



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

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ABSTRACT

Background Patients with bladder cancer (BC) who are cisplatin ineligible or have unresectable disease have limited treatment options. Previously, we showed targeting programmed death-ligand 1 (PD-L1) with durvalumab (durva) and radiation therapy (RT) combination was safe in BC. We now report results from a phase II study evaluating the toxicity and efficacy of durva and RT in localized BC.

Methods This is a single-arm, multi-institutional phase II study; N=26. Enrolled patients had pure or mixed urothelial BC (T2-4 N0-2 M0) with unresectable tumors and were unfit for surgery or cisplatin ineligible. Patients received durva concurrently with RT ×7 weeks, followed by adjuvant durva × 1 year.

Primary endpoints: (A) progression-free survival (PFS) at 1 year and (B) disease control rate (DCR) post adjuvant durva. Key secondary endpoints: (A) complete response (CR) post durvaRT (8 weeks), (B) overall survival (OS), (C) PFS and (D) toxicity. Correlative studies included evaluation of baseline tumor and blood (baseline, post durvaRT) for biomarkers.

Results Median follow-up was 27 months. Evaluable patients: 24/26 post durvaRT, 22/26 for DCR post adjuvant durva, all patients for PFS and OS. Post adjuvant durva, DCR was seen in 72.7%, CR of 54.5%. 1-year PFS was 71.5%, median PFS was 21.8 months. 1-year OS was 83.8%, median OS was 30.8 months. CR at 8 weeks post durvaRT was 62.5%. Node positive (N+) patients had similar median PFS and OS. DurvaRT was well tolerated. Grade ≥3 treatment-related adverse events: anemia, high lipase/amylase, immune-nephritis, transaminitis, dyspnea (grade 4-COPD/immune), fatigue, rash, diarrhea and scleritis. No difference in outcome was observed with PD-L1 status of baseline tumor. Patients with CR/PR or SD had an increase in naïve CD4 T cells, a decrease in PD-1+CD4T cells at baseline and an increase in cytokine-producing CD8 T cells, including interferon gamma (IFN γ) producing cells, in the peripheral blood.

Conclusion Durva with RT followed by adjuvant durva was safe with promising efficacy in localized BC patients with

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cisplatin-based neoadjuvant chemotherapy is the preferred option for patients with muscle invasive bladder cancer or locally advanced bladder cancer. However, a significant proportion of patients diagnosed with this cancer are elderly and are unable to tolerate cisplatin-based chemotherapy or surgery due to underlying comorbidities. Checkpoint inhibitors have demonstrated efficacy in bladder cancer, but approval has been restricted to BCG refractory non-muscle invasive bladder cancer, muscle invasive bladder cancer postsurgery in the adjuvant setting and in the metastatic urothelial cancer. Radiation therapy (RT), though effective in bladder cancer, needs to be combined with chemotherapy to enhance the antitumor effect. Studies are needed to evaluate the safety and efficacy of combining RT with concurrent and adjuvant immunotherapy in bladder cancer.

comorbidities, including N+ patients. Larger randomized studies, like S1806 and EA8185, are needed to evaluate the efficacy of combining immunotherapy and RT in BC.

Trial registration number NCT02891161.

INTRODUCTION

Cisplatin-based neoadjuvant chemotherapy (NAC), followed by radical cystectomy (RC), is the standard of care for patients with muscle invasive bladder cancer (MIBC).¹ A significant proportion of these patients have comorbidities prohibitive for NAC. Additionally, clinical trials incorporating NAC for MIBC often include a majority of N0 and few N1 patients with lymph nodes <2cm.^{1,2} Bladder preservation approach using radiation therapy (RT) is an effective alternative to RC for well-selected patients with MIBC. RT alone is considered inferior to

WHAT THIS STUDY ADDS

- ⇒ This is one of the first studies evaluating the safety and efficacy of combining definitive RT with durvalumab in muscle invasive and locally advanced bladder cancer patients with multiple comorbidities, or were cisplatin ineligible, had N1-2 status, had unresectable or were unfit for surgery.
- ⇒ We observed that durvalumab in combination with RT followed by adjuvant durvalumab was safe and demonstrated promising efficacy. The treatment-related adverse events were very similar to those previously observed with single-agent durvalumab. Notably, the addition of RT did not produce additional immune-related adverse events.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ The safety results from this study were used in the design of multiple studies, including a larger phase II National Clinical Trials Network study incorporating a bladder sparing approach and chemotherapy+RT with or without durvalumab (concurrent and adjuvant durvalumab) in patients with node positive bladder cancer. It also demonstrated the role of adjuvant durvalumab post-RT. Our data from correlative studies demonstrate the need to investigate blood-based biomarkers, such as cytokine producing T cells and CD4+PD-1+ T cells, which could have predictive value for bladder preservation approaches in patients with localized disease.

concurrent chemotherapy and RT.³ Numerous prospective clinical trials established the safety and efficacy of bladder preservation using maximal transurethral resection of bladder tumor (TURBT), followed by concurrent chemoradiation therapy,³⁻⁵ yet some patients are not candidates for chemotherapy. Hence, there is an unmet need for effective and tolerable non-chemotherapy regimens.

Checkpoint inhibitors (CPI) have efficacy in high risk, BCG refractory non-muscle invasive BC,⁶ metastatic BC⁷ and in the adjuvant setting.⁸ However, their role in the neoadjuvant setting^{9,10} and in combination with RT is still investigational. Preclinical data suggest improved synergy and efficacy when CPI are combined with RT.¹¹ Radiation induces immunogenic cell death that leads to upregulation of proinflammatory signals and activation of tumor-specific T-cells. These events play a key role in improved antitumor immunity.^{12,13} Durvalumab (durva) is a selective, high-affinity, human IgG₁ monoclonal antibody that blocks programmed death ligand 1 (PD-L1). This in turn allows 'PD-1 +T cells' to maintain antitumor function. Adjuvant durva showed efficacy in a stage III non-small cell lung cancer post chemoradiation therapy.¹⁴

Previously reported results from phase Ib of this study showed the combination of durva and RT was safe.¹⁵ Here, we report clinical and biomarker results of a phase II clinical trial of concurrent durvaRT in adult patients with localized BC.

METHODS

Study oversight and conduct

This is an investigator-initiated clinical trial (ClinicalTrials.gov number, NCT02891161) designed and

conducted by the study team within the Big Ten Cancer Research Consortium (BTCRC). Patients were enrolled between December 2016 and July 2019. The coauthors and the sponsor conducted data analyses and wrote the manuscript.

Study design and treatment

This is a phase Ib/II, multicenter, single-arm trial (figure 1A, Study Design). In both phases, patients were treated with durva 1500 mg every 4 weeks × 2 doses along with definitive intensity modulated RT (64.8 Gy in 36 fractions over 7 weeks to the bladder tumor region and involved lymph nodes, online supplemental protocol), followed by adjuvant durva 1500 mg every 4 weeks × 1 year. Hence, all patients from phase Ib were included in phase II. Response was evaluated with CT imaging based on modified RECIST V.1.1 and cystoscopy+biopsy post durvaRT (8 weeks) and during the adjuvant phase and follow-up. Phase II had coprimary endpoints: (A) progression-free survival (PFS) at 1 year and (B) disease control rate (DCR) post adjuvant durva. Secondary endpoints included: (A) complete response (CR) post durvaRT; (B) median PFS; and (C) median overall survival (OS). Correlative analyses included quantification of PD-L1 expression of the tumor at baseline and whole exome sequencing (WES) of tumor DNA.

Patients

The pertinent inclusion criteria for phase II are as follows: (A) T3-4 N0-2 M0 OR Tx N1-2 M0 OR T2 N1-2 M0 treatment naïve patients who were cisplatin-ineligible or unresectable or medically unfit for surgery; T2-3 N0 M0 patients were required to be cisplatin-ineligible; patients postneoadjuvant chemotherapy who were found to be unresectable OR medically unfit for surgery could be included; (B) maximal TURBT attempted prior to the start of treatment; (C) patients with pure or mixed urothelial histology; (D) ECOG (Eastern Cooperative Oncology Group) performance status 0–2; (E) glomerular filtration rate ≥30 mL/min by the Cockcroft-Gault formula. Pertinent exclusion criteria: (A) prior use of systemic immunotherapy; (B) presence of N3 or M1 and (C) small cell histology.

Study assessment

Toxicity was evaluated using NCI Common Terminology Criteria for Adverse Events V.4. Disease status post durvaRT was evaluated by imaging with CT scan utilizing RECIST 1.1 criteria and cystoscopy prior to starting adjuvant durvalumab at week 8 and thereafter every 12 weeks during adjuvant durvalumab. Biopsy for disease response confirmation was performed 2–3 weeks post durvaRT (week 8) and thereafter on an as needed basis during adjuvant therapy. Post 1 year, CT scans were performed every 6 months for year 2 and annually thereafter. Cystoscopy every 12 weeks for year 1 during adjuvant treatment and thereafter per discretion of urology as standard of care, which was every 3–6 months. Patients who

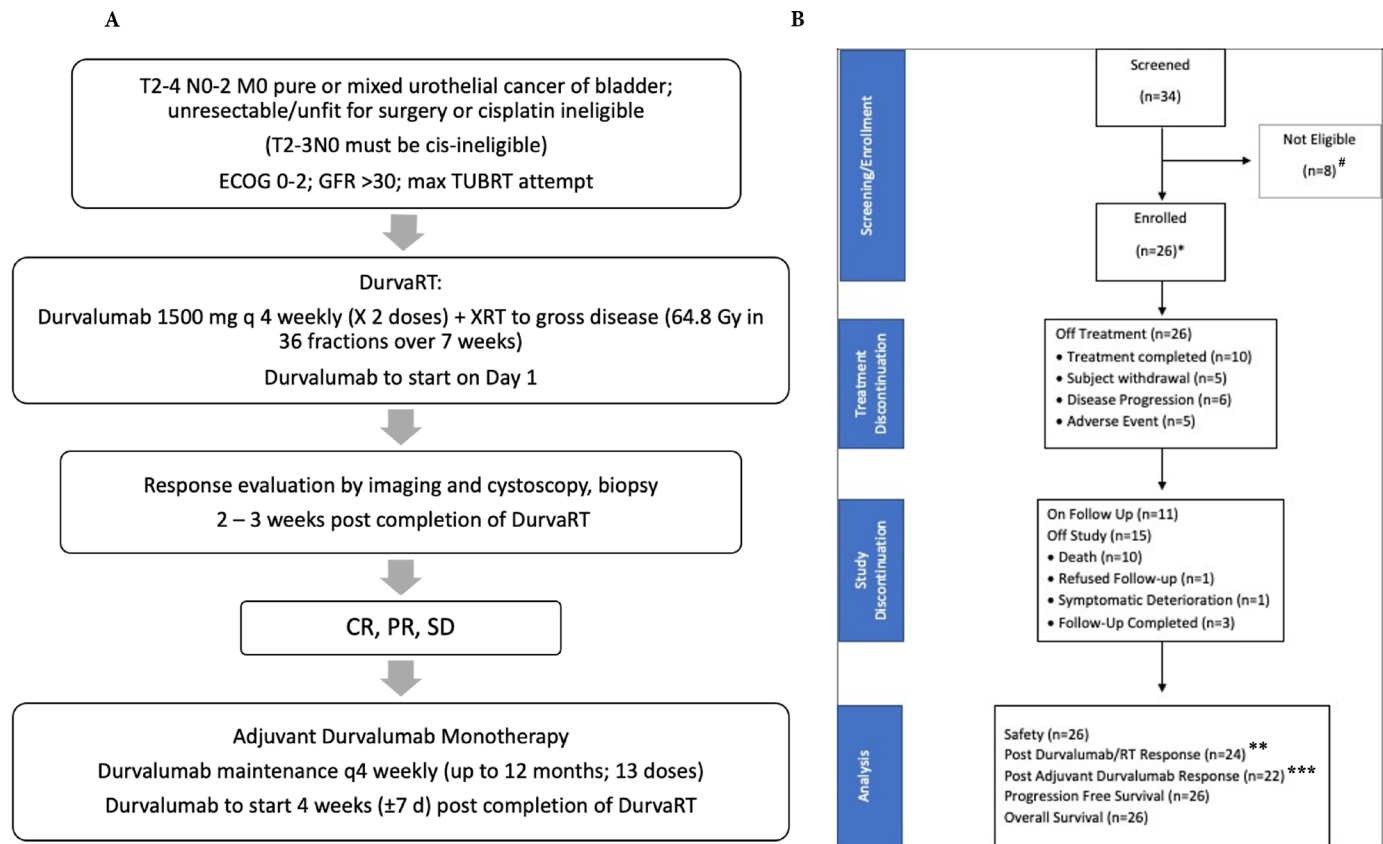


Figure 1 Study schema and CONSORT. (A) Study Schema. (B) CONSORT diagram. *Six patients from phase Ib were rolled over to phase II and 20 more patients were accrued. All patients were treated similarly. **One patient withdrew from active treatment post durvaRT, though had regular imaging done as follow-up and we included this patient for PFS, OS and subset analyses. One patient did not have cystoscopy as scheduled 8 weeks post durvalumab and RT. ***Three patients did not get adjuvant therapy and one patient was in CR on adjuvant therapy, but then decided to come off treatment and declined imaging; the response was not evaluated post-treatment. All patients had regular imaging and cystoscopy as planned except—two patients had limited number of follow-up cystoscopies (one patient had post durvaRT cystoscopy to document response and further surveillance cystoscopy was considered high risk by urologist; second patient was >90 years and no cystoscopy was done post durvaRT and this patient was excluded from early efficacy evaluation). Both had multiple imaging to document ongoing responses for long-term disease evaluation. #Eight patients were not eligible—due to hematuria, inability to get TURBT or did not meet all the inclusion criteria. OS, overall survival; PFS, progression-free survival; TURBT, transurethral resection of bladder tumor; GFR, glomerular filtration rate; XRT, radiation therapy; ECOG, Eastern Cooperative Oncology Group performance status; CONSORT, Consolidated Standards of Reporting Trials.

had no disease per cystoscopy with random biopsies and imaging were defined as CR. Disease control was defined as CR+partial response (PR)+stable disease (SD).

Correlative biomarkers

PD-L1 testing was performed on baseline tumor tissue from TURBT using the VENTANA PD-L1 (SP263) assay. Positive SP263 status was defined as ≥25% tumor cells with membranous staining; or percent of tumor area involved by immune cells ('immune cells present', ICP >1% and percent of immune cells in tumor positive for PD-L1 (IC+) ≥25%; or ICP=1% and IC+ = 100%).^{16 17} Other biomarkers included tumor DNA and RNA as well as markers from blood cytokine analyses. Variant histology was identified using any of the histomorphological patterns described as 'urothelial carcinoma with divergent differentiation'.¹⁸ Evaluation of PD-L1 expression by IHC and the presence of variant histology were determined by a single urological

pathologist (JW). Baseline tumor WES was performed by Caris LifeSciences.¹⁹

Peripheral blood mononuclear cells (PBMCs) were isolated from blood by density gradient centrifugation. Cells were frozen and stored in liquid nitrogen. PBMCs were thawed from liquid nitrogen and cultured overnight at 37°C, 5% CO₂ in 12-well plates using 3 mL of complete RPMI 1640 medium containing glutamax and 100 U/mL penicillin, 100 mg/mL streptomycin, 10 mM HEPES, 50 mM 2-mercaptoethanol, 25 mg/mL sodium pyruvate and 10% fetal bovine serum. Cells were counted and replated in 96-well round-bottom plates using complete RPMI 1640 and stimulated with 1 mg/mL phorbol myristate acetate and 20 ng/mL ionomycin in the presence of 1 mg/mL brefeldin A for 5 hour at 37°C, 5% CO₂. Cells were stained with a 1:100 dilution of antibodies from BD Biosciences targeting the following surface antigens: CD4

(BUV395), CD8 (BUV496), CD137 (BUV661), CD279 (BUV480), CD278 (BV711), C45RA (BV786), TIGIT (BB700), CD28 (PE-CF594), Tim-3 (Alexa647), CD197 (APC-R700) for 15 min at room temperature, washed and fixed/permeabilized using the BD cytofix/cytoperm kit (BD Biosciences) per the manufacturer's instructions. Permeabilized cells were stained for intracellular cytokines IFN γ (FITC), IL-2 (BUV737) and TNF α (BV421) for 15 min at room temperature. Stained cells were analyzed on a BD FACSsymphony A3 in the Penn State College of Medicine Flow Cytometry Core (RRID:SCR_021134), and the data were analyzed using FlowJo (V.10.8.1). For t-Distributed stochastic neighbor embedding (tSNE) analysis, samples were classified into four groups representing responders (CR, PR, SD) and progressors (PD) at week 1 or week 12. Clinical response was based on disease control status at treatment discontinuation. Individual .fcs files were combined into a concatenated file that was downselected to represent 7050 live CD3⁺ cells from each sample to give a total of 247 750 cells. The concatenated file was subjected to tSNE analysis using 1000 iterations in FlowJo using the compensated parameters. Data from subgated tSNE plots were summarized into pie charts using GraphPad Prism (V.9.4.1).

Statistical analyses

Design

This is a phase II single-arm trial. Six patients enrolled in the initial phase Ib part were rolled over to phase II and an additional 20 patients were enrolled. A total of 26 patients were used for efficacy evaluation. All patients received the same dose of the combination therapy and were evaluable for toxicity. Sample size considerations are described in the protocol (online supplemental protocol).

Analysis

Descriptive statistics are used to summarize the patients' characteristics and demographics. The 1-year, 2-year PFS rates and the median PFS are estimated using the Kaplan-Meier estimator. The DCR was estimated with 95% confidence limits. No adjustment for multiple testing was performed because of the small sample size. The secondary endpoint of OS was analyzed similarly. Associations of selected covariates with PFS/OS were examined using log-rank tests and Cox proportional hazards models, and associations with disease control status were analyzed using Fisher's exact test.²⁰ To compare the survival time of CR/PR versus SD/progressive disease (PD), we used landmark analysis as introduced by Anderson *et al.*^{21 22} CD4 and CD8 data measured at week 1 and week 12 were associated with the selected clinical outcomes using nonparametric Wilcoxon rank-sum tests or Cox proportional hazard models, as appropriate. The Wilcoxon rank sum test was applied to compare tumor mutation burden (TMB) values in groups defined by 1-year progression. TMB by WES measured the total number of non-synonymous, somatic mutations identified per megabase (Mb) of the genome coding area of

Table 1 Demographics

Characteristics	Total N=26 (N (%))
Median age (range), years	74 (range 51–94)
Sex	
Male	19 (73.1)
Female	7 (26.9)
Race	
White	24 (92.3)
Black	1 (3.8)
Unknown	1 (3.8)
TNM stage	
T2 N0 M0	10 (38.5)
T3 N0 M0	6 (23.1)
T4 N0 M0	2 (7.6)
T2-4 N1-2 M0	8 (30.8)
Unresectable	
Yes	9 (34.6)
No	17 (65.4)
Unfit for surgery	
Yes	13 (50)
No	13 (50)
Cisplatin ineligible	
Yes	23 (88.5)
No	3 (11.5)
ECOG performance status	
0–1	19 (73.1)
2	7 (26.9)
Extent of TURBT per urologist	
Complete	19 (73.1)
Incomplete	7 (26.95)
Presence of hydronephrosis	
Yes	7 (26.9)
No	19 (73.1)
Neoadjuvant chemotherapy exposure	
Yes	4 (15.4)
No	22 (84.6)
Tumor PD-L1 status	
Positive	10 (38.5)
Negative	16 (61.5)
Histology	
Pure urothelial	15 (57.7)
Mixed urothelial with variant*	11 (42.3)
*Mixed variants histology in addition to pure urothelial component included: squamous=3; sarcomatoid=1; micropapillary=1; glandular=4; sarcomatoid+squamous=1; micropapillary+glandular=1. Please refer to online supplemental table 1 for further details. ECOG, Eastern Cooperative Oncology Group; TURBT, transurethral resection of bladder tumor.	

Table 2 Treatment-related adverse events (TRAE)

SOC	CTCAE term	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	All grade n (%)
General disorders	Fatigue	9 (34.62)	5 (19.23)	1 (3.85)	0 (0)	15 (57.69)
Gastrointestinal disorders	Diarrhea	7 (26.92)	2 (7.69)	1 (3.85)	0 (0)	10 (38.46)
Infections and infestations	Urinary tract infection	0 (0)	6 (23.08)	0 (0)	0 (0)	6 (23.08)
	Kidney infection	0 (0)	0 (0)	1 (3.85)	0 (0)	1 (3.85)
Laboratory abnormalities	Serum amylase increased	3 (11.54)	1 (3.85)	1 (3.85)	0 (0)	5 (19.23)
	Lipase increased	0 (0)	0 (0)	2 (7.69)	1 (3.85)*	3 (11.54)
	Creatinine increased	3 (11.54)	1 (3.85)	0 (0)	0 (0%)	4 (15.38)
	Alanine aminotransferase increased	1 (3.85)	1 (3.85)	0 (0)	1 (3.85)*	3 (11.54)
	Platelet count decreased	4 (15.38)	0 (0)	0 (0)	0 (0)	4 (15.38)
	Anemia	1 (3.85)	0 (0)	1 (3.85)	0 (0)	2 (7.69)
	White blood cell decreased	2 (7.69)	0 (0)	1 (3.85)	0 (0)	3 (11.54)
	Lymphocyte count decreased	3 (11.54)	3 (11.54)	6 (23.08)	0 (0)	12 (46.15)
Metabolism and nutrition disorders	Anorexia	2 (7.69)	2 (7.69)	0 (0)	0 (0)	4 (15.38)
	Weight gain	0 (0)	0 (0)	1 (3.85)	0 (0)	1 (3.85)
	Dehydration	0 (0)	0 (0)	1 (3.85)	0 (0)	1 (3.85)
Renal and urinary disorders	Cystitis noninfective	2 (7.69)	7 (26.92)	0 (0)	0 (0)	9 (34.62)
	Urinary tract pain	4 (15.38)	0 (0)	0 (0)	0 (0)	4 (15.38)
	Acute kidney injury	1 (3.85)	1 (3.85)	1 (3.85)	0 (0)	3 (11.54)
	Nephritis	0 (0)	0 (0)	1 (3.85)	0 (0)	1 (3.85)
Skin and subcutaneous tissue disorders	Rash maculopapular	5 (19.23)	1 (3.85)	0 (0)	0 (0)	6 (23.08)
	Rash acneiform	1 (3.85)	0 (0)	1 (3.85)	0 (0)	2 (7.69)
Eye disorders	Scleral disorder	0 (0)	0 (0)	1 (3.85)	0 (0)	1 (3.85)
Respiratory disorders	Dyspnea	0 (0)	0 (0)	0 (0)	1 (3.85)†	1 (3.85)

Above TRAEs are >15% occurrence or any TRAE grade 3 or higher. No grade 5 events were reported.

*No clinical symptoms.

†Patient had underlying COPD (Chronic Obstructive Pulmonary Disease) and it was thought that his deterioration was related to COPD exacerbation but association to durvalumab could not be ruled out.

CTCAE, Common Terminology Criteria for Adverse Events; SOC, System Organ Class.

DNA. High TMB was defined as being ≥ 10 mutations per Mb.²³ All analyses were performed using R Programming Language V.4.2.1 (R Foundation).²⁰ All tests were two sided, and the statistical significance level used was 0.05. The Complex Heatmap R package²⁴ was employed to create an oncoprint illustrating mutations in select DNA damage response genes.^{25 26}

Role of the funding source

This is a BTCRC study, and the sponsor was the principal investigator. AstraZeneca provided research funds and free durva for patients on study but were not involved in the study conduct. AstraZeneca reviewed the manuscript

for medical accuracy only before journal submission. The study team, corresponding author and coauthors were involved in the study design and conduct, data interpretation, had full access to all the data in the study, the writing of the report, and had final responsibility for the decision to submit for publication.

RESULTS

A total of 26 patients were enrolled between December 2016 and July 2019 (table 1; online supplemental table 1 shows histological description). At the time of data cut-off

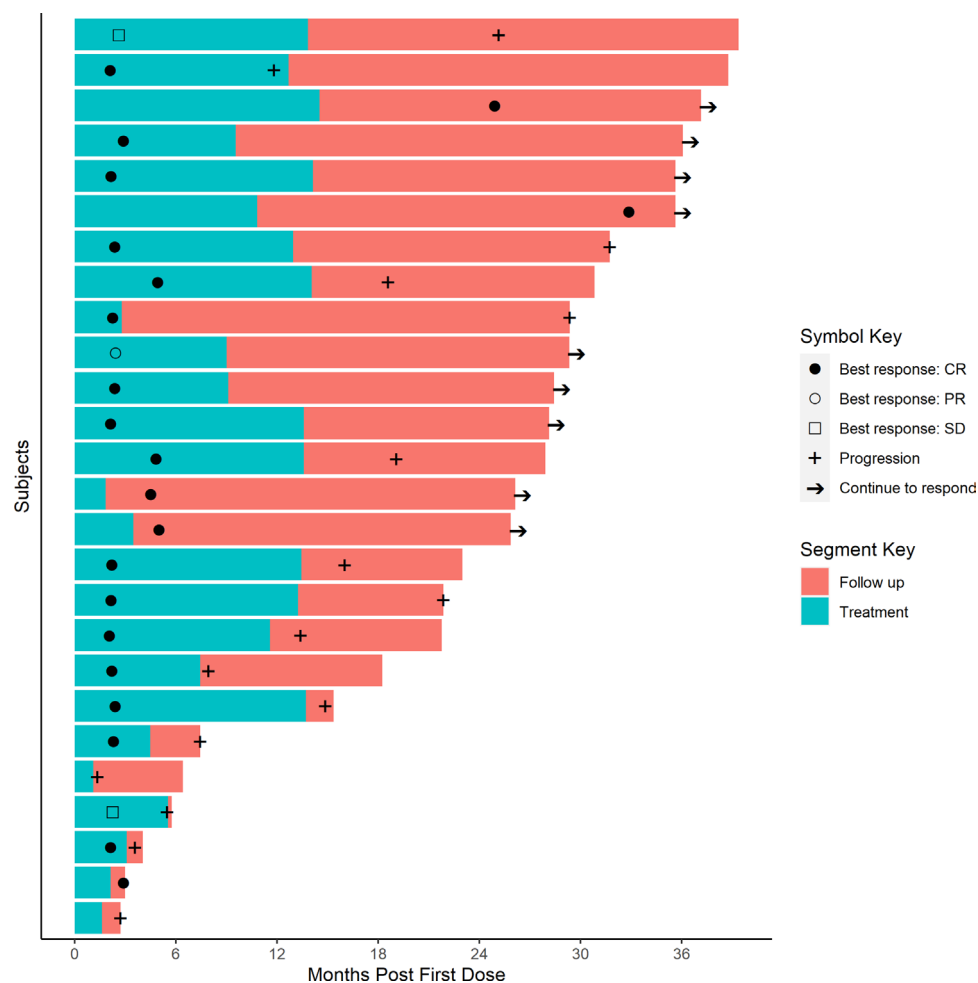


Figure 2 Swimmer plot for all 26 patients showing their best response on study. This figure shows best response during treatment or follow-up. The symbols represent different responses (CR, complete response; PR partial response; SD, stable disease), and time of progression during course of study. Two patients were censored for PFS before 12 months. One patient was in CR but declined follow up; PFS 2.9 months. Another patient declined to follow up for progression, but continued survival follow-up; PFS 5 months. PFS, progression-free survival.

(September, 2, 2022), 24/26 patients were evaluable for response post durvaRT (figure 1B) and 22/26 had DCR data available (3 patients did not get adjuvant therapy; 1 patient was in CR on adjuvant therapy but then decided to come off treatment and declined imaging; response was not evaluated post-treatment). Median duration of follow-up from day 1 of treatment was 27 months (ranging from 2.7 to 39.4 months). Median number of durvalumab cycles was 10.5 cycles from day 1, including concurrent and adjuvant (ranging from 1 to 15), and 8.5 cycles in the post durvaRT adjuvant period (ranging from 0 to 13).

Combination of durva and RT was safe and tolerable

No dose-limiting toxicities were observed during durvaRT treatment in phase Ib, as reported earlier.¹⁵ Table 2 shows treatment-related adverse events (TRAE) and online supplemental table 2 shows durva immune-related adverse events (IRAE). Treatment was tolerable. Fatigue was the most common TRAE, followed by diarrhea, which is thought to be related to RT. Five patients discontinued treatment, four of which due to IRAE, including nephritis, transaminitis, scleritis, questionable

pneumonitis (this was thought to be underlying COPD (Chronic Obstructive pulmonary Disease) exacerbation, but could not exclude IRAE). Radiation cystitis was reported in 23% (6 patients; 5 grade 1 and 1 grade 2). Proctitis was not reported. No treatment-related death was reported. Following disease progression, one patient had multiorgan failure, including stroke, and succumbed.

Combination of durva and RT demonstrated promising efficacy

At the time of adjuvant treatment discontinuation, overall response rate (ORR) was observed in 15/22 patients (68.2%). CR was observed in 12/22 (54.5%) and 3/22 (13.6%) had PR. One patient had SD accounting for DCR at 1 year of 72.7% (95% CI 49.8% to 89.3%) (figure 2). PFS probability at 1 year was 71.5% (95% CI 55.6% to 91.9%) (figure 3A). Median PFS was 21.8 months (95% CI 14.8 months to not reached (NR)). Median OS was 30.8 months (95% CI 22.9 to NR) and 1-year OS probability was 83.8% (95% CI 70.4% to 99.7%) (figure 3B).

Eight-week ORR post day 1 of durvaRT was observed in 20/24 patients (83%). Of these, 15/24 (62.5%) had

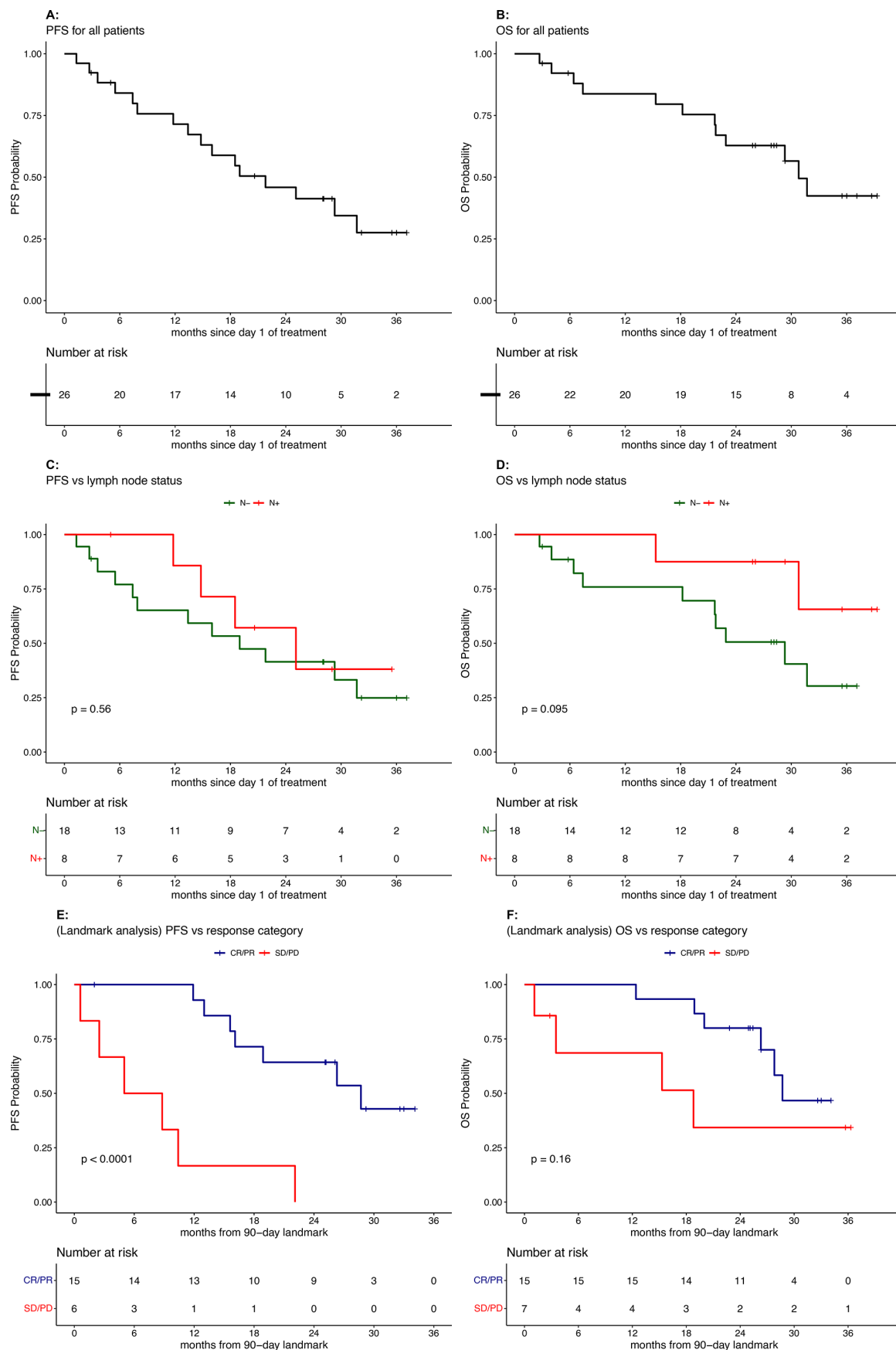


Figure 3 Progression-free survival (PFS) and overall survival (OS) outcomes. PFS analyses were assessed using the Kaplan-Meier estimator. The x-axis represents time period in months and the y-axis represents PFS probability. OS analyses were

Figure 3 (Continued)

assessed using the Kaplan-Meier estimator. The x-axis represents time period in months and the y-axis represents OS probability. (A) PFS for all patients. Median PFS was 21.8 months. A 1-year PFS probability was 71.5% (95% CI 55.6% to 91.9%), and 2-year PFS probability was 45.9% (95% CI 29.6% to 71.1%). (B) OS for all patients. Median OS was 30.8 months. A 1-year OS probability was 83.8% (95% CI 70.4% to 99.7%), and 2-year OS probability was 62.8% (95% CI 46.2% to 85.4%). (C) PFS for node positive cohort. Median PFS was 25.1 months. A 1-year PFS probability was 85.7% (95% CI 63.3% to 100%), and 2-year PFS probability was 57.1% (95% CI 30.1% to 100%). (D) OS for node positive cohort. Median OS NR (not reached). A 1-year OS probability was 100% (95% CI not applicable (NA)), and 2-year OS probability was 87.5% (95% CI 67.3%, 100%). (E) PFS for patients who had CR/PR versus SD/PD. Median PFS in patients who achieved CR/PR was 31.7 months (95% CI 21.8 months to NR) and it was significantly better than those who had SD/PR, median PFS 7.9 months (95% CI 3.6 months to NR), $p \leq 0.0001$. A 1-year PFS probability for CR/PR was 100% (95% CI NA); 1-year PFS probability for SD/PR was 28.6% (95% CI 8.86%, 92.2%). (F) OS for patients who had CR/PR versus SD/PD. Median OS was 31.7 months in CR/PR groups (95% CI 29.3 months to NR) and it was 21.7 months in SD/PR (95% CI 6.4 months to NR), $p = 0.16$. A 1-year OS probability for CR/PR was 100% (95% CI NA); 1-year OS probability for SD/PR was 68.6% (95% CI 40.3% to 100%). CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

clinical CR and 5/24 (20.8%) had PR. Three patients had SD accounting for DCR of 96%. Two patients deemed unevaluable due to lack of cystoscopy/biopsy post durvaRT had clinical CR on CT scan and were included for OS analysis. At the time of study closure, 15/26 patients had ongoing CR (57.7%), 1 had ongoing PR (3.8%) and 10 had PD (38.5%).

Patients with node positive (N+) disease ($n=8$) had median PFS of 25.1 months (95% CI 14.8 months to NR) and median OS was NR (95% CI 30.8 months to NR) (figure 3C,D). We also performed analyses to evaluate the differences in PFS and OS between patients who achieve CR/PR versus SD/PD at treatment discontinuation (figure 3E,F). We observed a significant difference ($p < 0.0001$) in PFS between CR/PR versus SD/PD patients. Median PFS in CR/PR patients was 31.7 months (95% CI 21.8 to NR), as opposed to 7.9 months (95% CI 3.6 to NR) in SD/PD patients. We observed a positive non-significant trend for OS favoring CR/PR patients (figure 3F). Neither the presence of hydronephrosis nor the extent of TURBT correlated with PFS OS (online supplemental figure 1A–D). In patients who received NAC ($n=4$), 1-year PFS was 33.3% with a median PFS of 11.8 months (95% CI 3.6 months to NR); 1-year OS was 66.7% and the median OS was NR.

No clinical correlation was observed with baseline PD-L1 status, but better PFS was observed with high TMB

Of pretreatment tumor samples, 10/26 (38.5%) were classified as PD-L1 positive (+). Variant histology was present in 11/26 (42.3%). PD-L1 status was significantly associated with variant histomorphology, $p = 0.0426$ (online supplemental figure 2). Specifically, 3/15 (20%) specimens of conventional urothelial carcinoma were PD-L1+ vs 7/11 (63.6%) for those with variant morphologies. However, PD-L1 status was not associated with DCR (75% in PD-L1+ vs 71.4% for PD-L1-), PFS or OS (online supplemental figure 3A,B). Because only normal urothelium with inflammation was observed post durvaRT, we did not perform PD-L1 staining on post durvaRT specimens. Presence of higher TMB was correlated with response (figure 4). As shown in figure 4, mutations were frequently observed in *TERT* (76%) *RBI* (32%), *KDM6A* (28%), *ATR* (24%), *PIK3CA* (24%) and *ARID1A* (24%).

Over 70% of samples exhibited mutations in at least one of these genes, suggesting dysregulated DNA damage response is common in this population, as previously reported.²⁷ Notably, around 58% of *TERT* mutant tumors had high TMB. Samples with low TMB had worse PFS and a trend for lower OS (log rank $p = 0.033$ and $p = 0.14$, respectively), and subjects with 1-year progression had lower TMB values (Wilcoxon two-sided $p = 0.083$).

Responders demonstrated high naïve CD4 T cells, low PD-1+ CD4 T cells and an increase in cytokine-producing CD8 T cells in their peripheral blood

PBMCs from baseline (week 1) and post-treatment (week 12) time points were stimulated in vitro to evaluate production of IFN γ , IL-2 and TNF α by CD4 and CD8 T cells using flow cytometry. Additional phenotypic markers were simultaneously evaluated, including CD45RA and CCR7, to define naïve, memory and effector cell differentiation, CPI receptors Tim3, TIGIT and PD-1 and the costimulatory receptors 4-1BB, ICOS and CD28. tSNE analysis was performed to visualize overall differences in total T cell subpopulations between responders (CR, PR, SD) and progressors (PD) at each time point (figure 5A–C). The areas of highest density varied between these two groups. Heatmaps of the individual markers overlaid onto the tSNE plots revealed well-defined CD4 and CD8 T cell clusters (figure 5D). In addition, distinct clusters of cytokine-expressing cells were visualized with some overlap in the positive populations. To better understand which populations were enriched by clinical response, CD4 and CD8 subpopulations were gated within the tSNE plots (figure 6A–C). Eleven CD4 and 12 CD8 subpopulations were identified. Within CD4 T cells, two populations of PD-1⁺ cells were enriched in patients that progressed (figure 6D, CD4-2, CD4-4). A minor population of IL-2 and TNF α -producing CD4 cells was enriched among responders (CD4-8) along with skewing toward cells with a naïve phenotype (CD45RA^{hi/int} CCR7⁺ CD28⁺; figure 6D, CD4-10, CD4-11). There was a corresponding decrease in memory phenotype cells in the responders (CD4-9). Thus, responders tended to have a larger naïve CD4 T cell population and fewer PD-1⁺ cells at both time points. CD8 T cells from the responders showed enrichment for multiple cytokine producing cell subsets (CD8-1, CD8-2,

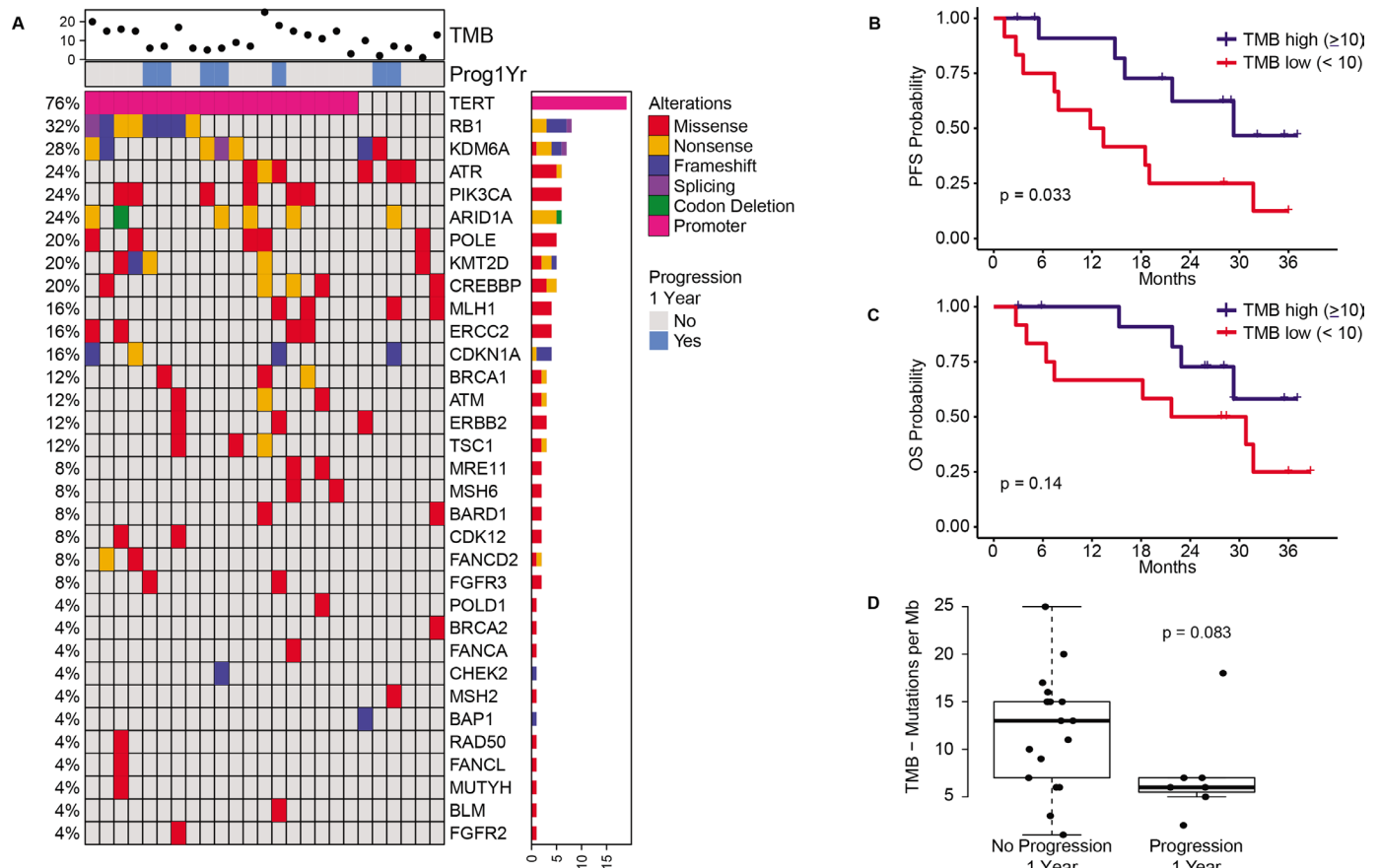


Figure 4 Tumor mutational analyses. Somatic mutations and tumor mutational burden in the DUART cohort. (A) The oncoprint display shows variant types and mutation frequencies of select DNA damage response genes as well as other genes that are frequently mutated in bladder cancer. (B, C) Kaplan-Meier plots illustrating progression-free survival (PFS) and overall survival (OS) in groups defined by tumor mutational burden (TMB). High TMB $n=13$; low TMB $n=12$. (D) Boxplot display of TMB values for subjects who did and did not have tumor progression within 1 year. DUART, durvalumab and radiation therapy.

and CD8-12; [figure 6E](#)) and a decrease in one major non-cytokine producing effector T cell population (CD8-6). In contrast, one population of IL-2⁺TNF α ⁺ central memory-like cells was enriched in the progressor patients (CD8-7; [figure 6E](#)). Thus, patients with better outcomes tended to have increased proportions of cytokine-producing CD8 T cells, including those producing IFN γ .

Flow cytometry data were also evaluated using manual gating as shown in online supplemental figure 4 to define the frequency of CD4 and CD8 cells that were naïve (CD45RA^{hi}CCR7⁺), central memory (CD45RA^{lo}CCR7⁺), effector/effector memory (CD45RA^{lo}CCR7⁺) or effector memory RA⁺ (CD45RA^{hi}CCR7⁺) as well as the frequency expressing Tim3, TIGIT, PD-1, 4-1BB, ICOS or CD28. We also determined the frequency of CD4 and CD8 T cells expressing intracellular IFN γ , IL-2 and TNF α , or a combination of cytokines. Each frequency was correlated with DCR status at treatment discontinuation. Those values showing a statistically significant difference in responders and progressors are shown in [figure 6F–K](#). We found that CD4 PD-1⁺ cells at baseline were significantly increased in the progressor patients ([figure 6F](#); $p=0.0465$), supporting our observations from the tSNE analysis. Additionally, naïve CD4 T cells were enriched in responder patients

([figure 6G](#); $p=0.027$), while those with a central memory phenotype were significantly decreased at baseline ([figure 6H](#); $p=0.0465$). CD8 T cells expressing IFN γ were positively correlated with clinical response at baseline (week 1; [figure 6I](#); $p=0.0356$) and week 12 ([figure 6J](#); $p=0.0263$, [figure 6K](#); $p=0.0264$), supporting the conclusion from the tSNE analysis that cytokine-producing CD8 T cells were enriched in the responder patients. Taken together, these results suggest that responder patients showed an increase in naïve CD4 T cells, and a decrease in PD-1⁺ CD4 T cells at baseline and an increase in cytokine-producing CD8 T cells in the circulation at both time points, pre and post durvaRT.

DISCUSSION

We report the safety and efficacy of combining CPI with definitive RT followed by adjuvant CPI in localized BC patients with significant comorbidities. Durva given concurrently with RT followed by adjuvant durva was tolerable with minimal clinically significant IRAEs.

The combination of RT with durva showed promising efficacy at both early (post durvaRT) and late (post adjuvant) time points, with meaningful benefits seen in

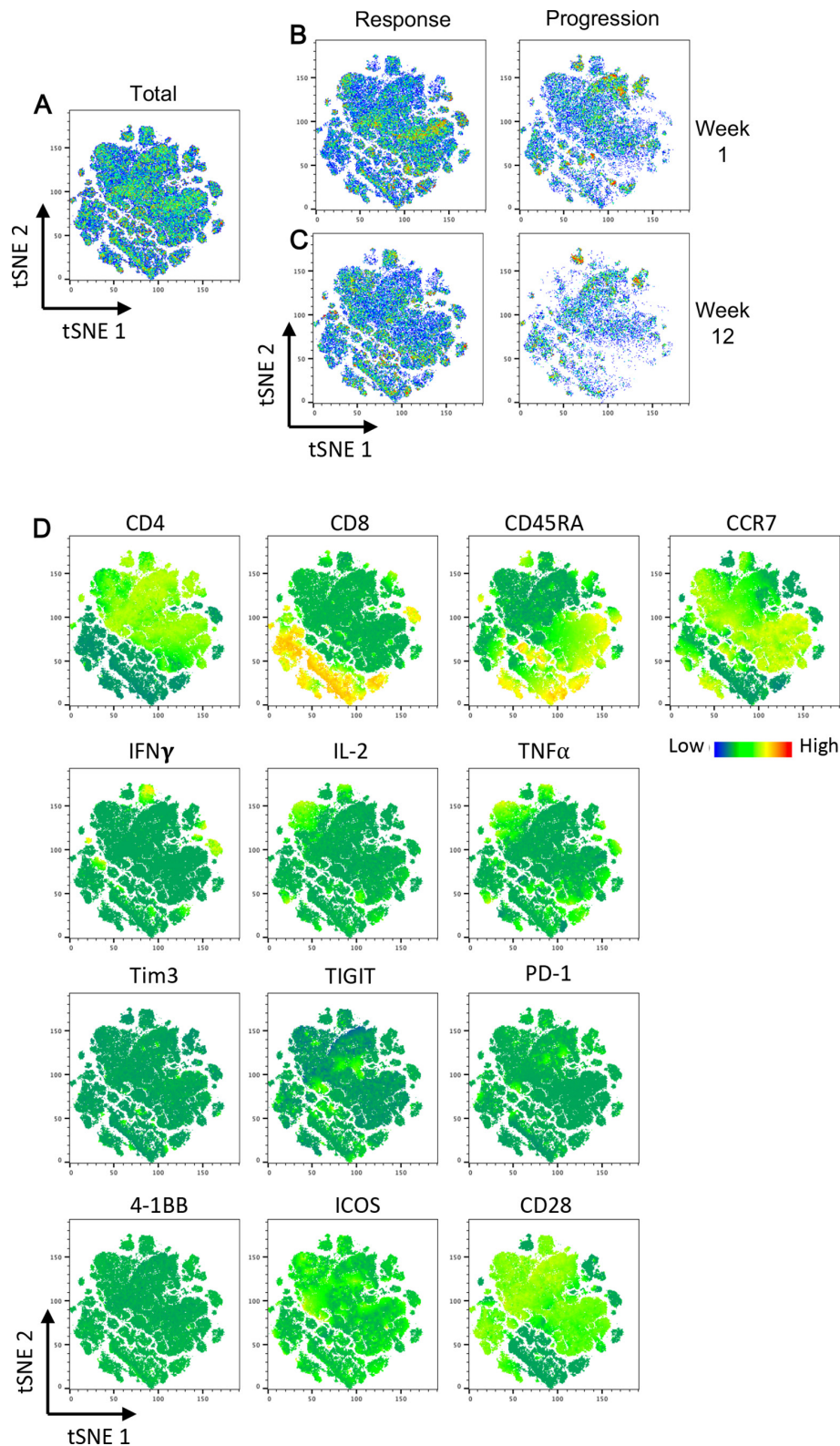


Figure 5 tSNE evaluation of PBMC-derived T cells reveals shifts in T cell phenotype between responders and progressors patients. PBMCs were stimulated in vitro with PMA/ionomycin for 5 hours in the presence of brefeldin A followed by staining for both surface antigens and intracellular cytokines. (A) Flow cytometric data were subjected to tSNE analysis to identify major cell clusters. (B, C) tSNE data were stratified by patient response at treatment discontinuation (PD (progression) vs CR, PR, SD (response)) and by time point to reveal relative differences in population intensities between groups. (D) Heatmap representation showing the localization of each evaluated marker within the tSNE plots. Intensity scale is indicated. For (B), $n=15$ for response and $n=6$ for progression at week 1. For (C), $n=11$ for response and $n=3$ for progression at week 12. CR, complete response; PBMC, peripheral blood mononuclear cell; PD, progression disease; PR, partial response; SD, stable disease. tSNE, t-Distributed stochastic neighbor embedding.

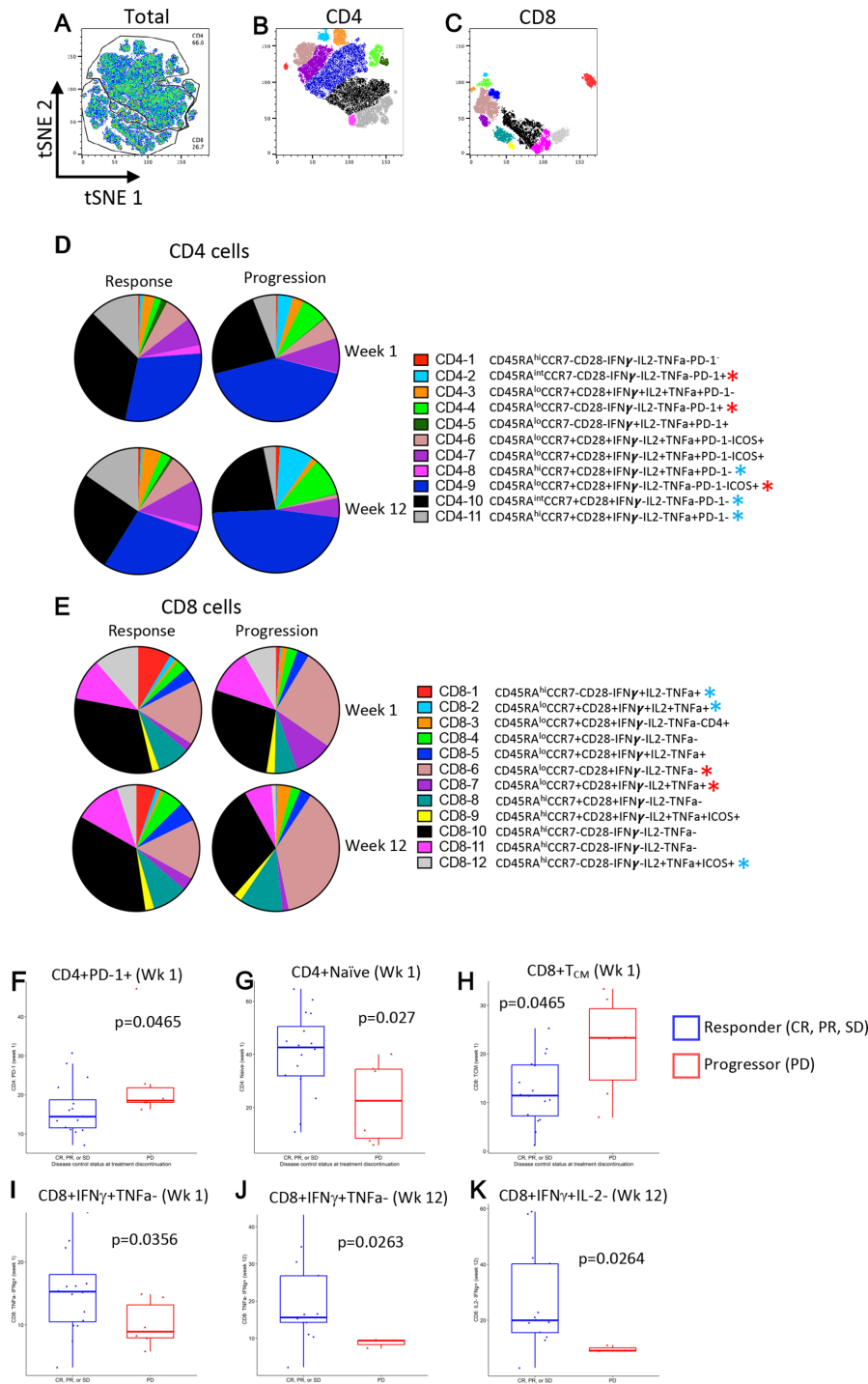


Figure 6 Skewing of peripheral CD4 and CD8 T cell subpopulations in patients with progressive disease. PBMCs were stimulated *in vitro* with PMA/ionomycin for 5 hours in the presence of brefeldin A followed by staining for both surface antigens and intracellular cytokines. Flow cytometric data were subjected to tSNE analysis. (A) Gates indicating the total CD4 and CD8 T cell populations within the tSNE plot of total cells. (B, C) Subgating of prominent subpopulations of CD4 and CD8 expressing cells, respectively. (D, E) The phenotype of subpopulations from B and C was determined by evaluating expression of individual markers within each subpopulation using FlowJo. Pie charts show the relative proportions of each tSNE subpopulation for CD4 and CD8 cells, segregated by response at treatment discontinuation (PD (progression) vs CR, PR, SD (response)) and at time of collection (week 1=baseline). Colors in D correspond to those in B and colors in E correspond to those in C. The identity of the 11 CD4 and 12 CD8 subpopulations are indicated beside the legends in D and E, respectively. Red asterix=subpopulations increased in progressors and blue asterix=subpopulations skewed in responders. (F–K) T cell subpopulations identified using the gating strategies shown in online supplemental figure 4 were correlated with the disease control status at treatment discontinuation. P values are indicated. CR, complete response; PBMC, peripheral blood mononuclear cell; PD, progression disease; PMA, phorbol myristate acetate; PR, partial response; SD, stable disease. tSNE, t-Distributed stochastic neighbor embedding.

both PFS and OS. Similar efficacy has been reported in other bladder preservation studies. Balar *et al.* reported preliminary results from phase II study in MIBC patients (T2-T4aN0M0)²⁸ where patients were treated with neoadjuvant pembrolizumab followed by pembrolizumab+gemcitabine in combination with hypofractionated RT. This combination showed 88% bladder intact disease-free survival (BDFS) at 1 year with 35% of patients having \geq grade 3 AEs.²⁸ Another study reported 81% CR post durva+tremelimumab combined with RT for T2-T4aN0 patients and 76% 1-year DFS with 1-year BDFS of 73% in 31 patients.²⁹ Our study did not report the BDFS as the majority of our patients were unfit for surgery, but we did report PFS at 1 year, which could be comparable to 1-year BDFS. Although cross-trial comparisons are difficult, results reported by these studies are comparable to ours (83% ORR post durvaRT with no \geq grade 3 radiation cystitis or proctitis). Only 15% of patients needed treatment discontinuation due to IRAEs. Despite a small sample size, 31% of our patients had N1-2+ (n=8; 3 had biopsy+) and one patient with bulky lymph nodes had the longest durable response lasting >30 months. Importantly, median PFS and OS were similar in the N+ cohort when compared with the overall cohort, suggesting patients with N+ disease could benefit from this approach. This is comparable to results reported from IMPART study that showed median OS of 22.8 months for patients with N+ or N- high-risk BC.³⁰ This requires further evaluation in a larger clinical trial. Our results are also comparable to chemoRT results in patients with poor prognosis as reported by Efsthathiou *et al.*³¹ Although our interpretation is limited due to the small sample size, we did not observe differences in PFS or OS relative to presence of hydronephrosis or TURBT extent, which indicate advanced disease.^{31–33} Our findings suggest that CPI in combination with RT could demonstrate an antitumor immune response even in patients who have presence of hydronephrosis or incomplete TURBT. We did observe superior PFS in patients who achieved CR/PR when compared with those who had SD/PD. However, there was no difference in OS. The latter could be explained by the underlying comorbidities.

Perhaps due to small sample size, we did not observe any significant correlation between baseline PD-L1 expression and clinical outcome. In addition, we were unable to analyze tumor PD-L1 status following treatment due to the lack of tumor in biopsy specimens post durvaRT. However, we did observe a trend for better OS and PFS in patients with high TMB (≥ 10). Previous studies show high TMB is of potential predictive value for response to CPI treatment^{34,35} and our results support that hypothesis. We also observed that patients who had CR/PR or SD had an increase in naïve CD4 T cells, a decrease in PD-1+CD4 T cells at baseline in PBMCs and an increase in cytokine-producing CD8 T cells, including IFN γ producing cells, in the circulation. Researchers in Canada reported a similar finding in immune profiling from peripheral blood from patients with advanced urothelial and renal

cancer.³⁶ Although our patient populations are different, they also found higher naïve CD4+T cells in the blood and lower PD-1+CD4 T cells at baseline in the patients who responded to immunotherapy. More studies are needed to explore the predictive and prognostic implications of these subpopulations in BC patients treated with immunotherapy and RT approach.

Our study had several limitations, including small sample size, single-arm design and slow accrual. We initially had planned to have a larger sample size but given slow accrual the sample size was adjusted to 26 patients. Additionally, not all patients completed the planned adjuvant therapy. Perhaps having a full year of adjuvant durva may have been too intensive for patients with comorbidities, especially after achieving a good response, as evidenced by the withdrawal of treatment by five patients. Future studies could consider shorter adjuvant treatment after the concurrent phase with RT. Despite good responses in N+ patients, given the small sample size our results should be interpreted with caution.

There is a dearth of treatment options for patients with unresectable disease or those who are unfit for surgery because the majority of these patients are also not fit to undergo cisplatin-based chemotherapy. Studies like ABACUS and PURE-01 have shown efficacy in early phase clinical trials with immune CPI alone as neoadjuvant therapy with pathological CR of 31%–37% in operable patients. However, there is still unmet need for patients who are not fit for surgery.^{7,24} Our results provide strong rationale to conduct future clinical trials with CPI and RT in locally advanced BC. The results of our study provided the rationale for the recently opened study, EA8185, for N+ BC where patients are being randomized to chemo-RT \pm durvalumab, followed by adjuvant durvalumab (NCT04216290).

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Patient consent for publication Consent obtained directly from patient(s).

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