastinal Disorders	1 (2.9)	1 (2.9)	0	0	0	2 (5.7)
Dyspnoea	1 (2.9)	0	0	0	0	1 (2.9)
Pneumonitis	0	1 (2.9)	0	0	0	1 (2.9)
Vascular Disorders	1 (2.9)	1 (2.9)	0	0	0	2 (5.7)
Hot Flush	1 (2.9)	0	0	0	0	1 (2.9)
Hypotension	0	1 (2.9)	0	0	0	1 (2.9)
Hepatobiliary Disorders	0	0	1 (2.9)	0	0	1 (2.9)
Immune-Mediated Hepatitis	0	0	1 (2.9)	0	0	1 (2.9)
Infections And Infestations	1 (2.9)	0	0	0	0	1 (2.9)
Urinary Tract Infection	1 (2.9)	0	0	0	0	1 (2.9)
Metabolism And Nutrition Disorders	0	1 (2.9)	0	0	0	1 (2.9)
Hyperglycaemia	0	1 (2.9)	0	0	0	1 (2.9)
Not Coded	0	1 (2.9)	0	0	0	1 (2.9)
Not Coded	0	1 (2.9)	0	0	0	1 (2.9)

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Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Investigators and site personnel collected data, which were housed on CG Oncology's database. Clinical data was collected using Zelta Merative EDC.
Data analysis	Statistical analyses utilized SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
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Clinical data from this study used to support this publication will be made available upon request from a qualified medical or scientific professional for the specific purpose laid out in that request and may include de-identified individual participant data. Requests for secondary use of this data will require a data use agreement created with CG Oncology and submitting a data access request to Kirk A. Keegan at pat.keegan@cgoncology.com

Data from this trial may be used in the future to support regulatory approval and as such, will not be made publically available.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Findings in this study apply to sex, which was determined based on self-reporting and confirmed through the medical history taking. The baseline clincopathologic data, including sex, are included in Table 1.
Reporting on race, ethnicity, or other socially relevant groupings	Race was included as a variable in the baseline clinicopathologic characteristics as shown in Table 1; ethnicity or other socially relevant groupings were not used in our reporting.
Population characteristics	The covariate relevant population characteristics of the human research participants are presented in Table 1 and described in the manuscript.
Recruitment	Patient fulfilling the inclusion and exclusion criteria of the clinical trial were recruited based on their availability and accessibility to any of the participating centers. The study may be subjected to self-selection bias and thus may not be generalizable to the broader population.
Ethics oversight	The study was conducted under the IRB at each of the individual clinical trial sites, including at Moffitt Cancer Center IRB, Mayo Clinic IRB, UC San Diego IRB, Pusan National University Yangsan Hospital IRB, University of Pennsylvania IRB, BCG Oncology IRB, UC Irvine IRB, Fox Chase Cancer Center IRB, SUNY Upstate IRB, Northwestern University IRB, Chesapeake IRB, Spokane Urology IRB, Korea University Anam Hospital IRB, CHonnam National University Hwasun Hospital IRB

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size calculation was based on the previously observed 12-month CR rate of 19% using pembrolizumab in patients with BCG-unresponsive CIS from the KEYNOTE-57 trial. For the initial study design hypothesis, a CR rate at 12-month of 19% (H0) for the experimental regimen was set as the a priori futility threshold while a CR rate of 36% (Ha) or higher was considered suitable for further investigation. The study used a single-stage design with standard one-sided normal statistical test of a single binomial proportion with no interim analysis. The primary analysis of the proportion of CR at the 12-month time point tested the null hypothesis H0: $\pi \leq 0.19$ versus Ha: $\pi > 0.19$ using a single proportion asymptotic normal test statistic, with one-sided alpha set at 0.10. With the pre-specified sample size there was approximately 81% power to test if the true population proportion achieved 36%. Using these operating characteristics for study design, a total of 30 evaluable patients were estimated to be required. An additional 5 patients were added to account for potential non-evaluable patients (e.g., losses to follow-up or withdrawal of consent prior to the first response assessment) resulting in a total study population of 35 patients. In the intention-to-treat (ITT) assessment, patients who enrolled in the study after completing baseline screening assessment but failed to reach the 12-month evaluation were considered to be in non-response.
Data exclusions	No data was excluded.
Replication	The data generated from each patient enrolled on trial cannot possibly be replicated, giving individual baseline clinicopathologic characteristics.
Randomization	Per FDA guidance, CIS cannot be completely resected at the time of diagnosis, and if left alone, will lead to cancer progression. As such, randomized control trials using a placebo arm would be unethical. As there is no effective bladder sparing alternative, randomized control trials are currently considered unfeasible.
Blinding	This study was an open labeled study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT0438746
Study protocol	Full protocol provided as Supplementary information.
Data collection	Investigators and site personnel collected data, which were housed on CG Oncology's database. Patients were accrued from November 4, 2020, to August 16, 2022.
Outcomes	The primary endpoint was the proportion of patients with CR at the 12-month assessment, incorporating mandatory mapping bladder biopsies. CR was defined as negative biopsy, cystoscopy, cytology, and axial imaging. An isolated positive urine cytology, in the absence of abnormal cystoscopy, prompted a repeat urine cytology for confirmation. If the repeated urine cytology remained positive or suspicious, a mandatory mapping biopsy of multiple bladder locations was performed. Secondary endpoints included CR at any timepoint, duration of response (DoR), defined as time from first CR to documented recurrence, progression, or death. Additionally, proportional and time to event analyses evaluating HG-Recurrence Free Survival (as a measure of duration of response), RC Free survival, Progression Free Survival, and Overall Survival were assessed. Safety endpoints included type, incidence, relatedness, and severity of AEs; and number of SAEs as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. A Data Monitoring Committee (DMC) oversaw the study conduct to ensure the safety of trial patients.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*