Abbreviated Title: Durvalumab & Vicineum for NMIBC
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Title: A Phase I Single-Arm Study of the Combination of Durvalumab (MEDI4736) and Vicineum (oportuzumab monatox, VB4-845) in Subjects with High-Grade Non-Muscle-Invasive Bladder Cancer Previously Treated with Bacillus Calmette-Guérin (BCG)

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Drug Name:	Durvalumab (MEDI4736)	Vicineum™ (oportuzumab monatox, VB4-845)
IND Number:	136199	136199
Sponsor:	Center for Cancer Research (CCR)	Center for Cancer Research (CCR)
Manufacturer:	AstraZeneca	Sesen Biotherapeutics
Supplier:	AstraZeneca	Sesen Biotherapeutics

PRÉCIS

Background:

In 2016, it is estimated that there will be 76,960 new cases of bladder cancer and 16,390 deaths associated with bladder cancer.[1] Bladder cancer is associated with the highest costs among all types of cancer, due to the need for lifelong routine monitoring and treatment.[2] Approximately 70% of cases are non-muscle invasive bladder cancer (NMIBC) at presentation and are treated by transurethral resection of bladder tumor (TURBT) followed by intravesical treatment with BCG (Bacillus Calmette-Guerin) or mitomycin C. However, in the setting of high grade disease, these therapies can become ineffective over time in up to two-thirds of patients and disease progression to muscle invasive bladder cancer (MIBC) can occur.[3] In patients who present with CIS (carcinoma in situ) rates of progression are greater than 50%.[4] Progression to MIBC portends a poor outcome as only 50% of patients will survive five years despite undergoing radical cystectomy.[5] Clearly, there is a large unmet need in therapeutic options for NMIBC that recurs or progresses.

Vicineum™ is a recombinant fusion protein, VB4-845, that contains a humanized single-chain antibody fragment specific for the epithelial cell adhesion molecule (EpCAM) antigen linked to ETA (252-608), a truncated form of Pseudomonas exotoxin A (ETA). EpCAM is overexpressed on the surface of urothelial carcinoma cells and therefore represents a good target for Vicineum™ to bind to. In a previous phase II study in BCG refractory or BCG intolerant patients with high grade bladder cancer, 16% of patients treated with induction and maintenance therapy with Vicineum™ remained disease-free at 1 year. As a result, Vicineum™ is currently being evaluated as a single agent in a phase III trial.

Pre-clinical work with a drug called Proxinium, an earlier version of Vicineum™, demonstrated an abscopal effect and synergy with the use of a checkpoint blockade inhibitor. Although it was done in a NSCLC model, the results were impressive in causing tumor shrinkage. Durvalumab is a human monoclonal antibody (MAb) that inhibits binding of programmed cell death ligand 1 (PD-L1) (B7 homolog 1 [B7-H1], cluster of differentiation [CD]274) to programmed cell death 1 (PD-1; CD279) and CD80 (B7-1). Durvalumab has been demonstrated to have activity against advanced metastatic urothelial bladder cancer whose tumor has progressed during or after one standard platinum-based regimen in a phase I trial.

Therefore, this trial will take two agents with single agent activity against urothelial cancer and combine them in a Phase I trial for patients with high-grade NMIBC Previously Treated with BCG.

Objectives:

Primary Objectives:

- To evaluate the safety and tolerability of durvalumab and Vicineum when administered in combination to subjects with BCG-refractory high-grade NMIBC

Eligibility:

- Subjects must have a histologically-confirmed high-grade non-muscle invasive urothelial carcinoma (transitional cell carcinoma) of the bladder as follows:
    - Carcinoma-in-situ (CIS) with or without papillary tumors
    - High-grade Ta or T1 disease based on a biopsy/TURBT performed within 12 weeks of the initial dose of study treatment. If multiple bladder biopsies/TURBTs are required to confirm eligibility, the timing of the last bladder biopsy to the initial dose of study treatment must be within 12 weeks.
- Subjects with BCG unresponsive disease as defined by the Society of Urologic Oncology and the FDA[6,7]: Subjects must have received at least two courses of intravesical BCG (at least 5 of 6 induction doses of BCG and at least 2 of 3 maintenance doses of BCG under a maintenance regimen or at least 2 doses of a repeat induction course). See exception below for persistent T1 disease below. There is no upper limit on the amount of prior BCG a subject may have received.
- Patients with persistent T1 high grade disease on TURBT following a single induction course of BCG (at least 5 of 6 doses) may also be eligible for this trial provided that the patient is surgically unfit for cystectomy as deemed by the investigator or the patient declines cystectomy

Design:

- This is a Phase I, open-label study of the combination of durvalumab and Vicineum in subjects with high-grade NMIBC previously treated with BCG.
- All subjects will receive Vicineum intravesically and durvalumab systemically at the standard doses for both drugs as determined by Phase II trials for each drug, as no synergy or additive effect is expected for adverse events.
- Vicineum is administered in a 12-week Induction Phase followed by a Maintenance Phase for at least one year with an option for a total of up to 2 years of treatment. During the Induction Phase, Vicineum is administered once weekly for 12 weeks. During the Maintenance Phase, Vicineum is administered every other week. The dose of Vicineum is 30 mg in 50 mL of saline.
- Durvalumab 1500 mg is administered intravenously (IV) once every 4 weeks for 12 months with an option to continue therapy for an additional 12 months (total of 24 months) provided that patient is tolerating therapy and remains free of recurrent high grade NMIBC (see Treatment Period below). The dose of durvalumab is 1500 mg. If optional maintenance therapy continued in the second year, durvalumab 1500 mg will be administered intravenously once every 3 months to provide an immune boost.
- Vicineum will be given as monotherapy for 1 week followed by treatment with the combination of Vicineum and durvalumab starting week 2.
- In the initial six patients, three subjects at a time will enroll at these doses and schedules. Dose-liming toxicity (DLT) for each subject will be determined during the initial 6-week

period that the subject is on treatment (i.e., the DLT period). When all subjects in the initial cohort have been on treatment through the DLT period, all available safety data will be considered in decisions to enroll additional subjects at this dose level, or to de-escalate the dose(s) of study drug(s), based on a standard "3 + 3" design. There will be no dose-escalations in this study. The dose of durvalumab will remain at 1500 mg every 4 weeks, and the dose of each intravesical Vicineum treatment can be reduced to 20 mg if the initial doses in combination induce DLTs.

- After the first six patients, an additional 18 subjects will be enrolled at the initial doses or at the reduced doses (if DLTs resulted in the first 6 patients) in order to obtain additional safety data, biomarker data and preliminary anti-tumor activity.
- Each subject's course will consist of the following periods:
    - Screening/Baseline Period: The subject is consented and undergoes screening assessments to determine eligibility for the study.
    - Treatment Period: The subject is treated and monitored for safety. Biomarker data will be obtained prior to treatment and at periodic intervals during treatment. Subjects who remain free of high-grade NMIBC after 12 months of study treatment may continue to receive treatment for an additional 12 months until they develop recurrent high-grade disease, disease progression, or intolerable toxicity, or meet another withdrawal criterion (e.g., consent withdrawal, pregnancy).
    - Post-Treatment. The subject will return to the study site monthly for up to 90 days after the last dose of immunotherapy for end-of-treatment assessments. Subjects with ongoing clinically-significant related AEs or SAEs will have additional follow-up after the initial post-treatment visit.

SCHEMA

Please note, at the end of each maintenance cycle there is a 2 week window in order to allow the patients sufficient time to recover from biopies prior to continuing treatment.

INDUCTION

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14
Durvalumab													**3 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum														

MAINTENANCE #1

	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27
Durvaluma b												**6 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

 = each symbol indicates the number of treatments for that week

MAINTENANCE #2

	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38	Week 39	Week 40
Durvaluma b												**9 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

MAINTENANCE #3

	Week 41	Week 42	Week 43	Week 44	Week 45	Week 46	Week 47	Week 48	Week 49	Week 50	Week 51	Week 52	Week 53
Durvalumab												**12 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

 = each symbol indicates the number of treatments for that week

OPTIONAL MAINTENANCE #4

	Week 54	Week 55	Week 56	Week 57	Week 58	Week 59	Week 60	Week 61	Week 62	Week 63	Week 64	Week 65	Week 66
Durvalumab												**15 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

 = each symbol indicates the number of treatments for that week

OPTIONAL MAINTENANCE #5

	Week 67	Week 68	Week 69	Week 70	Week 71	Week 72	Week 73	Week 74	Week 75	Week 76	Week 77	Week 78	Week 79
Durvalumab												**18 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

OPTIONAL MAINTENANCE #6

	Week 80	Week 81	Week 82	Week 83	Week 84	Week 85	Week 86	Week 87	Week 88	Week 89	Week 90	Week 91	Week 92
Durvalumab												**21 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

 = each symbol indicates the number of treatments for that week..

OPTIONAL MAINTENANCE #7

	Week 93	Week 94	Week 95	Week 96	Week 97	Week 98	Week 99	Week 100	Week 101	Week 102	Week 103	Week 104	Week 105
Durvalumab												**24 Month Evaluation (Cysto, Cytol, Biopsy)**	
Vicineum													

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To evaluate the safety and tolerability of durvalumab and Vicineum when administered in combination to subjects with BCG-refractory high-grade NMIBC

1.1.2 Secondary Objectives

- To assess potential predictive biomarkers and biomarkers of the immune response in bladder tissue, urine, and peripheral blood to durvalumab and Vicineum when administered in combination to subjects with BCG-refractory high-grade NMIBC
- To assess the pharmacokinetics of Vicineum with urine samples
- To assess if urinary EpCAM correlates with response to therapy
- To assess the preliminary antitumor activity of durvalumab and Vicineum when administered in combination to subjects with BCG-refractory high-grade NMIBC
- Assess PD-L1 expression and PD-1 expressing T cells in paired bladder biopsies pre and post treatment with durvalumab and Vicineum as a marker of response/benefit

1.2 BACKGROUND AND RATIONALE

Bladder cancer is the 6th most common cancer in the United States, affecting more men than women. It is the 3rd most common cancer in men and the 11th most common in women. (Bladder cancer treatment (PDQ® 2015) Approximately 75% of bladder cancers are of the non-muscle invasive type.[8] Non-muscle invasive bladder cancers (NMIBCs) can be categorized as Ta (non-invasive papillary carcinoma), T1 (tumor invades lamina propria or subepithelial connective tissue), and Tis (carcinoma in situ). Ta tumors are the most common, representing about 70% of NMIBCs, but only about 7% of these are categorized as high-grade.[9] About 20% of NMIBCs are T1 tumors.[10] T1 tumors are more aggressive than Ta tumors, and considered high-risk.[8] Flat, high-grade tumors confined to the mucosa (non-invasive) are characterized as carcinoma in situ (CIS),[8] and these represent approximately 10% of the NMIBCs.[10]

The usual first treatment for NMIBC (Ta, T1, and CIS) is transurethral resection of the bladder tumors (TURBT), followed by intravesical immunotherapy, most commonly with bacillus Calmette-Guérin (BCG).[8] In patients with T1 tumors, a second TURBT is recommended.[8] Induction therapy with BCG is usually dosed as 6 once-weekly instillations.[8] In patients with high-grade Ta, T1, and CIS, maintenance therapy with BCG of at least 1 year is recommended,[8,10] although the optimal dose, dosing schedule, and duration of treatment are unknown. Local and systemic side effects are common with intravesical BCG therapy, causing discontinuation of treatment in approximately 20% of patients.[11] Approximately 75% of patients experience local side effects (including cystitis, irritative voiding symptoms, and hematuria), while 40% report systemic side effects, including general malaise and fever.[11] Intravesical BCG failure occurs in up to 40% of patients.[11] In ~20-35% of cases that failed an initial induction course of BCG, a second course of induction BCG may be beneficial, but patients who fail two

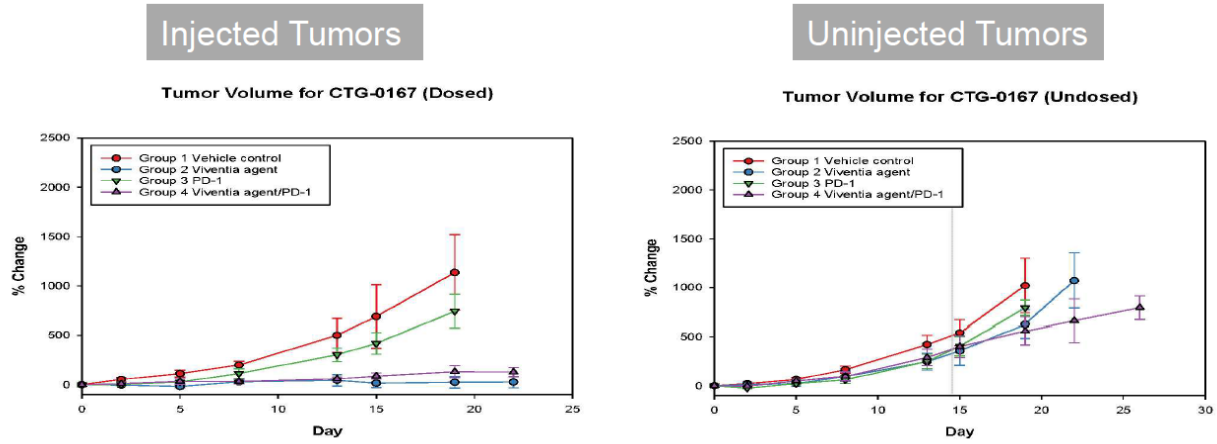
courses are often best served by radical cystectomy.[3,12,13] Therefore, cystectomy is recommended for CIS and high-grade Ta and T1 patients who experience disease recurrence following intravesical therapy.[8] However, Shabsigh et al. reported that 64% of patients who undergo cystectomy suffer at least one complication within the first 90 days after surgery[14] and the operation has been reported to have at least a 2.5% perioperative mortality rate even at a high-volume center.[5] For patients unable or unwilling to undergo cystectomy, treatment options are limited.

High grade non-muscle invasive bladder cancer tends to respond well to BCG and when it does not, there is evidence to suggest an incomplete or inefficient immune response. Immune responses directed against tumors are one of the body's natural defenses against the growth and proliferation of cancer cells. However, over time and under pressure from immune attack, cancers develop strategies to evade immune-mediated killing allowing them to develop unchecked. One such mechanism involves upregulation of surface proteins that deliver inhibitory signals to cytotoxic T cells.

Programmed cell death ligand 1 (PD-L1) is one such protein, and is upregulated in a broad range of cancers with a high frequency, with up to 88% expression in some tumor types. In a number of these cancers, including lung,[15] renal,[16-18] pancreatic,[19-21] ovarian cancer,[22] and hematologic malignancies,[23,24] tumor cell expression of PD-L1 is associated with reduced survival and an unfavorable prognosis. Programmed cell death ligand 1 is part of a complex system of receptors and ligands that are involved in controlling T-cell activation. PD-L1 acts at multiple sites in the body to help regulate normal immune responses and is utilized by tumors to help evade detection and elimination by the host immune system tumor response. In the lymph nodes, PD-L1 on antigen-presenting cells binds to PD-1 or CD80 on activated T cells and delivers an inhibitory signal to the T cell.[25,26] This results in reduced T-cell activation and fewer activated T cells in circulation. In the tumor microenvironment, PD-L1 expressed on tumor cells binds to PD-1 and CD80 on activated T cells reaching the tumor. This delivers an inhibitory signal to those T cells, preventing them from killing target cancer cells and protecting the tumor from immune elimination.[27] Therefore, targeting the PD-1/PD-L1 protein has emerged as an interesting strategy in bladder cancer especially given recent FDA approval of atezolizumab in the treatment of metastatic urothelial cancer. Durvalumab, a novel PD-L1 inhibitor, has not been investigated in NMIBC but has demonstrated activity in metastatic urothelial cancer as well.

Limited agents have demonstrated response in the setting of BCG-refractory disease. One such agent is Vicineum™. Vicineum™ consists of a recombinant fusion protein, VB4-845, that contains a humanized single-chain antibody fragment specific for the epithelial cell adhesion molecule (EpCAM) antigen linked to ETA (252-608), a truncated form of Pseudomonas exotoxin A (ETA). In 135 bladder cancer patients with CIS, TaG2-3, or T1G2-3 screened for trial enrollment for BCG-refractory disease, EpCAM staining was performed and revealed that 132 (98%) patients had some EpCAM staining. In fact, two of the 3 patients with negative staining, did not actually have tumor on the specimen analyzed. Ninety-four percent of all tumors had 2+ or 3+ staining. Therefore, EpCAM appears to be a good target in BCG-refractory bladder cancer. In a previous phase II study in patients with high grade bladder cancer previously treated with BCG, the 3-month complete response rate was 40% and 16% of patients treated with induction and maintenance therapy with Vicineum™ remained disease-free at 1 year.

Given activity of Vicineum™ in such patients and activity of durvalumab in metastatic urothelial cancer, the combination of both drugs may be more effective than either drug alone. PD-1/PD-L1 checkpoint inhibitors do not directly induce anti-tumor immune responses. Instead they function by taking the breaks off of existing T cell clones with a specificity for antigens expressed on the surface of tumor T cells. Therefore, in order for checkpoint inhibitor therapy to be effective, the checkpoint inhibitors should be used in conjunction with agents that are capable of inducing a cellular anti-tumor immune response. It is generally accepted that a type of cell death known as immunogenic cell death (ICD) is associated with effective stimulation of these cellular host immune responses. ICD can be identified by the presence of damage associated molecular patterns or DAMPS – these are characterized by distinct signals such as the release of ATP and HMGB1 (High Mobility Group Box 1) and cell surface translocation of calreticulin, an endoplasmic reticulum chaperone protein. Preclinical work demonstrates that Vicineum (VB4-845) mediated killing of tumor cells results in the presence of these DAMPS, thereby suggesting that it could promote the desired anti-tumor cellular immune responses. Additionally, as bladder cancer is one of the cancers associated with higher levels of somatic mutations, it follows that targeted killing of EpCAM positive cancer cells should present these antigens to the immune system, thereby providing targets for the cellular immune response. Therefore, use of both agents may lead to synergistic killing of tumors.



Based on the thoughts of synergy, an in vivo study was performed with a drug called Proxinium, an earlier version of Vicineum™, demonstrating an abscopal effect and synergy with the use of a checkpoint blockade inhibitor. Although it was done in a NSCLC model, the results were impressive in causing tumor shrinkage. In this experiment, a humanized mouse model was used with an engrafted immune system (myeloablated and engrafted with human bone marrow stem cells) and a human EpCAM-expressing NSCLC PDX tumor placed in the flanks bilaterally. The mice had circulating human T cells and B cells. Four treatment groups were used. In group 1, vehicle alone was given to serve as a control group. Group 2 was treated with direct injection of VB4-845 (Proxinium/Vicineum) unilaterally into only one of the flank-bearing tumors. Group 3 was treated with PD-1 checkpoint inhibitor alone (nivolumab) and finally group 4 was treated with the combination of VB4-845 and the PD-1 checkpoint inhibitor. The left graph shows the tumor size of the injected tumor in the four groups while the right graph shows the tumor size of the uninjected tumor in the four groups. As can be seen in the left graph, intratumoral treatment of the EpCAM positive subcutaneous PDX tumor with VB4-845 led to cessation of tumor

growth. PD-1 inhibitor by itself or in combination with VB4-845 was not that effective in reducing the size of the injected tumor. However, intratumoral treatment of the EpCAM positive subcutaneous PDX tumor with VB4-845 increased the ability of a PD-1 checkpoint inhibitor to decrease the growth rate of an uninjected contralateral PDX tumor as compared to treatment with the checkpoint inhibitor alone as shown on the right graph. The small number of animals per cohort and the use of a rapidly growing PDX model decreased the impact of the combination but interestingly the flattening of the growth rate of the uninjected tumors in the combination group was not observed until a sufficient period of time passed to allow for the induction of a cellular immune response. The preclinical study was performed with Vicineum in combination with a PD-1 inhibitor targeting the receptor, rather than durvalumab which is a PD-L1 inhibitor that targets the ligand of the PD-1/PD-L1 interaction. However, there is no reason to believe that durvalumab would act differently. It would be difficult to truly predict the safety of the combination of the two drugs in a preclinical model as neither antibody cross reacts with mouse homologues. Therefore, this trial is evaluating the safety and tolerability of the combination of Vicineum and durvalumab in BCG-refractory NMIBC. A secondary endpoint would be 12-month recurrence-free survival (RFS) with the combination. Historical data suggests that the 12-month recurrence-free survival (RFS) rate is approximately 20-35% for patients who failed one previous induction course of BCG and are treated again with BCG alone.

1.2.1 Durvalumab Background

Durvalumab is being developed as a potential anticancer therapy for patients with advanced solid tumors. Durvalumab is a human monoclonal antibody (MAb) of the immunoglobulin G1 kappa (IgG1κ) subclass that inhibits binding of programmed cell death ligand 1 (PD-L1) (B7 homolog 1 [B7-H1], cluster of differentiation [CD]274) to programmed cell death 1 (PD-1; CD279) and CD80 (B7-1). Durvalumab is composed of 2 identical heavy chains and 2 identical light chains, with an overall molecular weight of approximately 149 kDa. Durvalumab contains a triple mutation in the constant domain of the immunoglobulin (Ig) G1 heavy chain that reduces binding to complement protein C1q and the fragment crystallizable gamma (Fcγ) receptors involved in triggering effector function.

1.2.1.1 Summary of non-clinical experience

The non-clinical experience is fully described in the current version of the durvalumab Investigator's Brochure.

Durvalumab binds with high affinity and specificity to human PD-L1 and blocks its interaction with PD-1 and CD80. *In vitro* studies demonstrate that durvalumab antagonizes the inhibitory effect of PD-L1 on primary human T cells, resulting in their restored proliferation and release of interferon gamma (IFN-γ). Additionally, durvalumab demonstrated a lack of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) in cell-based functional assays. In vivo studies show that durvalumab inhibits tumor growth in a xenograft model using pancreatic and melanoma human cancer cell lines via a T lymphocyte (T-cell) dependent mechanism. Moreover, an anti-mouse PD-L1 antibody demonstrated improved survival in a syngeneic tumor model when given as monotherapy and resulted in complete tumor regression in > 50% of treated mice when given in combination with chemotherapy. Combination therapy (dual targeting of PD-L1 and cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4]) resulted in tumor regression in a mouse model of colorectal cancer.

1.2.1.2 Summary of clinical experience

Clinical experience with durvalumab is fully described in the current version of the durvalumab Investigator's Brochure.

As of the DCO date (12 July 2016), across the entire clinical development program, an estimated 5225 patients have been exposed to 1 or more doses of durvalumab in AstraZeneca- or MedImmune-sponsored studies, either as monotherapy or in combination, including 2878 patients in open label trials, and 2347 patients as an estimate based on the random scheme in studies where the treatment arm is blinded. Additionally, more than 1700 patients have been exposed to 1 or more doses of durvalumab in ESR/IITs. Of the 2878 patients exposed to durvalumab in ongoing AstraZeneca- or MedImmune-sponsored open label studies, 1744 patients received durvalumab monotherapy, 808 patients received durvalumab in combination with tremelimumab, 140 patients received durvalumab in combination with other investigational products and 186 patients received durvalumab in combination with approved products. No studies have been completed and no study has terminated prematurely due to toxicity. For a summary of the different studies including study design, dosing regimen, study populations and exposure, please see IB.

Pharmacokinetics and Product Metabolism

Study CD-ON-durvalumab-1108:

As of 24 July 2016, PK data were available for 977 patients who have been treated with durvalumab 0.1 to 10 mg/kg Q2W or 15 mg/kg Q3W (dose escalation), 10 mg/kg Q2W (dose expansion), and 20 mg/kg Q4W (dose-exploration) durvalumab administered as an iv infusion over 60 minutes. Following the first iv dose, durvalumab exhibited nonlinear PK at doses <3 mg/kg Q2W likely due to saturable target-mediated CL and exhibited linear PK at doses ≥3 mg/kg Q2W. The AUC_{0-14} increased dose-proportionally at doses of 3 to 20 mg/kg and more than dose proportionally at doses of <3 mg/kg, likely due to saturable target-mediated CL. Cmax increased in a dose proportional manner within the dose range examined. The steady state was achieved at approximately Week 16. Accumulation of durvalumab was observed following repeated dosing.

As of 24 July 2016, a total of 790 patients provided samples for ADA analysis. Only 25 of 790 patients (1 patient each in 0.1 and 3 mg/kg cohorts, 17 patients from 10 mg/kg cohort) were ADA positive. Based on population PK covariate analysis, ADA positive status was not associated with a clinically relevant reduction of exposure to durvalumab. At the 10 mg/kg Q2W dose, sPD-L1 suppression in ADA positive patients was similar to that observed in ADA negative patients. The relevance of ADA on safety and efficacy is unknown given the small number of ADA positive patients.

Safety

The safety profile of durvalumab as monotherapy and combined with other anticancer agents was consistent with the pharmacology of the target and other agents in the immune checkpoint inhibitor class. No tumor types appeared to be associated with unique AEs. Immune-related AEs (irAEs), which are important risks of immune checkpoint inhibitors, have been observed with durvalumab and include colitis, pneumonitis, hepatitis/hepatotoxicity, neuropathy/neuromuscular toxicity, endocrinopathy, dermatitis, and nephritis. In addition, pancreatitis is an important

potential risk particularly with durvalumab and tremelimumab combination therapy. These events are manageable by available/established treatment guidelines as described in the study protocols.

Safety data have been pooled for 4 durvalumab monotherapy studies (CD-ON-MEDI4736-1108, D4190C00002, ATLANTIC and D4193C00001 [HAWK]) for patients who received a durvalumab dose of 10 mg/kg Q2W; a total of 1645 patients are included in this pooled data set.

- Overall, AEs reported in ≥10% of patients were fatigue (31.1%), decreased appetite (22.5%), nausea (20.5%), dyspnoea (17.9%), constipation (17.8%), cough (17.4%), diarrhoea (16.0%), anaemia (15.3%), pyrexia (15.0%), vomiting (13.4%), back pain (12.5%), pruritus (11.0%), arthralgia (10.6%) and abdominal pain (10.2%).
- AEs that were considered by the investigator as related to durvalumab in ≥5% of patients were fatigue (14.5%); nausea (7.3%); diarrhoea (6.9%); hypothyroidism (6.6%); pruritus (6.4%); decreased appetite (6.0%) and rash (5.2%).
- A total of 820 patients (49.8%) reported AEs of Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or higher: of these, 487 patients (29.6%) had events of Grade 3, 63 patients (3.8%) had events of Grade 4 and 270 patients (16.4%) had Grade 5 (fatal) events. AEs of Grade 3 or higher considered related to durvalumab were reported in 164 patients (10.0%): of these, 144 patients (8.8%) had events of Grade 3, 12 patients (0.7%) had events of Grade 4 and 8 patients (0.5%) had Grade 5 (fatal) events.
- Grade 3 events occurring in ≥1% of patients were: anaemia (5.5%); dyspnoea (4.4%); hyponatraemia (4.1%); fatigue (2.9%); gamma-glutamyltransferase (GGT) increased (2.7%); abdominal pain (2.0%); decreased appetite and back pain (1.9% each); pneumonia (1.8%); aspartate aminotransferase (AST) increased and dehydration (1.6% each); hypertension (1.3%); blood alkaline phosphatase (ALP) increased, hypokalaemia, urinary tract infection and vomiting (1.2% each); alanine aminotransferase (ALT) increased and pleural effusion (1.1% each); bilirubin increased, asthenia, nausea and pulmonary embolism (1.0% each). Grade 3 events considered related to durvalumab occurring in ≥0.5% patients were fatigue (1.2%), GGT increased (0.8%) and AST increased (0.6%).
- The most commonly reported Grade 4 event was sepsis (15 patients [0.9%]). Other commonly reported Grade 4 events were: GGT increased (9 patients [0.5%]); dyspnoea, hypercalcaemia and respiratory failure (7 patients each [0.4%]) and pneumonia (5 patients [0.3%]). All other Grade 4 events were reported in less than 5 patients each. Grade 4 events considered related to durvalumab occurring in ≥2 patients were GGT increased and pneumonitis (0.1% each).
- Grade 5 events occurred in the system organ class (SOC) of ‘neoplasms benign, malignant and unspecified (including cysts and polyps)’ for 172 of the 270 patients with Grade 5 events (63.7%), with the highest number of events occurring for NSCLC (40 patients). Amongst the other SOCs, the most common Grade 5 events were general physical health deterioration (12 patients), respiratory failure (8 patients); Grade 5 events of pneumonia and sepsis occurred in 5 patients each with the remainder of the Grade 5

events occurring in ≤4 patients for each event. The only Grade 5 event considered related to durvalumab occurring in ≥2 patients was pneumonitis (0.1%).

- A total of 134 patients (8.1%) discontinued from study treatment due to an AE. The most common events leading to treatment discontinuation were: general physical health deterioration (10 patients); pneumonitis (7 patients); pneumonia (6 patients); dyspnoea and NSCLC (5 patients each); all other discontinuation events occurred in ≤4 patients.
- A total of 89 patients (5.4%) had serious treatment-emergent AEs (TEAEs) that were considered by the investigator as related to durvalumab. The most common were: pneumonitis (12 patients [0.7%]); fatigue (5 patients [0.3%]); colitis, infusion related reaction and ILD (4 patients each [0.2%]); dehydration, diarrhoea, nausea and nervous system disorder (3 patients each [0.2%]); abdominal pain, acute kidney injury, adrenal insufficiency, AST increased, bilirubin increased, dyspnoea, hepatic function abnormal, nephritis, transaminases increased, tumor haemorrhage and vomiting (2 patients each [0.1%]).
- A total of 854 patients (51.9%) experienced an AESI during the study. The most common grouped term AESI was diarrhoea (263 patients [16.0%]; of whom 12 patients [0.7%] had events of Grade ≥3). Other common AESIs (grouped term) were: selected hepatic events (248 patients [15.1%]; of whom 113 patients [6.9%] had events of Grade ≥3); dermatitis (237 patients [14.4%]; of whom 3 patients [0.2%] had events of Grade ≥3); rash (199 patients [12.1%]; of whom 7 patients [0.4%] had events of Grade ≥3); hypothyroidism (170 patients [10.3%]; of whom 2 patients [0.1%] had events of Grade ≥3); hyperthyroidism (93 patients [5.7%]; of whom 1 patient [<0.1%] had events of Grade ≥3); and select renal events (87 patients [5.3%]; of whom 16 patients [1.0%] had events of Grade ≥3). There were 6 patients who had AESIs of CTCAE Grade 5 (fatal events): three patients had hepatic events (autoimmune hepatitis; hepatic failure and hyperbilirubinemia); two patients had pneumonitis and 1 patient had immune thrombocytopenic purpura.

Efficacy

Study CD-ON-durvalumab-1108:

Urothelial carcinoma

A phase 1/2 multicenter, open-label study conducted in patients with inoperable or metastatic solid tumors, reported the results a urothelial carcinoma expansion cohort.[28] Durvalumab (10 mg/kg every 2 weeks) was administered intravenously for up to 12 months. The primary end-point was safety, and objective response rate (ORR, confirmed) was a key secondary end point. A total of 61 patients (40 PD-L1–positive, 21 PD-L1–negative), 93.4% of whom received one or more prior therapies for advanced disease, were treated (median duration of follow-up, 4.3 months). The most common treatment-related adverse events (AEs) of any grade were fatigue (13.1%), diarrhea (9.8%), and decreased appetite (8.2%). Grade 3 treatment-related AEs occurred in three patients (4.9%); there were no treatment-related grade 4 or 5 AEs. One treatment-related AE (acute kidney injury) resulted in treatment discontinuation. The ORR was 31.0% (95% CI, 17.6 to 47.1) in 42 response-evaluable patients, 46.4% (95% CI, 27.5 to 66.1) in the PD-L1–positive subgroup, and 0% (95% CI, 0.0 to 23.2) in the PD-L1–negative subgroup.

Responses are ongoing in 12 of 13 responding patients, with median duration of response not yet reached (range, 4.1+ to 49.3+ weeks).

1.2.2 Vicineum Background

Vicineum™ (Vicineum) contains the active pharmaceutical ingredient VB4-845, which is a recombinant fusion protein produced in Escherichia coli (E. coli) that expresses a humanized single-chain antibody fragment (scFv) specific for the epithelial cell adhesion molecule (EpCAM) antigen linked to ETA(252-608). ETA(252-608), which is a truncated form of Pseudomonas exotoxin A (ETA) that lacks the cell binding domain, is a single polypeptide fusion protein produced by continuous translation of a single construct.

The mechanism of action is dependent upon the 2 components of Vicineum. Once bound to the EpCAM antigen on the surface of carcinoma cells, Vicineum is internalized through an endocytic pathway. Furin contained within the endosomal compartment cleaves a proteolytic site on the surface of ETA(252-608), releasing ETA(252-608). The ETA(252-608) induces cell death by irreversibly blocking protein synthesis through adenosine diphosphate (ADP)-ribosylation of a post-translationally modified histidine residue of elongation factor-2 (EF-2), called diphthamide. (Oppenheimer 1981) The truncated version of ETA, ETA(252-608), has been engineered to retain the active domains necessary to induce cell death, but the cell binding domain has been eliminated thereby preventing the ETA(252-608) moiety from entering the cell in the absence of some alternate vehicle, such as via antibody-mediated internalization. Binding to EpCAM must occur to result in ETA(252-608)-mediated effects.

The ETA(252-608) component of the Vicineum fusion protein can cause an immunogenic response when administered systemically to humans. Therefore, Vicineum is being developed as a locally targeted therapeutic in order to limit its systemic exposure and to maximize the concentration of the drug in its target cells. By administering Vicineum via intravesical instillation, the probability of systemic exposure and subsequent generation of neutralizing antibodies is decreased. Furthermore, the high local concentrations of Vicineum maximize the likelihood of achieving a therapeutic benefit.

Clinical experience with Vicineum is fully described in the current version of the Vicineum Investigator's Brochure (Version 8.0).

1.2.2.1 Preclinical Studies

Preclinical study data have shown that Vicineum exhibits potent activity [inhibitory concentration 50% (IC50) = 0.001 - 10 pM] against numerous EpCAM-positive cell lines, with selectivity for EpCAM-expressing tumors. In vivo pharmacology demonstrated that Vicineum effectively inhibits tumor growth in several human xenograft animal models. Studies in rats found that the toxicological effects of Vicineum occur at doses 1,000-fold greater than the IC50 for activity on tumor cells, with a safety margin of at least 5- to 100-fold.

1.2.2.2 Clinical Studies

VB4-845-02-I

A Phase 1 trial (VB4-845-02-I) of Vicineum evaluated doses up to 30.16 mg administered intravesically to 64 subjects with Grade 2 or 3, stage Ta or T1 transitional cell carcinoma (TCC) or CIS, either refractory to or intolerant of BCG therapy.[29] No dose-limiting toxicities (DLTs)

were observed. Vicineum was well tolerated, and the majority of adverse events (AEs) were mild. The most frequently reported treatment-related AEs were renal and urinary disorders, with dysuria (14.1%) and hematuria (10.9%) most commonly reported. Of the systemic AEs, fatigue was reported by 7.8% of subjects, while fever/chills and loss of appetite were each reported by 6.2% of subjects. The frequency of treatment-related AEs did not increase with increasing doses of Vicineum. There was 1 serious adverse event reported (death due to cardiac failure), which occurred 3 weeks after stopping study drug; the Investigator assessed the event to be unrelated to Vicineum treatment, attributing it to the subject's long-standing history of cardiovascular disease. Exploratory efficacy assessment at 3 months showed that of 61 evaluable subjects, a complete response (defined as non-positive urinary cytology and either normal cystoscopy or abnormal cystoscopy with negative biopsy) was achieved by 24 subjects (39%). Of the 17 subjects with Tis, 29% achieved a complete response, while complete responses were observed in 44% and 43% of the subjects with T1 and Ta, respectively.

Blood samples for assessing humoral immune reactivity to Vicineum were taken prior to dosing on days 1, 8, 15, 22, 29, 36, and at the final study visit. Antibody titers to the scFv and ETA252–608 portions of the fusion protein (human antihuman antibodies (HAHA) and human anti-Pseudomonas antibodies (HAPA)) were measured using an enzyme-linked immunosorbent assay. HAPA response was more vigorous, as patients developed a measurable titer earlier; the majority of patients exhibited HAPA by day 29, with 77% (47/61) having a measurable titer at final visit. In contrast, only 16% (10/61) of patients had HAHA by the end of the study. HAPA titers were also generally higher, with a mean maximum titer of 20,512 versus 3373 for HAHA responses. A comparison of mean titers of the HAPA and HAHA responses measured in samples taken on the final visit showed no significant difference between responders and nonresponders.

VB4-845-02-IIA

A Phase 2 study (VB4-845-02-IIA) evaluated once-weekly instillations of Vicineum 30 mg in two different induction schedules: weekly x 6 weeks or weekly x 12 weeks, followed by up to 3 maintenance cycles (3 once-weekly instillations followed by a 9-week drug-free period) in 46 subjects with histologically-confirmed TCC of the bladder and residual CIS with or without concurrent Ta or T1 who were refractory or intolerant to BCG.[30] Some subjects in the 6-weekly induction arm received a second induction cycle. Forty-six subjects were considered evaluable for response. A complete response (defined as no histological evidence of disease and negative urine cytology at the 3-monthly evaluations) was achieved by 44% (20/45) of subjects, and 16% (7/45) of subjects remained disease-free at the 1-year end-of-study (EOS) assessment. A post-study assessment found that these subjects were still disease-free at 18-25 months. The median time to recurrence was 134 days longer in subjects who received 12 weeks of induction therapy compared to 6 weeks (408 vs 274 days, respectively; log rank test p=0.1708). The most frequently reported treatment-related AEs were renal and urinary disorders, including 50% with dysuria (61% of these were of mild intensity) and 13% with hematuria. Overall, most AEs were of mild severity. There were 3 severe events, all of which resolved without sequelae. No subject discontinued from the study due to AEs and there were no deaths or other serious AEs.

Based on the preliminary positive therapeutic results and favorable tolerability profile, the present Phase 3 trial will evaluate the efficacy of Vicineum induction therapy administered for 12 weeks, and the long-term effectiveness of Vicineum maintenance therapy (up to 24 months treatment in total) in preventing disease recurrence.

VB4-845-02-IIIA

An open-label, single–arm Phase 3 trial of Vicineum (VB4-845-02-IIIA) is evaluating Vicineum in subjects who have received a least 2 courses of BCG and whose disease is considered BCG-unresponsive. One hundred thirty-three subjects will enroll of whom 77 will have CIS with relapsed/refractory disease within 6 months of their last dose of BCG.

Based on data in the database as of 03 October 2016, the most frequent adverse events regardless of causality were UTIs, dysuria, hematuria, fatigue, micturition, urgency, pollakiuria, diarrhea, and arthralgia.

Two subjects were reported to have suspected unexpected serious adverse reactions (SUSARs). A 90 year-old patient with an elevated lipase developed acute renal failure and LFT elevations. The patient required dialysis. Cholestatic hepatitis progressed. The investigator judged these events as possibly related to study treatment. After a few weeks of dialysis, the patient elected to stop further dialysis and go to hospice care. After stopping dialysis, the patient passed away.

A 74-year-old patient also developed acute kidney injury with LFT elevations. The LFT elevations resolved quickly upon stopping Vicineum treatment. The kidney function improved with hospitalization and intravenous fluids.

Trial update – Investigator Brochure version date: 06/10/2020

Efficacy

Vicineum demonstrated efficacy in CR rate at 3 months with sustained CR and duration of response, as well as CR rate at 6, 9, and 12 months, cystectomy free days, event-free survival, progression-free survival, overall survival, recurrence-free rates in papillary disease subjects, and time to disease recurrence in papillary disease subjects, for the treatment of BCG-unresponsive subjects who are resistant to currently available therapy. Subjects that responded to Vicineum (CIS subjects with CR and papillary subjects who were recurrence-free at 3 months) were 5 times more likely to remain cystectomy-free at 2 years than those who did not respond.

The CR rate at 3 months was 39% and 40% for the primary efficacy population (mITT) and all evaluable CIS subjects, respectively. Of those mITT subjects with a CR at post-induction phase (3 months), 69%, 53%, and 42% continued to have a complete response at 6, 9, and 12 months, respectively. All CIS subjects with fewer BCG cycles had a higher CR rate than those CIS subjects treated with more courses of BCG.

1.2.3 US FDA withdrawal of metastatic Urothelial Carcinoma (mUC) indication for Imfinzi (durvalumab) granted under the accelerated approval pathway

On 5/1/17, the FDA had granted accelerated approval to durvalumab for the treatment of locally advanced or metastatic urothelial carcinoma with disease progression after failing platinum-based chemotherapy or disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-based chemotherapy.

As of February 2021, AstraZeneca has voluntarily withdrawn the indication for Imfinzi (durvalumab) in the US for the treatment of adult patients with locally advanced or metastatic urothelial carcinoma who:

– Have disease progression during or following platinum-containing chemotherapy, or

– Have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

This decision was made in consultation with the US Food and Drug Administration (FDA) based on their broad evaluation of accelerated approvals. The voluntary withdrawal was for lack of efficacy and not for safety concerns.

The urothelial carcinoma indication was granted Accelerated Approval from the US FDA, based on tumor response rate and duration of response from Study 1108 and the significant unmet needs of patients.2 Accelerated approval is a specific regulatory path whereby confirmatory data is required to convert an accelerated approval to full approval. In this case, continued approval for this indication in the US was contingent upon positive results from the Phase III DANUBE trial of Imfinzi or Imfinzi plus tremelimumab versus chemotherapy as first-line therapy for patients with unresectable, locally advanced or metastatic urothelial carcinoma. The DANUBE trial did not meet either of its co-primary endpoints of improving overall survival versus standard of care chemotherapy for Imfinzi monotherapy in patients whose tumor cells and/or tumor-infiltrating immune cells express high levels (≥25%) of programmed cell death ligand-1 (PD-L1) or for Imfinzi plus tremelimumab in patients regardless of their PD-L1 expression.

We would like to continue the drug-combination trial since our preliminary interim data analysis shows a 40% 12 week response rate (5/12 patients) with half of them showing a >12 month sustained response. We hypothesize that the prolonged response might be related to the systemic therapy (Durvalumab) administration. Also, most of the treatment related AEs are irritative voiding symptoms and most of them are grade 3 or lower, suggesting that the treatment is tolerated well thus far.Combination Therapy Dose Justification

In the single-agent phase I Vicineum study, there were no dose-limiting toxicities (DLTs) and no MTD was determined. Only 2 subjects of 64 had detectable plasma Vicineum levels and these were very near the lower level of quantitation. Durvalumab has been extensively studied in Phase I and Phase II and the recommended dose and safety profile has been established. For these reasons, the initial dose level for the combination will be the standard doses for both drugs. The FDA approved dose for durvalumab is currently 10mg/kg every 2 weeks and this amounts to 750 mg every 2 weeks for an average 75 kg individual. Based on pre-clinical data, there is no difference in exposure between weight based Q2W dosing and fixed dose Q4W dosing. Therefore, we are administering 1500 mg every 4 weeks instead of 750 mg every 2 weeks for ease and dosing convenience.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically or cytologically confirmed by NCI Laboratory of Pathology as high grade non-muscle invasive urothelial (transitional cell carcinoma) of the bladder as follows:

- Carcinoma-in-situ (CIS) with or without papillary tumors

- High-grade Ta or T1 disease based on a biopsy/TURBT performed within 12 weeks of the initial dose of study treatment. If multiple bladder biopsies/TURBTs are required to confirm eligibility, the timing of the last bladder biopsy to the initial dose of study treatment must be within 12 weeks.
- Patients with persistent T1 high grade disease on TURBT following a single induction course of BCG (at least 5 of 6 doses) may also be eligible for this trial provided that the patient is surgically unfit for cystectomy as deemed by the investigator or the patient declines cystectomy.

2.1.1.2 Subjects with BCG unresponsive disease as defined by the Society of Urologic Oncology and the FDA[6,7]: Subjects must have received at least two courses of intravesical BCG (at least 5 of 6 induction doses of BCG and at least 2 of 3 maintenance doses of BCG under a maintenance regimen or at least 2 doses of a repeat induction course). Please note exception above for persistent T1 disease. There is no upper limit on the amount of prior BCG a subject may have received.

2.1.1.3 Patients who have met eligibility criterion **2.1.1.2** must have received last BCG dose within a year of enrollment.

2.1.1.4 The investigator must document that he/she believes the subject would not benefit from additional BCG treatment at the time of study entry.

2.1.1.5 Age ≥ 18 years at time of signing the informed consent form (ICF). Because no dosing or adverse event data are currently available on the use of Vicineum in combination with durvalumab in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials. Furthermore, NMIBC does not occur in children.

2.1.1.6 Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 (**Appendix A**)

2.1.1.7 Adequate organ and marrow function as defined below:

- Hemoglobin ≥ 9.0 g/dL
- Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L (> 1500 per mm^3)
- Platelet count ≥ 75 x 10^9/L (>75,000 per mm^3)
- Serum bilirubin ≤ 1.5 x institutional upper limit of normal (ULN).
- AST (SGOT)/ALT (SGPT) ≤ 2.5 x institutional ULN
- Creatinine CL>40 mL/min by the Cockcroft-Gault formula (Cockcroft and Gault 1976) or by 24-hour urine collection for determination of creatinine clearance:

Males:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

Females:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}} \times 0.85$$

2.1.1.8 Female subjects must either be of non-reproductive potential (i.e., post-menopausal as described below) OR history of surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry.

- Women <50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and if they have luteinizing hormone and follicle-stimulating hormone levels in the post-menopausal range for the institution
- Women ≥50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments, had radiation-induced menopause with last menses >1 year ago, or had chemotherapy-induced menopause with last menses >1 year ago

2.1.1.9 The effects of Vicineum and durvalumab on the developing human fetus are unknown. Females of childbearing potential and and men who are sexually active must use a highly effective method of contraception (including abstinence, intrauterine system or intrauterine device contraceptive implant, hormonal, female or partner sterilization or menopause as defined in **2.1.1.8**) and a second form of barrier contraception during study treatment and for 120 days after the last dose of study drug. Note: partner's use of the same methods of contraception (barrier, hormonal, intrauterine device [IUD], surgical sterilization) is also acceptable. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.10 Written informed consent obtained from the subject prior to performing any protocol-related procedures

2.1.1.11 Subject is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations including follow up.

2.1.1.12 Body weight > 30 kg

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents.

2.1.2.2 QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥470 ms. [Any clinically significant abnormalities detected require triplicate ECG results and a mean

QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥470 ms calculated from 3 ECGs.]

2.1.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Vicineum or durvalumab or other agents used in the study.

2.1.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Urinary tract infections (UTIs) are excluded from being an exclusion criterion for treatment unless they are grade 3 or higher.

2.1.2.5 Pregnant women are excluded from this study because it is unknown whether Vicineum and/or durvalumab have any teratogenic effects. In nursing mothers, breastfeeding should be discontinued as these medications may have the potential risk for adverse events in nursing infants secondary to treatment of the mother.

2.1.2.6 Any previous treatment with a PD1 or PD-L1 inhibitor, including durvalumab

2.1.2.7 Evidence of non-bladder urothelial (transitional cell) carcinoma by biopsy, cytology, or radiological imaging within the past 2 years of treatment (e.g. upper tract transitional cell carcinoma, urethral urothelial carcinoma).

2.1.2.8 Subjects with hydronephrosis, except for those subjects where hydronephrosis has been longstanding (i.e., predates the diagnosis of the CIS, Ta, or T1 by more than 2 years) and diagnostic evaluation at screening shows no evidence of tumor causing the hydronephrosis.

2.1.2.9 Any other anticancer therapy (e.g., chemotherapy, biologic therapy, immunotherapy, targeted therapy, endocrine therapy, radiation therapy, intravesical therapy, investigational agent) within 28 days of the first dose of study therapy (and within 6 weeks for nitrosourea or mitomycin C) other than a single dose of intravesical chemotherapy which is permitted between 28 days and 14 days prior to the first dose of study treatment.

2.1.2.10 The subject has a diagnosis of another malignancy within 2 years before the first dose of study treatment, except for superficial skin cancer, localized prostate cancer on active surveillance, or localized solid tumors deemed cured by surgery and not treated with systemic anticancer therapy and not expected to require anticancer therapy in the next 2 years i.e., while the subject may be taking study treatment.

2.1.2.11 Current or prior use of immunosuppressive medication within 28 days before the first dose of durvalumab, with the exceptions of intranasal and inhaled corticosteroids or systemic corticosteroids at physiological doses, which are not to exceed 10 mg/day of prednisone, or an equivalent corticosteroid.

2.1.2.12 Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease [e.g. colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangiitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc.]). The following are exceptions to this criterion:

- Subjects with vitiligo or alopecia
- Subjects with hypothyroidism (e.g. following Hashimoto syndrome) stable on hormonal replacement
- Any chronic skin condition that does not require systemic therapy
- Subjects without active disease in the last 5 years may be included but only after consultation with the Principal Investigator
- Subjects with celiac disease controlled by diet alone

2.1.2.13 History of primary immunodeficiency.

2.1.2.14 History of allogeneic organ transplant.

2.1.2.15 History of hypersensitivity to durvalumab or any excipient

2.1.2.16 History of hypersensitivity to Vicineum or its components

2.1.2.17 Active infection with tuberculosis (clinical evaluation that includes clinical history, physical examination, and radiographic findings, and PPD testing if indicated), hepatitis B (known positive HBV surface antigen (HBsAg) result, hepatitis C, or human immunodeficiency virus (positive HIV 1/2 antibodies). Patients with HIV are excluded from participating on this clinical trial because their immunodeficiency would confound the evaluation of adverse events which would hinder meeting the primary objective. Subjects with a past or resolved HBV infection (defined as the presence of hepatitis B core antibody [anti-HBc] and absence of HBsAg) are eligible. Subjects positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA.

2.1.2.18 History of leptomeningeal carcinomatosis

2.1.2.19 Receipt of live attenuated vaccination within 30 days prior to the first dose of Vicineum or durvalumab

2.1.2.20 Any condition that, in the opinion of the investigator, would interfere with evaluation of study treatment or interpretation of patient safety or study results

2.1.2.21 Subjects with uncontrolled seizures

2.1.2.22 Any unresolved toxicity NCI CTCAE Grade ≥2 from previous anticancer therapy with the exception of alopecia, vitiligo, and the laboratory values defined in the inclusion criteria

- Subjects with Grade ≥2 neuropathy will be evaluated on a case-by-case basis after consultation with the Principal Investigator.

  Subjects with irreversible toxicity not reasonably expected to be exacerbated by treatment with durvalumab may be included only after consultation with the Principal Investigator.

2.1.3 Recruitment Strategies

This study will be posted on NIH websites and on NIH social media forums. Additionally, this study will be listed on available websites (www.clinicaltrials.gov and others) and participants will be recruited from the current patient population at NIH.

2.2 SCREENING EVALUATION

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols). Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

Evaluations to be performed within 12 weeks prior to starting treatment:

- History and physical examination (including medications, medical history, surgical history, previous treatments for bladder cancer with dates, allergies, and demography)
- ECOG performance status
- Formal review of available previous bladder tumor specimens by the Laboratory of Pathology, NCI, to confirm the bladder cancer diagnosis prior to enrollment into the study.
    - If outside bladder tumor specimens are inadequate to accurately stage the patient's disease, then additional biopsies will be performed at the NCI as part of standard of care therapy. Tissues obtained at the NCI will be used to confirm diagnosis and for study of immune-related markers. With preoperative patient permission, extra tissue not sent to Laboratory of Pathology, NCI, will be frozen for future analysis of immune-related markers and tumor infiltrates. This additional harvested tissue will be used for research purposes only and is not required for a candidate to participate in this protocol.
    - If outside bladder tumor specimens are adequate to accurately stage the patient's disease, cystoscopy with or without biopsy and/or TURBT to confirm diagnosis and resect and remove any residual papillary disease and fulgurate any CIS may be performed as part of standard of care therapy. With preoperative patient permission, extra tissue not sent to the Laboratory of Pathology, NCI, will be frozen for the future analysis of immune-related markers and tumor infiltrates. This additional harvested tissue will be used for research purposes only and is not required for a candidate to participate in this protocol.Blue light cystoscopy can be performed at urologist's discretion.
- Laboratory studies
    - Hematology (Complete blood count plus differential and platelet count)
    - Serum chemistries and liver function tests (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, pre-albumin, amylase, lipase
    - Coagulation parameters (PT, PTT)

  - Viral Studies (Hepatitis B and C; HIV)
  - Thyroid function tests
- Urinalysis
- 24 hour urine (only if CrCL will not be calculated)
- PPD if indicated
- Computerized Tomography (CT) urogram or Magnetic Resonance Imaging (MRI) urogram. If urogram protocol not available or contrast allergy/poor renal function preclude such imaging, then CT or MRI of the abdomen/pelvis without contrast will suffice and investigator may perform retrograde pyelogram at their discretion.
- Electrocardiogram (ECG) for QT interval – Mean QT interval corrected for heart rate using Fridericia's formula (QTcF) will be calculated from a single ECG. If QTcF ≥ 470ms, then the patient must be excluded from trial.
  - Resting 12-lead ECGs will be recorded according to the assessment schedule. ECGs should be obtained after the patient has been in a supine position for 5 minutes and should be recorded while the patient remains in that position.
  - ECG will be recorded at Screening, in Week 2 (pre-durvalumab treatment) and as clinically indicated.
  - In case of clinically significant ECG abnormalities, including a QTcF value >470 ms, 2 additional 12-lead ECGs should be obtained over a brief period (e.g., 30 minutes and at least 5 minutes apart) to confirm prolongation
- Serum or urine beta-HCG for women of child-bearing age

See section **3.4**, **Study Calendar** for more details.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Run in Cohort	Subjects with high grade non-muscle invasive urothelial (transitional cell carcinoma) of the bladder. Up to 12 subjects
2	Expansion Cohort	Subjects with high grade non-muscle invasive urothelial (transitional cell carcinoma) of the bladder. Up to 24 subjects enrolled after the run in cohort is filled.

Arms

Number	Name	Description
1	Run In	Durvalumab + Vicineum, escalating doses. Up to 2 dose levels will be evaluated in the first 6 – 12 subjects
2	Expansion	Durvalumab + Vicineum, at the MTD. Up to 24 subjects

Subjects in cohort 1 will be directly assigned to arm 1. Subjects in cohort 2 will be directly assigned to arm 2.

2.4 BASELINE EVALUATION

See section **3.4**, Study Calendar.

2.4.1 Baseline studies

Performed within 7 days before starting treatment. If Clinical evaluation laboratory work was also performed within 7 days before starting treatment, then those values can be used for baseline evaluation.

- Hematology (Complete blood count plus differential and platelet count)
- Acute care panel (Na+, K+, Cl-, CO2, glucose, BUN, creatinine)
- LDH
- Pre-albumin
- Pancreatic enzymes (amylase, lipase)
- Hepatic panel (alkaline phosphatase, ALT, AST, total bilirubin)
- Mineral panel (albumin, calcium, magnesium, phosphorus)
- Thyroid function tests

2.4.2 Urine studies (within 7 days prior to initiation of study therapy)

- Urine cytology
- Urinalysis

- Urine culture and sensitivity

2.4.3 Research Assessments at Baseline (within 7 days prior to initiation of study therapy)

Please refer to section **5.2**.

2.4.4 Other Assessments

- Weight
- Physical Exam, ECOG status, and medications

3 STUDY IMPLEMENTATION

This is a single arm study. A run in cohort of up to 12 evaluable subjects and an expansion cohort of up to 24 evaluable will be evaluated. No randomization of stratification will be performed.

3.1 STUDY DESIGN

See **SCHEMA**.

3.1.1 Dose Limiting Toxicity (DLT)

A DLT will be defined as any Grade 3 or higher toxicity that occurs during the initial 6-week period the subject is on treatment (i.e., the DLT evaluation period). Toxicity that is clearly and directly related to the primary disease or to another etiology is excluded from this definition. Since this trial also involves immunotherapy, immune-related AEs (irAEs) are defined as AEs of an immune nature (i.e. inflammatory) in the absence of a clear alternative etiology. In the absence of a clinically significant abnormality, repeat laboratory testing will be conducted to confirm significant laboratory findings prior to designation as a DLT. The following will be DLTs:

- Inability to reduce prednisone dose below 10 mg within 6 weeks (rather than 12 weeks) due to ongoing immune-related adverse reaction of Grade >1
- Any Grade 4 irAE not attributed to local tumor response (e.g., inflammatory reaction attributed to local tumor response or inflammatory reaction at sites of metastatic disease or lymph nodes)
- Any $\geqslant$ Grade 3 colitis
- Any Grade encephalopathy
- Any $\geqslant$ Grade 3 neurotoxicity
- Any $\geqslant$ Grade 3 peripheral neuromotor syndromes
- Any Grade 2 or higher noninfectious pneumonitis irrespective of duration
- Any Grade 3 irAE, excluding colitis that does not downgrade to Grade 2 within 3 days after onset of the event despite optimal medical management including systemic corticosteroids or does not downgrade to $\leqslant$ Grade 1 or baseline within 14 days

- Total bilirubin >1.5 - 3.0 x ULN (Grade 2 Hyperbilirubinemia)
- ALT or AST >8x ULN
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and
- ALT or AST >3x ULN and INR >1.5
- ALT or AST >3x ULN with appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
- Any Grade ≥3 non-irAE toxicity that does not downgrade to Grade ≤1 or baseline status (for patients who entered the study with an existing laboratory abnormality) within 14 days
- Inability to receive ⩾ 75% or more of scheduled study treatment (both Vicineum and durvalumab) during the first six weeks of the trial
- Persistent Grade 2 events possibly related to study drugs such as persistent nausea, vomiting, diarrhea, or fatigue that are refractory to maximal intervention or are severely limiting to the patient's ability to carry out activities of daily living.

The definition excludes the following conditions:

- Grade 3 endocrine disorder (thyroid, pituitary, and/or adrenal insufficiency) that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the subject is asymptomatic
- Concurrent vitiligo or alopecia of any AE grade
- Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management
- Isolated Grade 3 electrolyte abnormalities that are not associated with clinical signs or symptoms and are reversed with appropriate maximal medical intervention within 3 days.
- Grade 3 inflammatory reaction attributed to a local antitumor response (e.g., inflammatory reaction at sites of metastatic disease, lymph nodes, etc.)

3.1.2 Dose De-Escalation / Cohort Expansion

- This trial will begin with a fixed dose of Vicineum 30 mg intravesical (Dose Level 1) administered once weekly for the first 12 weeks. Durvalumab administration will begin after 1 week of Vicineum monotherapy, at 1500mg IV once every 4 weeks.
- In the initial six patients, three subjects at a time will enroll at these doses and schedules (Dose Level 1). Dose-limiting toxicity (DLT) for each subject will be determined during the initial 6-week period that the subject is on treatment (i.e., the DLT period). When all subjects in the initial cohort have been on treatment through the DLT period, all available safety data will be considered in the decision to enroll additional subjects at this dose level, or to de-escalate the doses of the study drugs to Dose Level -1, based on a standard "3 + 3" design (see table below). There will be no dose-escalations in this study.

- After the first six to twelve patients, an additional 18 subjects will be enrolled at the highest dose level at which fewer than 2 of 6 subjects experienced DLT in order to obtain additional safety data and preliminary anti-tumor activity. Treatment at this level will continue as long as the cumulative fraction of patients experiencing DLT is < 1/3.

Dose De-Escalation Schedule	
Dose Level	Dose of IND Agent
Level 1	• Durvalumab 1500mg IV every 4 weeks • Vicineum 30 mg intravesically per dose*
Level -1	• Durvalumab 1500mg IV every 4 weeks • Vicineum 20 mg intravesically per dose*
* Vicineum administered weekly during the first 12 weeks of the study, then every 2 weeks thereafter.	

Dose de-escalation will follow the rules outlined in the Table below.

If 2 or more DLTs occur at dose level -1, then accrual to the trial will end pending an amendment to consider other dosing options to explore for safety before proceeding to explore efficacy.

Number of Patients with DLT at a Given Dose Level	**De-Escalation Decision Rule**
0 out of 3	Enter an additional 3 patients at same dose level
1 of 3	Enter an additional 3 patients at Dose Level 1 • If 0 of these 3 patients experience DLT, enter additional patients at Dose Level 1 • If 1 or more of this group suffer DLT then enter up to 3 patients at the next lowest dose level. Reduce by 1
≥2	• Enter up to 3 patients at the next lowest dose level.

3.2 DRUG ADMINISTRATION

In this trial, patients will receive both drugs in certain weeks as specified in the schema. The order of administration will be determined by drug delivery by pharmacy to the day hospital on

the day of administration. Therefore, patients can receive either both drugs concurrently or sequentially without a particular order.

3.2.1 Durvalumab

3.2.1.1 Durvalumab doses and treatment regimens

The initial dose of durvalumab (Cohort 1) is 1500 mg IV every 4 weeks for up to 52 weeks. Please note, at the end of each maintenance cycle there is a 2 week window in order to allow the patients sufficient time to recover from biopsies prior to continuing treatment. Refer to the schema for a detailed description of Durvalumab administration weeks.

Study treatment should be discontinued prior to 12 months if there is confirmed PD, initiation of alternative cancer therapy, unacceptable toxicity, withdrawal of consent, or if other reasons to discontinue study treatment occur.

Subjects who have a dose interruption due to toxicity at any point in the first 12 months of treatment may resume treatment and complete the 12-month treatment period. For patients without recurrent high grade disease and who are tolerating therapy well, optional maintenance therapy may be continued in the second year. The maintenance dose will be durvalumab 1500 mg administered intravenously once every 3 months to provide an immune boost. During that second year, as per standard of care for high grade disease, cystoscopy and urine cytology will be performed every 3 months. This is depicted graphically in the schema in the optional maintenance section.

No incompatibilities between durvalumab and polyvinylchloride or polyolefin IV bags have been observed. Dose of 1500mg durvalumab (for patients >30 kg in weight) will be administered using an IV bag containing 0.9% (w/v) saline or 5% (w/v) dextrose, with a final durvalumab (MEDI4736) concentration ranging from 1 to 20 mg/mL, and delivered through an IV administration set with a 0.2- or 0.22-µm in-line filter.

Add 30.0 mL of durvalumab (MEDI4736) (ie. 1500 mg of durvalumab (MEDI4736)) to the IV bag. The IV bag size should be selected such that the final concentration is within 1 to 20 mg/mL. Mix the bag by gently inverting to ensure homogeneity of the dose in the bag.

For patients who lose weight (<30 kg) during the course of the trial and maintain their enrollment ECOG status, further treatment with durvalumab will be weight based. The dose volume of durvalumab and number of vials needed for the subject to achieve the accurate dose will be calculated according to **Appendix B**.

Durvalumab will be administered at room temperature (approximately 25°C) by controlled infusion via an infusion pump into a peripheral or central vein. Following preparation of durvalumab, the entire contents of the IV bag should be administered as an IV infusion over approximately 60 minutes (±5 minutes), using a 0.2, or 0.22-µm in-line filter. Less than 55 minutes is considered a deviation.

The IV line will be flushed with a volume of IV solution (0.9% [w/v] saline) equal to the priming volume of the infusion set used after the contents of the IV bag are fully administered, or complete the infusion according to institutional policy to ensure the full dose is administered and document if the line was not flushed.

Standard infusion time is 1 hour. However, if there are interruptions during infusion, the total allowed time should not exceed 4 hours at room temperature. The table below summarizes time allowances and temperatures.

Durvalumab hold and infusion times

Maximum time from needle puncture to start of administration	4 hours at room temperature, 24 hours at 2°C to 8°C
Maximum time for IV bag infusion, including interruptions	8 hours at room temperature

In the event that either preparation time or infusion time exceeds the time limits outlined in the table above, a new dose must be prepared from new vials. Durvalumab does not contain preservatives, and any unused portion must be discarded.

3.2.1.2 Monitoring of dose administration

Subjects will be monitored before, during and after the infusion with assessment of vital signs at the times specified in the Schedule of Assessment. Subjects are monitored (pulse rate, blood pressure) every 30 minutes during the infusion period (including times where infusion rate is slowed or temporarily stopped).

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and re-initiated at 50% of the initial rate until completion of the infusion. For subjects with a ≤Grade 2 infusion-related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion-related reaction is Grade 3 or higher in severity, study drug will be discontinued. The standard infusion time is one hour, however if there are interruptions during infusion, the total allowed time from infusion start to completion of infusion should not exceed 4 hours at room temperature, with maximum total time at room temperature not exceeding 4 hours (otherwise requires new infusion preparation).

As with any antibody, allergic reactions to dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit subjects to an intensive care unit if necessary.

3.2.2 Vicineum™

3.2.2.1 Doses And Treatment Regimens

The initial dose of vicineum is 30 mg in 50 mL of saline (Dose level 1). Vicineum will be instilled through a catheter into the bladder. Subjects will be instructed to hold study drug in the bladder for 2 hours, and asked to refrain from fluid intake to reduce urine flow during this period. After complete administration of the Vicineum dose, the catheter may be clamped and remain in for the 2-hour dwell time, or may be removed, per the discretion of the investigator.

Subjects will be encouraged, when physically possible, to be upright (sitting or standing), prone, supine, and in the left and right lateral decubitus positions, for at least 15 minutes each, in any order. If physically unable, this should be recorded in the CRF. At the end of 2-hour period, the catheter will be unclamped and the bladder will be drained (if the catheter remained in place) or the subject will void to empty the bladder.

Study Phase	Treatment Regimen
Induction Phase (Weeks 1-12)	One intravesical dose of Vicineum in 50 mL of saline instilled once weekly for a total of 12 weeks.
Maintenance Phase (up to Week 104)	One intravesical dose of Vicineum 50 mL saline instilled once every other week for 12 months from the start of the Induction phase. Please note, at the end of each maintenance cycle there is a 2 week window in order to allow the patients sufficient time to recover from biopsies prior to continuing treatment. Refer to the schema for a detailed description of Vicineum administration weeks. If remain disease-free, therapy can be continued for up to 24 months (Week 104) from the start of the Induction Phase.

3.3 DOSE MODIFICATIONS

3.3.1 Durvalumab

Following any biopsy or TURBT, study drug must be interrupted for a maximum of 2 weeks before restarting Durvalumab treatment.

For adverse events (AEs) that are considered at least partly due to administration of durvalumab outside of the DLT evaluation period, the following dose adjustment guidance may be applied:

- Treat each of the toxicities with maximum supportive care (including holding the agent suspected of causing the toxicity where required).
- If the symptoms promptly resolve with supportive care, consideration should be given to resuming the same dose of durvalumab along with appropriate continuing supportive care.
- If medically appropriate, dose modifications are permitted for durvalumab. All dose modifications should be documented with clear reasoning and documentation of the approach taken (see **Appendix C**).

In addition, there are certain circumstances in which durvalumab should be permanently discontinued (see **Appendix C)**. In circumstances where subjects cannot tolerate durvalumab or do not meet retreatment criteria to receive either drug, subjects will be taken off protocol therapy.

Following the first dose of durvalumab, subsequent administration of durvalumab can be modified based on toxicities observed (see **Appendix C**).

Based on the mechanism of action of durvalumab leading to T-cell activation and proliferation, there is the possibility of observing immune related Adverse Events (irAEs) during the conduct of this study. Potential irAEs include immune-mediated enterocolitis, dermatitis, hepatitis, and endocrinopathies. Subjects should be monitored for signs and symptoms of irAEs. In the absence of an alternate etiology (e.g., infection or PD) signs or symptoms of enterocolitis, dermatitis, hepatitis, and endocrinopathy should be considered to be immune-related.

Dose modification recommendations and toxicity management guidelines for immune-mediated reactions, for infusion-related reactions, and for non-immune-mediated reactions are detailed in **Appendix C**.

3.3.2 Vicineum

Following any biopsy or TURBT, study drug must be interrupted for a minimum of 1 week and maximum of 2 weeks before restarting Vicineum treatment. Patients who develop low grade papillary disease will be allowed to stay on trial. In November 2016, the FDA published a draft guidance for trials involving patients with BCG-unresponsive disease in which only high-risk disease counts as a recurrence or an event. In this clinical setting, low-risk disease would lead to transurethral resection while high-risk disease would lead to cystectomy, which is a much different clinical outcome. Therefore, in this trial, low grade recurrences will be documented but will not count as a trial-ending recurrence or event. Furthermore, low grade recurrences are considered to be downstaging of disease since the inclusion criteria requires patients with high grade disease.

Study drug administration must be postponed if a subject develops any Grade 3 or greater adverse event. Study treatment may resume when the AE returns to ≤ Grade 1 or baseline or sooner if there is agreement between the investigator and the sponsor. Study treatment may be postponed at the discretion of the investigator for unrelated AEs, if this is considered in the best interest of the subject. If the causal relationship between Vicineum and an AE is initially uncertain, study drug should be interrupted until that determination is made. Also study treatment may be postponed for lower grade AEs at the investigator discretion if considered in the best interest of the subject.

The following specific criteria will be used for holding Vicineum dosing based on serum creatinine and calculation of creatinine clearance:

Baseline Creatinine	Criteria for Holding Vicineum Treatment*	
	Serum Creatinine	Creatinine Clearance
<1.0 mg/dl or <88 µmol/L	>1.5 mg/dl or >133 µmol/L	<40 ml/min or a decrease of 25% from baseline
≥1.0 mg/dl or ≥88 µmol/L	Increase to >2 x Baseline Value	<40 ml/min or a decrease of 25% from baseline

*Vicineum dosing should be held if either the serum creatinine or the creatinine clearance criteria, as defined in this table, are met.

If changes in renal function are noted, the investigator should evaluate the subject for the etiology of these changes. Vicineum treatment may be resumed upon improvement or stabilization at creatinine of ≤ 2 x baseline or ≤ 2 x ULN.

- Vicineum dosing must be held for any subject whose AST or ALT doubles from their baseline value and is ≥2xULN; OR for a bilirubin ≥2mg/dl (≥34 µmol/L). However, if Grade 2 hyperbilirubinemia occurs during the DLT evaluation period, then a DLT is present and treatment will be permanently discontinued and patient will be removed from study. The investigator should evaluate subjects for the etiology of any changes in these values. Vicineum treatment may be resumed upon improvement or stabilization of AST and ALT of ≤ 2 x baseline or ≤ 2 x ULN; OR for a bilirubin ≤ 2mg/dl (≤34 µmol/L) provided that Grade 2 hyperbilirubinemia did not occur.
- The investigator should consider holding Vicineum treatment for patients with a clinically significant UTI requiring antibiotic therapy until improvement. Investigators should also consider holding Vicineum treatment for patients with grade II hematuria.

Missed doses should not be made up. In the Maintenance Phase, dosing should be restarted as soon as there is AE resolution i.e., treatment should not be postponed longer in order to align with the initial every-other-week schedule.

If study treatment is postponed longer than 3 weeks, the investigator should consider whether the subject should remain on treatment.

In circumstances where subjects cannot tolerate Vicineum or do not meet retreatment criteria to receive either drug, subjects will be taken off protocol therapy.

The reasons and details relevant to the subject postponement and/or discontinuation of study treatment must be recorded on the CRF. The Investigator must notify Sesen Biotherapeutics if study treatment is discontinued.

3.4 STUDY CALENDAR

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Written informed consent/assignment of subject identification number		X						
Inclusion/Exclusion criteria	X							
Demography	X							
Medical and surgical history	X							

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Previous treatments for bladder cancer including dates/doses of prior BCG	X							
Confirmation of diagnosis by NCI LP	X							
Cystoscopy	X							X (every 3 months as per **SCHEMA**)
Bladder biopsies / TURBTs	X							X (every 3 months as indicated as per **SCHEMA**)

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Urine cytology		X						X (every 3 months preceding each cystoscopy as per **SCHEMA**)
EpCAM determination		X						Last biopsy on trial
Tissue PD-L1 assay		X						Last biopsy on trial
Hepatitis B and C; HIV	X							
Urine hCG or serum βhCG[2]	X[3]			Week 4		Week 8	X	Prior to each durvalumab dose

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Durvalumab administration[3]				Starting Week 2		Week 6 Week 10		Every 4 weeks resuming in week 15 and as per **SCHEMA**. Every 3 months if optional maintenance performed after first 12 months.
Vicineum administration[4]			X	X	X	X	X	Every 2 weeks resuming in week 15 and as per **SCHEMA**.

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Physical examination including weight[5]	X	X		Week 2		Weeks 6, 10	X	Targeted physical examination as needed.
Vital signs (pre-during and post-durvalumab infusion; pre- and post-Vicineum drainage)[6]	X	X	All treatment visits					
Electrocardiogram[7]	X			Week 2		If clinically indicated		If clinically indicated

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Adverse event/serious adverse event assessment			All visits					
Concomitant medications		X	All visits					
ECOG performance status	X	X			X			
Acute care panel[7]	X	X	X	X	X	X	X	Every 2 weeks
LDH	X	X	X	X	X	X	X	Every 2 weeks
Pre-albumin		X						
Pancreatic enzymes (amylase, lipase)	X	X	X	X	X	X	X	Every 2 weeks

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Hepatic panel	X	X	X	X	X	X	X	Every 2 weeks
Mineral panel	X	X	X	X	X	X	X	Every 2 weeks
Thyroid function tests (TSH and fT3 and fT4)[8]	X	X		Week 2		Week 6 Week 10		Prior to each durvalumab dose
Hematology[7]	X	X		Week 2 Week 4	X	Week 8	X	Prior to each durvalumab dose
Urinalysis	X	X	X	X (weekly)	X	X (weekly)	X	X (prior to each Vicineum treatment)
Urine culture and sensitivity		X						

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
24 hour urine (only if CrCl to be measured rather than calculated)	X							
PPD testing (if indicated)	X							
Coagulation parameters	X		As clinically indicted					
Urine cytokines		X					X	6 months
Serum circulating cytokine profile		X		Weeks 2, 3, and 5				

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
sPD-L1 concentration (to assess target engagement)		X		Weeks 2, 3, and 5				
Gene expression analysis		X						
Tissue immune correlates (IHC and possibly RNAseq)		X						Last biopsy on trial

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Flow cytometry of PBMCs for immune subsets and antigen-specific responses (T-cell clonal expansion if feasible)		X					X	6 months
Immunogenicity assessment		X				Week 8: ADA	Week 12: AVA	
Tumor CT/CT urogram or MRI/MRI urogram of abdomen and pelvis[9]	X							Every 12 months while on study

SCHEDULE OF STUDY ASSESSMENTS: Screening (Weeks -12 to -1), Induction Phase (Weeks 1 – 12), and Post-Induction Evaluation (All assessments can be performed within +/-7 days of scheduled assessment to accommodate holidays and scheduling difficulties)								
Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject[15].	**Screening**	**Baseline**	**All assessments to be performed pre-infusion unless stated otherwise**					
			Week 1	**Weeks 2 - 5**	**Week 6**	**Weeks 7 - 11**	**Week 12**	**Post-Induction and Maintenance**
Day	**-84 to -1**	**-7 to 0**						
Week	**-12 to -1**	**-1**						
Urinary PK for Vicineum		X	X[10,11,]	X[12]				
Urinary EpCAM levels		X	X[10,11]	X[12]	X (before Vicineum)	Week 10 (before Vicineum)	X (before Vicineum)	
Follow-up Safety Visits for patients who come off treatment or complete study[13, 14]								Every month up to 90 days post the last dose of immunotherapy[14]

1. May be performed up to 12 weeks prior to enrollment.
2. Pre-menopausal female subjects of childbearing potential only.
3. Dose can be administered within +/- 7 days of scheduled administration to accommodate schedule and holidays.
4. Once weekly intravesical administration for weeks 1-12; Dose can be administered within +/- 7 days of scheduled administration to accommodate schedule and holidays.
5. Full physical examination at screening and/or baseline; targeted physical examination at other timepoints and when clinically indicated.

6. Vital signs will be obtained at a single timepoint at screening and baseline. Subjects will have their blood pressure and pulse measured before, during and after the infusion at the following times (based on a 60-minute infusion):
    - At the beginning of the infusion (at 0 minutes)
    - At 30 minutes during the infusion (±5 minutes)
    - At the end of the infusion (at 60 minutes ±5 minutes)
    - In the 1 hour observation period post-infusion: 30 and 60 minutes after the infusion (ie, 90 and 120 minutes from the start of the infusion) (±5 minutes) – for the first infusion only and then for subsequent infusions as clinically indicated.
    - If the infusion takes longer than 60 minutes then blood pressure and pulse measurements should be collected every 30 minutes (±5 minutes) and as described above or more frequently if clinically indicated.
7. ECG during screening required to measure QTc as specified above and 1 ECG is needed while on treatment (pre-durvalumab infusion) and as clinically indicated. ECGs should be obtained after the patient has been in a supine position for 5 minutes and should be recorded while the patient remains in that position. In case of clinically significant ECG abnormalities, including a QTcF value >470 ms, 2 additional 12-lead ECGs should be obtained over a brief period (e.g., 30 minutes) to confirm prolongation. If screening laboratory assessments are performed within 3 days prior to Day 1, they do not need to be repeated at Day 1.
8. Free T3 and free T4 will only be measured if TSH is abnormal. They should also be measured if there is clinical suspicion of an adverse event related to the endocrine system. During maintenance phases 4-7, TSH will be drawn prior to each durvalumab dose and subsequently 4 weeks and 8 weeks after durvalumab administration.
9. Can be given without contrast if severe contrast allergy or renal function does not allow contrast administration per institutional recommendations. MRIs will be done per PI discretion and when clinically indicated.
10. First void after Vicineum administration in Week 1.
11. First void after the next morning after Vicineum administration in Week 1.
12. Day 8 void prior to Vicineum administration in Week 2.
13. H&P, Labs including renal, liver, complete blood count + platelets, coagulation studies monthly for up to 90 days after last immunotherapy.
14. End of treatment visit will occur 90 days after the last dose of immunotherapy. If clinically indicated, patients may continue to be followed by phone or clinic visits beyond that time and up to a year at the NIH/NCI or their local urologist.
15. Per PI discretion, a remote evaluation may be done by phone by a member of the study team. This evaluation will be conducted in compliance with NIH guidelines and FDA regulations. A patient may be asked to come to the NIH Clinical Center or may be referred to their local provider for an in-person assessment, if deemed necessary by the PI. In the case of any visits with participants' local providers, records will be obtained.

3.5 COST AND COMPENSATION

3.5.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by their insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.5.2 Compensation

Participants will not be compensated on this study.

3.5.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete monthly safety visits (+/- 7 days) for 90 days following the last dose of study therapy. These visits will consist of H&P and labs (including renal, liver, complete blood count + platelets, coagulation studies). Patients will revert to standard of care once coming off study. If clinically indicated, patients may continue to be followed by phone or clinic visits beyond that time and up to a year at the NIH/NCI or their local urologist.

3.6.1 Criteria for Removal from Protocol Therapy

Subjects are free to withdraw from further treatment or to withdraw consent to participate in this study at any time and without prejudice to further treatment. For subjects who withdraw their consent for study participation, no further study specific assessments will be performed and no additional study data will be collected. For subjects who withdraw from further treatment only will remain on study for further evaluations but receive no additional study treatment. The investigator may also withdraw a subject from study treatment or from the study if he or she deems it to be in the best interest of the subject or if the subject is unable to comply with the study. All subjects withdrawing only from further treatment should have enter the Post-Treatment Period and have the appropriate assessments performed 90 days after the last dose of study drug, unless the subject withdraws consent for the procedures. If clinically indicated, patients may continue to be followed by phone or clinic visits beyond that time and up to a year at the NIH/NCI or their local urologist. The reasons and details relevant to subject withdrawal must be recorded in the source documents and in the CRFs.

In addition, an individual subject will not receive any further investigational product if any of the following occur in the subject in question:

- Completion of protocol therapy

- Unacceptable toxicity as defined in section **3.3** and **Appendix C**
- Adverse event that, in the opinion of the investigator, contraindicates further dosing of either study drug
- Subject experiences a DLT during the DLT evaluation period (see section **3.1.1**)
- Requirement for prohibited medications/therapies
- Subject met eligibility criteria at screening but then subsequently is determined to have met one or more of the exclusion criteria for study participation and continuing investigational therapy might constitute a safety risk
- Subjects refuses to use contraception as required by the protocol
- Disease progression (as defined in section **6.2.4.3**) or confirmation that the subject is no longer benefiting from study treatment
- Grade 2 or greater hyperbilirubinemia
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined as any serious adverse event that is unexpected relative to the known safety profile of the investigational agents in the opinion of the investigator
- Sponsor or Manufacturer terminates the study
- The FDA requests that the sponsor terminate treatment of a specific subject or subjects or all subjects
- Subject noncompliance that, in the opinion of the investigator or sponsor, warrants withdrawal; e.g. refusal to adhere to scheduled visits
- Investigator discretion
- Positive pregnancy test or intent to become pregnant or intent to breast-feed or is breastfeeding
- Disease recurrence with high-grade bladder cancer

3.6.2 Off-Study Criteria

The following are off study criteria that will preclude further follow-up:

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Death
- Patient is lost to follow-up
- Patient enrolled into another clinical study

3.6.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PERMITTED CONCOMITANT MEDICATIONS

Investigators may prescribe concomitant medications or treatments (e.g., acetaminophen, diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care except for those medications identified as "excluded" as listed in Section **4.2**.

4.2 EXCLUDED CONCOMITANT MEDICATIONS

The following medications are considered exclusionary during the study.

1. Any investigational drugs
2. Any concurrent chemotherapy, radiotherapy (except palliative radiotherapy), immunotherapy, biologic or hormonal therapy, including investigational therapy for cancer treatment. Concurrent use of hormones for noncancer-related conditions (e.g., insulin for diabetes and hormone replacement therapy) is acceptable. Subjects who enter the study with low risk prostate cancer and then progress to require androgen-deprivation therapy (ADT) when already enrolled on this trial may continue on trial as per investigator discretion.
3. Immunosuppressive medications including, but not limited to systemic corticosteroids at doses not exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and TNF-α blockers. Use of immunosuppressive medications for the management of investigational product-related AEs or in subjects with contrast allergies is acceptable. In addition, use of inhaled and intranasal corticosteroids is permitted.
4. Live attenuated vaccines within 30 days of durvalumab dosing (i.e., 30 days prior to the first dose, during treatment with durvalumab and for 30 days post discontinuation of durvalumab. Inactivated vaccines, such as the injectable influenza vaccine, are permitted.)

Prohibited & concomitant meds

Prohibited medication/class of drug:	**Usage:**
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
mAbs against CTLA-4, PD-1, or PD-L1 other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment. (Concurrent use of hormones for non-cancer-related conditions [e.g., insulin for diabetes and hormone replacement therapy] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable [e.g., by local surgery or radiotherapy])
Live attenuated vaccines	Should not be given through 30 days after the last dose of IP

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Blood studies

5.1.1.1 Serum circulating/Blood cytokine profile

Refer to the table in section **5.2** for volume, tube types and sample collection schedule. Samples will be assessed for:

- IL-1B, IL–2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-18, IFN-y, IL-6, TNF-alpha, and IL-10. This will be done with a commercial ELISA kit such as V-plex in collaboration with Liang Cao, William D. Figg, and Dr. Andrea Apolo.
- Soluble PD-L1 (total and free unbound PD-L1)

For Serum collection of samples for cytokines and soluble PDL-1, please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred). For sample pickup, page 102-11964. For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number). For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.1.1.2 PBMCs for immune analysis

Samples will be collected at the timeframes indicated in the table in section **5.2**

- Flow cytometry of immune subsets (include CD4+ and CD8+ T-cells, Tregs, NK cells, and MDSCs and PD-L1 expression on immune cells) – This will be processed

in Dr. Schlom's laboratory as described below. Dr. Schlom's laboratory now has the ability to analyze 123 peripheral immune cell subsets[31] as shown in **Appendix D**.

- T-cell clonal expansion assay if appropriate funding and material is available
- Antigen-specific responses of cytotoxic lymphocytes for tumor associated antigens (e.g. brachyury, EPCAM)
- Immunogenicity Assessment (anti-durvalumab antibodies and anti-vicineum antibodies) – Samples will be collected at baseline and stored in Dr. Linehan's Lab
    i. Week 8 for ADA and Week 12 for AVA (separate blood collection at the weeks specified – Stored in Dr. Linehan's Lab

5.1.1.2.1 Sample processing

For PBMCs, green top tubes will be obtained and then processed at the Clinical Services Program (CSP) NCI Frederick Cancer Research and Development Center, PO Box B, Frederick, MD 21702 in collaboration with Dr. Schlom's research group. On days samples are drawn, Jen Bangh at CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). Once a patient's treatment schedule has been determined, Caroline Jochems should be notified at jochemscm@mail.nih.gov for planning purposes. Dr. Schlom and colleagues recently published their ability to analyze PBMCs for 123 subsets of peripheral immune cells using 30 unique markers in 5 immune flow cytometry panels. These subsets are subsets of CD4+ T cells, CD8+ T cells, Tregs, B cells, NK cells, NK-T cells, conventional dendritic cells (cDCs), plasmacytoid DCs (pDCs), and MDSCs. A full list is enclosed in **Appendix D**.

5.1.2 Research urine studies

5.1.2.1 Urine cytokines

Urine collection at the timepoints indicated in the table in section **5.2** for correlative/exploratory research aims (e.g. inflammatory cells, cytokines) at baseline. This will be performed through a commercial vendor, Luminex, using a platform of 12 urinary cytokines.[32]

5.1.2.2 Urine EpCAM, Pharmacokinetics and Pharmacodynamics

This will be done in direct collaboration with Dr. Cao's laboratory

- Evaluate PK for urinary Vicineum by testing for VB4-845 – see table in section **5.2** for PK timepoints
- Evaluate pharmacodynamics (PD) for EpCAM shedding in the urine [baseline, first void after drug administration in Week 1, first void the next morning after treatment, and the day 8 void prior to start of week 2 treatment, Week 6 before Vicineum, Week 10 before Vicineum, Week 12 before Vicineum - – see table in section **5.2** for PD timepoints
- Evaluate EpCAM in the urine for the ability to monitor or predict response to therapy - see table in section **5.2** for PK timepoints

5.1.2.3 Sample Processing

For urine collection, it will be processed according to standard protocol (refer to **Appendix E**) and stored in the Urologic Oncology Branch in Dr. Linehan's lab. Samples will be batched at the end of the trial and then analyzed.

5.1.3 Tissue studies

Samples will be obtained pre-and post treatment as indicated in the table in section **5.2** to:

- Perform immunohistochemistry (IHC) or cytogenetic analysis of baseline tissue (FFPE or frozen tissue specimen) for EpCAM, PD-1/PD-L1, TILS, CD4, CD8, Tregs, MDSCs, Macrophages, and monocytes. In addition, mesenchymal markers such as vimentin, TWIST, Snail, Slug, and Sip1 may also be assessed by immunohistochemistry of baseline tissue. This can be compared to similar IHC of tissue obtained from the last biopsy on trial. This analysis will be performed by the NCI Laboratory of Pathology upon accruing at least 4 patients or by PI's discretion. Baseline FFPE slides will be obtained from paraffin-embedded blocks stored within NCI's Laboratory of Pathology at a future date when analysis will be performed. In the event that patients have had baseline biopsy at an outside institution, every effort will be made to obtain the paraffin-embedded blocks in order to generate baseline FFPE slides for further analysis.

  In particular to the current study, we would like to study the T-cell infiltration pattern within bladder tumors pre- and post-treatment. Specifically, we will look at:

  1. The presence of CD4 and CD8 T cells in bladder tumor specimens pre- and post-treatment
  2. The presence of regulatory T cells (Tregs) by double staining with FoxP3 and CD4 in bladder tumor specimens pre- and post-treatment
  3. The presence of myeloid derived suppressor cells (MDSC) in bladder tumor specimens pre- and post-treatment
  4. The presence of myeloid derived suppressor cells (MDSC) in bladder tumor specimens pre- and post-treatment
  5. The presence of PD-L1 staining pre- and post-treatment as specified below in section **5.1.3.2**.
  6. The presence of EpCAM and mesenchymal markers.

- If sufficient tissue is available, cell lines may be generated. Cell lines may be characterized by karyotyping, interphase fluorescence in situ hybridization (FISH) mapping, immunohistochemistry, and/or flow cytometry. Differences between cell lines will be manipulated to study the various molecular pathways active in nutrient sensing, cell cycle, cell signaling, proliferation, apoptosis, angiogenesis, and metastatic potential in urothelial cancer.

- If adequate tissue and funding available, then may perform mutational load analysis through a targeted panel (RNAseq or DNA mutation panel) of tumor tissue. This analysis will be performed by the NCI CORE facility, NCI collaborator (Paul Meltzer), or outside entity (e.g. Foundation Medicine)

5.1.3.1 Tissue Collection Procedures

If tissue is clinically available, the Urologic Oncology Branch procedures for tissue collection will be followed. This is usually done with standard of care procedures such as such as transurethral resection of bladder tumor. CT guided biopsy is not a procedure by which to procure non-muscle invasive bladder tumor and so it will not be performed on this protocol. A procurement form will be completed. The tissue procurement nurse or a UOB-designated procurement staff member will work with an NCI pathologist following resection of the specimen to obtain tumor tissue from the resected specimen. this will be done using standard of care procedures This will then be delivered by the procurement nurse or UOB Procurement staff member to the Urologic Oncology Branch Research Laboratory for individual processing. Specimens will be placed in cryovials and also be snap frozen in OCT for frozen tissue sections. Tissue sent to pathology will be processed in standard fashion but when formalin-fixed paraffin embedded slides made, they will be modified as below in section **5.1.3.2** to allow for PD-L1 testing in a standardized fashion.

Pathologic material, such as fixed tissue blocks from surgery performed at the NIH Clinical Center, outside surgeries or autopsy materials, may be obtained at the request of, and with written permission from the subject, or appropriate relatives. These samples may undergo pathologic analysis by individuals in the NCI Laboratory of Pathology to confirm diagnosis and may undergo histo-immunologic or other analysis.

5.1.3.2 Tissue PD-L1 Testing

To ensure comparability of data across all studies of durvalumab and to gain real world experience on the performance of this assay, it is strongly encouraged that all studies that include PD-L1 testing utilize the Ventana SP263 assay. Testing should be restricted to the Ventana SP263 assay and should be performed in accordance with the package insert on the Ventana Benchmark platform (Ultra or XT).

The Ventana SP263 assay is fully analytically validated test characterized through to the completion of reader precision studies in the non-small cell lung cancer (NSCLC) and squamous cell carcinoma of the head & neck (SCCHN). For these tumors, the Ventana SP263 assay has a fully reproducibility data package supporting cut-off and scoring algorithm. Following completion of ATLANTIC and HAWK clinical trials, the assay will be associated with clinical utility. In other cancer types (bladder, pancreatic, gastric, hepatocellular, triple negative breast, ovarian, esophageal, nasopharyngeal, glioblastoma, soft tissue sarcoma, cholangiocarcinoma, small cell lung, melanoma and cervical HPV+ cancers), the Ventana SP263 assay has only limited clinical performance data.

5.1.3.2.1 Sample collection for PD-L1 testing

- The preferred tumor sample for the determination of a patient's PD-L1 status is the one taken following the completion of the most recent prior line of therapy. Samples taken at this time reflect the current PD-L1 status of the tumor and considered clinically most relevant.
- In AstraZeneca studies, the preferred sample for PD-L1 testing was less than or equal to 3 months old. In cases where a sample a less than 3 months old was not available, patients

were asked to undergo a new biopsy if considered clinically appropriate by their treating physician.

- Samples should be collected via a core needle of 18 gauge or larger or be collected by an incisional or excisional tumor biopsy. Where institutional practice uses a smaller gauge needle, samples should be evaluated for tumor cell quantity (i.e. >100 tumor cells) to allow for adequate PD-L1 immunohistochemistry analyses.
- When the collection of a new sample is not clinically appropriate, archival samples may be utilized provided the specimen it is not older than 3 years of age. When archival samples are used to assess PD-L1 status, the age of the sample / date of collection should be captured.
- Samples submitted for PD-L1 testing should be formalin fixed and embedded in paraffin. Samples from fine needle aspirates (FNA) or decalcified bone are not appropriate for PD-L1 analysis.

5.1.3.2.2 Sample data collection for PD-L1 testing

The following fields of data should be collected from the site/institution collecting and if, indicated shipping of the samples:

- Patient identifier (unique identifier)
- Specimen identifier (written on the specimen)
- Site identifier
- Specimen collection date
- Type of specimen submitted
- Quantity of specimen
- Date of sectioning
- Archival of fresh tumor
- Tumor type
- Primary tumor location
- Metastatic tumor location (if applicable)
- Fixative

The following fields of data should be collected from PD-L1 testing laboratory:

- Are the negative and positive controls stained correctly
- Is the H&E material acceptable
- Is morphology acceptable
- Total percent positivity of PD-L1 in tumor cells
- PD-L1 status (positive, negative or NA) in tumor cells
- Total percent positivity of PD-L1 in infiltrating immune cells

The Ventana SP263 assay to measure PD-L1 in tumors is experimental. As with all tests, there is a chance of false positive (the test shows high PD-L1 when it is not there) or false negative (the test does not show PD-L1 when it is there) results may occur.

5.1.3.2.3 Sample processing for PD-L1 testing

Preparing Stored samples for testing

- Where samples already exist, they should be retrieved from the Bio-Bank storage location. These blocks should undergo quality review, prior to evaluation or shipment. Where it is not possible or indicated to ship the block to a testing laboratory, unstained slides should be prepared from the paraffin-embedded tumor sample block (described below) prior to evaluation or shipment.

Preparing newly acquired samples for PD-L1 testing

- If patients are undergoing a biopsy procedure that provides the option to submit newly acquired samples, this sample should be used to determine PD-L1 status. Where clinically acceptable, a minimum of 2 core biopsies should be collected and processed to FFPE in a single block. The provision of 2 cores is advised in order to provide sufficient tissue for PD-L1 assessment.
- It is recommended that core needle tumor biopsies are collected using an 18 gauge or larger needle and the process should be image-guided. Excisional or incisional samples are also adequate. If this is not per the institutions normal practice and a smaller gauge needle is used then the number of cores collected should be increased to allow sufficient material for successful PD-L1 testing (>100 tumor cells) and embedded in the same block. If available, a single excisional biopsy of at least 4 mm in diameter may substitute for all core biopsies.

Fixation of biopsy samples for PD-L1 testing

- Previously frozen tissue is not acceptable for processing to FFPE for PD-L1 testing. To fix newly acquired tissue, place immediately (within 30 min of excision) into an adequate volume of 10% v/v neutral buffered formalin (NBF). Samples should remain in fixative for 24 – 48 hours at room temperature.
- It is vital that there is an adequate volume of fixative relevant to the tissue (at least a 10 volume excess) and that large specimens (if any) are incised prior to fixation to promote efficient tissue preservation.

Embedding in paraffin for PD-L1 testing

- An overnight processing schedule into paraffin wax is recommended
- Below is the suggested routine overnight processing schedule

Storage of tumor blocks for PD-L1 testing

- FFPE blocks should be stored at ambient temperature and protected from light until shipment by courier at ambient temperature. FFPE blocks are stable under these conditions for an indefinite period.

Quality control of samples to be used for PD-L1 testing

- Tissue should be assessed by the site pathologist prior to PD-L1 testing.
- Each sample should be reviewed for:
    - Adequate fixation
    - Good preservation of morphology
    - Presence of tumor tissue

- Histopathology consistent with indication
- Greater than 100 tumor cells are required to determine PD-L1 status – tumor cell content must be reviewed prior to testing in order for PD-L1 obtain a valid result.

If indicated, shipping samples to a PD-L1 testing laboratory

- When submitting sample to for PD-L1 testing the recommendation is to ship the block in order for sectioning to occur at the laboratory. Blocks should be shipped - containing enough material to be provided to allow a minimum of 5, and preferably 10, sections to be cut (each 4 micron thick) to be used for PD-L1 testing.

Sectioning instructions

- Where it is not possible or indicated to ship the block to laboratory for PD-L1 testing, unstained slides should be prepared from the paraffin-embedded tumor sample block as described below:
  - A minimum of 5-10 x 4 micron (μm) thick, unstained sections should be provided for PD-L1 testing
  - A new disposable microtome blade must be used for each block to prevent contamination between Slides are stable under these conditions for 6 months.
  - patient samples
  - Apply one section per slide to positively-charged Superfrost glass slides
  - The sections should be dried overnight between room temperature and 37°C. Do not dry sections at temperatures above 37°C.

Sections should be stored at ambient temperature and protected from light until use or shipment to testing lab by courier at ambient temperature. It is recommended that slides are cut freshly prior to PD-L1 testing and they are used within 90 days of being cut to obtain PD-L1 status

Any additional FFPE tissue obtained that is not used for PD-L1 testing can be used for the following additional correlative assays:

1. Tissue Immune Correlates - (FFPE or frozen tissue specimens) - pre-treated and post-treated samples can be analyzed by IHC (FFPE) for Epcam, TILs, CD4, CD8, Tregs, MDSCs, Macrophages, and monocytes. In addition, mesenchymal markers such as vimentin, TWIST, Snail, Slug, and Sip1 may also be assayed by IHC.
2. Frozen tissue specimens or a single FFPE slide can be analyzed later for tumor mutational load through targeted sequencing panel.

Frozen tissue specimens or a single FFPE slide for gene expression using HT Seq immune-oncology panel of 500+ genes and/or microRNA analysis.

5.2 SAMPLE COLLECTION SCHEDULE

Test/assay	Volume blood (approx)	Type of tube [a]	Collection point (+/- 7 days)	Location of specimen analysis
Urine collection for Urinary Cytokines (Section **5.1.2**)	50-100 mLs	Urine specimen cup	Baseline, 3 months, 6 months	Stored and processed in Linehan Lab (**Appendix E**) using Luminex Assay
Serum collection for cytokines (section **5.1.1**)	20 mLs	2 Red Top (SST) Tubes	Baseline, 2 weeks, 3 weeks, 5 weeks	Stored in Dr. Figg's Lab and processed by Dr. Liang Cao's lab
Plasma collection for soluble PD-L1 (secion 5.1.1)	6 mLs	EDTA	Baseline, 2 weeks, 3 weeks, 5 weeks	Stored in Dr. Figg's Lab and processed by Dr. Liang Cao's lab
Urinary PK for Vicineum (Section **5.1.2.2**)	50-100 mLs	Urine specimen cup	Baseline, First void after Week 1 Vicineum, First void morning after Week 1 Vicineum, Day 8 void prior to Week 2 Vicineum	Stored in Linehan Lab (**Appendix E**) and processed by Dr. Liang Cao's Lab
Urinary EpCAM (Section **5.1.2.2**)	50-100 mLs	Urine specimen cup	Baseline, First void after Week 1 Vicineum, First void morning after Week 1 Vicineum, Day 8 void prior to Week 2 Vicineum, Week 6 before Vicineum, Week 10 before Vicineum,	Stored in Linehan Lab (**Appendix E**) and processed by Dr. Liang Cao's Lab

Test/assay	Volume blood (approx)	Type of tube [a]	Collection point (+/- 7 days)	Location of specimen analysis
			Week 12 before Vicineum	
Gene Expression (see section **5.4.1**)	Single FFPE slide or frozen tissue	N/A	Baseline	Stored in Linehan Lab and processed by Dr. Jane Trepel's lab or NCI CORE
Tissue IHC (see section **5.1.2**)	Single FFPE slide	N/A	Baseline, Last Biopsy on Trial	Will be obtained from tissue blocks in Laboratory of Pathology and analyzed by Dr. Merino (Laboratory of Pathology)
RNAseq and/or DNA mutational panel for mutational load (see section **5.1.2**)	Single FFPE slide or frozen tissue	N/A	Baseline	NCI CORE facility or outside entity (Foundation Medicine)
PBMCs for immune subsets, antigen specific responses (section **5.1.1.2**)	60 mL	6 green top Tubes	Baseline, 3 months, 6 months	Schlom Laboratory (Deliver to Frederick Lab)
Immunogenic Assessment (ADA, AVA) (section **5.1.1.2**)	8 mL	2 lithium heparin (4 mL each) tubes	Baseline, Week 8 (ADA), Week 12 (AVA)	Linehan Laboratory in collaboration with AZ and Sesen Biotherapeutics

a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside the NIH without appropriate approvals and/or agreements, if required.

5.3.1 UOB Research Laboratory

All specimens obtained for research purposes will be coded upon arrival in the UOB research laboratory to maintain patient confidentiality. All samples will be accessioned and entered into a database (such as LabMatrix) with access limited to the PI and UOB research data managers. The location of all samples will be carefully tracked in the database. All stored samples will be coded and identifying patient information will not be placed on sample containers. Stored samples will be kept in freezers/refrigerators or secure containers located in the Urologic Oncology Branch research laboratories or in approved storage facilities. The research samples collected will be used for the correlative/biomarker studies mentioned above in Section **5.1**. Tissue slides/blocks may be obtained in order to determine protocol eligibility. Tissues are submitted to the NCI Laboratory of Pathology at the NIH Clinical Center for clinical determination of histologic features. Specialized stains/assays may be used to clarify tissue phenotype. After completion of analysis, tissue slides/blocks are either returned to the originating pathology department or archived in the Laboratory of Pathology or Urologic Oncology Branch and tracked in the UOB database (LabMatrix). Archived material will be retained for the duration of the study unless the originating pathology department requests return.

Coded/anonymous samples with minimal clinical information may be shared with qualified NIH and non-NIH researchers who have IRB-approved protocols with similar research objectives. No personal identifying information such as name, address, or date of birth will be provided to the non-UOB investigator. In the event a subject is eligible to participate in a clinical trial based on tumor characteristics, coded tissue samples may be sent to an outside laboratory.

5.3.2 Samples (serum samples for cytokines) Stored with Clinical Pharmacology Program (Figg Laboratory)

5.3.2.1 Procedures for sample data collection for the Clinical Pharmacology Program:

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

All samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in the Labmatrix utilized by the CPP. This is a secure program, with access to Labmatrix limited to defined CPP personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the Clinical Center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.3.2.2 Procedures for sample storage at the Clinical Pharmacology Program:

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20°C or - 80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2.1**.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.3.3 Clinical Services Program

All data associated with the patient samples is protected by using a secure database. All samples drawn at the NIH Clinical Center will be transported to the NCI Frederick Central Repository by couriers.

Samples will be tracked and managed by the Central Repository database. All samples will be stored in liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

NCI Frederick Central Repositories (managed under a subcontract) store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

The subcontractor's role is limited to clinical research databases and repositories containing patient specimens. The subcontractor neither conducts nor has any vested interest in research on human subjects, but does provide services and supports the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of the subcontractor to accept only codedsamples and sample information. If the coded data are linked, the code key will not be accepted.To the best of our

ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory (BSI) System II. This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.3.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open as long as sample or data analysis continues. Samples will be stored until they are no longer of scientific value or until a subject withdraws consent for their continued use, at which time they will be destroyed. If the patient withdraws consent, the participants data will be excluded from future distributions but data that have already been distributed for approved research use will not be able to be retrieved.

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples, provided they have an IRB-approved protocol and patient consent or an exemption from OHSRP.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Proposed Genetic Analysis

Protein, DNA mutational analysis, and miRNA/mRNA for immune gene expression profile analysis (quantitative polymerase chain reaction [Q-PCR]) of known and putative targets obtained from tumor tissue at baseline if adequate tumor tissue is available for such an analysis. The analysis will be performed through one of the following platforms:

- Targeted DNA mutational panel from frozen tissue or FFPE via NCI Core facility, collaborator (Paul Meltzer), or outside entity (e.g. Foundation Medicine) if adequate funding and tissue available
- HT seq from single FFPE slide for immune-oncology panel of 500+ genes or microRNA analysis – NCI CORE facility or Jane Trepel
- Nanostring panel for immune oncology related genes obtained from tumor tissue

5.4.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Using a computerized inventory system (LabMatrix), each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected but personal identifiers will be removed.

For each specimen, the corresponding subject demographics and clinical information will be assigned in the database as described in section **6.1** and only be accessible by the PI and research nurses within the UOB through a password-protected database.

Samples provided to other researchers will be bar coded and these researchers will not have access to any information in LabMatrix. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

Data from genomic analysis will be deposited in dbGAP.

5.4.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects will be contacted at this time with a request to provide a sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis**.**

Note: incidental findings considered clinically relevant will be discussed among Senior Scientific Staff at meetings such as the weekly UOB conference prior to being communicated to subjects.

5.4.4 Genetic counseling

Genetic counseling will be recommended should any clinically relevant incidental findings be discovered and consultation with the NCI Genetics Branch will be offered.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Document AEs from the first study intervention, Week 1/Day 1, through 90 days after the last administration of the study intervention. After 90 days, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository. Insert name or names: clinicaltrials.gov, dbGaP.
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared*?*

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.2.3 Response Criteria

Patients will be evaluated every 3 months as per standard of care with cystoscopy. Biopsy will be done at PI discretion pending cystoscopic findings. Any biopsies done will be for clinical reasons to rule out tumor recurrence, however, extra biopsy material if available will be used for research purposes.

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with vicineum or durvalumab. All observed toxicities will be tabulated using CTCAE v5.0.

6.2.4 Evaluable for Objective Secondary Endpoints/Response

Only those patients who have biopsy-proven disease present at baseline, have received at least one intravesical dose of Vicineum therapy and one intravenous dose of durvalumab, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

6.2.4.1 Recurrence-free survival

The recurrence-free survival duration will be measured from the start of Vicineum therapy until disease recurrence, progression, or death due to any cause. Recurrence is suspected and/or determined by urine cytology and/or cystoscopic exam and then confirmed pathologically after a TURBT. Positive cytology in the absence of pathologic confirmation is not considered to be a recurrence. Low grade papillary tumor will not be considered recurrent disease.

6.2.4.2 Complete Response Rate for CIS

In patients with any component of CIS upon study entry, the complete response (CR) rate will be determined at 3 months, 6 months, and 12 months from the start of Vicineum therapy. Complete response will be defined as the absence of CIS upon follow-up biospies. These rates can be compared to historical rates of CR for CIS at these various time points.

6.2.4.3 Disease Progression & Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first. The duration of progression-free survival is measured from the start of vicineum therapy until progression or death due to any cause. Disease progression is defined as upstaging from a lower stage to a higher stage (e.g., Ta to T1-T4 or T1 to T2-4; CIS to T1 or CIS to T2-T4; or any N+ or M+ in these high grade tumors).

6.2.4.4 Time to Tumor Recurrence

The duration of time measured from the start of Vicineum therapy until recurrence is noted.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32).

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.6 Adverse Events of Special Interest (AESI)

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring and rapid communication by the investigator to the sponsor. An AESI may be serious or non-serious. The rapid reporting of AESIs allows ongoing surveillance of these events in order to characterize and understand them in association with the use of this investigational product.

AESIs for durvalumab include but are not limited to events with a potential inflammatory or immune-mediated mechanism and which may require more frequent monitoring and/or interventions such as steroids, immunosuppressants and/or hormone replacement therapy. These AESIs are being closely monitored in clinical studies with durvalumab monotherapy and combination therapy. An immune-mediated adverse event (imAE) is defined as an AESI that is

associated with drug exposure and is consistent with an immune-mediated mechanism of action and where there is no clear alternate etiology. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an imAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the imAE.

In the event of imAE or suspected imAE, the AstraZeneca study team may request relevant clinical information (including images) for those subjects who demonstrate the event, and may request the independent review by external experts based on the acquired clinical information.

AESIs observed with durvalumab include:

- Diarrhea / Colitis and intestinal perforation
- Pneumonitis / ILD
- hepatitis / transaminase increases
- Endocrinopathies (i.e. events of hypophysitis/hypopituitarism, adrenal insufficiency, hyper- and hypothyroidism and type I diabetes mellitus)
- Rash / Dermatitis
- Nephritis / Blood creatinine increases
- Pancreatitis / serum lipase and amylase increases
- Myositis / Polymyositis
- Neuropathy / neuromuscular toxicity (e.g. Guillain-Barré, and myasthenia gravis)
- Intestinal Perforations

Other inflammatory responses that are rare/less frequent with a potential immune-mediated etiology include, but are not limited to:

- Myocarditis
- Pericarditis
- Sarcoidosis
- Uveitis
- Other events involving the eye and skin
- Hematological events
- Rheumatological events
- Vasculitis
- Non-infectious meningitis
- Non-infectious encephalitis
- Pemphigoid

It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs. In addition, infusion-related reactions and hypersensitivity/anaphylactic reactions with a different underlying pharmacological aetiology are also considered AESIs.

Further information on these risks (e.g., presenting symptoms) can be found in the current version of the durvalumab Investigator's Brochure. More specific guidelines for their evaluation and treatment are described in detail in the Dosing Modification and Toxicity Management Guidelines (see **Appendix C**). These guidelines have been prepared by the Sponsor to assist the Investigator in the exercise of his/her clinical judgment in treating these types of toxicities. These guidelines apply to AEs considered causally related to the study drug/study regimen by the reporting investigator.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets a protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

8.4.1 Safety Reporting Criteria to AstraZeneca

8.4.1.1 Safety Reports sent by OSRO

All SAEs will be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). The reporting period for SAEs is the period immediately following the time that written informed consent is obtained through 90 days after the last dose of durvalumab or until the initiation of alternative anticancer therapy. The investigator and/or Sponsor are responsible for informing the Ethics Committee and/or the Regulatory Authority of the SAE as per local requirements.

The investigator and/or sponsor must inform the FDA, via a MedWatch/AdEERs form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to AstraZeneca. A copy of the MedWatch/AdEERs report must be emailed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the sponsor to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

* A cover page should accompany the MedWatch/AdEERs form indicating the following:

- "Notification from an Investigator Sponsored Study"
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name/title and AstraZeneca ISS reference number (ESR-##-#####)

* Sponsor must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

* Send SAE report and accompanying cover page by way of email to AstraZeneca's designated mailbox: AEMailboxClinicalTrialTCS@astrazeneca.com

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

8.4.1.2 Safety Reports sent by the Study Team

Serious adverse events that do not require expedited reporting to the FDA still need to be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events. This information should be reported on a monthly basis and under no circumstance less frequently than quarterly.

In the case of blinded trials, AstraZeneca will request that the Sponsor either provide a copy of the randomization code/ code break information or unblind those SAEs which require expedited reporting.

On a monthly basis, non-expedited SAEs will be provided to Collaborator Patient Safety Department via fax at: (301) 398-4205 and email to: drugsafety@medimmune.com and AEMailboxClinicalTrialTCS@astrazeneca.com.

8.4.2 Safety Reporting Criteria to Sesen Biotherapeutics

As soon as the investigator becomes aware that an adverse event meets the criteria for being a serious adverse event (SAE) the following is required:

- The investigator will notify Sesen Biotherapeutics within twenty-four (24) hours of becoming aware of the SAE by submitting a completed SAE form and any supporting documentation to Sesen Biotherapeutics.
- The SAE form and supporting documentation should be scanned and emailed to Sesen Biotherapeutics at with joycelyn.entwistle@sesenbio.com and rachelle.dillon@sesenbio.com.
- Follow-up information must be submitted in a timely fashion

All suspected unexpected serious adverse reactions (SUSARs), also called serious and unexpected suspected adverse reaction (i.e., serious, unexpected and related SAEs), must be reported to all appropriate regulatory authorities and IRBs/Ethics Committees as required by 21 CFR 312.32 utilizing the MedWatch 3500A form or the CIOMS-1. The final forms for the initial and all follow-up reports must be submitted within two (2) business days of submission to the FDA or other health authorities. These reports should be scanned and emailed to Sesen Biotherapeutics at joycelyn.entwistle@sesenbio.com and rachelle.dillon@sesenbio.com.

8.5 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.5.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.5.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last dose of Durvalumab and Vicineum.

Pregnancy of the patient's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 120 days after the last dose should, if possible, be

followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.7 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING PLAN

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

Primary endpoints:

The primary objective of this trial is to determine whether the use of a combination of Vicineum and durvalumab in patients with high grade NMIBC previously treated with BCG is safe and tolerable as determined by a 3+3 dose de-escalation trial design. The primary endpoint will be the fraction of patients who have adverse events.

Secondary endpoints:

A secondary endpoint will be to determine if the combination of the two agents can be associated with an increase in the disease-free survival (DFS) probability in patients with high grade NMIBC previously treated with BCG. If accrual is rapid, an amendment may be made to accrue additional patients to evaluate DFS as described in section **10.2**.

A secondary objective is whether the combination is associated with changes in a set of immune parameters obtained from blood and biopsies.

A secondary objective is whether the pharmacokinetics of Vicineum can be determined from urine samples obtained within the first 7 days of Vicineum treatment.

A secondary objective is whether urinary EpCAM levels correlate with response to therapy.

A secondary objective is to assess PD-L1 expression and PD-1 expressing T cells in paired bladder biopsies pre- and post-treatment with durvalumab and Vicineum as a marker of response/benefit.

10.2 SAMPLE SIZE DETERMINATION

The trial will begin with a 3-patient run-in at the proposed doses of Vicineum and durvalumab (1500mg; 30 mg) and if 0/3 or 1/3 patients have a DLT, then the next 3 patients will also be treated at the full doses of the agents (1500mg durvalumab; 30 mg Vicineum). If 0/6 or 1/6 has a DLT, then the remaining 18 patients will be treated at the full doses if the cumulative fraction of patients experiencing a DLT is <1/3.

If 2 or more patients among 3 to 6 have a DLT at 1500mg durvalumab and 30mg Vicineum, then 3 patients will be enrolled at the lower dose level (1500mg durvalumab and 20mg Vicineum), and if 0/3 or 1/3 patients have a DLT, then the next 3 patients will also be treated at the reduced doses of the agents. If 1/6 has a DLT, then the remaining patients will be treated at the lower dose level of the agents (1500mg durvalumab and 20mg Vicineum) if the cumulative fraction of patients experiencing a DLT is <1/3.

As the secondary endpoint, all the evaluable patients will have determinations of many immune parameters at baseline, 3 months, and 6 months. The changes in the parameters obtained from blood samples will be determined at baseline vs. 3 months, and baseline vs. 6 months. The changes in the parameters obtained from biopsies will be obtained from baseline vs. a single second biopsy at 6 months. A very large number of parameters may be evaluated, and unless more parameters than anticipated are to be evaluated, we will assume that 24 patients will be adequate to evaluate changes in these parameters based on the following. As an illustration, we will assume that a total of 25 paired tests will be considered of equal, primary importance. Paired results from 24 evaluable patients, including those used in the initial run-in portion who are treated at the highest safe dose, will provide 90% power to detect a change from baseline of 1.0 SD of the change (effect size=1.0) using a two-sided 0.002 significance level paired t-test, to allow an overall 0.05 significance level test, applying a very conservative Bonferroni adjustment to the set of approximately 25 paired comparisons. In practice, comparisons of the paired values will likely be performed using a Wilcoxon signed rank test instead of a paired t-test, and a Hochberg adjustment may be used instead of an overly stringent Bonferroni adjustment.

If accrual is sufficiently rapid, that is, if the 24 evaluable patients can be accrued within 2.5 years, then these 24 patients will be included in an expansion cohort, opened by an amendment to the study, designed to evaluate DFS, as follows that would allow for the enrollment of an additional 23 patients:

It has been estimated from published trials that the 6 month DFS in patients with high grade NMIBC previously treated with BCG is approximately 50%, and that it is 35% at 12 months, and 25% at 18 months. In this expansion cohort, the goal is to determine if use of a combination of Vicineum and durvalumab can be potentially associated with a 50% 1-year DFS probability. With 47 evaluable patients receiving the proposed therapy, including those in the initial 6 patients at the intended dose level as well as those in the evaluation of biologic endpoints, assuming accrual would take place over approximately 4 years, and that there would be at least 2 years of additional potential follow-up after the last patient has begun the therapy, there would be 80% power to determine whether there is a difference between a 35% 1 year DFS and an

improved 50% 1-year DFS, with a one sided 0.10 alpha level test, using the method of Brookmeyer and Crowley.[33] In practice, Kaplan-Meier curves and appropriate confidence intervals at selected time points will be provided to help interpret results relative to the expected results.

It is anticipated that one patient per month may enroll onto this trial; thus, allowing for 6 to 12 separate patients as a run-in if needed, and 24 evaluable patients at the intended dose level, approximately 24-30 months would be the approximate accrual duration for the secondary endpoint based on evaluating biologic correlates. To allow for a small number of invaluable patients, the accrual ceiling will be set at 40. If the expansion to evaluate DFS will be undertaken, this will be modified by an amendment.

10.3 POPULATIONS FOR ANALYSIS

Modified intention to treat: all patients who receive at least one dose of the two drugs will be included in the statistical analyses performed.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

For the toxicity evaluation, the fraction of patients with toxicity will be determined and reported at each of the dose levels explored. The immune parameters will be obtained at baseline, 3 months, and 6 months and for each parameter, the change between baseline and the later time points will be calculated and evaluated for the statistical significance of the change. If the disease-free survival (DFS) is evaluated, a Kaplan-Meier curve for DFS will be constructed.

10.4.2 Analysis of the Primary Endpoint

All patients will be evaluated for the grades of toxicity identified, and the number of patients will DLTs at each dose level will be reported. The MTD will be identified based on being the dose level at which 0 or 1 patient in 6 has a DLT. All toxicities will be tabulated for all 24 or more patients to be enrolled onto the trial.

10.4.3 Analysis of the Secondary Endpoints

Efficacy: A DFS curve will be created using the Kaplan-Meier method based on all patients considered to be evaluable based on having received protocol treatment, provided that adequate patients are enrolled in order to address this following an amendment to increase accrual.

The response to treatment will be determined for patients who receive treatment; the response rate for those patients who are evaluable for response, along with a 95% two-sided confidence interval will be reported.

As another secondary endpoint, all the evaluable patients will have determinations of many immune parameters at baseline, 3 months, and 6 months. The changes in the parameters obtained from blood samples will be determined at baseline vs. 3 months, and baseline vs. 6 months. The changes in the parameters obtained from biopsies will be obtained from baseline vs. a single second biopsy at 6 months. As an illustration, we will assume that a total of 25 paired tests will be considered of equal, primary importance. Paired results from 24 evaluable patients, including those used in the initial run-in portion who are treated at the highest safe dose, will be formed and tested for a difference from zero using a two-sided 0.002 significance level paired t-test, to

allow an overall 0.05 significance level test, applying a very conservative Bonferroni adjustment to the set of approximately 25 paired comparisons. In practice, comparisons of the paired values will likely be performed using a Wilcoxon signed rank test instead of a paired t-test, and a Hochberg adjustment may be used instead of an overly stringent Bonferroni adjustment.

Another secondary objective is to evaluate the pharmacokinetic parameters of Vicineum obtained by urine samples. This will be a descriptive analysis and will report sample statistics such as mean, standard error, median, minimum, maximum and other descriptive measures only.

Urinary EpCAM will be measured and will be compared between patients who have a clinical response to therapy vs. those who do not respond. The results will be presented for both response categories using descriptive statistics such as mean, standard error, median, minimum, maximum and other descriptive measures. Although it is expected to have low power, a comparison of the EpCAM levels may be compared between the two response categories using a Wilcoxon rank sum test, with the resulting p-value intended to help describe the differences noted.

PD-L1 and PD-1 levels will be obtained at baseline and after treatment with both agents. The change in levels will be determined between the two measurements, and these changes will be compared between responders and non-responders, as well as between those who respond or have SD (clinical benefit=CR+PR+SD) and those with PD. Although it is expected to have low power, in each case the comparisons between the two response categories will be made using a Wilcoxon rank sum test, with the resulting p-value intended to help describe the differences noted.

10.4.4 Safety Analyses

All patients will be evaluated for the grades of toxicity identified, and the number of patients will DLTs at each dose level will be reported. The MTD will be identified based on being the dose level at which 0 or 1 patient in 6 has a DLT. All toxicities will be tabulated.

10.4.5 Baseline Descriptive Statistics

Demographic and clinical characteristics of all patients will be reported.

10.4.6 Planned Interim Analyses

None will be performed.

10.4.7 Subgroup Analyses

None will be performed.

10.4.8 Tabulation of Individual Participant Data

None will be provided.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

This Protocol will be carried out under a collaboration between NCI, Sesen Biotherapeutics Inc. and AstraZeneca AB. Sesen Biotherapeutics will provide Vicineum (VB4-845) under NCI CRADA #3149, and AstraZeneca will provide Durvalumab (MEDI 4736) under NCI CRADA #3155.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial/ethnic groups and both genders are eligible for this study if they meet the eligibility criteria.

12.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible for participation in this study based on the fact that patients under 18 are unlikely to have this disease and there are unknown toxicities in pediatric patients.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **12.4**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subject and to identify an LAR.

Please see section **12.5.1** for the consent procedure.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients may benefit from the study as treatment may delay or avoid having the bladder removed.

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. Patients will be examined and evaluated prior to enrollment. All evaluations to monitor the treatment of patients will be recorded in the patient chart. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland.

Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. In all publications and presentations resulting from this trial, patients' anonymity will be protected to the maximum extent possible. Authorized personnel from the National Cancer Institute (NCI) and Food and Drug Administration (FDA) or other regulatory authorities may have access to research files in order to verify that patients' rights have been safeguarded. In addition, patient names will be given to the Central Registration to register and verify patients' eligibility.

12.4.1 Study Agents Risks

Potential adverse reactions attributable to the administration of the study agents utilized in this trial are discussed in sections **14.1.2** and **14.2.2**. Additional information is located in the investigator brochures.

12.4.2 Research Blood Sampling Risks

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.4.3 Risks from Bladder Procedures

Risks associated with cystoscopy, TURBT, biopsies and catheterization include: burning when passing urine, blood-tinged urine, bladder infection or the need to urinate frequently following the procedures. The insertion of a catheter into the bladder through the urethra may cause discomfort. There is also a small risk that these procedures, particularly cystoscopy, TURBT and biopsies, could damage the lining of the bladder or cause a perforation of the bladder. This could lead to urine or drugs leaking out of the bladder causing severe side effects.

12.4.4 Non-Physical Risks of Genetic Research

- Risk of receiving unwanted information: Anxiety and stress may arise as a result of the anticipation of unwanted information regarding disease. Subjects will be clearly informed that the data related to genetic analysis is coded, investigational and will not be shared with family members or health care providers. However, as specified above in Section **5.4.3**, subjects who have indicated a desire to be informed of these data will be given an opportunity.
- Risk related to possibility that information may be released: This includes the risk that data related to genotype or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies.
- Risk to family or relatives: Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems.

12.4.5 Radiation Risks

The risk of radiation exposure is expected from up to 1 CT scan a year. The total estimated effective dose for one year is approximately 1.4 rem.

12.4.6 CT Contrast Risks

Itching, hives or headaches are possible risks associated with contrast agents that may be used during CT imaging. Symptoms of a more serious allergic reaction include shortness of breath and swelling of the throat or other parts of the body. Very rarely, the contrast agents used in CT can cause kidney problems for certain patients, such as those with impaired kidney function.

12.4.7 Electrocardiogram Risks

Other than possibly experiencing some minor skin irritation from the electrodes, there are no anticipated risks related to complete the electrocardiogram and/or the echocardiogram.

12.5 Consent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) (e.g. legally authorized representative [LAR] if participant is an adult unable to consent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to

review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or on the electronic document. Signatures on electronic documents are described below. Note: FDA only regulates electronic signatures (i.e., an electronic timestamp is generated at the time of signature) in FDA regulated research.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

12.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in section **12.5**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory

authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 DURVALUMAB (IND #: 136199)

14.1.1 Source

Durvalumab will be supplied by the Investigational Products supply section of AstraZeneca/MedImmune under NCI CRADA #3155. There are no manufacturing differences between the clinical supply that will be used in this study and the supply of marketed agent.

14.1.2 Toxicity

For additional information, refer to the investigator brochure.

Table 1. Frequency of ADRs for Durvalumab Monotherapy From Pooled Data Across Multiple Tumour Types (N=3006)

SOC	PT/medical concept MedDRA (v24.0)	CIOMS frequency indicator [a]/ overall frequency all CTC grades	All CTCAE Grades n (%)	Grade 3 or 4 n (%)
Respiratory, thoracic, and mediastinal disorders	Cough/productive cough [b]	Very common	646 (21.5)	11 (0.4)
	Pneumonitis [b,c]	Common	114 (3.8)	26 (0.9)
	Dysphonia	Common	93 (3.1)	2 (<0.1)
	ILD	Uncommon	18 (0.6)	4 (0.1)
Hepatobiliary disorders	ALT increased/AST increased [b, c]	Common	244 (8.1)	69 (2.3)
	Hepatitis [b, c]	Uncommon	25 (0.8)	12 (0.4)
Gastrointestinal disorders	Diarrhea	Very common	491 (16.3)	19 (0.6)
	Abdominal pain [b]	Very common	383 (12.7)	53 (1.8)
	Colitis [b]	Uncommon	28 (0.9)	10 (0.3)
	Pancreatitis [b]	Uncommon	6 (0.2)	5 (0.2)
Endocrine disorders	Hypothyroidism [b]	Very common	345 (11.5)	5 (0.2)
	Hyperthyroidism [b]	Common	164 (5.5)	0
	Thyroiditis [b]	Uncommon	23 (0.8)	2 (<0.1)
	Adrenal insufficiency	Uncommon	18 (0.6)	3 (<0.1)
	Type 1 diabetes mellitus	Rare	1 (<0.1)	1 (<0.1)
	Hypophysitis/Hypopituitarism [b]	Rare	2 (<0.1)	2 (<0.1)
	Diabetes insipidus	Rare	1 (<0.1)	1 (<0.1)
Renal and urinary disorders	Blood creatinine increased	Common	105 (3.5)	3 (<0.1)
	Dysuria	Common	39 (1.3)	0
	Nephritis [b]	Uncommon	9 (0.3)	2 (<0.1)
Skin and subcutaneous tissue disorders	Rash [b]	Very common	480 (16.0)	18 (0.6)
	Pruritus	Very common	325 (10.8)	1 (<0.1)
	Night sweats	Common	47 (1.6)	1 (<0.1)
	Dermatitis [b]	Uncommon	22 (0.7)	2 (<0.1)
	Pemphigoid [b]	Rare	3 (<0.1)	0
Cardiac disorders	Myocarditis	Rare	1 (<0.1)	1 (<0.1)
General disorders and administration site conditions	Pyrexia	Very common	414 (13.8)	10 (0.3)
	edema peripheral [b]	Common	291 (9.7)	9 (0.3)

SOC	PT/medical concept MedDRA (v24.0)	CIOMS frequency indicator [a]/ overall frequency all CTC grades	All CTCAE Grades n (%)	Grade 3 or 4 n (%)
Infections and infestations	Upper respiratory tract infections [b]	Very common	407 (13.5)	6 (0.2)
	Pneumonia [b,c]	Common	269 (8.9)	106 (3.5)
	Oral candidiasis	Common	64 (2.1)	0
	Dental and oral soft tissue infections [b]	Common	50 (1.7)	1 (<0.1)
	Influenza	Common	47 (1.6)	2 (<0.1)
Musculoskeletal and connective tissue disorders	Myalgia	Common	178 (5.9)	2 (<0.1)
	Myositis [b]	Uncommon	6 (0.2)	1 (<0.1)
	Polymyositis [d]	Not determined	-	-
Nervous system disorders	Myasthenia gravis [b, e]	Not determined	-	-
	Encephalitis [b, f]	Not determined	-	-
Injury, poisoning, and procedural complications	Infusion related reaction [b]	Common	49 (1.6)	5 (0.2)
Blood and lymphatic system disorders	Immune thrombocytopenia [c]	Rare	2 (<0.1)	1 (<0.1)

[a] The corresponding frequency category for each ADR is based on the CIOMS III convention and is defined as: (1) very common (≥1/10); (2) common (≥1/100 to <1/10); (3) uncommon (≥1/1000 to <1/100); (4) rare (≥1/10000 to <1/1000); (5) very rare (<1/10000); not determined (cannot be estimated from available data).

[b] Denotes a medical concept. See Table 31 of investigator brochure for individual PTs.

[c] Fatal events have been reported.

[d] Polymyositis (fatal) was observed in a patient treated with durvalumab from an ongoing AstraZeneca-sponsored clinical study outside of the pooled dataset.

[e] Reported frequency from AstraZeneca-sponsored clinical studies outside of the pooled dataset is rare, with no events at Grade >2.

[f] Reported frequency from ongoing AstraZeneca-sponsored clinical studies outside of the pooled dataset is rare and includes 2 events of encephalitis, onefatal (PT: Immune-mediated encephalitis) and one Grade 2 (PT: Autoimmune encephalitis).

ADR = adverse drug reaction; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CIOMS = Council for International Organisations of Medical Sciences; CTCAE = Common Terminology Criteria for Adverse Events; ILD = interstitial lung disease; MedDRA = Medical Dictionary for Regulatory Activities; n = number of patients; PT = preferred term; SOC = system organ class.

Table 2. Serious Adverse Reactions for Durvalumab Monotherapy Considered Expected for Safety Reporting Purposes

MedDRA (v25.0) SOC	PT	Number (%) of subjects exposed (N=5208)		
		Suspected SARs n (%) [a]	Occurrence of life-threatening suspected SARs n (%) [b]	Occurrence of fatal suspected SARs n (%) [b]
Respiratory, thoracic, and mediastinal disorders	Pneumonitis	43 (0.8)	0	0
	Interstitial lung disease	10 (0.2)	0	0
Injury, poisoning, and procedural complications	Infusion related reaction	7 (0.1)	0	0
Infections and infestations	Pneumonia	16 (0.3)	0	0
	Pneumocystis jirovecii pneumonia	2 (<0.1)	0	0
Gastrointestinal disorders	Colitis	12 (0.2)	0	0
	Diarrhoea	13 (0.2)	0	0
	Abdominal pain	3 (<0.1)	0	0
	Enterocolitis	3 (<0.1)	0	0
	Pancreatitis	2 (<0.1)	0	0
	Proctitis	2 (<0.1)	0	0
Endocrine disorders	Adrenal insufficiency	6 (0.1)	0	0
	Hyperthyroidism	3 (<0.1)	0	0
	Hypopituitarism	3 (<0.1)	0	0
	Hypothyroidism	3 (<0.1)	0	0
Hepatobiliary disorders	Hepatitis	5 (<0.1)	0	0
	Autoimmune hepatitis	3 (<0.1)	0	0
Renal and urinary disorders	Nephritis	3 (<0.1)	0	0
	Blood creatinine increased	2 (<0.1)	0	0
Investigations	AST increased	6 (0.1)	0	0
	ALT increased	5 (<0.1)	0	0
	Hepatic enzyme increased	2 (<0.1)	0	0
	Transaminases increased	2 (<0.1)	0	0

MedDRA (v25.0) SOC	PT	Number (%) of subjects exposed (N=5208)		
		Suspected SARs n (%) [a]	Occurrence of life-threatening suspected SARs n (%) [b]	Occurrence of fatal suspected SARs n (%) [b]
General disorders and administration site conditions	Pyrexia	5 (<0.1)	0	0
Musculoskeletal and connective tissue disorders	Myositis	4 (<0.1)	0	0
Skin and subcutaneous tissue disorders	Rash	4 (<0.1)	0	0
Blood and lymphatic system disorders	Immune thrombocytopenia	2 (<0.1)	0	0
Metabolism and nutrition disorders	Type 1 diabetes mellitus	2 (<0.1)	0	0
Nervous system disorders	Myasthenia gravis	2 (<0.1)	0	0

[a] n = number of patients who have experienced the SAR.

[b] All fatal and all life-threatening events for durvalumab monotherapy are considered unexpected for reporting purposes and are excluded from this table. Pooled dataset from studies CD-ON-MEDI4736-1108, ATLANTIC, MYSTIC, EAGLE, ARCTIC, CONDOR, HAWK, PACIFIC, D4190C00002, BISCAY, STRONG, PACIFIC 6, DANUBE, HIMALAYA, KESTREL, NeoCOAST, D4190C00007, D4190C00021, D4190C00022, and D4198C00001.

Only patients exposed to 10 mg/kg Q2W or 20 mg/kg Q4W durvalumab, or equivalent fixed dosing of durvalumab 750 mg Q2W or 1500 mg Q4W schedule, are included.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; DCO = data cut-off; MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; Q2W = every 2 weeks; Q4W = every 4 weeks; SAR = serious adverse reaction; SOC = system organ class.

14.1.2.1 Other Potential Risks for Durvalumab Monotherapy and the Durvalumab Plus Tremelimumab Combination

Hypersensitivity Reactions and Anaphylaxis

Hypersensitivity reactions and anaphylaxis are considered potential risks for durvalumab monotherapy and the durvalumab + tremelimumab combination. In the durvalumab monotherapy pooled dataset (N=3006), hypersensitivity/anaphylactic reactions (grouped term including

reported PTs of hypersensitivity, drug hypersensitivity, drug eruption, anaphylactic shock, and anaphylactic reaction), were uncommonly reported in 28 patients (0.9%); 5 patients had Grade 3 or 4 events. Of the 28 patients, 16 patients had events of drug hypersensitivity (1 patient had a Grade 4 event), 5 patients had events of drug eruption (2 patients had Grade 3 events), 4 patients had events of hypersensitivity (Grade 1 or 2 events), 3 patients had events of anaphylactic reaction (1 patient had a Grade 4 event), and 1 patient had a Grade 2 event of anaphylactic shock.

Subcutaneous Injection Site Reaction

Subcutaneous injection site reactions can occur via immune or non-immune mechanisms.
A potential immunogenic mechanism could be due to antigen presentation and processing by lymph node and migratory cutaneous dendritic cells in the subcutaneous space. Non-immune mechanisms by which injection site reactions may arise can be related to biophysical characteristics of the drug and preservative/stabilizing agents. Patients should be closely monitored for signs and symptoms of subcutaneous injection site reactions including, but not limited to pain, blistering, induration, burning, and erythema at the site of injection.

Immunogenicity

As with the administration of any therapeutic protein, there is a potential for an immune response. The incidence of durvalumab ADA-positive patients in clinical studies is low. Overall, of the 4045 patients who were treated with durvalumab monotherapy 10 mg/kg Q2W, 20 mg/kg Q4W, or 1500 mg Q4W, 3069 patients were evaluable for the presence of ADAs, of which 6.2% (191/3069) patients were ADA-positive at any visit and 2.7% (84/3069) patients were treatment-emergent ADA-positive. Neutralising antibodies against durvalumab were detected in 0.5% (16/3069) patients. The presence of ADAs did not have a clinically relevant effect on PK. The presence of durvalumab ADA did not have an influence on durvalumab exposure metric, and there was no apparent effect on the safety of durvalumab.

Other Rare or Less Frequent Immune-mediated Adverse Events

Events with an inflammatory or immune mediated mechanism could occur in nearly all organs. Potential risks with an immune-mediated aetiology that are rare or less frequent include, but are not limited to, Guillain-Barre Syndrome, pericarditis, sarcoidosis, uveitis, cholangitis sclerosing, immune-mediated cystitis and other events involving the eye (eg, keratitis and optic neuritis), skin (eg, scleroderma and vitiligo), haematological (eg, hemolytic anaemia and immune-mediated neutropenia), rheumatological events (polymyalgia rheumatica and autoimmune arthritis), vasculitis, non-infectious meningitis and psoriasis.

14.1.3 Formulation, packaging and storage

Durvalumab will be supplied by AstraZeneca as a 500 mg vial solution for infusion after dilution. The solution contains 50 mg/mL durvalumab, 26 mM histidine/histidine hydrochloride, 275 mM trehalose dihydrate, and 0.02% (weight/volume) polysorbate 80; it has a pH of 6.0. The nominal fill volume is 10 mL. Investigational product vials are stored at 2°C to 8°C (36°F to 46°F) and must not be frozen. Durvalumab must be used within the individually assigned expiration date on the label.

14.1.4 Study Drug Preparation

The dose of durvalumab for administration must be prepared by the Investigator's or site's designated IP manager using aseptic technique. Total time from needle puncture of the durvalumab vial to the start of administration should not exceed:

- 24 hours at 2°C to 8°C (36°F to 46°F)
- 4 hours at room temperature

If in-use storage time exceeds these limits, a new dose must be prepared from new vials. Infusion solutions must be allowed to equilibrate to room temperature prior to commencement of administration.

14.1.5 Study Drug Administration

See section **3.2.1**

14.1.6 Incompatibilities

There are no incompatibilities for durvalumab.

14.1.7 Accountability and dispensation

The investigator is responsible for keeping accurate records of the clinical supplies received, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

14.1.8 Disposition of unused investigational study drug

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

14.2 VICINEUM (IND #: 136199)

14.2.1 Source

Vicineum (investigational supply) will be supplied by Sesen Biotherapeutics under NCI CRADA #3149. Commercial supply will be provided for the study after the 31st Aug 2021.

14.2.2 Toxicity

14.2.2.1 Phase 1/2 clinical trial (Study No. VB4-845-02-I)

The most frequently reported treatment-related AEs were those associated with renal and urinary disorders (23.4% of subjects). Within this class, dysuria (14.1% of subjects) and hematuria (10.9% of subjects) were reported most frequently. All other AEs assessed as possibly, probably, or definitely related to Vicineum occurred in less than 10% of the subject population. Of the related systemic AEs, fatigue was reported as an AE by 7.8% of subjects and myalgia was reported by 4.7% of subjects.

14.2.2.2 Phase 2 trial (Study No. VB4-845-02-IIA)

The most commonly reported treatment-emergent adverse events regardless of causality were associated with renal and urinary disorders, including dysuria (60.9% of subjects), hematuria (26.1% of subjects), pollakiuria (23.9% of subjects), micturition urgency (21.7% of subjects), nocturia (15.2% of subjects), incontinence (urge incontinence and urinary incontinence; 13.0% of subjects), urinary tract infections (13.0% of subjects), and bladder pain (10.9% of subjects). Other treatment related AEs that occurred in 10% or more of subjects were fatigue (21.7%), flu-like symptoms (10.9%), fever (10.9%), joint pain (13%), and dizziness (10.9%).

14.2.2.3 Phase 3 trial (Study No. VB4-845-02-IIIA)

Table 3. Treatment-Emergent Adverse Events Considered Related to Vicineum occurring in ≥5% of subjects, VB4-845-02-IIIA

Adverse Event	**Grade 1 – 2**	**Grade 3 – 4**	**Grade 5**	**Total**
Any Event Related to Vicineum	**61 (46%)**	**4 (3%)**	**1 (1%)**	**66 (50%)**
Renal and Urinary Disorders	**45 (34%)**	**1 (1%)**	**1 (1%)**	**47 (35%)**
Dysuria	18 (14%)	0	0	18 (14%)
Haematuria	17 (13%)	0	0	17 (13%)
Pollakiuria	15 (11%)	0	0	15 (11%)
Micturition Urgency	15 (11%)	0	0	15 (11%)
General Disorders and Administration Site Conditions	**12 (9%)**	**0**	**0**	**12 (9%)**
Fatigue	10 (8%)	0	0	10 (8%)
Gastrointestinal Disorders	**9 (7%)**	**0**	**0**	**9 (7%)**
Infections and Infestations	**15 (11%)**	**2 (2%)**	**0**	**17 (13%)**
Urinary Tract Infection	14 (11%)	2 (2%)	0	16 (12%)
Investigations	**7 (5%)**	**3 (2%)**	**0**	**10 (8%)**

Note: Treatment-emergent adverse events were assessed as possibly, probably, or definitely related to study treatment per Investigator
Data Source: VB4-845-02-IIIA Interim CSR Table 28

Table 4. Serious Adverse Events, VB4-845-02-IIIA

Age/ Gender	**Adverse Event (MedDRA Preferred Term)**	**Relationship to Study Drug[a]**	**Severity**	**Day of Onset[b]**	**Duration (days)**	**Outcome**
70 / M	Pyelonephritis	Not related	Gr. 3	Day 17	4	Recovered/Resolved
76 / M	Asthma	Not related	Gr. 3	Day 264	9	Recovered/Resolved
91 / M	Hepatitis cholestatic	Related	Gr. 4	Day 46	Ongoing	Not recovered/Not resolved
	Renal failure	Related	Gr. 5	Day 46	24	Fatal

81 / M	Small intestinal obstruction	Not related	Gr. 3	Day 212	5	Recovered/Resolved
59 / M	UTI	Not related	Gr. 3	Day 72	3	Recovered/Resolved
74 / M	Acute kidney injury	Related	Gr. 3	Day 20	3	Recovered/Resolved
	Acute kidney injury	Related	Gr. 3	Day 29	6	Recovered/Resolved
70 / M	Esophageal obstruction	Not related	Gr. 3	Day 140	3	Recovered/Resolved
	Fall	Not related	Gr. 3	Day 407	3	Recovered/Resolved
82 / M	Hematuria	Not related	Gr. 2	Pre-drug	4	Recovered/Resolved
	Urinary retention	Not related	Gr. 2	Pre-drug	10	Recovered/Resolved
	UTI	Not related	Gr. 2	Pre-drug	25	Recovered/Resolved
	Pericardial effusion	Not related	Gr. 3	Day 61	4	Recovered/Resolved
	UTI	Not related	Gr. 3	Day 61	4	Recovered/Resolved
	Small intestinal obstruction	Not related	Gr. 1	Day 75	3	Recovered/Resolved
86 / M	Hematuria	Not related	Gr. 1	Day 475	3	Recovered/Resolved
73 / M	Lung neoplasm surgery	Not related	Gr. 4	Day 287	15	Recovered/Resolved with sequelae
70 / M	Pyrexia	Related	Gr. 2	Day 32	2	Recovered/Resolved
75 / F	Acute kidney injury	Not related	Gr. 3	Day 121	3	Recovered/Resolved
	Small intestinal obstruction	Not related	Gr. 3	Day 380	3	Recovered/Resolved
83 / M	Rib fracture	Not related	Gr. 3	Day 85	Ongoing	Not recovered/Not resolved
77 / M	Hematuria	Not related	Gr. 3	Pre-drug	2	Recovered/Resolved
	Acute kidney injury	Not related	Gr. 2	Day 32	3	Recovered/Resolved
	Cystitis	Not related	Gr. 3	Day 32	4	Recovered/Resolved
75 / F	Urinary retention	Not related	Gr. 2	Day 102	5	Recovered/Resolved
89 / M	Hematuria	Not related	Gr. 3	Day 214	2	Recovered/Resolved
76 / M	Tachycardia	Not related	Gr. 3	Day 17	4	Recovered/Resolved
82 / M	Aortic valve disease	Not related	Gr. 4	Day 694	2	Recovered/Resolved with sequelae
77 / F	Pelvic pain	Not related	Gr. 3	Day 36	3	Recovered/Resolved

F = female; M = male; MedDRA = Medical Dictionary for Regulatory Activities; UTI = urinary tract infection
[a] Investigator's assessment
[b] Initial dose of Vicineum = Day 1
Data source: VB4-845-02-IIIA Interim CSR Table 26

14.2.3 Formulation, packaging and storage

Vicineum™ (oportuzumab monatox, VB4-845) is a clear, colorless solution for intravesical administration. It is provided as a frozen product in a glass vial, and then thawed at room temperature prior to preparation for administration. Cloudiness, a change in color and/or the presence of particulate matter may indicate the product has deteriorated. If this occurs, Sesen

Biotherapeutics should be contacted immediately and the product must not be used. Vicineum is packaged in a 10 mL glass borosilicate vial containing 7 mL of study drug at a concentration of 5 mg/mL. Each vial is sealed with grey butyl stoppers and covered with an aluminum cap center tear-off seal.

Vicineum will be mixed with phosphate buffered saline prior to intravesical administration. Refer to the Vicineum Dose Preparation and Administration Manual for details.

14.2.4 Product preparation

Refer to the Vicineum Dose Preparation and Administration Manual for details.

14.2.5 Administration procedures

See section **3.2.2** and Vicineum Dose Preparation and Administration Manual.

14.2.6 Incompatibilities

Vicineum is administer intravesically via a catheter. Only material indicated as qualified in the Vicineum Dose Preparation and Administration Manual in Section 6 should be used.

14.2.7 Accountability and dispensation

The Investigator agrees that the study drug will be administered only to subjects who have provided written informed consent, have met all entry criteria, and are enrolled in the study. The Investigator must not loan or dispense clinical study material to another Investigator or site.

Drug accountability records will be maintained by the Investigator or authorized site staff for all clinical trial supplies (includes Vicineum and phosphate buffered saline). Sesen Biotherapeutics . or designee will provide the Investigator with drug accountability logs to document receipt and dispensing. Any discrepancy and/or deficiency must be recorded, reported to Sesen Biotherapeutics or designee, and a plan for resolution documented. If at any point in the conduct of the study a vial is deemed unacceptable for use, the site will document the damage on the drug accountability log and immediately notify Sesen Biotherapeutics or designee. Sesen Biotherapeutics or designee will monitor the completeness and accuracy of all accountability records throughout the study and verify product accountability against site documentation.

14.2.8 Disposition of unused investigational study drug

Any expired Vicineum, or Vicineum vial deemed unacceptable for use or any Vicineum supply remaining at the end of the study should be destroyed at each clinical site in accordance with the site's drug destruction policy.

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16 APPENDICES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

16.2 APPENDIX B: DURVALUMAB DOSE CALCULATIONS

Durvalumab Dosing

The durvalumab dosing should be done depending on subject weight (if subject is < 30kg)):

1. Cohort dose: X mg/kg

2. Subject weight: Y kg

3. Dose for subject: XY mg = X (mg/kg) × Y (kg)

4. Dose to be added into infusion bag:

Dose (mL) = XY mg / 50 (mg/mL)

where 50 mg/mL is durvalumab nominal concentration

The corresponding volume of durvalumab should be rounded to the nearest tenth mL (0.1 mL). Dose adjustments for each cycle only needed for greater than 10% change in weight.

5. The theoretical number of vials required for dose preparation is the next greatest whole number of vials from the following formula:

Number of vials = Dose (mL) / 10 (mL/vial)

Example:

1. Cohort dose: 10 mg/kg

2. Subject weight: 30 kg

3. Dose for subject: 300 mg = 10 (mg/kg) × 30 (kg)

4. Dose to be added into infusion bag:

Dose (mL) = 300 mg / 50 (mg/mL) = 6.0 mL

5. The theoretical number of vials required for dose preparation:

Number of vials = 6.0 (mL) / 10.0 (mL/vial) = 1 vials

16.3 APPENDIX C: DURVALUMAB DOSING MODIFICATION AND TOXICITY MANAGEMENT GUIDELINES

These guidelines are standardized for durvalumab trials. However, these guidelines are superseded and should be superceded by any protocol-specific DLTs as as specified in section 3.1.1. This is specifically the case for toxicities such as hyperbilirubinemia, encephalopathy, neurotoxicity, peripheral neuromotor syndromes, and transaminase elevation as well as others.

General Considerations Regarding Immune-Mediated Reactions

These guidelines are provided as a recommendation to support investigators in the management of potential immune-mediated adverse events (imAEs).

Immune-mediated events can occur in nearly any organ or tissue, therefore, these guidelines may not include all the possible immune-mediated reactions. Investigators are advised to take into consideration the appropriate practice guidelines and other society guidelines (e.g., National Comprehensive Cancer Network (NCCN), European Society of Medical Oncology (ESMO)) in the management of these events. Refer to the section of the table titled "Other -Immune-Mediated Reactions" for general guidance on imAEs not noted in the "Specific Immune-Mediated Reactions" section.

Early identification and management of imAEs is essential to ensure safe use of the study drug. Monitor patients closely for symptoms and signs that may be clinical manifestations of underlying imAEs. Patients with suspected imAEs should be thoroughly evaluated to rule out any alternative etiologies (e.g., disease progression, concomitant medications, infections). In the absence of a clear alternative etiology, all such events should be managed as if they were immune-mediated. Institute medical management promptly, including specialty consultation as appropriate. In general, withhold study drug/study regimen for severe (Grade 3) imAEs. Permanently discontinue study drug/study regimen for life-threatening (Grade 4) imAEs, recurrent severe (Grade 3) imAEs that require systemic immunosuppressive treatment, or an inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks of initiating corticosteroids.

Based on the severity of the imAE, durvalumab and/or tremelimumab should be withheld and corticosteroids administered. Upon improvement to Grade ≤ 1, corticosteroid should be tapered over ≥ 28 days. More potent immunosuppressive agents should be considered for events not responding to systemic steroids. Alternative immunosuppressive agents not listed in this guideline may be considered at the discretion of the investigator based on clinical practice and relevant guidelines. With long-term steroid and other immunosuppressive use, consider the need for glucose monitoring.

Dose modifications of study drug/study regimen should be based on severity of treatment-emergent toxicities graded per NCI CTCAE version in the applicable study protocol.

Considerations for Prophylaxis for Long Term use of Steroids for Patients Receiving Immune Checkpoint Inhibitor Immunotherapy

- Infection Prophylaxis: Pneumocystis jirovecii pneumonia (PJP), antifungal and Herpes Zoster reactivation
- Gastritis: Consider prophylaxis for patients at high risk of gastritis (e.g. NSAID use, anticoagulation) when the patient is taking steroid therapy
- Osteoporosis: Consider measures for prevention and mitigation of osteoporosis .

General Considerations Regarding Immune-Mediated Reactions

Relevant Society Guidelines for Management of imAEs

These society guidelines are provided as references to serve in support of best clinical practice and the TMGs. Please note, these were the current versions of these guidelines at the time of updating TMGs. Please refer to the most up to date version of these guidelines.

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Pediatric Considerations Regarding Immune-Mediated Reactions

Dose Modifications	Toxicity Management
The criteria for permanent discontinuation of study drug/study regimen based on CTCAE grade/severity is the same for pediatric patients as it is for adult patients, as well as to permanently discontinue study drug/study regimen if unable to reduce corticosteroid ≤ a dose equivalent to that required for corticosteroid replacement therapy **within 12 weeks of** initiating corticosteroids.	– All recommendations for specialist consultation should occur with a pediatric specialist in the specialty recommended. – The recommendations for steroid dosing (i.e., mg/kg/day) provided for adult patients should also be used for pediatric patients. – The recommendations for intravenous immunoglobulin (IVIG) and plasmapheresis use provided for adult patients may be considered for pediatric patients. – The infliximab 5 mg/kg IV one time dose recommended for adults is the same as recommended for pediatric patients ≥ 6 years old. For subsequent dosing and dosing in children < 6 years old, consult a pediatric specialist. – For pediatric dosing of mycophenolate mofetil, consult a pediatric specialist. – With long-term steroid and other immunosuppressive use, consider need for PJP prophylaxis, gastrointestinal protection, and glucose monitoring.

Specific Immune-Mediated Reactions			
Adverse Events	**Severity Grade of the Event**	**Dose Modifications**	**Toxicity Management**
Pneumonitis/Interstitial Lung Disease (ILD)	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology with similar clinical presentation (e.g. infection, progressive disease). – Monitor patients for signs (e.g. tachypnoea) and symptoms of pneumonitis or ILD (new onset or worsening shortness of breath or cough). Evaluate patients with imaging and pulmonary function tests, including other diagnostic procedures as described below. – Suspected pneumonitis should be confirmed with radiographic imaging and other infectious and disease-related etiologies excluded, and managed as described below. – Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up (including clinically relevant culture specimens to rule out infection), and high- resolution computed tomography (CT) scan. – Consider Pulmonary and Infectious Diseases consults.
	Grade 1	No dose modifications required. However, consider holding study drug/study regimen dose as clinically appropriate and during diagnostic work-up for other etiologies.	**For Grade 1** – Monitor and closely follow up in 2 to 4 days for clinical symptoms, pulse oximetry (resting and exertion), and laboratory work-up, and then as clinically indicated.
	Grade 2	Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1. • If toxicity improves to Grade ≤1, then the decision to reinitiate study drug/study regimen will be based upon treating physician's clinical judgment and after	**For Grade 2** – Monitor symptoms daily and consider hospitalization as clinically indicated.

		completion of steroid taper (≤10 mg prednisone or equivalent).	– Consider Pulmonary and Infectious Diseases Consults. – Promptly start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent). – Consider HRCT or chest CT with contrast, Repeat imaging as clinically indicated. – If no improvement within 2 to 3 days, additional workup should be considered and prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day started. – If no improvement within 2 to 3 days despite IV methylprednisolone at 2 to 4 mg/kg/day, promptly start immunosuppressive therapy. such as tumor necrosis factor (TNF) inhibitors (e.g., infliximab at 5 mg/kg IV once, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Consider discussing with Clinical Study Lead.
	Grade 3 or 4	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Hospitalize the patient – Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent. – Obtain Pulmonary and Infectious Diseases Consults; consider discussing with Clinical Study Lead, as needed. – Consider starting anti-infective therapy if infection is still a consideration on the basis of other diagnostic testing despite negative culture results – Supportive care (e.g., oxygen).

			– If no improvement within 2 days, additional workup should be considered and prompt treatment with additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: rule out sepsis and refer to infliximab label for general guidance before using infliximab.
Diarrhea/Colitis	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections), including testing for *Clostridium difficile* toxin, etc. – Monitor for symptoms that may be related to diarrhea/enterocolitis (abdominal pain, cramping, or changes in bowel habits such as increased frequency over baseline or blood in stool) or related to bowel perforation (such as sepsis, peritoneal signs, and ileus). – Consider further evaluation with imaging study with contrast. – Consult a gastrointestinal (GI) specialist for consideration of further workup – **WHEN SYMPTOMS OR EVALUATION INDICATE AN INTESTINAL PERFORATION IS SUSPECTED, CONSULT A SURGEON EXPERIENCED IN ABDOMINAL SURGERY IMMEDIATELY WITHOUT ANY DELAY.** – **PERMANENTLY DISCONTINUE STUDY DRUG FOR ANY GRADE OF INTESTINAL PERFORATION.** – Steroids should be considered in the absence of clear alternative etiology, even for low-grade events, in order to prevent potential

			progression to higher grade events, including intestinal perforation. – Use analgesics carefully; they can mask symptoms of perforation and peritonitis.
	Grade 1	No dose modifications.	**For Grade 1** – Monitor closely for worsening symptoms. – Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), loperamide, and other supportive care measures. – If symptoms persist, consider checking lactoferrin; if positive, treat as Grade 2 below. If negative and no infection, continue Grade 1 management.
	Grade 2	Hold study drug/study regimen until resolution to Grade ≤1 • If toxicity improves to Grade ≤1, then study drug/study regimen can be resumed after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** – Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), and loperamide and/or budesonide. – Consider further evaluation with imaging study with contrast. – Consider consult of a gastrointestinal (GI) specialist for consideration of further workup. – Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If no improvement within 3 days despite therapy with 1 to 2 mg/kg IV prednisone equivalent, reconsult GI specialist and, if indicated, promptly start additional immunosuppressant agent such as infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines. **Caution:** it is important to rule out bowel perforation and refer to infliximab

			label for general guidance before using infliximab. – **If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.** – Consider, as necessary, discussing with Clinical Study Lead if no resolution to Grade ≤1 in 3 to 4 days.
	Grade 3 or 4	**Grade 3** • For patients treated with durvalumab monotherapy, hold study drug/study regimen until resolution to Grade ≤1; study drug/study regimen can be resumed after completion of steroid taper (≤10 mg prednisone per day, or equivalent). • For patients treated with durvalumab in combination with other products (not tremelimumab), decision to be made at the discretion of the study investigator, in discussion with AstraZeneca Clinical Study Lead. • **<u>For patients treated with durvalumab in combination with tremelimumab or tremelimumab monotherapy,</u> permanently discontinue both durvalumab and tremelimumab for 1) Grade 3 diarrhea colitis or 2) Any grade of intestinal perforation.** **Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Urgent GI consult and imaging and/or colonoscopy as appropriate. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – Monitor stool frequency and volume and maintain hydration. – If still no improvement within 2 days, continue steroids and promptly add further immunosuppressants. (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution**: Ensure GI consult to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. – **If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.**
Hepatitis **Infliximab should not be used for management of immune-related hepatitis.**	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., viral hepatitis, disease progression, concomitant medications). – Monitor and evaluate transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], and total bilirubin.

	ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN	• No dose modifications. • If it worsens, then consider holding therapy.	– Continue transaminase and total bilirubin monitoring per protocol.
	ALT or AST > 3 ≤ 5 x ULN or total bilirubin > 1.5 ≤ 3 x ULN	• Hold study drug/study regimen dose until ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN. Resume study drug/study regimen after completion of steroid taper (<10 mg prednisone or equivalent). • Permanently discontinue study drug/study regimen for any case meeting Hy's law laboratory criteria (AST or ALT ≥3 × ULN AND bilirubin ≥2 × ULN without initial findings of cholestasis (i.e., elevated ALP) and in the absence of any alternative cause.	– Regular and frequent checking of transaminases and total bilirubin (e.g., every 1 to 2 days) until LFT elevations improve or resolve. – If no resolution to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN in 1 to 2 days, consider discussing with Clinical Study Lead, as needed. – If event is persistent (>2 to 3 days) or worsens, promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent.
	ALT or AST > 5-≤ 10 x ULN	• Hold study drug/study regimen . Resume study drug/study regimen if elevations downgrade to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN after completion of steroid taper (<10 mg prednisone, or equivalent). • **If in combination with tremelimumab, do not restart tremelimumab.**	– Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. – Perform Hepatology Consult, abdominal workup, and imaging as appropriate – If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant.(e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not available. **Infliximab should NOT be used.**
	Concurrent ALT or AST > 3 x ULN and	Permanently discontinue study drug/study regimen.	– Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day

	total bilirubin > 2 x ULN [d] **ALT or AST > 10 x ULN OR total bilirubin > 3 x ULN**		or equivalent. – If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant.(e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not available. **Infliximab should NOT be used.** – Perform Hepatology Consult, abdominal workup, and imaging as appropriate.
Hepatitis (elevated transaminases and total bilirubin) **Infliximab should not be used for management of immune-related hepatitis.** **THIS shaded area is guidance *only* for management of "Hepatitis (elevated LFTs)" in HCC patients (or secondary tumour involvement of the liver with abnormal baseline values [BLV])**	**Any Elevations of AST, ALT, or T. Bili as Described Below**	**General Guidance**	**For Any Elevations Described** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., viral hepatitis, disease progression, concomitant medications, worsening of liver cirrhosis [e.g., portal vein thrombosis]). – Monitor and evaluate liver function test: AST, ALT, ALP, and T. Bili. – For hepatitis B (HBV) + patients: evaluate quantitative HBV viral load, quantitative Hepatitis B surface antigen (HBsAg), or Hepatitis B envelope antigen (HBeAg). – For hepatitis C (HCV) + patients: evaluate quantitative HCV viral load. – Consider consulting Hepatology or Infectious Diseases specialists regarding changing or starting antiviral HBV medications if HBV viral load is >2000 IU/ml. – Consider consulting Hepatology or Infectious Diseases specialists regarding changing or starting antiviral HCV medications if HCV viral load has increased by ≥2-fold.

See instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either **increasing bilirubin or signs of DILI/liver decompensation**			– For HCV+ with Hepatitis B core antibody (HBcAb) +: Evaluate for both HBV and HCV as above.
	Isolated AST or ALT >ULN and ≤2.5 BLV	• No dose modifications. • If ALT/AST elevations represents significant worsening based on investigator assessment, then treat as described for elevations in the row below. • For all transaminase elevations , see instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either **increasing bilirubin or signs of DILI/liver decompensation**	
	AST or ALT >2.5 BLV and ≤5×BLV and ≤ 20 ULN	• Hold study drug/study regimen dose until resolution to AST or ALT ≤2.5×BLV. • If toxicity worsens, then treat as described for elevations in the rows below. If toxicity improves to AST or ALT ≤2.5×BLV, resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	– Regular and frequent checking of Transaminases and total bilirubin (e.g., every 1 to 3 days) until elevations of these are improving or resolved. – Recommend consult hepatologist; consider abdominal ultrasound, including Doppler assessment of liver perfusion. – Consider, as necessary, discussing with Clinical Study Lead. – If event is persistent (>2 to 3 days) or worsens, and investigator suspects toxicity to be an imAE, start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day of prednisone PO or IV equivalent, consider additional workup. If still no improvement within 2 to 3 days despite 2mg/kg/day of IV methylprednisolone, consider additional abdominal workup (including liver biopsy) and imaging (i.e., liver ultrasound), and consider starting additional immunosuppressants. (e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss Clinical Study Lead if mycophenolate

			mofetil is not available. **Infliximab should NOT be used.**
	ALT or AST >5-7X BLV and ≤ 20X ULN OR concurrent 2.5-5X BLV and ≤20XULN and total bilirubin > 1.5 - < 2 x ULN [d]	• Withhold durvalumab and permanently discontinue tremelimumab • Resume study drug/study regimen if elevations downgrade to AST or ALT ≤2.5×BLV and after completion of steroid taper (<10 mg prednisone, or equivalent). • Permanently discontinue study drug/study regimen if the elevations do not downgrade to AST or ALT ≤2.5×BLV within 14 days	– Regular and frequent checking of LFTs (e.g., every 1-2 days) until elevations of these are improving or resolved. – Consult hepatologist (unless investigator is hepatologist); obtain abdominal ultrasound, including Doppler assessment of liver perfusion; and consider liver biopsy. – Consider discussing with Clinical Study Lead, as needed. – If investigator suspects toxicity to be immune-mediated, promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. – If no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, obtain liver biopsy (if it has not been done already) and promptly start treatment with an additional immunosuppressant. (e.g. mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with a hepatologist or relevant practice guidelines). Discuss with Study Clinical Lead if mycophenolate is not available. **Infliximab should NOT be used.**
	ALT or AST > 7 X BLV OR > 20 ULN whichever occurs first OR bilirubin > 3ULN	Permanently discontinue study drug/study regimen.	**Same as above (except recommend obtaining liver biopsy early)**
Nephritis and/or renal dysfunction	**Any Grade** (Refer to NCI CTCAE applicable version in	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g.,

	study protocol for defining the CTCAE grade/severity)		disease progression, infections, recent IV contrast, medications, fluid status). – Consider Consulting a nephrologist. – Consider imaging studies to rule out any alternative etiology. – Monitor for signs and symptoms that may be related to changes in renal function (e.g., routine urinalysis, elevated serum BUN and creatinine, decreased creatinine clearance, electrolyte imbalance, decreased urine output, or proteinuria). – Follow urine protein/creatinine ratio every 3-7 days.
	Grade 1	No dose modifications.	**For Grade 1** – Monitor serum creatinine weekly and any accompanying symptoms. • If creatinine returns to baseline, resume its regular monitoring per study protocol. • If creatinine worsens, depending on the severity, treat as Grade 2, 3, or 4. – Consider hydration, electrolyte replacement, and diuretics, as clinically indicated. – Consider nephrologist consult if not resolved within 14 days, or earlier as clinically indicated
	Grade 2	Hold study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity improves to Grade ≤1 or baseline, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** – Consider including hydration, electrolyte replacement, and diuretics as clinically indicated. – Follow urine protein/creatinine ratio every 3-7 days. – Carefully monitor serum creatinine and as clinically warranted. – Consult nephrologist and consider renal biopsy if clinically indicated.

			– Start prednisone 0.5 – 1 mg/kg/day if other causes are ruled out. – If event is persistent beyond 5 days or worsens, increase to prednisone up to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 5 days or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup. When event returns to baseline, resume study drug/study regimen and routine serum creatinine monitoring per study protocol.
	Grade 3 or 4	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Carefully monitor serum creatinine daily. – Follow urine protein/creatinine ratio every 3-7 days. – Consult nephrologist and consider renal biopsy if clinically indicated. – Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 3 to 5 days of steroids or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup and prompt treatment with an immunosuppressant.
Rash or Dermatitis **(Including Pemphigoid)**	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for definition of severity/grade depending on type of skin rash)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology. – Monitor for signs and symptoms of dermatitis (rash and pruritus). – **HOLD STUDY DRUG IF GRADE 3 PEMPHIGOID OR SEVERE CUTANEOUS ADVERSE REACTION (SCAR) IS SUSPECTED.** – **PERMANENTLY DISCONTINUE STUDY DRUG IF SCAR OR GRADE 3 PEMPHIGOID IS CONFIRMED.**

	Grade 1	No dose modifications.	**For Grade 1** – Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., emolient, lotion, or institutional standard).
	Grade 2	For persistent (>1 week) Grade 2 events, hold scheduled study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity improves to Grade ≤1 or baseline, then resume drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** – Consider dermatology consult and skin biopsy, as indicated. – Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy – Consider moderate-strength topical steroid. – If no improvement of rash/skin lesions occurs within 1 week or is worsening despite symptomatic treatment and/or use of moderate strength topical steroid, consider discussing with Clinical Study Lead, as needed, and promptly start systemic steroids such as prednisone 1 to 2 mg/kg/day PO or IV equivalent.
	Grade 3	**For Grade 3** • Hold study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity improves to Grade ≤1 or baseline, then resume drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 3** – Reconsult dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – Consider hospitalization. – Monitor the extent of rash [Rule of Nines]. – Consider, as necessary, discussing with Clinical Study Lead.
	Grade 4	**For Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 4** – Reconsult a dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – Consider hospitalization. – Monitor the extent of rash [Rule of Nines]. Consider, as necessary, discussing with Clinical Study

Endocrinopathy (e.g., hyperthyroidism, thyroiditis, hypothyroidism, type 1 diabetes mellitus, hypophysitis, hypopituitarism, and adrenal insufficiency)	**Any Grade** (Depending on the type of endocrinopathy, refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression including brain metastases, or infections). – Consider consulting an endocrinologist for endocrine events. – Consider discussing with Clinical Study Lead, as needed. – Monitor patients for signs and symptoms of endocrinopathies. (Non-specific symptoms include headache, fatigue, behaviour changes, mental status changes, photophobia, visual field cuts, vertigo, abdominal pain, unusual bowel habits, polydipsia, polyuria, hypotension, and weakness.) – Depending on the suspected endocrinopathy, monitor and evaluate thyroid function tests: thyroid stimulating hormone (TSH), free T3 and free T4 and other relevant endocrine and related labs (e.g., blood glucose and ketone levels, hemoglobin A1c (HgA1c)). If a patient experiences an AE that is thought to be possibly of autoimmune nature (e.g., thyroiditis, pancreatitis, hypophysitis, or diabetes insipidus), the investigator should send a blood sample for appropriate autoimmune antibody testing. – Investigators should ask subjects with endocrinopathies who may require prolonged or continued hormonal replacement, to consult their primary care physicians or endocrinologists about further monitoring and treatment after completion of the study.

	Grade 1	No dose modifications.	**For Grade 1** – Monitor patient with appropriate endocrine function tests. – For suspected hypophysitis/hypopituitarism, consider consulting an endocrinologist to guide assessment of early-morning adrenocorticotropin hormone (ACTH), cortisol, TSH and free T4; also consider gonadotropins, sex hormones, and prolactin levels, as well as cosyntropin stimulation test (though it may not be useful in diagnosing early secondary adrenal insufficiency). – If TSH < 0.5 × LLN, or TSH >2 × ULN, or consistently out of range in 2 subsequent measurements, include free T4 at subsequent cycles as clinically indicated and consider consultation of an endocrinologist.
	Grade 2, 3, or 4	– For Grade 2-4 endocrinopathies <u>other than hypothyroidism and type 1 diabetes mellitus (T1DM)</u>, consider holding study drug/study regimen dose until acute symptoms resolve. – Study drug/study regimen can be resumed once patient stabilizes and after completion of steroid taper (<10 mg prednisone, or equivalent). – Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen if the patient is clinically stable as per investigator or treating physician's clinical judgement.	**For Grade 2, 3, or 4** – Consult endocrinologist to guide evaluation of endocrine function and, as indicated by suspected endocrinopathy and as clinically indicated, consider pituitary scan. – For all patients with abnormal endocrine work up, except those with isolated hypothyroidism or T1DM, and as guided by an endocrinologist, consider short-term corticosteroids (e.g., 1 to 2 mg/kg/day methylprednisolone or IV equivalent) and prompt initiation of treatment with relevant hormone replacement. – **Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids.** – **Isolated T1DM may be treated with appropriate diabetic therapy, and without corticosteroids. <u>Only hold study drug/study regimen in setting of</u>**

			hyperglycemia when diagnostic workup is positive for diabetic ketoacidosis. – For patients with normal endocrine workup (laboratory assessment or magnetic resonance imaging (MRI) scans), repeat laboratory assessments/MRI as clinically indicated.
Amylase/Lipase increased	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g. disease progression, viral infection, concomitant medications, substance abuse). – For modest asymptomatic elevations in serum amylase and lipase, corticosteroid treatment is not indicated as long as there are no other signs or symptoms of pancreatic inflammation. – Assess for signs/symptoms of pancreatitis – Consider appropriate diagnostic testing (e.g., abdominal CT with contrast, MRCP if clinical suspicion of pancreatitis and no radiologic evidence on CT) – If isolated elevation of enzymes without evidence of pancreatitis, continue immunotherapy. Consider other causes of elevated amylase/lipase – If evidence of pancreatitis, manage according to pancreatitis recommendations
	Grade 1	No dose modifications.	
	Grade 2, 3, or 4	**For Grade 2, 3, or 4** In consultation with relevant gastroenterology specialist, consider continuing study drug/study regimen if no clinical/radiologic evidence of pancreatitis ± improvement in amylase/lipase.	
Acute Pancreatitis	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology. – Consider Gastroenterology referral

	Grade 2	**Consider holding study drug/regimen.**	**Grade 2** – Consider IV hydration – Consider Gastroenterology referral
	Grade 3, or 4	**For Grade 3** Hold study drug/study regimen until resolution of elevated enzymes and no radiologic findings. If no elevation in enzymes or return to baseline values, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent). **For Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 3, or 4** – Promptly start systemic steroids prednisone 1 to 2 mg/kg/day PO or IV equivalent. – IV hydration
Nervous System Disorders			
Aseptic Meningitis	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance** – Symptoms may include headache, photophobia, and neck stiffness, nausea/ vomiting which may resemble an infectious meningitis. – Patients may be febrile. – Mental status should be normal	**For Any Grade** – Consider neurology consult – Consider MRI brain with and without contrast with pituitary protocol and a lumbar puncture for diagnosis. – Exclude bacterial and viral infections. (ie HSV) – Consider IV acyclovir until polymerase chain reactions are available
	Any Grade	Permanently discontinue study drug/study regimen	**For Any Grade** – Consider neurology consult – Consider MRI brain with and without contrast with pituitary protocol and a lumbar puncture for diagnosis. – Exclude bacterial and viral infections. (ie HSV) – Consider IV acyclovir until polymerase chain reactions are available – Consider, as necessary, discussing with Clinical Study Lead.(Last bullet)

			− Consider hospitalization. − Once infection has been ruled out promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent.
Encephalitis	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	− Symptoms may include Confusion, altered behaviour, headaches, seizures, short-term memory loss, depressed level of consciousness, focal weakness, and speech abnormality.	**For Any Grade** − Consider neurology consult − Consider testing including MRI of the brain with and without contrast, lumbar puncture, electroencephalogram (EEG) to evaluate for subclinical seizures, ESR, CRP, antineutrophil cytoplasmic antibody (ANCA) (if vasculitic process suspected), thyroid panel including TPO and thyroglobulin and additional autoantibodies to rule out paraneoplastic disorders. − Exclude bacterial and viral infections. (i.e. − HSV) Consider IV acyclovir until polymerase chain reactions are available.
	Grade 2	**For Grade 2** Permanently discontinue study drug/study regimen.	**For Grade 2** − Consider, as necessary, discussing with the Clinical Study Lead. − Once infection has been ruled out methylprednisolone 1–2 mg/kg/day − For progressive symptoms or if oligoclonal bands are present consider methylprednisolone 1 g IV daily for 3–5 days plus IVIG or plasmapheresis
	Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** − Consider, as necessary, discussing with Clinical Study Lead. − Consider hospitalization. − Once infection is ruled out, start methylprednisolone 1 g IV daily for 3–5 days for progressive symptoms consider adding IVIG or plasmapheresis

Transverse Myelitis	**Any Grade**	**General Guidance** – Permanently discontinue immunotherapy – Consider MRI of the spine and brain – Once imaging is complete, consider lumbar puncture Consider testing to rule out additional aetiologies: B12, HIV, rapid plasma reagin (RPR), ANA, anti-Ro/La antibodies, aquaporin-4 IgG, myelin oligodendrocyte glycoprotein (MOG) IgG, paraneoplastic panel for anti-Hu and anti-CRMP5/CV2	**For Any Grade** – Consider neurology consult – Inpatient care – Consider prompt initiation of high methylprednisolone pulse dosing – Strongly consider IVIG or plasmapheresis
Peripheral neuropathy	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be evaluated to rule out any alternative etiology for neuropathy (e.g., disease progression, infections, metabolic syndromes or medications). It should be noted that the diagnosis of immune-mediated peripheral neuromotor syndromes can be particularly challenging in patients with underlying cancer, due to the multiple potential confounding effects of cancer (and its treatments) throughout the neuraxis. Given the importance of prompt and accurate diagnosis, it is essential to have a low threshold to obtain a neurological consult. – Neurophysiologic diagnostic testing (e.g., electromyogram and nerve conduction investigations) are routinely indicated upon suspicion of such conditions and may be best facilitated by means of a neurology consultation.
	Grade 1	No dose modifications.	**For Grade 1** – Consider discussing with the Clinical Study Lead, as needed. – Monitor symptoms for interference with ADLS, gait difficulties, imbalance, or autonomic dysfunction

	Grade 2	Hold study drug/study regimen dose until resolution to Grade ≤1.	**For Grade 2** − Consult a neurologist. − Consider EMG/NCS − Consider discussing with the Clinical Study Lead, as needed. − Observation for additional symptoms decompensation or consider initiating prednisone 0.5–1 mg/kg orally − − If progression, initiate methylprednisolone 2–4 mg/kg/day and treat as GBS. − Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin or duloxetine).
	Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** − Consider discussing with Clinical Study Lead, as needed. − Recommend hospitalization. − Monitor symptoms and consult a neurologist. − **Treat per Guillain-Barré Syndrome recommendations**
Guillain-Barré Syndrome (GBS)		General Guidance	− Recommend hospitalization − Obtain neurology consult − Obtain MRI of spine to rule out compression lesion − Obtain lumbar puncture − Antibody tests for GBS variants − Pulmonary function tests − Obtain electromyography (EMG) and nerve conduction studies − Frequently monitor pulmonary function tests and neurologic evaluations − Monitor for concurrent autonomic dysfunction

			– Initiate medication as needed for neuropathic pain
	Grade 2-4	Grade 2-4 Permanently discontinue	**Start IVIG or plasmapheresis in addition to methylprednisolone 1 gram daily for 5 days, then taper over 4 weeks.**
Myasthenia gravis		General Guidance	– Obtain neurology consult – Recommend hospitalization – Obtain pulmonary function tests – Obtain labs: ESR, CRP, creatine phosphokinase (CPK), aldolase and anti-striational antibodies – Consider cardiac exam, ECG, troponin, transthoracic echocardiogram for possible concomitant myocarditis – Obtain electromyography (EMG) and nerve conduction studies – Consider MRI of brain/spine to rule out CNS involvement by disease – Avoid medications that might exacerbate MG (e.g. beta blockers, some antibiotics, IV magnesium)
	Grade 2	Permanently discontinue	– Consider pyridostigmine 30mg three times daily and gradually increase based on symptoms (max dose 120mg four times daily) – Consider starting low dose prednisone 20mg daily and increase every 3-5 days. (Target dose 1mg/kg/day. Max dose 100mg daily)
	Grade 3-4	Permanently discontinue	– Start methylprednisolone 1-2mg/kg/day. Taper steroids based on symptom improvement – Start plasmapheresis or IVIG – Consider rituximab if refractory to plasmapheresis or IVIG – Frequent PFT assessments – Daily neurologic evaluations

Myocarditis	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance** Discontinue drug permanently if biopsy-proven immune-mediated myocarditis.	**For Any Grade** – Initial work-up should include clinical evaluation, B-type natriuretic peptide (BNP), cardiac enzymes, electrocardiogram (ECG), echocardiogram (ECHO), monitoring of oxygenation via pulse oximetry (resting and exertion), and additional laboratory work-up as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed. – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections) – The prompt diagnosis of immune-mediated myocarditis is important, particularly in patients with baseline cardiopulmonary disease and reduced cardiac function. – Consider discussing with the Clinical Study Lead, as needed. – Monitor patients for signs and symptoms of myocarditis (new onset or worsening chest pain, arrhythmia, shortness of breath, peripheral edema). As some symptoms can overlap with lung toxicities, simultaneously evaluate for and rule out pulmonary toxicity as well as other causes (e.g., pulmonary embolism, congestive heart failure, malignant pericardial effusion). Consult a cardiologist early, to promptly assess whether and when to complete a cardiac biopsy, including any other diagnostic procedures. – as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed.

	Grade 2, 3 or 4	– If Grade 2-4, permanently discontinue study drug/study regimen.	**For Grade 2-4** – Monitor symptoms daily, hospitalize. – Consider cardiology consultation and a prompt start of high-dose/pulse corticosteroid therapy – Supportive care (e.g., oxygen). – If no improvement consider additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab), IVIG or plasmapheresis or other therapies depending on the clinical condition of the patient, based on at the discretion of the treating specialist consultant or relevant practice guidelines. **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. Infliximab is contraindicated for patients who have heart failure.**
Myositis/ Polymyositis	**Any Grade** (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections). – Monitor patients for signs and symptoms of poly/myositis. Typically, muscle weakness/pain occurs in proximal muscles including upper arms, thighs, shoulders, hips, neck and back, also difficulty breathing and/or trouble swallowing can occur and progress rapidly. Increased general feelings of tiredness and fatigue may occur, and there can be new-onset falling, difficulty getting up from a fall, and trouble climbing stairs, standing up from a seated position, and/or reaching up. – If poly/myositis is suspected, a Neurology consultation should be obtained early, with prompt guidance on diagnostic procedures. Myocarditis may co-occur with poly/myositis; refer to guidance under Myocarditis. Given breathing complications, refer to guidance under Pneumonitis/ILD. Given possibility of an existent (but previously unknown) autoimmune disorder, consider Rheumatology consultation. – Consider, as necessary, discussing with the Clinical Study Lead.

	Grade 1	– No dose modifications.	**For Grade 1** – Monitor and closely follow up in 2 to 4 days for clinical symptoms and initiate evaluation as clinically indicated. – Consider Neurology consult. – Consider, as necessary, discussing with the Clinical Study Lead.
	Grade 2	– Hold study drug/study regimen dose until resolution to Grade ≤1. – Permanently discontinue study drug/study regimen if it does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	**For Grade 2** – Monitor symptoms daily and consider hospitalization. – Consider Rheumatology or Neurology consult, and initiate evaluation. – Consider, as necessary, discussing with the Clinical Study Lead. – If clinical course is rapidly progressive (particularly if difficulty breathing and/or trouble swallowing), promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant – If clinical course is *not* rapidly progressive, start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent); if no improvement within 2 to 3 days, continue additional work up and start treatment with IV methylprednisolone 2 to 4 mg/kg/day – If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 3 days, consider additional immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab) IVIG or plasmapheresis, or other therapies based on the discretion of the specialist consultant or relevant practice guideline. **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.**

	Grade 3	**For Grade 3** – Hold study drug/study regimen dose until resolution to Grade ≤1. – Permanently discontinue study drug/study regimen if Grade 3 imAE does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	**For Grade 3** – Monitor symptoms closely; recommend hospitalization. – Consider Rheumatology or Neurology consult – Consider discussing with the Clinical Study Lead, as needed. – Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. – If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.** – Consider whether patient may require IV IG, plasmapheresis.

	Grade 4	**For Grade 4** Permanently discontinue study drug/study regimen.	**Grade 4** – Monitor symptoms closely; recommend hospitalization. – Consider Rheumatology and/or Neurology consult – Consider discussing with the Clinical Study Lead, as needed. – Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. – If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.**

1 SCAR terms include Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), Erythema Multiforme, Acute Generalized Exanthematous Pustulosis, Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) and Drug-induced hypersensitivity syndrome.

Other–Immune-Mediated Reactions

Severity Grade of the Event (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	Dose Modifications	Toxicity Management
Any Grade	Note: It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs, some of them are not noted specifically in these guidelines (e.g. immune thrombocytopenia, haemolytic anaemia, uveitis, vasculitis).	– Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections). – The Clinical Study Lead may be contacted for immune-mediated reactions not listed in the "specific immune-mediated reactions" section – Consultation with relevant specialist – Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Monitor as clinically indicated
Grade 2	• Hold study drug/study regimen until resolution to ≤Grade 1 or baseline. • If toxicity worsens, then treat as Grade 3 or Grade 4. • Study drug/study regimen can be resumed once event stabilizes to Grade ≤1 after completion of steroid taper. • Consider whether study drug/study regimen should be permanently discontinued in Grade 2 events with high likelihood for morbidity and/or mortality when they do not rapidly improve to Grade <1 upon treatment with systemic steroids and following full taper	**For Grade 2, 3, or 4** Treat accordingly, as per institutional standard, appropriate clinical practice guidelines, and society guidelines. (See page 4).
Grade 3	Hold study drug/study regimen	
Grade 4	Permanently discontinue study drug/study regimen	

Note: As applicable, for early phase studies, the following sentence may be added: "Any event greater than or equal to Grade 2, please discuss with Clinical Study Lead."

Infusion-Related Reactions

Severity Grade of the Event (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	Dose Modifications	Toxicity Management
Any Grade	General Guidance	**For Any Grade** – Manage per institutional standard at the discretion of investigator. – Monitor patients for signs and symptoms of infusion-related reactions (e.g., fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnea or chest discomfort, or skin rashes) and anaphylaxis (e.g., generalized urticaria, angioedema, wheezing, hypotension, or tachycardia).
Grade 1 or 2	**For Grade 1** The infusion rate of study drug/study regimen may be decreased by 50% or temporarily interrupted until resolution of the event. **For Grade 2** • The infusion rate of study drug/study regimen may be decreased 50% or temporarily interrupted until resolution of the event. • Subsequent infusions may be given at 50% of the initial infusion rate.	**For Grade 1 or 2** – Acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator. – Consider premedication per institutional standard or study protocol prior to subsequent doses. – Steroids should not be used for routine premedication of Grade ≤2 infusion reactions.
Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Manage severe infusion-related reactions per institutional standard, appropriate clinical practice guidelines, and society guidelines.

Non-Immune-Mediated Reactions

Severity Grade of the Event (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	Dose Modifications	Toxicity Management
Any Grade	Note: Dose modifications are not required for AEs not deemed to be related to study treatment (i.e., events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant.	Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Treat accordingly, as per institutional standard.
Grade 2-3	Hold study drug/study regimen until resolution to ≤Grade 1 or baseline.	Treat accordingly, as per institutional standard.
Grade 4	Discontinue study drug/study regimen (Note: For Grade 4 labs, decision to discontinue should be based on accompanying clinical signs/symptoms, the Investigator's clinical judgment, and consultation with the Sponsor.).	Treat accordingly, as per institutional standard.

Note: As applicable, for early phase studies, the following sentence may be added: "Any event greater than or equal to Grade 2, please discuss with Clinical Study Lead."

16.4 APPENDIX D: COMPLETE LIST OF PERIPHERAL IMMUNE CELL SUBSETS[31]

Complete list of 123 peripheral immune cell subsets analyzed by flow cytometry. Nine classic subsets were identified as well as 114 refined subsets relating to maturation and function within the classic subsets.

1. Total CD4$^+$ T cells
- PD-L1$^+$ CD4
- PD-1$^+$ CD4
- EOMES$^+$ CD4
- TCR$^+$ CD4
- Tbet$^+$ CD4
- BATF$^+$ CD4
- CTLA-4$^+$ CD4
- Tim-3$^+$ CD4
- ICOS$^+$ CD4
  - PD-L1$^+$ ICOS$^+$ CD4
  - PD-1$^+$ ICOS$^+$ CD4
- Total naïve (CCR7$^+$CD45RA$^+$) CD4
  - PD-L1$^+$ naïve CD4
  - PD-1$^+$ naïve CD4
  - CTLA-4$^+$ naïve CD4
  - Tim-3$^+$ naïve CD4
- Total central memory (CCR7$^+$ CD45RA$^-$) CD4
  - PD-L1$^+$ CM CD4
  - PD-1$^+$ CM CD4
  - CTLA-4$^+$ CM CD4
  - Tim-3$^+$ CM CD4
- Total effector memory (CCR7$^-$ CD45RA$^-$) CD4
  - PD-L1$^+$ EM CD4
  - PD-1$^+$ EM CD4
  - CTLA-4$^+$ EM CD4
  - Tim-3$^+$ EM CD4
- Total EMRA (CCR7$^-$CD45RA$^+$) CD4
  - PD-L1$^+$ EMRA CD4
  - PD-1$^+$ EMRA CD4
  - CTLA-4$^+$ EMRA CD4
  - Tim-3$^+$ EMRA CD4

2. Total CD8$^+$ T cells
- PD-L1$^+$ CD8
- PD-1$^+$ CD8
- EOMES$^+$ CD8
- TCR$^+$ CD8
- Tbet$^+$ CD8
- BATF$^+$ CD8
- CTLA-4$^+$ CD8
- Tim-3$^+$ CD8
- Total naïve (CCR7$^+$CD45RA$^+$) CD8
  - PD-L1$^+$ naïve CD8
  - PD-1$^+$ naïve CD8
  - CTLA-4$^+$ naïve CD8
  - Tim-3$^+$ naïve CD8
- Total central memory (CCR7$^+$CD45RA$^-$) CD8
  - PD-L1$^+$ CM CD8
  - PD-1$^+$ CM CD8
  - CTLA-4$^+$ CM CD8
  - Tim-3$^+$ CM CD8
- Total effector memory (CCR7$^-$ CD45RA$^-$) CD8
  - PD-L1$^+$ EM CD8
  - PD-1$^+$ EM CD8
  - CTLA-4$^+$ EM CD8
  - Tim-3$^+$ EM CD8
- Total EMRA (CCR7$^-$CD45RA$^+$) CD8
  - PD-L1$^+$ EMRA CD8
  - PD-1$^+$ EMRA CD8
  - CTLA-4$^+$ EMRA CD8
  - Tim-3$^+$ EMRA CD8

3. Total Tregs
- PD-L1$^+$ Tregs
- PD-1$^+$ Tregs
- CTLA-4$^+$ Tregs
- ICOS$^+$ Tregs
- CD45RA$^+$ Tregs
- CD49d$^-$ Tregs

4. Total B cells
- PD-L1$^+$ B cells
- PD-1$^+$ B cells
- CTLA-4$^+$ B cells
- Tim-3$^+$ B cells

5. Total NK
- PD-L1$^+$ NK
- PD-1$^+$ NK
- Tim-3$^+$ NK
- Total mature (CD16$^+$ CD56^{dim}) NK
  - PD-L1$^+$ mature NK
  - PD-1$^+$ mature NK
  - Tim-3$^+$ mature NK
- Total functional intermediate (CD16$^+$ CD56^{br}) NK
  - PD-L1$^+$ functional intermediate NK
  - PD-1$^+$ functional intermediate NK
  - Tim-3$^+$ functional intermediate NK
- Total immature (CD16$^-$ CD56^{br}) NK
  - PD-L1$^+$ immature NK
  - PD-1$^+$ immature NK
  - Tim-3$^+$ immature NK
- Total unconventional (CD16$^-$ CD56^{dim}) NK
  - PD-L1$^+$ unconventional NK
  - PD-1$^+$ unconventional NK
  - Tim-3$^+$ unconventional NK

6. Total NK-T
- PD-L1$^+$ NK-T
- PD-1$^+$ NK-T
- Tim-3$^+$ NK-T

7. Total cDC
- PD-L1$^+$ cDC
- PD-1$^+$ cDC
- CD83$^+$ cDC
- Tim-3$^+$ cDC

8. Total pDC
- PD-L1$^+$ pDC
- PD-1$^+$ pDC
- CD83$^+$ pDC
- Tim-3$^+$ pDC

9. Total MDSC
- PD-L1$^+$ MDSC
- PD-1$^+$ MDSC
- CD16$^+$ MDSC
- Total monocytic (CD14$^+$ CD15$^-$) MDSC
  - PD-L1$^+$ mMDSC
  - PD-1$^+$ mMDSC
  - CD16$^+$ mMDSC
- Total granulocytic (CD14$^-$ CD15$^+$) MDSC
  - PD-L1$^+$ gMDSC
  - PD-1$^+$ gMDSC
  - CD16$^+$ gMDSC
- Total lineage negative (CD14$^-$ CD15$^-$) MDSC
  - PD-L1$^+$ lin neg MDSC
  - PD-1$^+$ lin neg MDSC
  - CD16$^+$ lin neg MDSC

BATF, basic leucine zipper transcription factor ATF-like; cDC, conventional dendritic cells; CM, central memory; CTLA-4, cytotoxic T lymphocyte-associated protein-4; EM, effector memory; EMRA, terminally differentiated effector memory; EOMES, eomesodermin; FoxP3, forkhead box P3; gMDSCs, granulocytic mononuclear derived suppressor cells; ICOS, inducible T cell co-stimulator; lin neg MDSCs, lineage negative MDSCs; mMDSCs, monocytic MDSCs; NK, natural killer; pDC, plasmacytoid DC; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; Tbet, T box expressed in T cells; TCR, T cell receptor; Tim-3, T cell immunoglobulin and mucin domain-3; Tregs, regulatory T cells.

16.5 APPENDIX E: URINE COLLECTION

The following protocol is used in the Linehan Laboratory:

Urine Protocol

A. Urine will be collected at room temperature from patients through voiding or instrumentation and transported to laboratory staff on ice. Collection will be 30-100 mL of urine in a sterile urine container.
B. Centrifuge urine at 1200xg (2491 rpm for 20 minutes using our 5810R Eppendorf Centrifuge) at 4°C in 50 mL conical tube.
C. Filter the supernatant (cell-free urine) with .45 µm filter to remove large debris. Save cell pellet.
D. Store filtered urine as cell-free, filtered urine in 6 vials (1.8 mL cryovial each) at -80°C without any cryopreservative.
E. Resuspend urine cell pellet in 1 mL of 5% DMSO (95% MEM media) and transfer to 1.8 mL cryovial tube.
F. Spin this cryovial at 1200xg for 20 minutes at 4°C and leave cell pellet packed.
G. Do slow freeze of cell pellet with Mr. Frosty and then store at -80.
H. Schematic: