Bladder Preservation with Durvalumab plus Tremelimumab and Concurrent Radiotherapy in Patients with Localized Muscle-Invasive Bladder Cancer (IMMUNOPRESERVE): A Phase II Spanish Oncology GenitoUrinary Group Trial

Xavier Garcia-del-Muro[1], Begoña P. Valderrama[2], Ana Medina-Colmenero[3], Olatz Etxaniz[4], Regina Gironés Sarrió[5], María José Juan-Fita[6], Marcel Costa-García[7], Rafael Moreno[7], Isabel Miras Rodríguez[2], Irene Ortiz[1], Andrés Cuéllar[1], Ferran Ferrer[8], Francesc Vigués[9], Roberto de Haro Piedra[10], Arturo Candal Gomez[11], Salvador Villà[12], José Luis Pontones[13], Yasmina Murria[14], Guillermo Lendínez-Cano[15], and Ramon Alemany[7]

ABSTRACT

Purpose: The combination of radiation and immunotherapy potentiated antitumor activity in preclinical models. The purpose of this study is to explore the feasibility, safety, and efficacy of a bladder-preserving approach, including dual immune checkpoint blockade and radiotherapy, in patients with muscle-invasive bladder cancer (MIBC).

Patients and Methods: Patients with localized MIBC underwent transurethral resection, followed by durvalumab (1,500 mg) plus tremelimumab (75 mg) every 4 weeks for three doses and concurrent radiotherapy (64–66 Gy to bladder). Patients with residual or relapsed MIBC underwent salvage cystectomy. The primary endpoint was complete response, defined as the absence of MIBC at post-treatment biopsy. Secondary endpoints were bladder-intact disease-free survival, distant metastasis–free survival, and overall survival.

Results: Thirty-two patients were enrolled at six centers. Complete response was documented in 26 (81%) patients. Two patients had residual MIBC, and four patients were not evaluated. After a median follow-up of 27 months, 2 patients underwent salvage cystectomy. The 2-year rates for bladder-intact disease-free survival, distant metastasis–free survival, and overall survival were 65%, 83%, and 84%, respectively. The 2-year estimates of non–muscle-invasive bladder relapse, MIBC, and distant metastasis were 3%, 19%, and 16%, respectively. Grade 3 to 4 toxicities were reported in 31% of patients, with diarrhea (6%) and acute kidney failure (6%) being the most frequent.

Conclusions: This multimodal approach including durvalumab plus tremelimumab with concurrent radiotherapy is feasible and safe, showing high efficacy in terms of response and eliciting bladder preservation in a large number of patients. Further research on this approach as an alternative to cystectomy is warranted.

Introduction

Radical cystectomy has long been considered the standard treatment for patients with muscle-invasive bladder cancer (MIBC). Nevertheless, the potential morbidity of this major surgical procedure, as well as its impact on the quality of life, has led to the search for therapeutic bladder-sparing alternatives. Several studies have shown that long-term bladder preservation can be achieved in a considerable proportion of patients with a multimodal approach. Trimodal therapies, including transurethral resection of the bladder tumor (TURBT) followed by radiotherapy and concurrent chemotherapy, are the most extensively studied (1–6). In these studies, patients who achieve a complete response (CR) of the MIBC with the treatment are candidates for bladder preservation. Salvage cystectomy is recommended for the remaining patients (7). These studies demonstrated that a selective multimodal bladder-sparing approach is feasible and constitutes an alternative to standard radical surgery for patients with surgical contraindication or cystectomy

[1]Medical Oncology, Institut Català d'Oncologia (ICO), IDIBELL, University of Barcelona, Barcelona, Spain. [2]Medical Oncology, Hospital Universitario Virgen del Rocío, Sevilla, Spain. [3]Medical Oncology, Fundación Centro Oncológico de Galicia, A Coruña, Spain. [4]Medical Oncology, Institut Català d'Oncologia (ICO), Hospital Germans Trias i Pujol, Badalona, Spain. [5]Medical Oncology, Hospital Universitari i Politècnic la Fe, Valencia, Spain. [6]Medical Oncology Service, Instituto Valenciano de Oncología, Valencia, Spain. [7]Cancer Immunotherapy Group, Oncobell and iProCURE Programs, IDIBELL-Institut Català d'Oncologia, l'Hospitalet de Llobregat, Barcelona, Spain. [8]Radiotherapy Oncology, Institut Català d'Oncologia (ICO) L'Hospitalet del Llobregat, IDIBELL, University of Barcelona, Barcelona, Spain. [9]Urology Service, Hospital Universitari de Bellvitge, Barcelona, Spain. [10]Radiotherapy Oncology, Hospital Universitario Virgen del Rocío, Sevilla, Spain. [11]Radiotherapy Oncology, Fundación Centro Oncológico de Galicia, A Coruña, Spain. [12]Radiation Oncology Department, Catalan Institute of Oncology, Barcelona, Spain. [13]Oncology Urology Department, Hospital Universitari i Politècnic la Fe, Valencia, Spain. [14]Radiation Oncology Department, Fundación Instituto Valenciano de Oncología (I.V.O.), Valencia, Spain. [15]Urology Department, Hospital Universitario Virgen del Rocío, Sevilla, Spain.

Prior Presentation: Presented as oral communication at the ASCO Annual Meeting 2021.

Corresponding Author: Xavier Garcia-del-Muro, Medical Oncology, Institut Català d'Oncologia (ICO), Avenue Gran Via de l'Hospitalet, 199-203, L'Hospitalet del Llobregat, Barcelona 08908, Spain. E-mail: garciadelmuro@iconcologia.net

Clin Cancer Res 2025;31:659–66

doi: 10.1158/1078-0432.CCR-24-2636

Translational Relevance

IMMUNOPRESERVE is the first study combining radiotherapy with dual immune checkpoint inhibition using durvalumab plus tremelimumab. Our results show that the combined treatment results in a high rate of complete responses (81%) and a significant number of patients with bladder preserved (2-year bladder-intact disease-free survival of 65%). Moreover, this clinical benefit was maintained over time, with a 2-year overall survival rate of 84%. Adverse events were mostly low grade and manageable. Adverse events of grade 3 to 4 related to therapy were reported in 31% of patients.

This study constitutes a proof of concept that a combined-modality approach including immunotherapy with durvalumab plus tremelimumab with concurrent radiotherapy is feasible and safe, avoiding some of the limitations and toxicities usually associated with chemotherapy. This approach shows high efficacy in terms of response rate, elicits bladder preservation in a large number of patients, and warrants further investigation.

refusal. In the absence of randomized clinical trials comparing bladder-preserving approaches with radical cystectomy (8), a recent propensity score–matched and –weighted study has shown similar survival outcomes between both treatments in a large multicenter series of patients (9).

In recent years, the incorporation of immune checkpoint inhibitors (ICI) has transformed the treatment of metastatic urothelial carcinoma, substantially improving the survival of patients (10). Moreover, some recent studies have suggested that these agents may also be effective in earlier stages of the disease (11–13). However, the role of immunotherapy in patients with MIBC eligible for bladder preservation strategies remains unexplored. Several preclinical studies suggest an interaction between immunotherapy and radiotherapy (14, 15). In particular, a study showed that the combination of radiation with a cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) inhibitor and a PD-L1 inhibitor activates nonredundant immune mechanisms, potentiating antitumor activity in preclinical models (16). Therefore, the present study aimed to explore the feasibility, efficacy, and toxicity of a multimodal bladder-sparing approach combining double immune checkpoint inhibition and radiotherapy, and avoiding chemotherapy, in patients with localized MIBC.

Patients and Methods

Study design and patients

IMMUNOPRESERVE is a multicenter, single-arm, open-label, phase II trial. Patients ages ≥18 years, with a histologic diagnosis of urothelial MIBC in clinical stages T2 to 4a N0 M0, who were ineligible for radical cystectomy owing to medical reasons or patient refusal, were eligible. The urothelial component had to be predominant, although mixed histology was acceptable. Patients were required to have Eastern Cooperative Oncology Group performance status of 0 or 1, adequate bone marrow and hepatic function, and creatinine clearance >40 mL/minute. Main exclusion criteria were prior treatment with radiotherapy to the bladder, systemic chemotherapy or immune-mediated therapy except intravesical Bacillus Calmette–Guerin therapy, active or prior autoimmune or inflammatory disorders, active primary immunodeficiency, active infection, and use of immunosuppressive therapy other than ≤10 mg/day of prednisone.

Written informed consent was obtained from all patients before study entry. The study protocol was approved by the independent Ethics Committee of Hospital Universitari de Bellvitge (reference 15/18) on September 9, 2018, the competent authority in Spain (Agencia Española de Medicamentos y Productos Sanitarios), and the participating centers. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The study was included in the European registry for clinical trials (EudraCT: 2017-003159-44) and in www.clinicaltrials.gov (NCT03702179) on August 10, 2018.

Treatment schedule and assessments

The treatment consisted of initial maximal TURBT, with multiple random biopsies of the normal-appearing bladder urothelium, followed by intravenous durvalumab 1,500 mg plus tremelimumab 75 mg, every 4 weeks for a total of three doses. Two weeks after the initiation of immunotherapy, normofractionated external-beam radiotherapy with high-energy photons was administered concurrently. The planned volume for pelvis radiation was contoured from common iliac bifurcation to the obturator node area, with a 0.8 cm margin, to deliver 46 Gy at 2 Gy per fraction. Bladder volume included the proximal urethra and bladder with a 1 cm isometric margin. Delivered dose to this volume reached 64 to 66 Gy at 2 Gy per fraction. Pelvis and bladder volumes were treated together till 46 Gy, and then sequentially, radiation was delivered to the bladder volume till 64 to 66 Gy. A safety run-in cohort was performed in the first five patients included to assess potential dose-limiting toxicities caused by the combination of immunotherapy and radiation.

Response was assessed by cystoscopy and biopsy of the tumor bed and any residual lesion, performed 6 weeks after the end of radiotherapy. Patients with CR preserved their bladders, whereas those with residual MIBC were considered for salvage radical cystectomy. Patients with preserved bladders who developed an isolated bladder MIBC relapse during follow-up were also candidates for salvage cystectomy, whereas those developing a non–muscle-invasive bladder cancer (NMIBC) in the preserved bladder were managed with TURBT and intravesical therapy. Adverse events were graded using the Common Terminology Criteria for Adverse Events, version 4.03.

At baseline, patients underwent CT of the chest, abdomen, and pelvis and were followed up with CT and urine cytology every 3 months the first year, every 4 months the second year, and every 6 months thereafter. In patients with conserved bladders, cystoscopy was performed every 6 months. Additional cystoscopy and bladder biopsy will be performed in case of detection abnormalities in the cytology or imaging studies according to investigator criteria and local standard practice.

PD-L1 expression in archived formalin-fixed tumor biopsies obtained from baseline TURBT was centrally assessed using the PD-L1 VENTANA SP263 (AB_2819099) IHC assay (Ventana Medical Systems). PD-L1 combined positive score of 1 or greater was considered positive.

Outcomes

The primary endpoint of the study was the CR rate, defined as the absence of MIBC (≤T1) at the posttreatment evaluation based on a biopsy via cystoscopy. The biopsy should include muscle tissue to

ensure optimal assessment. Secondary endpoints included bladder-intact disease-free survival (BIDFS), defined as the time from the start of immunotherapy to either the date of cystectomy due to any cause, bladder MIBC recurrence, distant metastasis, or death; distant metastasis–free survival (DMFS), defined as the time from the start of immunotherapy to either the date of distant metastasis or death; overall survival (OS), defined as the time from the start of immunotherapy to the date of death due to any cause; and safety profile and tolerability of the combined-modality treatment.

Statistical analyses

The study was conducted using a two-stage sequential Simon optimal design, using a one-sided test with a type I error of 0.10 and power of 80% one-sided test. Using the assumption that the treatment would be considered ineffective if it had a response rate of 50% (P_0: 0.5) but would be of interest if it had a response rate of 70% of more (P_1: 0.7), the sample size requirement was 12 patients for the first stage and 20 additional patients for the second stage. Six or more responses in the first stage were required for continuation to second-stage accrual.

Continuous variables are summarized as *n*, median, and range. Categorical variables are summarized as frequency counts and percentages. The median follow-up time was calculated using the reverse Kaplan–Meier method. Time-to-event endpoints, including BIDFS, DMFS, and OS, were estimated using the Kaplan–Meier method. HRs and 95% confidence intervals (CI) for OS and DFS were calculated using stratified Cox proportional hazards and log-rank tests. Patients without events (cystectomy, MIBC recurrence, distant metastasis, or death for BIDFS; distant metastasis or death for DMFS; and death for OS) were censored on their last tumor assessment for BIDFS and DMFS or on their last contact for OS. Cumulative incidences of NMIBC, MIBC, and distant metastasis relapses were estimated using competing risk analysis. Statistical comparisons were two-tailed (P <0.05). Statistical analyses were performed using R [version 4.3.1 (2023); RStudio: integrated development environment for R. Posit Software, PBC, http://www.posit.co/, RRID: SCR_001905], and figures and tables were generated using RStudio (version 1.2.5033 2009-2019 RStudio, Inc., RRID: SCR_000432).

Biomarker analyses

Blood samples were obtained at the following timepoints: before the first immunotherapy dose (baseline), 1 week after the first dose, at the end of treatment (EOT), and 1 month after the EOT. Plasma samples were obtained using EDTA tubes. Total blood samples were collected using citric acid, citrate and dextrose in solution A (ACD-A) tubes (BD Biosciences), and peripheral blood mononuclear cells were isolated after a Ficoll gradient (Rafer). Due to the high rate of CRs obtained in the study, it was not possible to perform a biomarker comparison between responders and nonresponders, so a cohort of patients treated with the standard of care (SOC) was analyzed to identify potential differential trends potentially specific to ICI. The SOC cohort comprised 10 consecutive patients diagnosed with MIBC at clinical stages T2 to 4a N0 M0, who were eligible for cisplatin chemotherapy and radical cystectomy. These patients were treated at the Catalan Institute of Oncology between October 2020 and February 2021. The baseline characteristics, treatment, and response of these patients are presented in Supplementary Table S1. The analysis of the SOC cohort was completely exploratory and planned *post hoc*. The SOC patients also provided informed consent for the analysis of their samples with translational research objectives in urothelial cancer.

Peripheral blood mononuclear cell samples were analyzed using a flow cytometer (Flow Cytometry Platform from IDIBELL, RRID: SCR_025671) using five different antibody panels (detailed in Supplementary Table S2). Surface staining was performed following standard protocols. For intracellular cytoplasmic staining, the Cytofix/Cytoperm fixation/permeabilization kit (BD Biosciences, RRID: AB_2869008) was used. The FoxP3 Staining Buffer Set (Miltenyi Biotec) was used for intranuclear staining. All samples were acquired using the Gallios cytometer (Beckman Coulter, RRID: SCR_019639) and analyzed using FlowJo (Tree Star, RRID: SCR_008520) and R software (4.2.0 version, RRID: SCR_001905). For multiplex assay, plasma samples were analyzed using a ProcartaPlex Mix&Match 29-Plex kit (Thermo Fisher Scientific) on a MAGPIX multiplex reader (RRID: SCR_025645) and xPONENT 4 software (RRID: SCR_025653). Data were analyzed using R software (RRID: SCR_001905).

Data availability

Patient data will be provided upon reasonable request. Any request should be sent to the corresponding author with a detailed description of the research protocol. Access will be provided to the data after the proposal has been approved by the sponsor and an independent review committee established for this purpose.

Results

Patient characteristics and treatment administered

Between January 2019 and August 2020, 32 patients were enrolled at 6 Spanish centers. Baseline characteristics are listed in **Table 1** and representativeness contextualized in Supplementary Table S3. Ten (31%) patients were classified as unfit for cisplatin according to the Galsky criteria. Only one patient had mixed histology. No dose-limiting toxicities were observed in the first five patients, who were included in the safety run-in cohort; therefore, remaining patients were also treated at full doses. All patients received at least two cycles of immunotherapy, and 23 of them received the complete planned regimen. The third cycle of immunotherapy was not administered in nine patients, mainly owing to toxicity. The median dose of radiotherapy administered was 64 Gy (range 50–66).

Efficacy

In the pre-planned interim analysis, including the first 12 patients, all patients had a CR, eliciting the accrual expansion with the second stage. In total, the CR rate at the posttreatment biopsy was documented in 26 (81%; 80% CI, 69–90) patients (25 presented with T0 disease and 1 with T1), 2 (6%) patients had residual MIBC, and 4 (13%) were not evaluated owing to rejection, pneumonia, death from coronavirus disease 2019, and treatment-related death, respectively (**Table 2**). The lack of cystoscopic evaluation for the primary endpoint in four patients was considered a failure for this endpoint. The CR rate represented 93% of the patients evaluated by cystoscopy. No immediate salvage cystectomies were performed because the 2 nonresponders developed metastasis. After a median follow-up of 27 months (range, 14–41 months), two patients underwent salvage cystectomy owing to isolated bladder MIBC relapse at 9 and 21 months, respectively. Only one NMIBC relapse was observed. Overall, seven patients died: three from progressive disease, three from intercurrent diseases, and one from toxicity.

The estimated 2-year rate for BIDFS was 65% (95% CI, 50%–84%). The events were as follows: Late salvage cystectomies were

Table 1. Patient characteristics.

Characteristic, units	IMMUNOPRESERVE (*N* = 32)
Median age (range), years	71 (49–91)
Sex, *n* (%)	
Male	25 (78)
Female	7 (22)
Race, *n* (%)	
Caucasian	32 (100)
ECOG PS	
0	25 (78)
1	7 (22)
Histology, *n* (%)	
Urothelial carcinoma	31 (97)
Mixed urothelial carcinoma	1 (3)
Clinical T stage, *n* (%)	
T2	28 (88)
T3	3 (9)
T4	1 (3)
Cisplatin ineligible (Galsky), *n* (%)	
Yes	10 (31)
No	22 (69)
Creatinine clearance, *n* (%)	
≥60 mL/minute	23 (72)
<60 mL/minute	9 (28)
Non-MIBC history, *n* (%)	
Yes	14 (44)
No	18 (56)
Previous treatment, *n* (%)	
BCG	9 (64)
Mitomycin	1 (3)
PD-L1 expression, *n* (%)	
Positive	15 (56)
Negative	12 (44)
Unknown	5 (16)

Abbreviations: BCG, Bacillus Calmette–Guerin; ECOG PS, Eastern Cooperative Oncology Group performance status; MIBC, muscle invasive bladder cancer.

reported in 2 (6%) patients, bladder relapse or metastases in 7 (22%) patients, and death in 4 (13%) patients. The 2-year DMFS and OS rates were 83% (95% CI, 70%–98%) and 84% (95% CI, 73%–98%), respectively (**Fig. 1**). The 2-year cumulative incidence estimates of NMIBC relapse, MIBC relapse, and distant metastasis were 3% (95% CI, 0.2%–14%), 19% (95% CI, 8%–35%), and 16% (95% CI, 6%–31%), respectively (Supplementary Fig. S1). In total, seven patients died: three due to progression of the disease; three due to adverse events not related to study treatment that comprised one due to pneumonia, one due to coronavirus disease 2019, and one due to stroke; and one due to toxicity (see the Safety section for details).

The response rate in patients with PD-L1–positive expression was 65% (Supplementary Table S4). There was no observed association between response and immune-mediated toxicity. No correlation was found between time-to-event efficacy outcomes and the following clinical and molecular characteristics explored: age, sex, T stage, Eastern Cooperative Oncology Group performance status, creatinine clearance, and PD-L1 expression (Supplementary Figs. S2 and S3).

Safety

In total, 31 (97%) patients experienced adverse events of any grade related to radiotherapy and/or immunotherapy, with diarrhea (41%) and urinary disorders (38%) being the most common (**Table 3**). Grade 3 or 4 adverse events related to therapy were reported in 31% of patients, the most frequent being gastrointestinal toxicity including diarrhea (6%), acute kidney failure (6%), and hepatitis (3%). One patient experienced immune-related grade 3 panhypopituitarism, another grade 3 erythroderma, and another grade 3 cystitis. Two patients had immune-mediated colitis. One patient died owing to a suspected treatment-related colitis and peritonitis after two cycles of durvalumab and tremelimumab. The fatal event was related to both ICI and radiotherapy. In total, eight patients discontinued immunotherapy owing to treatment-related events.

Table 2. Pathologic response rate.

Outcome, *n* (%)	Number of patients (*N* = 32)	95% CI; binomial estimate
CR (≤T1)	26 (81)	(64–93)
T0	25 (78)	—
Non-MIBC (T1, Ta, and Tis)	1 (3)	—
Nonresponse (MIBC)	2 (6)	(1–21)
Not evaluated	4 (13)	(4–29)
Consent withdrawal	1 (3)	—
Clinical deterioration	1 (3)	—
Death from COVID-19	1 (3)	—
Toxic death from peritonitis	1 (3)	—

Abbreviation: COVID-19, coronavirus disease 2019.

Biomarker analyses

Analysis of immune populations in the patients treated in the study showed decreased cell counts for T and B lymphocytes and NK cells throughout treatment, being significant at the end of it, whereas monocyte levels were not altered with treatment (**Fig. 2A**). Normal lymphocyte levels recovered 4 weeks after the EOT. Notably, analysis in patients from the SOC cohort showed opposing immune population kinetics, with early monocyte depletion recovering at EOT and stable lymphocyte levels throughout treatment (Supplementary Fig. S4A). T-cell differentiation state distribution was analyzed (**Fig. 2B**), and a shift in CD4 T cells was observed from a naïve to effector (effector memory and terminal effector memory) phenotype in patients treated with ICI.

The main ICIs were also monitored, including those directly targeted in this study, PD1 and CTLA-4 (**Fig. 2B**). An increase in PD1 and T-cell immunoglobulin and mucin-domain containing-3 (TIM3) levels in CD8 and CD4 T cells was detected 1 week after the start of immunotherapy, reaching their highest levels at EOT. In addition, a peak in surface CTLA-4 expression was detected 1 week after the first dose of immunotherapy, which reduced at the EOT. Surface lymphocyte-activation gene 3 (LAG3) showed a moderate increase at the EOT in CD8 T cells (**Fig. 2B**). In contrast, the increase in CTLA-4 expression after 1 week of immunotherapy did not correlate with the expression of the other ICIs. These results are in contrast with the SOC cohort, which showed no variation in any of those receptors (Supplementary Fig. S4B). Finally, an increase in T-cell activation marker expression, particularly CD38 and human leukocyte antigen–DR isotype (HLA-DR) for CD8 and CD4 T cells, respectively, was observed (**Fig. 2B**). CD8 T cells clearly showed an increase in co-expression of two activation markers

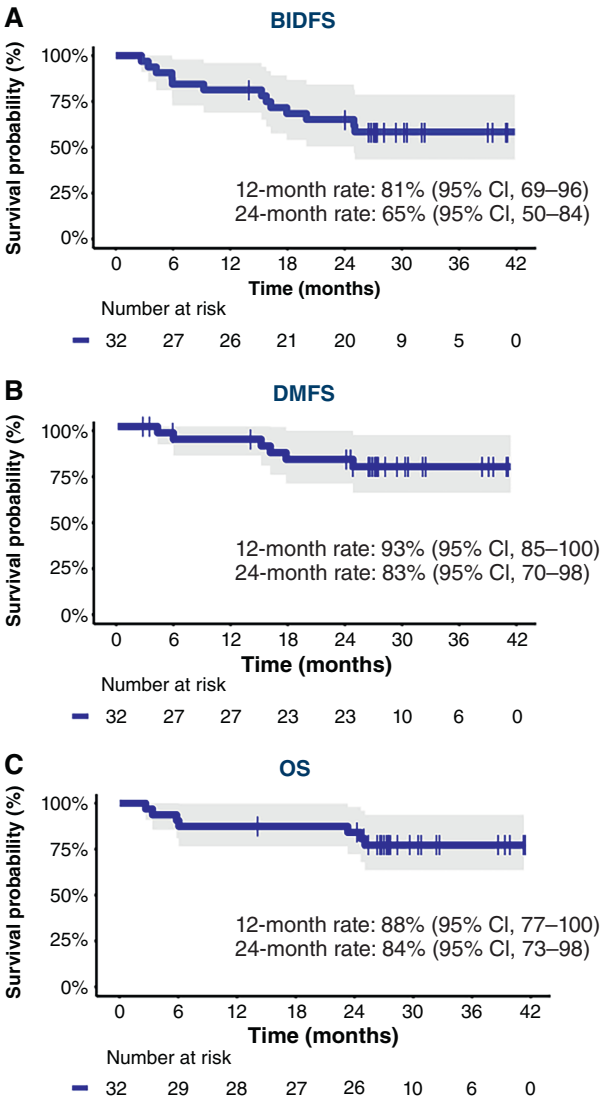

Figure 1.

Assessment of efficacy through time-to-event endpoints. **A,** BIDFS, defined as the time from the start of immunotherapy to either the date of cystectomy or the date of recurrence of MIBC or distant recurrence or death. **B,** DMFS, defined as the time from the start of therapy to distant recurrence or death. **C,** OS.

(CD38 and HLA-DR; **Fig. 2B**). A similar trend was observed in CD4 T cells, although reaching lower levels and favoring CD38 and HLA-DR co-expression. In the SOC cohort, a small tendency of CD38 upregulation was observed after the EOT, although reaching lower levels compared with the study cohort (Supplementary Fig. S4B).

The plasma concentration of 29 analytes related to inflammatory processes was measured through multiplex assay. In total, 22 samples from the study cohort and 18 samples from the SOC cohort were analyzed, comprising samples from baseline, 1 week after treatment start, and EOT. An increase in several molecules after the first week of immunotherapy, including IFNγ, IL1β, CD137 (4-1BB), PD1, TNFα, indoleamine 2,3-dioxygenase (IDO), B and T lymphocyte attenuator 4 (BTLA4), and interferon gamma-induced protein 10 (IP10), compared with baseline levels was determined (**Fig. 2C**). Although this variation was transient for some analytes, it was stable for others throughout the whole treatment. However, no such variation was observed in the SOC cohort (Supplementary Fig. S5).

Table 3. Safety profile and the most frequent treatment-related adverse events (threshold 10%).

Event, *n* (%)	Any grade	Grade ≥3
Any toxicity	31 (97)	10 (31)
Diarrhea	13 (41)	2 (6)
Renal and urinary disorders	12 (38)	—
Urinary frequency	10 (31)	—
Hyperthyroidism	8 (25)	—
Pruritus	7 (22)	—
Gastrointestinal disorder	6 (19)	3 (9)
Fatigue	6 (19)	—
Skin and subcutaneous tissue disorders	5 (16)	1 (3)
Hypothyroidism	4 (13)	—
ALT increased	4 (13)	1 (3)
Urinary tract pain	3 (9)	—
Rash	3 (9)	—
General disorders and administration site conditions	3 (9)	2 (6)
AST increased	3 (9)	1 (3)
Anemia	3 (9)	—

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Discussion

The present study constitutes an exploratory phase II trial, assessing the incorporation of immunotherapy instead of chemotherapy in a novel selective bladder-preserving combined-modality approach in patients with localized MIBC. The CR rate was chosen as the primary endpoint because it can be rapidly evaluated after multimodal treatment completion, and most studies of bladder preservation showed a good correlation with long-term bladder preservation and OS (1, 17). Our study showed a remarkably high CR rate. The 2-year DMFS and OS rates achieved in this study are consistent with the high activity observed, suggesting that this activity is maintained after the treatment. Notably, the study showed a promising BIDFS, a relevant parameter that reflects the long-term success of a bladder-preserving therapy in both aspects, survival and bladder preservation. During the follow-up period, only two late salvage cystectomies due to MIBC relapse were performed. The 2-year BIDFS rates observed in our study seem comparable with those reported in recent bladder preservation studies utilizing chemotherapy and radiotherapy. However, the relatively small sample size of our cohort raises the possibility that certain adverse characteristics frequently present in our patients, such as a prior history of NMIBC and ineligibility for cisplatin, may have negatively affected the long-term outcome in our study.

In this regard, our study shows an association between the treatment and a systemic impact on lymphocyte subpopulations, increasing its activation and immune checkpoint expression (i.e., PD1 and TIM3) and promoting differentiation of CD4 T cells toward an effector profile. This was accompanied by an initial release of inflammatory mediators in plasma (i.e., IFNγ and TNFα), and some mediators remained at higher concentrations throughout the treatment. Conversely, this immune activation was absent in a

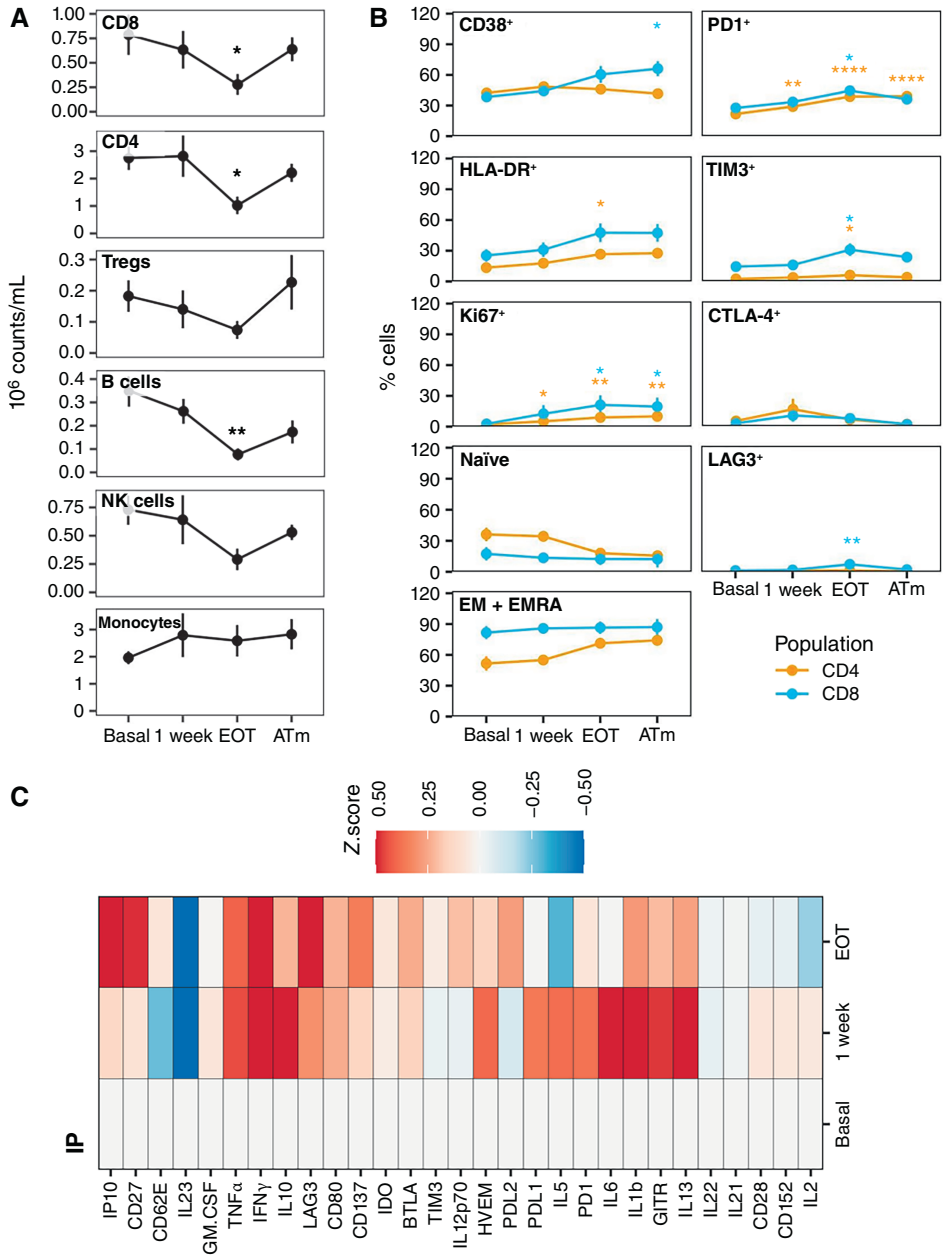

Figure 2.

Phenotype profiling of immune populations by flow cytometry. **A,** Percentage of immune cells, showing the mean number of cells/mL in blood for each immune population at baseline (basal), 1 week of treatment (1 week), EOT, and 1–3 months after the EOT (ATm). **B,** Percentage of cells within CD8 or CD4 T populations (mean + SE). Statistical comparisons between each timepoint and the basal levels (Mann–Whitney U test; *, <0.05; **, <0.01; ****, <0.0001). **C,** Measurement of soluble inflammatory mediators in peripheral blood by multiplex assay. Heatmap showing changes in the expression of each biomarker in the IMMUNOPRESERVE (IP) cohort. BTLA, B and T lymphocyte attenuator; EM, effector memory; EMRA, terminal effector memory; HVEM, herpesvirus entry mediator; IP10, IFNγ-induced protein 10; Treg, regulatory T cells.

control cohort treated with chemotherapy and surgery, even in those patients achieving CRs. This suggests different mechanisms of action for both therapies and a direct correlation of immunotherapy combined with radiation with systemic activation in early-stage MIBC.

An interesting observation was the low relapse rate of NMIBC in the preserved bladders. Although the follow-up was limited, only one patient experienced relapse, a low rate in comparison with the majority of series using chemotherapy regimens (3). We hypothesize that ICIs might play some protective role against this type of relapse, considering that they are active in the treatment of NMIBC (18).

The combination of pelvic radiotherapy with concurrent durvalumab and tremelimumab had a manageable safety profile, with no new or unexpected safety concerns. The incidence of adverse events was consistent with that observed in previous studies exploring dual ICIs and radiation separately, as no apparent increase in gastrointestinal and urinary toxicities due to the combination was observed. However, there was one toxic death due to intestinal perforation and peritonitis; thus, special caution should be paid to this potential complication. Notably, at least two of the three initially planned doses of immunotherapy were administered to all the patients.

Currently used trimodal bladder-preserving therapies include chemotherapy, cisplatin being the most used agent, although alternative schedules with fluorouracil or gemcitabine are also available. There is a wide experience with these regimens. A pooled analysis of patients treated in the Radiation Therapy Oncology Group (RTOG) trials reported a rate of CRs of 69% (3) and a large European series reported 72% (2). Our results compare favorably with these values,

suggesting that the combination of immunotherapy and radiation may be highly active for bladder preservation, although caution is needed because the number of patients is small and the follow-up is still limited. However, new regimens combining radiation and gemcitabine have also shown a promising high activity (19). In our study, cytology and MRI were not systematically included in the response assessment protocol. Our evaluation was primarily based on re-biopsy of the tumor bed and any residual lesions, along with CT imaging. As a result, we acknowledge the potential for a slight overestimation of the response rate in comparison with studies that incorporated these additional techniques into their assessment protocols. Moreover, the safety profile of the present regimen that spares chemotherapy could be an additional advantage over current chemotherapy-including bladder-preserving therapies. Approximately half of the patients with bladder cancer are considered unfit for cisplatin, and precisely, patients who are candidates for bladder-preserving therapies have often been discarded for cystectomy owing to comorbidity or advanced age. Thus, in patients with renal dysfunction or high risk of chemotherapy toxicity, immunotherapy-based regimens could be particularly useful.

Preclinical evidence of interaction between anti–CTLA-4 agents and radiation has long been reported (16–20). Moreover, PD1 or PD-L1 blockade combined with radiation has also shown improved activity in preclinical models (20). It is well known that radiotherapy can increase expression of tumor-associated antigens, release cytokines, stimulate recruitment of dendritic cells, and activate cytotoxic T-cell function, complementing the action of immunotherapy. However, the influence of the different radiation fractions remains to be clarified. Recently, a meta-analysis of available randomized trials showed that hypofractionated radiotherapy was superior to the conventional schedule in locoregional control of MIBC (21). Our study was designed before the meta-analysis was reported; thus, conventional fractionation was used. Nonetheless, further investigation on the combination of hypofractionated radiotherapy and ICI would be of interest.

A recent study evaluated the combination of cisplatin and gemcitabine plus nivolumab as organ-sparing therapy for MIBC (22). The treatment regimen, which lacked radiotherapy, was active and obtained 43% CRs. Nevertheless, the proportion of potential candidates for bladder preservation seems to be much lower than that attained with approaches including radiation as local therapy. Moreover, a long follow-up is required to determine if the absence of radiotherapy may also have an impact on long-term bladder preservation. The preliminary results of another phase II study combining pembrolizumab with chemoradiation showed promising results (23). Additionally, the combination of durvalumab as a single agent in combination with radiotherapy in the neoadjuvant/adjuvant setting also showed promising results (24, 25). Taken together, our results along with the abovementioned studies suggest that immunotherapy may play a significant role in multimodal bladder-preservation strategies.

In conclusion, a combined-modality approach including durvalumab plus tremelimumab with concurrent radiotherapy is feasible and safe, avoiding some of the limitations and toxicities usually associated with chemotherapy. This approach shows high efficacy in terms of response rate and elicits bladder preservation in a large number of patients. Nevertheless, caution is still required owing to the phase II nature of the trial, the small sample size, and limited follow-up. Moreover, subgroup analysis was exploratory and hypothesis-generating owing to the limitation in sample size. The future results of ongoing trials will help contextualize these results. Further research on this approach as an alternative to cystectomy, including larger series with longer follow-up, comparison with chemoradiation schedules, and identification of predictive factors for improving the selection of patients, is warranted.

Authors' Disclosures

X. Garcia-del-Muro reports a role as advisor for Pfizer, Bristol Myers Squibb, Ipsen, Roche, PharmaMar, GES, Merck, Eisai, Deciphera, MSD, Recordati, and Astellas Pharma; a role as speaker on behalf of Pfizer, Astellas Pharma, Eisai, and Recordati; funding for research from AstraZeneca and Incyte; and travel and accommodation payment from Pfizer, Roche, and Merck. B. P. Valderrama reports personal fees and nonfinancial support from Bristol Myers Squibb, MSD, Merck, and Astellas, nonfinancial support from Pfizer and Roche, and personal fees from Bayer, Advanced Accelerator Applications (AAA), AstraZeneca, Janssen, and Almirall Pharma outside the submitted work. A. Medina-Colmenero reports personal fees from AstraZeneca, Astellas, Johnson and Johnson, and Merck outside the submitted work. I. Miras Rodríguez reports nonfinancial support from pharma&, Eli Lilly and Company, and Deciphera Pharmaceuticals outside the submitted work. I. Ortiz reports personal fees from ICO L'Hospitalet, Pfizer, and Bristol Myers Squibb outside the submitted work, grants, personal fees, and nonfinancial support from GSK, nonfinancial support from MSD, and personal fees and nonfinancial support from Ipsen outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

X. Garcia-del-Muro: Conceptualization, resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **B.P. Valderrama:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **A. Medina-Colmenero:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **O. Etxaniz:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **R. Gironés Sarrió:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **M.J. Juan-Fita:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **M. Costa-García:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **R. Moreno:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **I. Miras Rodríguez:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **I. Ortiz:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **A. Cuéllar:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **F. Ferrer:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **F. Vigués:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **R. de Haro Piedra:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **A. Candal Gomez:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **S. Villà:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **J.L. Pontones:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **Y. Murria:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **G. Lendínez-Cano:** Resources, formal analysis, supervision, funding acquisition,

validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **R. Alemany:** Resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing.

Acknowledgments

This work was sponsored by the Spanish Oncology GenitoUrinary Group (SOGUG). AstraZeneca provided durvalumab and tremelimumab and awarded a grant to SOGUG and X. Garcia-del-Muro to pay the costs of the study. The funders did not play a role in the design or conduction of this study. The authors especially thank all patients and families involved in the IMMUNOPRESERVE study; the MFAR Clinical Research team for regulatory, monitoring, and quality assurance activities; Pau Doñate, PhD, for manuscript and language editing; and Emilio Pecharroman, PhD, for statistical calculations.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received August 20, 2024; revised October 30, 2024; accepted December 11, 2024; published first February 17, 2025.

References

1. James ND, Hussain SA, Hall E, Jenkins P, Tremlett J, Rawlings C, et al. Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. N Engl J Med 2012;366:1477–88.
2. Rödel C, Grabenbauer GG, Kühn R, Papadopoulos T, Dunst J, Meyer M, et al. Combined-modality treatment and selective organ preservation in invasive bladder cancer: long-term results. J Clin Oncol 2002;20:3061–71.
3. Mak RH, Hunt D, Shipley WU, Efstathiou JA, Tester WJ, Hagan MP, et al. Long-term outcomes in patients with muscle-invasive bladder cancer after selective bladder-preserving combined-modality therapy: a pooled analysis of Radiation Therapy Oncology Group protocols 8802, 8903, 9506, 9706, 9906, and 0233. J Clin Oncol 2014;32:3801–9.
4. Giacalone NJ, Shipley WU, Clayman RH, Niemierko A, Drumm M, Heney NM, et al. Long-term outcomes after bladder-preserving tri-modality therapy for patients with muscle-invasive bladder cancer: an updated analysis of the Massachusetts General Hospital Experience. Eur Urol 2017;71:952–60.
5. Arcangeli G, Arcangeli S, Strigari L. A systematic review and meta-analysis of clinical trials of bladder-sparing trimodality treatment for muscle-invasive bladder cancer (MIBC). Crit Rev Oncol Hematol 2015;94:105–15.
6. Ploussard G, Daneshmand S, Efstathiou JA, Herr HW, James ND, Rödel CM, et al. Critical analysis of bladder sparing with trimodal therapy in muscle-invasive bladder cancer: a systematic review. Eur Urol 2014;66:120–37.
7. Pieretti A, Krasnow R, Drumm M, Gusev A, Dahl DM, McGovern F, et al. Complications and outcomes of salvage cystectomy after trimodality therapy. J Urol 2021;206:29–36.
8. Huddart RA, Hall E, Lewis R, Birtle A; SPARE Trial Management Group. Life and death of spare (selective bladder preservation against radical excision): reflections on why the spare trial closed. BJU Int 2010;106:753–5.
9. Zlotta AR, Ballas LK, Niemierko A, Lajkosz K, Kuk C, Miranda G, et al. Radical cystectomy versus trimodality therapy for muscle-invasive bladder cancer: a multi-institutional propensity score matched and weighted analysis. Lancet Oncol 2023;24:669–81.
10. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee J-L, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 2017;376:1015–26.
11. Szabados B, Kockx M, Assaf ZJ, van Dam P-J, Rodriguez-Vida A, Duran I, et al. Final results of neoadjuvant atezolizumab in cisplatin-ineligible patients with muscle-invasive urothelial cancer of the bladder. Eur Urol 2022;82:212–22.
12. Necchi A, Anichini A, Raggi D, Briganti A, Massa S, Lucianò R, et al. Pembrolizumab as neoadjuvant therapy before radical cystectomy in patients with muscle-invasive urothelial bladder carcinoma (PURE-01): an open-label, single-arm, phase II study. J Clin Oncol 2018;36:3353–60.
13. van Dijk N, Gil-Jimenez A, Silina K, Hendricksen K, Smit LA, de Feijter JM, et al. Preoperative ipilimumab plus nivolumab in locoregionally advanced urothelial cancer: the NABUCCO trial. Nat Med 2020;26:1839–44.
14. Wang Y, Liu Z-G, Yuan H, Deng W, Li J, Huang Y, et al. The reciprocity between radiotherapy and cancer immunotherapy. Clin Cancer Res 2019;25:1709–17.
15. Sharabi AB, Lim M, DeWeese TL, Drake CG. Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy. Lancet Oncol 2015;16:e498–509.
16. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015;520:373–7.
17. Garcia del Muro X, Condom E, Vigués F, Castellsagué X, Figueras A, Muñoz J, et al. p53 and p21 Expression levels predict organ preservation and survival in invasive bladder carcinoma treated with a combined-modality approach. Cancer 2004;100:1859–67.
18. Balar AV, Kamat AM, Kulkarni GS, Uchio EM, Boormans JL, Roumiguié 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.
19. Coen JJ, Zhang P, Saylor PJ, Lee CT, Wu C-L, Parker W, et al. Bladder preservation with twice-a-day radiation plus fluorouracil/cisplatin or once daily radiation plus gemcitabine for muscle-invasive bladder cancer: NRG/RTOG 0712-A randomized phase II trial. J Clin Oncol 2019;37:44–51.
20. Demaria S, Golden EB, Formenti SC. Role of local radiation therapy in cancer immunotherapy. JAMA Oncol 2015;1:1325–32.
21. Choudhury A, Porta N, Hall E, Song YP, Owen R, MacKay R, et al. Hypofractionated radiotherapy in locally advanced bladder cancer: an individual patient data meta-analysis of the BC2001 and BCON trials. Lancet Oncol 2021;22:246–55.
22. Galsky MD, Daneshmand S, Izadmehr S, Gonzalez-Kozlova E, Chan KG, Lewis S, et al. Gemcitabine and cisplatin plus nivolumab as organ-sparing treatment for muscle-invasive bladder cancer: a phase 2 trial. Nat Med 2023;29:2825–34.
23. Tissot G, Xylinas E. Efficacy and safety of pembrolizumab (MK-3475) in combination with chemoradiotherapy versus chemoradiotherapy alone in muscle-invasive bladder cancer: the MK-3475-992/KEYNOTE-992 trial. Eur Urol Focus 2023;9:227–8.
24. Joshi M, Tuanquin L, Zhu J, Walter V, Schell T, Kaag M, et al. Concurrent durvalumab and radiation therapy (DUART) followed by adjuvant durvalumab in patients with localized urothelial cancer of bladder: results from phase II study, BTCRC-GU15-023. J Immunother Cancer 2023;11:e006551.
25. Powles T, Catto JWF, Galsky MD, Al-Ahmadie H, Meeks JJ, Nishiyama H, et al. Perioperative durvalumab with neoadjuvant chemotherapy in operable bladder cancer. N Engl J Med 2024;391:1773–86.