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Original Article

The SUB-urothelial DUrvalumab InjEction-1 (SUBDUE-1) trial: first-in-human trial in patients with bladder cancer

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Objectives

To assess the safety of sub-urothelial injection of durvalumab and examine the impact on tissue and circulating immune cell populations.

Patients and Methods

The patients were chemotherapy and immunotherapy naïve (bacille Calmette-Guérin allowed) with non-metastatic muscle-invasive bladder cancer or non-muscle-invasive bladder cancer planned for radical cystectomy (RC). The study was a Phase Ib 3 + 3 dose-escalation design with sub-urothelial injection of durvalumab at three pre-determined doses (25, 75, 150 mg) diluted in 25 mL normal saline, injected at 25 locations (25 × 1 mL injections), at least 2 weeks before RC.

Results

A total of 11 patients were recruited (10 male, one female). No significant changes were reported on American Urological Association Symptom Score or O'Leary Interstitial Cystitis Scale. In all, 14 adverse events (AEs) were reported (10 Grade 1, three Grade 2, one Grade 3), none considered immune-related. No Grade 4 or 5 AEs were recorded. All the patients underwent RC. Tissue immune populations changed following durvalumab injection ($P = 0.012$), with a statistically significant increase in M2-macrophage (CD163) when comparing the 25–150 mg dose ($P = 0.021$). Basal/mixed cancers showed a larger CD163 increase than luminal cancers ($P = 0.033$).

Conclusion

Sub-urothelial injection of durvalumab is feasible and safe without immune-related AEs and shows local immunological effects.

Keywords

durvalumab, immunotherapy, SUBDUE-1, sub-urothelial injection, urothelial carcinoma, anti-PD-L1, bladder cancer, clinical trial

Introduction

Bladder cancer features amongst the 10 most prevalent cancers worldwide [1]. At diagnosis, 75% of patients have non-muscle-invasive bladder cancer (NMIBC) [2] but these patients experience high rates of disease recurrence and progression to muscle-invasive bladder cancer (MIBC) [3]. Management of NMIBC has hardly changed in three decades, with recommended treatment including transurethral tumour resection, instillation of postoperative chemotherapy and, for high-grade tumours (Ta/T1) or carcinoma *in situ* (CIS), subsequent intravesical BCG [2]. Patients with BCG-unresponsive tumours have limited treatment options. Radical cystectomy (RC) is the preferred treatment but has a high morbidity and may overtreat many patients [2].

Bladder cancer sub-typing is well established with core luminal, basal, and mixed sub-types [4]. Basal sub-types often associate with lower overall and disease-specific survival [5]. Tumour-infiltrating lymphocytes (TILs) and tumour-associated macrophages (TAMs) are linked to the anti-tumour immunological response, with levels of these often associated with stage and overall survival [6].

Immune checkpoint inhibitors (ICIs) have revolutionised treatment of many tumour types. Interference with the programmed cell death 1/ programmed death-ligand 1 (PD-1/PD-L1) axis by ICIs prevents cancer immune escape by boosting immune activation and overcoming T-cell exhaustion [7]. ICIs are employed in the treatment of advanced urothelial cancer [8], but have found less utility to date in early disease. In 2021, pembrolizumab was approved for the NMIBC BCG-unresponsive cohort in the United States, demonstrating a 41% 3-month complete response rate, although two-thirds of patients had treatment-related adverse events (TRAEs) and longer-term control was uncommon [9]. Small studies have also reported intravesical delivery of ICIs alone without toxicity; however, demonstrable efficacy was lacking, possibly related to poor urothelial penetration due to large molecular mass [10–12].

Sub-urothelial injection of ICIs is untested but could prove beneficial for several reasons: a sub-urothelial administration route would facilitate maximal urothelial penetrance; the procedure does not require surgical upskilling; local ICI administration may be safer than systemic delivery with less immune-related AEs (IRAEs); and it may be effective in BCG-unresponsive bladder cancer.

Durvalumab, a human monoclonal anti-PD-L1 antibody, blocks the PD1/PD-L1 interaction [13], with systemic administration showing benefit in various cancer types [14,15]. Systemic durvalumab for NMIBC has been delivered in a multi-arm trial, although few patients received durvalumab alone making efficacy assessment difficult [16]. In the present study, we explored the safety of sub-urothelial durvalumab injection, and the impact on tissue and circulating immune cell populations.

Patients and Methods

Study Design and Patients

This Phase Ib 3 + 3 dose-escalation trial administered increasing doses of durvalumab via sub-urothelial injection at least 2 weeks prior to RC. The patients were recruited from a single tertiary institution and were eligible if they had high-grade NMIBC (Ta, T1 and/or CIS) or non-metastatic MIBC, with no prior chemo- or immunotherapy (BCG allowed) and planned for RC. The complete inclusion and exclusion criteria and methodological detail are outlined in the previously published trial protocol [17] and are also in the Appendix S1.

The primary objective was to assess the safety of sub-urothelial durvalumab injection, using patient-reported outcome measures (PROMs), and quantify AEs graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Dose-limiting toxicities (Appendix S1) were defined as any serious AEs at least possibly related to sub-urothelial durvalumab administration.

The secondary objective was to assess the impact of sub-urothelial durvalumab on tissue and circulating immune cell populations (see Section 'Pathological and Immunohistochemical Analysis').

The trial protocol [17] was approved by the South Metropolitan Health Service Human Research Ethics Committee (RGS0000003534) and registered on the Australia and New Zealand Clinical Trials Registry (ANZCTR; ACTRN12620000063910).

Study Procedures and Dose-Escalation Scheme

Dose-escalation was according to a 3 + 3 design with durvalumab doses set at 25, 75 and 150 mg. Consensus was reached within the trial group that 10% of the systemic dose of durvalumab was sufficient to assess effect given the likely vastly increased local tissue concentration of drug achieved compared to systemic administration.

Informed consent was obtained from eligible patients. Baseline assessments included routine laboratory blood tests, body mass index , electrocardiogram, and Eastern Cooperative Oncology Group (ECOG) score assessment.

The patients underwent cystoscopy under general anaesthetic at least 2 weeks before planned RC. Findings were recorded and four quadrant 'cold cup' biopsies of the bladder (and tumour, if present) were taken (pre-injection biopsies). Sub-urothelial injection of durvalumab using a BoNee® needle (Coloplast A/S, Humlebaek, Denmark) was then performed.

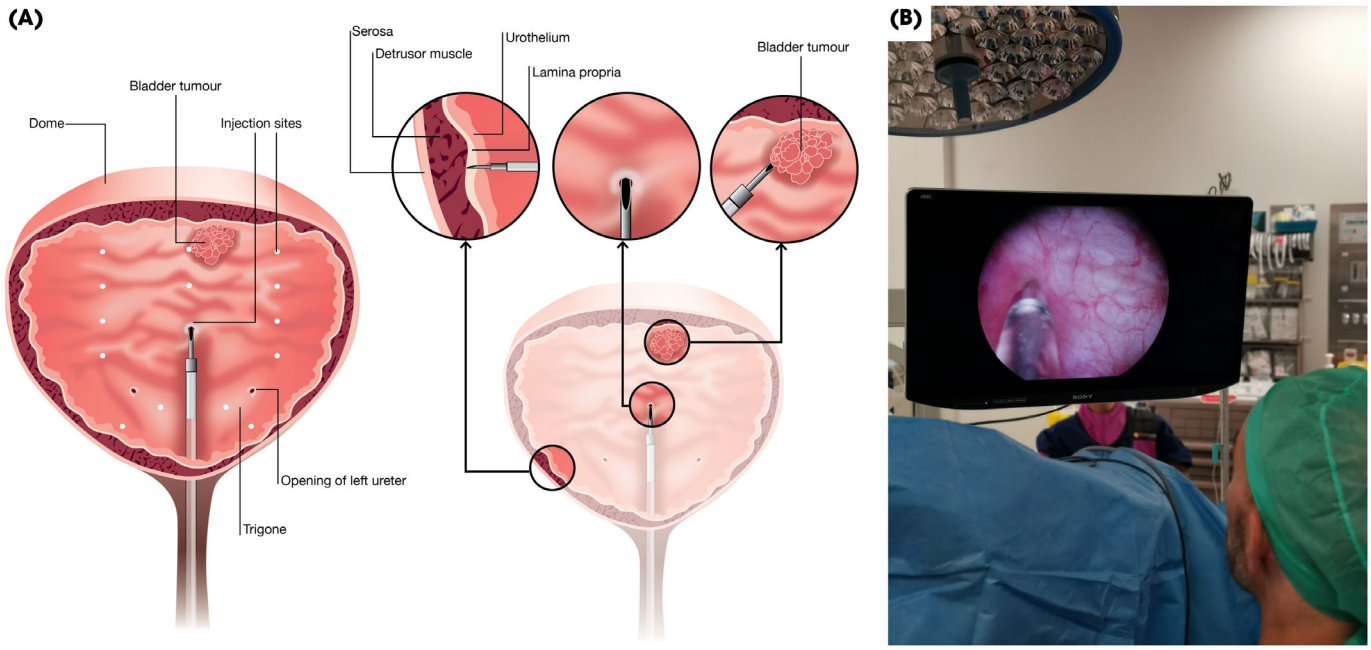
The durvalumab was diluted with 25 mL normal saline and injected in 1-mL aliquots across 25 locations (25 × 1 mL injections) throughout the bladder. Injections were distributed in a grid-like pattern (including the trigone) to achieve bladder-wide distribution. Where tumour was present, durvalumab was injected at the base (Fig. 1A,B). The patients were discharged home on the same day.

Immediately before RC, the patients underwent repeat cystoscopy, four-quadrant 'cold cup' and tumour biopsies (post-injection biopsies).

Outcome Measures

The PROMs used included the AUA Symptom Score [18] and O'Leary International Cystitis Scale [19], recorded a day

Fig. 1 Sub-urothelial injection of durvalumab, with: (**A**) Diagram illustrating injection method and locations; (**B**) Cystoscopic view of injection of durvalumab into bladder. Note (A) is a diagrammatic representation only—not all injection sites are shown.



before durvalumab injection, 48 h after injection, fortnightly, and on the day of RC.

Blood and urine testing was performed at screening and at 2 weeks after durvalumab injection. Blood testing continued fortnightly until RC, on the day of RC, and 2 weeks after RC.

The AE assessment started from date of durvalumab injection and continues in follow-up. Clinical and radiological follow-up of patients is occurring at intervals according to the European Association of Urology (EAU) Guidelines for MIBC [3].

Pathological and Immunohistochemical Analysis

All biopsies and RC specimens underwent routine histopathological analysis with haematoxylin and eosin staining to determine tumour content. Tumours were graded and staged according to the WHO grading system [20] and TNM staging system [21].

Tumours were designated basal, luminal, or mixed molecular sub-type based on GATA-binding protein 3 (GATA3), cytokeratin (CK)20, CK5, and CK14 immunohistochemistry (IHC). Carcinomas were considered positive for GATA3, CK20, CK5 and CK14 if >20% of the tumour cells showed at least moderate intensity staining [22]. Tumours were classified as luminal if positive for GATA3 and/or CK20, or as basal if positive for CK5 and/or CK14. Tumours expressing both luminal and basal markers were regarded as mixed tumours.

The PD-L1 tumour immunostaining utilised the Ventana PD-L1 (SP263) assay (Ventana Medical Systems Inc., Marana, AZ, USA), according to manufacturer's methods [23]. The PD-L1 expression was scored as previously described [23], with tumours considered PD-L1 positive if either tumour or immune cells showed ≥25% staining.

To determine the quantity and distribution of TILs and TAMs before and after injection of durvalumab, IHC evaluation of $CD3^+$ (total lymphocytes) and $CD8^+$ (tumour-activated lymphocytes) was used for assessment of TILs, and CD68 (total macrophages) and CD163 (M2 macrophages) were used for assessment of TAMs, on pre- and post-injection biopsy material. For CD3, CD8, CD68 and CD163, IHC slides were scanned with a high-resolution scanner (ScanScope CS; Aperio Technologies Inc., Vista, CA, USA) and image analysis software (ImageScope Software version 12.1, with the Positive Pixel Count version 9 [PPCv9] algorithm; Aperio Technologies Inc) was used to quantify lymphocyte and macrophage sub-populations. Positivity was recorded in 'hotspots', with positivity scoring defined as the total number of positive pixels divided by total number of pixels: (NTotal − Nn)/(NTotal) [24]. Scores for each marker in each quadrant biopsy at each time point were recorded. These scores were combined to give a mean score for each cell population for each patient at each time point. A relative change in immune cells (RCI) score was assigned for each marker in each patient comparing the ratio of pre- and post-treatment immune cell levels, with an RCI score >1.0 used to

denote an increase in TILs or TAMs after durvalumab injection relative to pre-treatment.

The IHC was carried out on a BenchMark Ultra immunostainer (Roche Diagnostics, Basel, Switzerland) with the following antibodies: GATA3 (Biocare Medical, Pacheco, CA, USA; clone L50-823, 1:100 dilution), CK20 (DAKO, Glostrup, Denmark; clone Ks20.8, 1:500 dilution), CK5 (Leica Biosystems, Nussloch, Germany; clone XM26, 1:100 dilution), CK14 (Leica Biosystems; clone LL002, 1:100 dilution), CD3 (Agilent Technologies, Santa Clara, CA, USA/DAKO; polyclonal, 1:500 dilution), CD8 (Agilent/DAKO; clone C8/144B, 1:50 dilution), CD68 (Agilent/DAKO; clone KP1, 1:600 dilution) and CD163 (Leica Biosystems; clone 10D6, 1:100 dilution).

Statistics

Statistical analyses were performed with the IBM Statistical Package for the Social Sciences (SPSS®), version 29.0.0.0 (241) (IBM Corp., Armonk, NY, USA). Dose-dependent immune population changes were compared using unpaired one-way ANOVA with Bonferroni *post hoc* test correcting for multiple comparisons. Differences in immune populations between luminal and basal sub-types were explored using an independent samples *t*-test, with two-tailed *P* value. The statistician was blinded so as not to confound the analysis. Results were considered statistically significant for a $P < 0.05$.

Results

Treatment Cohort Composition

A total of 11 patients enrolled between December 2019 and May 2022, with a predominantly male cohort (91%). All the patients received sub-urothelial durvalumab at their pre-determined dose with all subsequently undergoing RC. The patients' demographics and previous therapy are described in Table 1. Two patients had delays undergoing RC due to non-trial factors—one contracted COVID-19; the other was found to have rectal cancer requiring further investigations and concurrent abdominoperineal resection with RC.

Adverse Events and Toxicity

There were no significant changes reported on AUA Symptom Score or O'Leary Interstitial Cystitis Scale at any timepoint. In total, six of the 11 patients reported AEs of any grade and causation (Table 2). One patient had a Grade 3 serious AE requiring admission for a pre-existing tachycardia occurring prior to durvalumab injection. There were only mild TRAEs as per investigator assessment and no IRAEs.

Two patients had a transient perioperative rise of thyroid-stimulating hormone (TSH) without corresponding thyroxine (T4) level change. Both levels normalised without intervention within 4 weeks. No imaging was deemed necessary by the advising endocrinologist. The Safety Review Committee determined these as unlikely to be related to durvalumab injection due to the transient and mild level of elevation. There were no other biochemical or haematological toxicities identified (Appendix S1).

Histopathological Results – Pathological Stage

Histopathology from original resection, pre- and post-injection biopsies, and RC specimen, and time from durvalumab injection to RC, are summarised in Table 3. Of note, one patient with BCG-unresponsive NMIBC with CIS on their pre-durvalumab biopsies had benign post-durvalumab biopsies and no disease in the bladder at RC.

Tumour Sub-Typing and PD-L1 Status

According to IHC-based molecular sub-typing; seven patients had luminal, two had basal, and two had mixed (double

Table 1 Patients' demographics.

	Cohort 1 (25 mg)	Cohort 2 (75 mg)	Cohort 3 (150 mg)	All patients
Number of patients	3	3	5	11
Age, years, mean (range)	73 (64–82)	65 (56–74)	75 (57–83)	72 (56–83)
Sex, *n*				
Male	3	3	4	10
Female	0	0	1	1
Prior intravesical therapy, *n*				
None	3	2	2	7
BCG	0	1	3	4
Gemcitabine	0	0	1	1
Mitomycin	0	0	1	1
BMI, kg/m^2, mean (range)	28.8 (26.6–30.5)	28.9 (26.8–31.5)	25.4 (23.4–26.9)	27.3 (23.4–31.5)
Initial bladder cancer pathological stage, *n*				
NMIBC	3	2	4	9
MIBC	0	1	1	2

BMI, body mass index.

Table 2 The AEs.

AEs, *n*	Cohort 1 (25 mg)	Cohort 2 (75 mg)	Cohort 3 (150 mg)	Total, *n*
Grade 1				
Nausea	1	0	1	10
Vomiting	0	0	1	
Dysuria	1	1	0	
Cough	1	0	0	
Light-headedness	0	0	1	
Pruritis	1	0	0	
Diarrhoea	1	0	1	
Grade 2				
Dysuria	0	1	1	3
Fatigue	1	0	0	
Grade 3				
Tachycardia	1	0	0	1
Grade 4	0	0	0	0
Grade 5	0	0	0	0
Total AEs	7	2	5	14

Table 3 Histopathology and sub-typing of tumours with biomarkers.

Patient	Original resection	Highest stage pathology on pre-durvalumab biopsies	Highest stage pathology on post-durvalumab biopsies	Final bladder histology on RC specimen	Time from durvalumab to RC, days	CK20	GATA3	CK5	CK14	PD-L1	Sub-type
1	T1HG + CIS	CIS	CIS	CIS	28	Pos	Pos	Neg	Neg	High	Luminal
2	T1HG	Benign	T1HG (nested variant)	T3bHG (nested variant)	22	Pos	Pos	Neg	Neg	Low	Luminal
3	T1HG	TaHG	CIS	T1HG + CIS	26	Pos	Pos	Neg	Neg	Low	Luminal
4	T2HG	T2HG	T1HG	T2HG (nested variant) + CIS	26	Neg	Pos	Pos	Neg	High	Mixed
5	T1HG + CIS	T1HG	Benign – mild chronic inflammation	T2HG	14	Neg	Pos	Pos	Neg	High	Mixed
6	T1HG (focal sq differentiation) + CIS	T1HG	Benign – mild chronic inflammation	T3aHG (sarcomatoid differentiation 20%) + CIS	25	Neg	Neg	Pos	Pos	High	Basal sq
7	T2HG + CIS (sq differentiation 70%)	Benign – cystitis cystica	Necrosis + HG urothelial cancer	T2bHG (extensive sq differentiation) + CIS	21	Neg	Neg	Pos	Pos	High	Basal sq
8	TaHG + CIS	CIS	Benign – mild to moderate chronic inflammation	T0 (bladder)	32	Pos	Pos	Neg	Neg	N/A	Luminal
9	CIS	Benign – chronic inflammation	Benign – chronic inflammation	CIS	25	Pos	Pos	Neg	Neg	N/A	Luminal
10	T1HG + CIS	TaHG	TaLG	TaHG + CIS	50*	Neg	Pos	Neg	Neg	Low	Luminal
11	T1HG	TaHG + CIS	T1HG + CIS	T3bHG + CIS	65†	Pos	Pos	Neg	Neg	Low	Luminal

*Sub-typing based on: luminal = CK20 and GATA3, Basal = CK5 and CK14. HG, high grade; LG, low grade; N/A, not applicable; Neg, negative; Pos, positive; sq, squamous; T, Tumour stage as defined by the American Joint Committee on Cancer (AJCC) TNM system. *Delayed due to incidental finding of low rectal cancer requiring further evaluation and concurrent rectal surgery. †Delayed due to patient contracting COVID-19.*

positive) sub-types. All basal or mixed tumour sub-types, but only one of seven luminal sub-types were PD-L1 positive (Table 3).

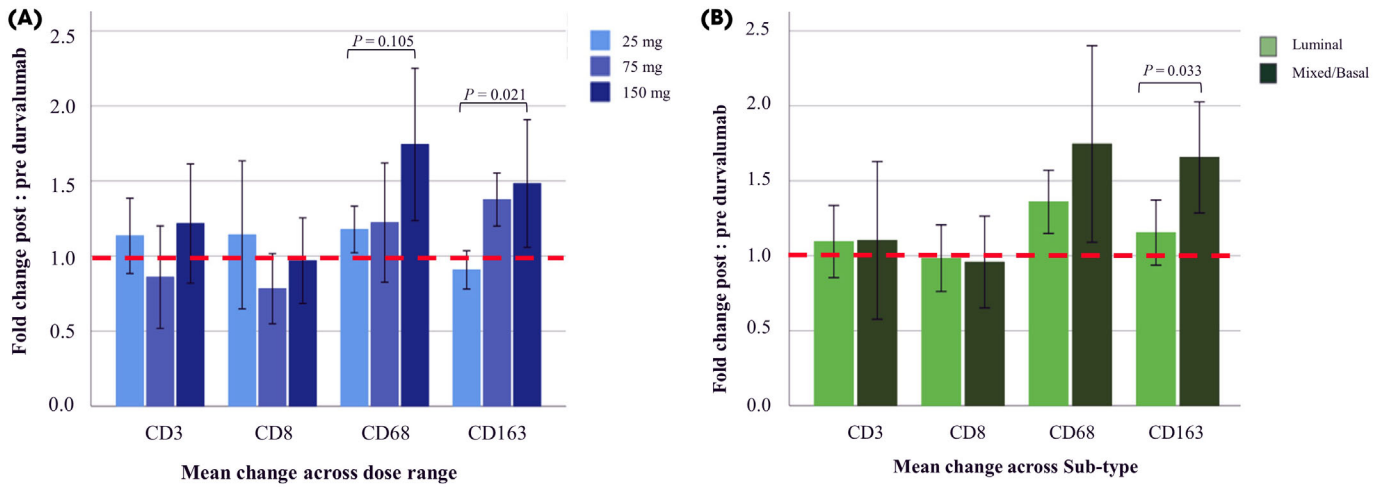
Assessment of Tissue Immune Cell Populations – TILs and TAMs

Overall, the RCI varied between cell types (CD68 > CD163 > CD3 > CD8; $P = 0.012$). When considering dose-dependent effects, we observed increases in CD68 total macrophages (non-significant trend) and CD163 M2 macrophages ($P = 0.021$, Fig. 2A). Basal and mixed cancers showed a significantly greater CD163 increase relative to luminal cancers post-durvalumab injection ($P = 0.033$, Fig. 2B). There was no significant change in lymphocyte populations irrespective of tumour sub-type.

Assessment of Circulating Immune Cell Populations

There were no significant alterations in circulating monocytes, neutrophils, or lymphocytes after durvalumab injection. There

Fig. 2 The RCI post-durvalumab injection according to: (**A**) dose of durvalumab, and (**B**) by bladder cancer sub-type. Error bars represent ±2 standard errors from the mean.



was a non-significant trend towards increased monocytes, especially at the 150 mg dose (Appendix S1).

Discussion

Given the success of systemic ICIs in advanced urothelial cancer, these treatments hold promise in localised disease. The confinement of disease to the bladder, which obviates the need for systemic exposure in NMIBC introduces the hope that lasting therapeutic benefit might also be achieved without the serious IRAEs associated with systemic ICIs.

The favourable overall toxicity profile we observed supports the hypothesis that sub-urothelial durvalumab is safe in bladder cancer. The absence of IRAEs likely arises from confinement of drug exposure to the bladder, an organ generally regarded to harbour a favourable immunological milieu. The only Grade 3 AE was related to a pre-existing condition and occurred prior to durvalumab. The mild transient TSH elevations were adjudged unlikely to be related to durvalumab injection although causality has not been excluded.

The lack of significant change in circulating immune cell populations suggests there may have been no substantive systemic immune activation, although larger and more detailed multi-dose studies are required to validate this. In contrast, alterations in tissue immune populations across treatment are consistent with the second hypothesis that sub-urothelial durvalumab would exert a local immunological effect. Specifically, the RCI for CD163 (M2 macrophages) increased between 25 mg and 150 mg doses (P = 0.021).

Changes in TAMs number in response to ICIs are not extensively reported elsewhere, although animal studies suggest TAMs number may increase with tumour progression [25]. Immune suppressive macrophages have also been associated with poorer immune responsiveness in patients. Interleukin 10-secreting macrophages were associated with exhausted $CD8^+$ T cells and a poorer prognosis in patients with MIBC and were more prevalent in basal sub-type tumours [26]. Consistent with this, macrophage changes were confined to the uniformly PD-L1 positive mixed and basal sub-type tumours in our study, although whether this difference is driven by the presence of the PD-L1 target or by other differential biology between sub-types will require further study in larger cohorts.

In the Phase II IMvigor210 trial (ClinicalTrials.gov identifier: NCT02108652), participants where M2 TAMs predominated were immunotherapy resistant [27]. Further investigation of sub-urothelial ICIs also may reveal that M2 TAMs induction predicts for PD-L1 blockade resistance, whereas lack of such response may correlate with efficacy.

Our study was not designed to detect direct anti-tumoral effect, with complete tumour resection occurring soon after durvalumab injection. However, notably one patient with BCG-unresponsive NMIBC with CIS on pre-durvalumab biopsies had benign post-durvalumab biopsies and no disease in the bladder at RC.

The role of infused intravesical ICIs is under investigation with promising safety profiles but as yet little demonstrated efficacy [28]. Intravesical pembrolizumab alone lacked toxicity or evidence of systemic absorption, although also lacked an efficacy signal [10]. Intravesical durvalumab also appeared safe but without confirmed efficacy [11]. Intravesical pembrolizumab co-administered with BCG has also been reported but demonstrated high recurrence rates and safety concerns [29]. Sub-urothelial injection of ICIs may prove better and warrants further investigation.

Limitations

Dose-escalation was to a planned maximum dose, as opposed to maximum tolerated dose [17]. This was for numerous reasons, including: systemically administered durvalumab is distributed without particular affinity for any tissue type, Phase I dose-escalation studies of durvalumab monotherapy have not reached maximum tolerated doses at 20 mg/kg, durvalumab toxicity has not generally been a function of dose or exposure, and higher systemic doses of PD-L1 inhibitors have not been found to improve efficacy [30]. Regardless, the adult bladder represents <1% of adult bodyweight and direct infiltration of 150 mg of durvalumab likely results in vastly higher tissue concentration than 1500 mg systemic administration.

Due to the low numbers of suitable patients for recruitment, prior BCG treatment was not balanced between cohorts, which could potentially influence the results.

For future potential therapy, repeated administration of this dose may be required, potentially provoking IRAEs. Phase II and dose distribution studies in these areas are planned.

Conclusions

This trial has demonstrated that sub-urothelial injection of 150 mg durvalumab is feasible and safe without associated IRAEs. Immune populations changes seen after sub-urothelial injection of durvalumab suggest establishment of a local immunological response. Further studies utilising this therapeutic modality should be pursued based on its tolerability, safety, potential efficacy, and the unmet need for effective treatment options in NMIBC.

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Disclosure of Interests

The following authors declare the following affiliations: Dickon Hayne – Advisory Board with honoraria with Merck, Pfizer, BMS, Urogen and Pacific Edge. Andrew Redfern – Advisory Board with honoraria with AstraZenica, Novartis, Pfizer, Roche, Gilead. Ciara Conduit – Honoraria from AstraZenica. Elizabeth Liow – Partial funding for attending Pfizer for attending 2017 American Society of Clinical Oncology (ASCO) Annual Meeting and 2020 ASCO Genitourinary Cancers Symposium, Virtual Conference Resources from BMS and Merck.

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Abbreviations: (IR)(TR)AE, (immune-related) (treatment-related) adverse event; CIS, carcinoma *in situ*; CK, cytokeratin; GATA3, GATA-binding protein 3; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; (N)MIBC, (non-)muscle-invasive bladder cancer; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; PROM, patient-reported outcome measure; RC, radical cystectomy; RCI, relative changes in immune cells; TAM, tumour-associated macrophage; TIL, tumour-infiltrating lymphocyte; TSH, thyroid-stimulating hormone.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Key inclusion and exclusion criteria.

Table S2 Patient blood test results.

Table S3 Dose-limiting toxicities categories and criteria.

Fig. S1 Changes in monocytes across different durvalumab dose.

Fig. S2 (**A**) The RCI post-durvalumab injection according to dose of durvalumab. (**B**) The RCI post-durvalumab injection according to bladder cancer subtype.