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RAPID COMMUNICATION

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

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ABSTRACT

Purpose
To determine the activity of pembrolizumab as neoadjuvant immunotherapy before radical cystectomy (RC) for muscle-invasive bladder carcinoma (MIBC) for which standard cisplatin-based chemotherapy is poorly used.

Patients and Methods
In the PURE-01 study, patients had a predominant urothelial carcinoma histology and clinical (c) T≤3bN0 stage tumor. They received three cycles of pembrolizumab 200 mg every 3 weeks before RC. The primary end point in the intention-to-treat population was pathologic complete response (pT0). Biomarker analyses included programmed death-ligand 1 (PD-L1) expression using the combined positive score (CPS; Dako 22C3 pharmDx assay), genomic sequencing (FoundationONE assay), and an immune gene expression assay.

Results
Fifty patients were enrolled from February 2017 to March 2018. Twenty-seven patients (54%) had cT3 tumor, 21 (42%) cT2 tumor, and two (4%) cT2-3N1 tumor. One patient (2%) experienced a grade 3 transaminase increase and discontinued pembrolizumab. All patients underwent RC; there were 21 patients with pT0 (42%; 95% CI, 28.2% to 56.8%). As a secondary end point, downstaging to pT<2 was achieved in 27 patients (54%; 95% CI, 39.3% to 68.2%). In 54.3% of patients with PD-L1 CPS ≥ 10% (n = 35), RC indicated pT0, whereas RC indicated pT0 in only 13.3% of those with CPS < 10% (n = 15). A significant nonlinear association between tumor mutation burden (TMB) and pT0 was observed, with a cutoff at 15 mutations/Mb. Expression of several genes in pretherapy lesions was significantly different between pT0 and non-pT0 cohorts. Significant post-therapy changes in the TMB and evidence of adaptive mechanisms of immune resistance were observed in residual tumors.

Conclusion
Neoadjuvant pembrolizumab resulted in 42% of patients with pT0 and was safely administered in patients with MIBC. This study indicates that pembrolizumab could be a worthwhile neoadjuvant therapy for the treatment of MIBC when limited to patients with PD-L1–positive or high-TMB tumors.

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INTRODUCTION

The recommended standard of care for muscle-invasive urothelial bladder carcinoma (MIBC) is radical cystectomy (RC) with bilateral pelvic lymph node dissection, preceded by the administration of neoadjuvant chemotherapy in patients who are eligible to receive cisplatin.[1] However, neoadjuvant chemotherapy has failed to become a widely used treatment for MIBC, as it is administered in only 20% of eligible patients. Approximately 50% of patients are ineligible to receive cisplatin as a result of established pre-existing contraindications, and a subset of patients refuse to receive any chemotherapy.[2,3]

RC in Europe usually occurs 6 to 8 weeks after the diagnosis of MIBC, with a delay in definitive surgery of more than 12 weeks associated with an increased mortality.[4] This time

ASSOCIATED CONTENT

Appendix
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Data Supplements
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frame offers a unique opportunity to test therapies in the neoadjuvant setting. Although neoadjuvant chemotherapy is active, residual high-risk disease (pT2 or higher) is present in more than 50% of patients and represents a poor prognostic factor.[5] These findings, coupled with the overall high rate of relapse and rapid development of metastatic disease in some patients, favor a multidisciplinary approach.[6] Moreover, pathologic downstaging with neoadjuvant therapy and especially the finding of a pathologic complete response (pT0) to neoadjuvant treatment are a well-recognized surrogate end point of overall survival.[7,8]

The introduction of immune checkpoint inhibitors has revolutionized the therapeutic landscape of urothelial carcinoma (UC). Among several anti–programmed cell death (PD)-1/ligand-1 (PD-L1) agents that have been approved for locally advanced or metastatic UC in the postplatinum setting, pembrolizumab is the only therapy to have been approved by both the US Food and Drug Administration and European Medicines Agency based on level 1 evidence. Pembrolizumab is also approved as first-line treatment in cisplatin-ineligible patients with high PD-L1 expression.[9-11] Integrating short courses of pre-RC immunotherapy in nonmetastatic MIBC has the potential to become a new strategy for neoadjuvant therapy.[12] The PURE-01 study was designed to assess the efficacy and to obtain biomarker results of single-agent, neoadjuvant pembrolizumab administration in patients with MIBC.

PATIENTS AND METHODS

Study Population and Trial Design

Eligible patients had a confirmed diagnosis of MIBC, were scheduled for RC, and had clinical (c)T2-3bN0M0 stage disease. Additional inclusion criteria included a predominant (ie, at least 50%) UC histology, Eastern Cooperative Oncology Group performance status of 0 to 2, and a renal function defined by a glomerular filtration rate of at least 20 mL/min. Patients were enrolled regardless of their cisplatin eligibility.

Patients were staged with thorax-abdomen computed tomography, fluorodeoxyglucose positron emission tomography–computed tomography scan and multiparametric magnetic resonance imaging (mpMRI) of the bladder to better assess residual disease after transurethral resection of the bladder (TURB; details of the mpMRI protocol are provided in the Data Supplement). Patients received three courses of pembrolizumab 200 mg intravenously every 3 weeks, followed by restaging via the same radiologic assessments 1 week later and by RC within a minimum of 1 week and a maximum of 3 weeks of the last dose, unless otherwise clinically indicated. In selected cases of initial treatment failure, on the basis of the investigator's decision, additional standard chemotherapy—three cycles of dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) —was administered before surgery. These patients were considered to have experienced treatment failure for the primary end point. Post-RC management followed European Association of Urology guidelines,[1] and survival data were collected until 2 years post-RC.

The primary end point was pT0 in the intention-to-treat population. Statistical assumptions were as follows: the H_1 was pT0 $\geq$ 25% and the H_0 (RC benchmark) was pT0 $\leq$ 15%.[13] In a Simon's MiniMax two-stage design, the study had a total sample size of 71 patients, with 43 patients included in the first stage. Of the 71 patients, 14 pT0 was the limit for H_0 rejection (with 80% power and a one-sided test of significance at the 10% level). Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events classification, version 5.0. The study was conducted in accordance with good clinical practice guidelines and the provisions of the Declaration of Helsinki. All patients provided written informed consent before enrollment.

Biomarker Analyses

The pathologic diagnosis of each patient was centrally reviewed. TURB and RC specimens were used to analyze biomarkers and match pre- and post-therapy results. PD-L1 expression was determined by immunohistochemistry (IHC; Dako 22C3 pharmDx assay; Agilent Technologies, Carpinteria, CA, USA) at a local laboratory, with expressions scored using the combined positive score (CPS) as previously described.[9,10] PD-L1 expression was evaluated on the hotspot region of the tumor slide and validated by two independent pathologists (M.C. and S.M.). Genomic profiling was performed with a hybrid capture-based next-generation sequencing assay (FoundationONE) in a single Clinical Laboratory Improvement Amendments–certified laboratory (Foundation Medicine, Cambridge, MA). Samples were assayed for all coding exons of 395 cancer-related genes plus select introns from 31 genes that are frequently rearranged in cancer. Sequencing was performed to a mean exon coverage depth of $>$ 500×. Resultant sequences were analyzed for all classes of genomic alterations, including short variant alterations, copy number alterations, and selected gene fusions or rearrangements, as previously described.[14] Microsatellite instability was determined on 114 loci. Tumor mutational burden (TMB; reported as mutations [mut]/Mb) was determined on 1.1 Mb of sequenced DNA for each sample on the basis of the number of somatic base substitution or indel alterations per Mb after filtering to remove known functionally oncogenic mutations.[15] Finally, using quantitative polymerase chain reaction (details in the Data Supplement and Appendix Table A1, online only), we analyzed relative gene expressions of 22 genes that had already been reported to be associated with immune response mechanisms.[16,17] In matched pre-pembrolizumab and post-pembrolizumab samples, we also investigated the expressions of six genes—*CCL2, CCL7, CCL8, IL10, VEGFA,* and *VEGFC*—that belong to the signature of innate resistance to anti–PD-1 therapy.[18]

Statistical Analyses

As the actual and planned sample sizes are different, 95% CI of pT0 accounted for the Shultz correction,[19] whereas exact (Clopper-Pearson) CI was used for the secondary end point. Comparisons between patient subgroups were made via Fisher's exact tests for categorical variables and *t* tests for continuous variables. For single-gene alterations, we used the Bonferroni adjustment for multiple hypothesis adjustment (on the basis of 40 genes with three or more alterations, with and without variants of unknown significance). Logistic models were also used to analyze associations between genomic alterations/TMB and pT0 response. TMB was modeled as a continuous variable by means of a three-knot restricted cubic spline. Reported *P* values were two sided, and the significance level was set at .05 for all analyses.

RESULTS

Patient Characteristics and Safety

Fifty patients were enrolled and treated from February 2017 to March 2018 at two centers in Milan, Italy. Baseline characteristics are listed in Table 1. Forty-six patients (92%) were eligible to receive cisplatin chemotherapy. The majority (n = 27; 54%) of tumors were cT3 stage, and two cisplatin-ineligible patients were included despite having evidence of lymph node involvement. Median total treatment period was 63 days (interquartile range [IQR], 57 to 70 days). Medical and surgical AEs are listed in Appendix Table A2 (online only) and Table 2. The most frequent all-grade AE was thyroid dysfunction (n = 9; 18%), and there were three patients (6%) with grade 3 AEs that caused pembrolizumab discontinuation for one patient (MVAC administration instead). Postsurgical complications were consistent with previously reported findings. Clavien Dindo grade $>$ II complications occurred

Table 1. Baseline Characteristics (N = 50)

Characteristic	No. (%)
Time frame of accrual	Feb 2017 to Mar 2018
Median age, years (IQR)	66 (60-72)
Gender	
Male	41 (82)
Female	9 (18)
Smoking status	
Nonsmoker	19 (38)
Former smoker	22 (44)
Current smoker	9 (18)
Clinical T stage	
T2N0	21 (42)
T3N0	27 (54)
T2-3N1	2 (4)
Hydronephrosis	9 (18)
History of previous non–muscle-invasive UC	7 (14)
Previous BCG intravesical instillations	5 (10)
Histology	
Pure UC	41 (82)
UC and squamous cell carcinoma component	6 (12)
Micropapillary variant	2 (4)
Lymphoepithelioma-like variant	1 (2)
Concomitant carcinoma in situ component	3 (6)
Median bladder tumor volume, cm^3 (range)*	0.7 (0.4-1.5)
Cisplatin eligibility (Galsky criteria)	
Yes	46 (92)
No	4 (8)
No. of cycles of pembrolizumab administered	
1	1 (2)
2	2 (4)
3	47 (94)
Type of RC	
RARC	32 (64)
ORC	18 (36)
Type of urinary diversion	
Neobladder	23 (46)
Ileal conduit	26 (52)
Ureterocutaneostomy	1 (2)
Adjuvant chemotherapy post-RC	3 (6)
Median time from end pembrolizumab-RC, days (IQR)	22 (15-30)
Total treatment period†	
Median No. of days (IQR)	63 (57-70)

Abbreviations: BCG, Bacillus Calmette-Guérin; IQR, interquartile range; ORC, open radical cystectomy; RARC, robot-assisted radical cystectomy; RC, radical cystectomy; UC, urothelial carcinoma.
*Assessed via multiparametric magnetic resonance of the bladder.
†Calculated from cycle 1, day 1 of pembrolizumab until the date of RC.

in 15 patients (30%), and the most frequent complications were sepsis (n = 10; 20%) and subocclusion (n = 8; 16%). However, there were a few delayed immune-related AEs, including pyrexia (n = 3; 6%), pruritus (n = 3; 6%), and xerostomia (n = 2; 4%). All of the latter AEs occurred within 2 months postoperatively, and three patients required corticosteroid treatment.

Efficacy Outcomes

All treated patients underwent RC. Twenty-one patients (42%; 95% CI, 28.2% to 56.8%) achieved a pT0 stage, and an additional six patients had residual pTa (n = 3), pTis (n = 2), or pT1 (n = 1) stage tumor, which resulted in 27 patients (54%; 95% CI, 39.3% to 68.2%) being downstaged to nonmuscle invasive tumors (Table 3). Ten patients (20%) showed pathologic lymph node involvement, and five patients experienced treatment failure because they were deemed to receive sequential MVAC chemotherapy (lack of radiologic response [n = 4] and pembrolizumab discontinuation for grade 3 transaminitis [n = 1]). Two of these patients were downstaged to pTis with chemotherapy. No Response Evaluation Criteria in Solid Tumors (RECIST) –measurable disease progressions occurred during the treatment. An example of a patient with an MIBC response to pembrolizumab, assessed with mpMRI, is shown in the Data Supplement. At the time of data cutoff (June 10, 2018) median follow-up was 6.2 months.

Biomarker Analyses

TURB samples from all patients were retrospectively analyzed. Thirty-five patients (70%) had PD-L1 CPS ≥ 10% on their TURB specimen, and median TMB for all patients was 11.4 mut/Mb (IQR, 7 to 14 mut/Mb), with all tumors being microsatellite stable. PD-L1 expression and TMB were not correlated, as shown in the Data Supplement (Pearson's r = −0.118; P = .416). pT0 was achieved in 19 patients (54.3%) with PD-L1 CPS ≥ 10% compared with only two patients (13.3%) with CPS < 10% (P = .011; Table 3 and Data Supplement). A significant (P = .022) nonlinear association between TMB and pT0 response was found and suggested that a meaningful cutoff might be placed at a value of TMB ≥ 15 mut/Mb, corresponding to the 80th quantile (Data Supplement).

A total of 26 patients (52%) had deleterious DNA damage response and repair (DDR) and/or *RB1* gene alterations (Fig 1), and median TMB in these patients was higher than in those without (median, 13.2 mut/Mb *v* median, 9.7 mut/Mb; P = .008). Logistic models demonstrated that, by adjusting for TMB, the

Table 2. Postcystectomy Complications (N = 50)

Characteristic	No. (%)
Median length of hospital stay, days (IQR)	
Total patients	16 (12-20)
RARC	15 (10.8-18.3)
ORC	17 (15-20)
Neobladder	18.5 (15-24)
Ileal conduit	13 (9-17)
Median intraoperative blood loss, mL (IQR)	300 (150-500)
30-day readmission	11 (22)
30-day surgical reintervention	5 (10)
Postoperative complications (Clavien Dindo) within 90 days	
0	25 (50)
II	10 (20)
IIIa	9 (18)
IIIb	5 (10)
IV	1 (2)
Type of postoperative complications	
Fever of unknown origin	4 (8)
Sepsis	10 (16)
Subocclusion	8 (20)
Ureteral anastomosis dehiscence	2 (4)
Ileal anastomosis dehiscence/fistula	3 (6)
Median No. of removed lymph nodes (IQR)	
Total patients	27 (22-31)
RARC	30 (26-39.3)
ORC	20.5 (18.3-25)
Positive margin status	0 (0)

Abbreviations: IQR, interquartile range; ORC, open radical cystectomy; RARC, robot-assisted radical cystectomy.

Table 3. Pathologic Response to Pembrolizumab

Response	All Treated Patients (N = 50)	PD-L1 CPS ≥ 10% (n = 35)	PD-L1 CPS < 10% (n = 15)
Primary end point			
Pathologic complete response, No. (%)	21 (42)	19 (54.3)	2 (13.3)
95% CI	28.2 to 56.8		
Secondary end point			
Pathologic downstaging to pT<2, No. (%)	27 (54)	23 (65.7)	4 (26.7)
95% CI*	39.3 to 68.2		
Treatment failure, No. (%)			
pT2N0	2 (3.8)		
pT3-4N0	6 (12)		
pTanyN+	10 (20)		
Additional MVAC chemotherapy†	5 (10)		
RECIST v1.1 PD	0		

Abbreviations: CPS, combined positive score; MVAC, methotrexate, vinblastine, doxorubicin, cisplatin; PD, disease progression; PD-L1, programmed death ligand-1.
*Including pTa (n = 3), pTis (n = 2), and pT1 (n = 1).
†As a result of investigator decision after the evidence of radiologic non-response to pembrolizumab (n = 4) or because of the onset of immune-related, grade 3 transaminase increase (n = 1). These patients achieved pTis (n = 2), pT2pN2 (n = 1), and pT3pN1 (n = 2) stage at radical cystectomy.

association between DDR and/or *RB1* gene alterations and pT0 response was weakened (Appendix Table A3, online only).

In analyzing associations between single-gene alterations and pT0 response, *PBRM1* was shown to be the only significant gene (seven of seven pT0; unadjusted $P = .001$; Data Supplement), but it was no longer significant after multiple hypothesis testing (full data provided in the Data Supplement). In addition, tumors that harbored *PBRM1* mutations had significantly higher median TMB values than did those without them (16.7 mut/Mb *v* 10.5 mut/Mb; $P = .001$). The logistic model could not be applied as a result of the small numbers.

Gene expression analyses demonstrated that 18 of 22 genes from pT0 patients had significantly higher expressions in pretherapy lesions compared with those from pT≥2 patients (Data Supplement). Informative genes were those involved in interferon gamma signaling, antigen presentation (HLA molecules and immunoproteasome subunits), T-cell functional differentiation to cytolytic effectors, chemokines and chemokine receptors, inhibitory receptors and ligands, and the immunosuppressive gene *IDO1*.

Fourteen patients among those with a postpembrolizumab pT2 stage tumor or higher had paired tissue samples that were evaluable for comparison after quality check. Postpembrolizumab TMB was significantly lower compared with the baseline TMB score. Median postpembrolizumab TMB was 5.7 mut/Mb (IQR, 4.4 to 8.3 mut/Mb) versus median pretherapy TMB of 11.0 mut/Mb (IQR, 6.1 to 11.4 mut/Mn; $P = .002$; Fig 2A). Conversely, PD-L1 CPS increased, although the increase was not significant ($P = .1402$; Fig 2B). Changes in $CD8^+$ cell frequency and PD-L1 expression postpembrolizumab could also be visualized with IHC, as shown in Figure 2C.

An overall increase in immune-gene expression was observed in post-therapy lesions compared with baseline lesions (Fig 3 and Data Supplement). These analyses included genes that are involved in the promotion of adaptive immunity, negative regulation, and innate resistance to anti–PD-1 therapy.

Finally, a mean of 71.7% of genomic alterations, including variants of unknown significance, were shared between pretherapy

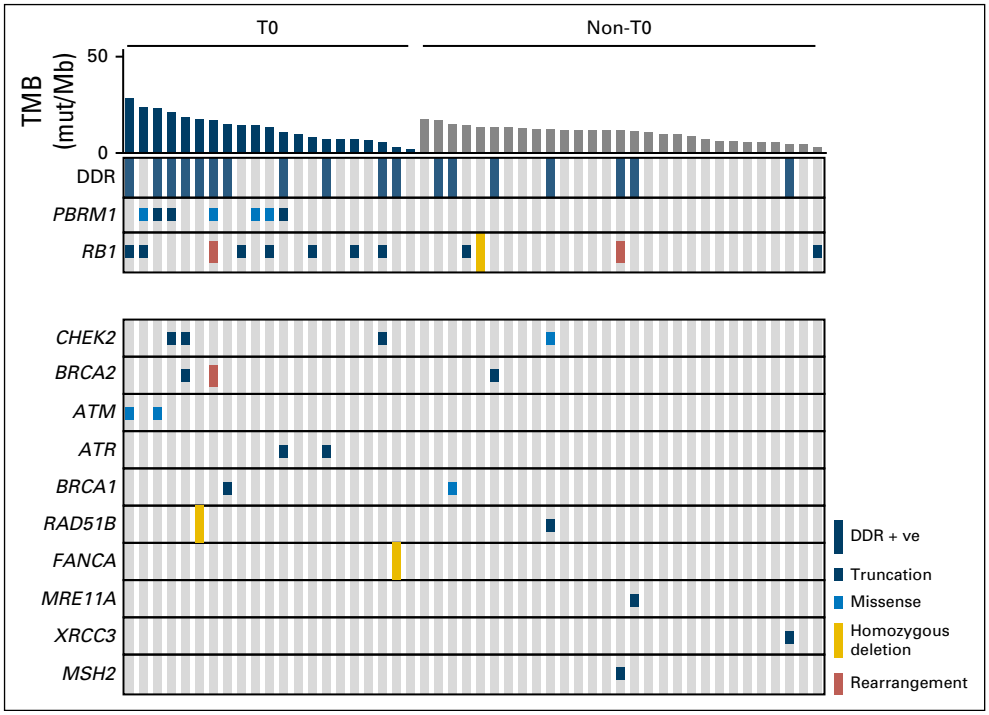

Fig 1. Oncoprint showing the spectrum of deleterious DNA damage response (DDR) and repair gene alterations observed in pretherapy lesions of all patients, divided according to the pathologic response to pembrolizumab. *PBRM1* gene alterations, which resulted in significant association with pT0 in unadjusted tests, are also shown. mut, mutation; T0, pathologic complete response; TMB, tumor mutation burden; +ve, positive.

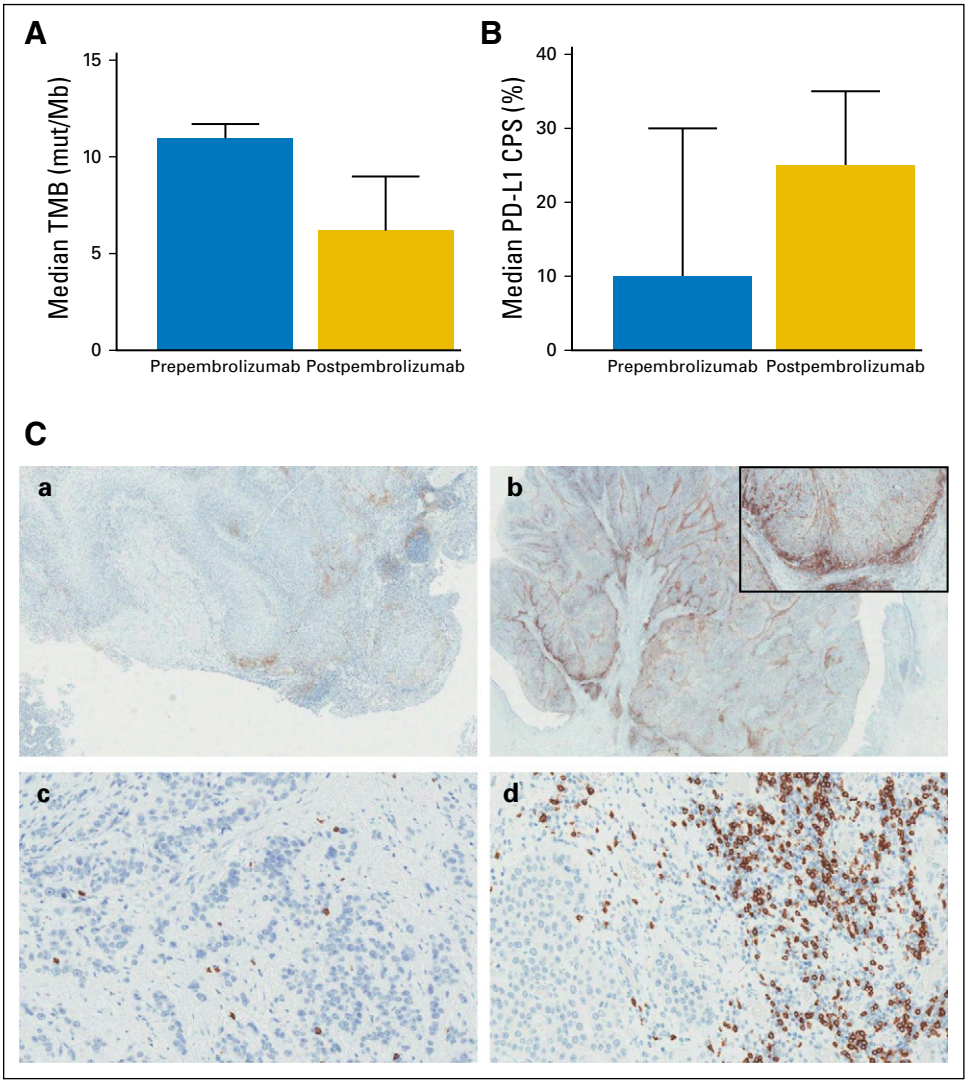

Fig 2. (A) Column bar graphs showing the median values (with interquartile range) of prepembrolizumab and postpembrolizumab tumor mutation burden (TMB) in the 14 patients with matched tumor samples sequenced via the FoundationONE assay. Paired *t* test value is reported in the text. (B) Column bar graphs showing the median values (with interquartile range) of prepembrolizumab and postpembrolizumab programmed death-ligand 1 (PD-L1) combined positive scores (CPSs) in the 14 patients with matched tumor samples assayed. Paired *t* test value is reported in the text. (C) Comparison of the immunohistochemical staining of the pretherapy tumor samples and that of the post-therapy tumor samples. (a) Immunohistochemistry for PD-L1 (Dako 22C3 pharmDx assay) on a pretherapy lesion showing CPS < 5% (reduced from ×4). (b) Immunohistochemistry for PD-L1 on a postpembrolizumab lesion showing CPS of 40% with PD-L1–expressing cells showing a distribution in the surrounding tumor areas (reduced from ×4), provided as low- and high-resolution (reduced from ×10, box) figures. (c) Immunohistochemical staining of $CD8^+$ cells on a pretherapy lesion (reduced from ×20). (d) Immunohistochemical staining of $CD8^+$ cells showing an increase of $CD8^+$ cells infiltrating the tumor stroma throughout the radical cystectomy specimen (reduced from ×20). Mut, mutation.

and post-therapy tumors, whereas a mean of 23.9% of cancer-related genomic alterations were lost and a mean of 7.2% were new after treatment (Data Supplement).

DISCUSSION

Three courses of pembrolizumab before RC in patients with MIBC resulted in an unprecedented proportion (42%) of patients with pT0, and the primary end point of the study was met far in advance of the planned accrual. These responses were significantly enriched in patients with PD-L1 CPS ≥ 10%, which constituted as much as 70% of the total enrolled patients—significantly more than the proportions reported in previous studies. This may be a consequence of our method of IHC assessment in which CPS was calculated on the hotspot region of the tumor section. No appreciable antitumor effects of pembrolizumab were observed in patients with CPS < 10%.

Pembrolizumab was associated with few immune-related AEs and did not delay planned surgery, and postsurgical complications recapitulated the most recent literature related to either open or robot-assisted procedures.[13] Furthermore, radical lymphadenectomy performance (the number of removed lymph nodes) was not compromised by neoadjuvant immunotherapy. A few noteworthy AEs, including delayed immune-related AEs that occurred post-cystectomy, were observed, which suggests that the follow-up schedule for postcheckpoint inhibitors and RC will require closer multidisciplinary monitoring.

The results of another window-of-opportunity study have been presented, allowing for the administration of two courses of neoadjuvant atezolizumab in cisplatin-ineligible patients with MIBC.[20] In this study, pT0 was observed in 40% of PD-L1–positive patients (evaluated on the tumor microenvironment with the Ventana anti–PD-L1 antibody) versus 16% in the PD-L1–negative cohort. In both investigator-initiated neoadjuvant trials, the chemotherapy-free regimen produced major pathologic responses in a substantial proportion of biomarker-selected cases, which indicates that the AEs of chemotherapy can be avoided in this setting when single-agent immunotherapy is used instead of cytotoxic drugs.

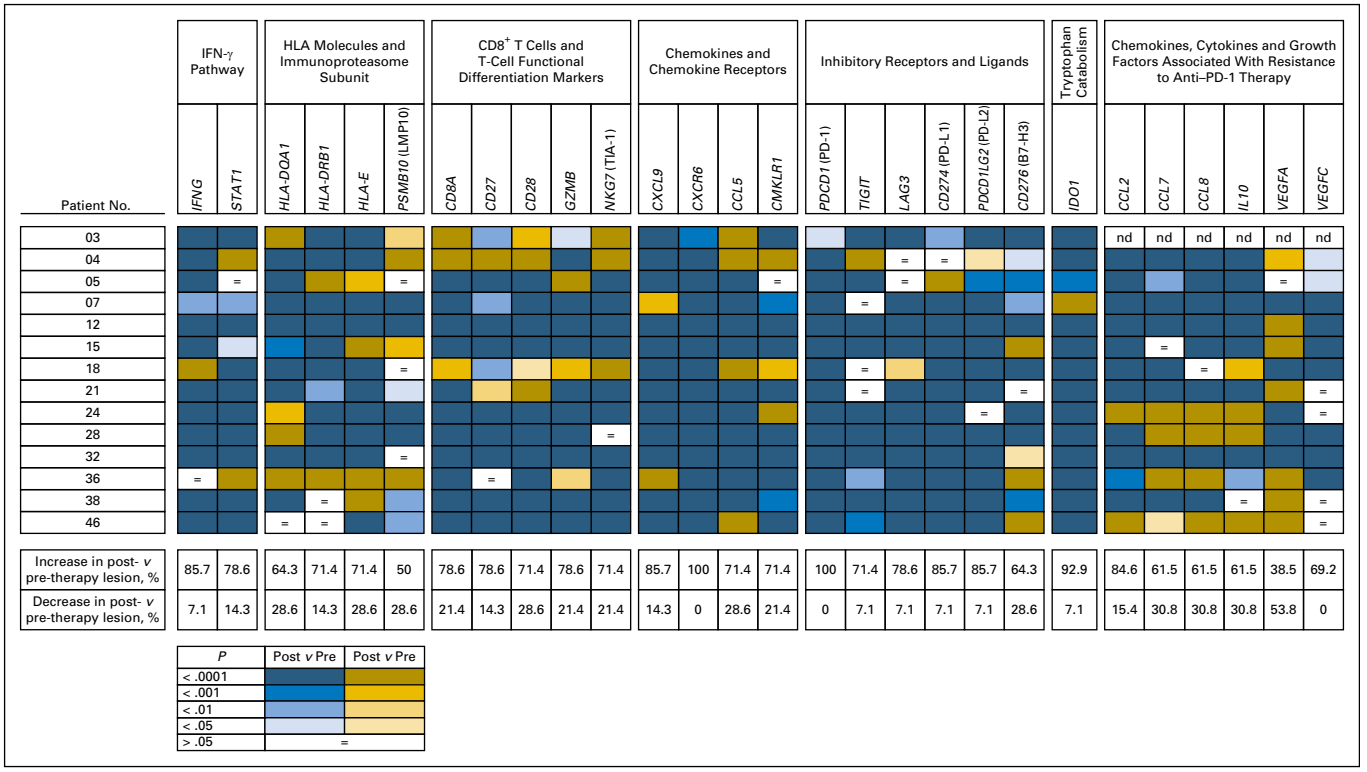

Fig 3. Neoadjuvant pembrolizumab modulates the expressions of immune-related genes associated with a response or resistance to programmed death 1 (PD-1) blockade. Formalin-fixed, paraffin-embedded sections from transurethral resection of the bladder and radical cystectomy samples of patients with pT≥2 tumor (n = 14) were compared using quantitative polymerase chain reaction (qPCR) for the expression of two sets of immune-related genes: a first set on the basis of the interferon gamma (IFN-γ)–related signature predicting response to the PD-1 blockade (from *IFNG* to *IDO1*), and a second set (from *CCL2* to *VEGFC*) on the basis of the signature of innate resistance to anti–PD-1. For each gene of interest, six qPCR replicate values obtained by analysis of the pretherapy (pre) and post-therapy (post) tissue samples in each patient are the result of independent technical procedures of reverse transcription, preamplification, and real-time PCR amplification, as described in Patients and Methods. Unpaired *t* test was used to compare gene expression in post- versus pretherapy lesions and corresponding *P* values are reported. Significant changes in gene expression observed in post- versus pretherapy lesions are shown by a color code on the basis of the *P* value, as shown by the legend (bottom; light blue to dark blue, significant increase in gene expression level in the post-therapy lesion compared with the pretherapy sample; light gold to dark gold, significant decrease in gene expression level in the post-therapy lesion compared with the pretherapy sample). Percentage of patients showing either an increase or a decrease in gene expression level in the post- versus pretherapy lesions are shown (bottom). nd, not done.

In the current study, the top scores of pretherapy TMB were associated with pT0. Likewise, the IMvigor210 study of patients with metastatic UC TMB demonstrated a nonlinear association with objective response and survival to atezolizumab, denoting a threshold effect at the top quartile.[21] Moreover, TMB emerged as another potentially dynamic biomarker of resistance to checkpoint inhibitors. Such a modulation (reduction) in the number of patients with residual high-risk disease, added to the gene expression data, may further support the hypothesis that an underlying immune editing process induced by pembrolizumab is able to select a less immunogenic tumor in nonresponders. Of note, the finding that the postpembrolizumab TMB decrease is associated with immunotherapy resistance and/or a lack of response conflicts with findings reported with nivolumab in patients with advanced melanoma.[22]

In our study, DDR gene alterations were associated with pT0 and higher TMB. DDR gene alterations have been associated with the clinical benefit of checkpoint inhibitors in advanced UC,[23] but are collinear with the mutational load. In comparing the mutational load with TMB, the latter offers refinements in calculation to ultimately better correlate with neoantigenic burden so that their results are correlated, but not directly comparable. Moreover, such results should be compared with those of a control gene group with similar megabase coverage to test whether DDR gene alterations truly preferentially associate with TMB or, rather, represent a passenger. Additional investigation is required to assess whether DDR gene lesions are a contributor to the checkpoint response or are predominantly a surrogate for the TMB.

Furthermore, DDR and *RB1* gene alterations have been linked to the pathologic response to neoadjuvant chemotherapy in MIBC.[24-26] Several phase II trials will already evaluate the role of an active surveillance/bladder-sparing approach in those patients who have DDR mutation–positive MIBC and show a clinical complete response to neoadjuvant chemotherapy or chemoimmunotherapy (ClinicalTrials.gov identifiers: NCT02710734, NCT03609216, and NCT03558087). Harmonization of the panels of DDR genes that are used to screen patients in similar trials is also needed.

PBRM1 mutations could be another biomarker of pT0 in the neoadjuvant immunotherapy setting. An extensive literature currently associates *PBRM1* genomic alterations with an objective response to immunotherapy in other tumor types, but additional investigation is needed in UC as it is possible that the lack of significance at multiple hypothesis testing is a result of the small numbers.[26,27]

In our study, on the basis of the immune-related gene profile, TURB lesions from patients who achieved pT0 featured characteristics

that are suggestive of a preexisting immunity that promoted a subsequent response to pembrolizumab. Increased expression of T-cell signature genes, as well as that of genes that encode for inhibitory molecules, is in fact a distinctive feature of T-cell inflamed–UC.[28]

The results of the matched pre–post-therapy analyses from the nonresponding tumors suggest that strong immune-mediated mechanisms of adaptive resistance may develop during neoadjuvant pembrolizumab administration and may contribute to the lack of tumor regression from RC, despite a concurrent promotion of adaptive immunity. The dynamic expression of several negative regulators of the immune response (eg, *IDO1*, *TIGIT*, and *VEGFC*) also suggests that combination immunotherapeutic approaches may further enhance the pathologic response. The few druggable gene alterations that were lost or newly identified in cystectomy specimens support the need for the reassessment of postimmunotherapy tissue before screening patients for trials with targeted agents.

Finally, we incorporated the use of advanced imaging tools to stage and evaluate the response to pembrolizumab in patients with MIBC. There are still major developments needed before these newer imaging methods can be routinely used to determine the pathologic stage of MIBC at diagnosis and after neoadjuvant treatments; however, our data will serve to prospectively validate newly proposed criteria to assess residual disease after neoadjuvant therapy and to predict pathologic response.[29]

Some limitations should be acknowledged in our study. The most relevant limitation is the lack of a mature follow-up period to present survival results. In particular, the hypothesis that pT0 response to immunotherapy may portend a survival benefit, as with chemotherapy, remains unproven. In this regard, the availability of a new nomogram for 12-month relapse-free survival quantification with neoadjuvant chemotherapy may represent a useful tool with which to retrospectively assess the benefit of single-agent pembrolizumab.[30] Several prospective, randomized, phase III studies that will compare chemotherapy with chemoimmunotherapy or combination immunotherapy are being planned and will potentially set new standard therapies for the treatment of MIBC. Another limitation is the exclusion of patients with cT4aN0M0 tumor from our study, which we applied for safety reasons (we included all comers regardless of cisplatin eligibility) and that may partly prevent comparability with chemotherapy outcomes. It should be noted, however, that patients with cT4a tumor represent less than 10% of the total enrolled patients in the majority of published studies.

In conclusion, neoadjuvant pembrolizumab therapy resulted in an impressively high proportion of pT0 in PD-L1–positive patients with MIBC. TMB scores of $\geq$ 15 mut/Mb in pretreatment tumors predicted an association with high pT0 frequencies. These results will encourage the clinical development of new neoadjuvant therapies and allow for more patients with MIBC to receive multimodality therapy. Pending the results of the next randomized studies, pembrolizumab may now be considered an option for cisplatin-ineligible patients with a PD-L1–expressing or high-TMB tumor.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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Appendix

Table A1. Taqman Gene Expression Assays

Gene	Assay ID
GAPDH	Hs00266705_g1
B2M	Hs99999907_m1
CCL2	Hs00234140_m1
CCL5	Hs00982282_m1
CCL7	Hs00171147_m1
CCL8	Hs00271615_m1
CD8A	Hs01555594_g1
CD27	Hs00386811_m1
CD28	Hs01007419_m1
CD274	Hs00204257_m1
CD276	Hs00987207_m1
CMKLR1	Hs01386064_m1
CXCL9	Hs00970537_m1
CXCR6	Hs00174843_m1
GZMB	Hs04261345_m1
HLA-DQA1	Hs03007426_mH
HLA-DRB1	Hs04192464_mH
HLA-E	Hs03045171_m1
IDO1	Hs00984148_m1
IFNG	Hs00989291_m1
IL10	Hs00961622_m1
LAG3	Hs00158563_m1
NKG7	Hs01120688_g1
PDCD1	Hs00355498_g1
PDCD1LG2	Hs00228839_m1
PSMB10	Hs00988194_g1
STAT1	Hs01013996_m1
TIGIT	Hs00545087_m1
VEGFA	Hs00173626_m1
VEGFC	Hs01099206_m1

Table A2. AEs Observed in the Intention-to-Treat Population (Common Terminology Criteria for Adverse Events v5.0; N = 50)

Treatment-Related AE	Grade 1-2	Grade 3-4	Total
Hyperthyroidism	6 (12)	—	6 (12)
Hypothyroidism	3 (6)	—	3 (6)
AST/ALT increase	3 (6)	1 (2)*	4 (8)
Iperkaliemia	2 (4)	1 (2)	3 (6)
Pruritus	3 (6)†	—	3 (6)
Pyrexia	3 (6)†	—	3 (6)
Xerostomia	2 (4)†	—	2 (2)
Pneumonitis	1 (2)	—	1 (2)
Myasthenia	1 (2)	—	1 (2)
Alopecia	2 (4)	—	2 (4)
Headache	1 (2)	—	1 (2)
Diarrhea	—	1 (2)	1 (2)
Cutaneous rash	1 (2)	—	1 (2)

NOTE. Data presented as No. (%).
Abbreviation: AE, adverse event.
*Causing pembrolizumab discontinuation and a switch to chemotherapy.
†Postcystectomy onset.

Table A3. Analysis With a Logistic Model of the Association Between Putative Genomic Biomarkers and Pathologic Response to Pembrolizumab

Variable	Statistics	Odds Ratio	95% CI	Wald Test *P*
TMB (mutation per megabase)	Continuous	Nonlinear effect; cutoff, ≥ 15 (80th quantile)		.0219
DDR and/or RB1 GA	Unadjusted	5.23	1.44 to 18.94	.0096
	Adjusted	3.41	0.76 to 15.24	.0989

Abbreviations: DDR, DNA damage response and repair; GA, genomic alterations; TMB, tumor mutation burden.