Phase 2 Trial of Tremelimumab in Patients with Metastatic Urothelial Cancer Previously Treated with PD-1/PD-L1 Blockade

Sponsor Investigator
Matthew D. Galsky, MD
Icahn School of Medicine at Mount Sinai
Tisch Cancer Institute

Co-Principal Investigator
Neeraj Agarwal, MD
University of Utah School of Medicine

Statistician
Menggang Yu, PhD

Trial Management Provided by
Hoosier Cancer Research Network, Inc.
500 N. Meridian, Suite 100
Indianapolis, IN 46204

Trial Supported by
AstraZeneca
ESR-17-12779

Investigational New Drug (IND) # 138594

Initial Protocol Version Date: 02JUL2018
Protocol Amendment Version Date:
28MAR2019

PROTOCOL SIGNATURE PAGE

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VERSION DATE: 28MAR2019

I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable institutional review board(s).

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Signature of Site Investigator Date

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Site Investigator Name (printed)

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Site Investigator Title

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Name of Facility

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Location of Facility (City and State)

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SYNOPSIS

TITLE	Phase 2 Trial of Tremelimumab in Patients with Metastatic Urothelial Cancer Previously Treated with PD-1/PD-L1 Blockade
SHORT TITLE	Phase 2 Trial of Tremelimumab in previously treated urothelial cancer
PHASE	2
OBJECTIVES	Primary Objective: Estimate the objective response rate (according to RECIST 1.1) with tremelimumab in subjects with metastatic urothelial cancer previously treated with PD-1/PD-L1 blockade Secondary Objectives: • Describe the safety of treatment • Describe the disease control rate (objective response + stable disease) • Describe the duration of response • Describe the progression-free survival • Describe the overall survival Correlative/Exploratory Objectives: • Explore the relationship between PD-L1 expression and response to treatment • Explore the relationship between gene expression signatures and response to treatment • Explore the relationship between genomic alterations including mutational load and response to treatment • Explore the impact of treatment on the peripheral blood immune cells and other circulating biomarkers • Explore the relationship between the microbiome and adverse events and outcomes with treatment
STUDY DESIGN	This is a phase II trial designed to estimate the activity of single agent tremelimumab in subjects with metastatic urothelial cancer with disease progression despite prior treatment with PD-1/PD-L1 blockade. The primary endpoint is objective response rate and the study will employ a Simon's 2-stage design.
KEY ELIGIBILITY CRITERIA; See Section 3 for ALL eligibility criteria	1. Histologically or cytologically documented urothelial cancer. Locally advanced (T4b, any N; or any T, N 2−3) or metastatic disease (M1, Stage IV) (also termed TCC or UCC of the urinary tract; including renal pelvis, ureters, urinary bladder, and urethra) Subjects with mixed histologies are eligible provided that the predominant component is urothelial cancer. Locally advanced bladder cancer

	must be inoperable on the basis of involvement of pelvic sidewall or adjacent viscera (clinical Stage T4b) or bulky nodal metastasis (N2−N3). 2. Representative formalin-fixed paraffin-embedded (FFPE) tumor specimens in paraffin blocks (blocks preferred) or at least 15 unstained slides. If archival tissue is not available and the subject is undergoing a standard of care biopsy, tissue from the biopsy is required to be submitted for correlative analyses. Subjects without adequate baseline tumor tissue may be considered for enrollment on a case by case basis after discussion with the sponsor-investigator. 3. Measurable disease according to RECIST 1.1 within 28 days prior to registration. At least 1 lesion, not previously irradiated, that can be accurately measured at baseline as ≥10 mm in the longest diameter (except lymph nodes, which must have short axis ≥15 mm) with computed tomography (CT) (preferred) or magnetic resonance imaging (MRI) scans, preferably with IV contrast, and that is suitable for accurate repeated measurements as per RECIST 1.1 guidelines; lesions in a previously irradiated field can be used as a measurable disease provided that there has been demonstrated progression in the lesion 4. A subject with prior brain metastasis may be considered if they have completed their treatment for brain metastasis at least 4 weeks prior to study registration, have been off of corticosteroids for ≥ 2 weeks, and are asymptomatic 5. Subjects must have progressed despite prior treatment with anti-PD-1/PD-L1 antibody therapy. Subjects must not have progressed within 2 months of starting prior anti-PD-1/PD-L1 antibody therapy. Subjects must have received at least 1 line of prior systemic therapy. Subjects must not have experienced a toxicity that led to permanent discontinuation of prior immunotherapy. All AEs while receiving prior immunotherapy must have completely resolved or resolved to baseline prior to screening for this study with the exception of endocrine related AEs that are stable on replacement therapy (e.g., steroids, thyroid hormone) which may be considered eligible but must be discussed with the sponsor-investigator. 6. Prior cancer treatment must be completed at least 28 days or 5 half-lives (whichever is shorter) prior to first dose of study drug. Subjects must have recovered from all reversible acute toxic effects of the regimen (other than alopecia) to ≤ Grade 1 or baseline. 7. Adequate organ function per screening labs

	8. Cannot have QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥470 ms calculated. Any clinically significant abnormalities detected require triplicate ECG results and a mean QTcF< 470 ms calculated from 3 ECGs obtained over a brief period (eg, 30 minutes)
STATISTICAL CONSIDERATIONS	A Simon's 2-stage design will be applied. The null hypothesis that the true response rate is 2% will be tested against a one-sided alternative. In the first stage, 18 subjects will be accrued. If there are 0 responses in these 18 subjects, enrollment will be stopped. Otherwise, 10 additional subjects will be accrued for a total of 28. The null hypothesis will be rejected if 3 or more responses (complete/partial) are observed in 28 subjects and further investigation will be warranted. This design yields a type I error rate of 0.02 and power of 80% when the true response rate is 15% or larger.
TOTAL NUMBER OF SUBJECTS	N = Approximately 28
ESTIMATED ENROLLMENT PERIOD	Estimated 12 months
ESTIMATED STUDY DURATION	Estimated 24 months

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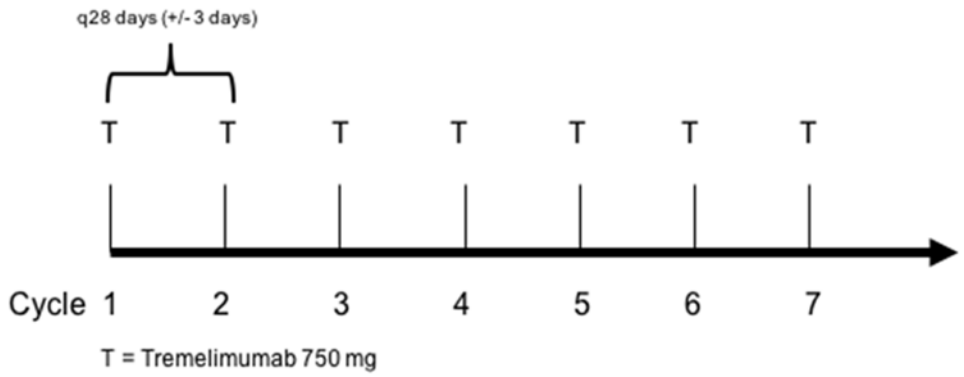
SCHEMA



1. BACKGROUND AND RATIONALE

1.1 Disease Background

Each year in the United States, over 60,000 patients succumb to metastatic urothelial cancer. Standard first-line treatment for metastatic urothelial cancer includes platinum-based chemotherapy, regimens characterized by a relatively high response proportion but for the majority of patients, insufficient response durations. Given that a large subset of patients with metastatic urothelial cancer treated with first-line platinum based chemotherapy regimens are candidates for further treatment, several novel therapeutic approaches have been explored over the last several decades. However, until 2016, there had been no treatments approved in this setting by the United States Food and Drug Administration. The development of immune checkpoint blockade has recently changed the landscape of treatment for metastatic urothelial cancer.

1.2 Immune checkpoint blockade in advanced urothelial cancer

1.2.1 PD-1/PD-L1 blockade in advanced urothelial cancer

Several phase 1-3 trials have now established the activity and safety of PD-1/PD-L1 blockade in patients with metastatic urothelial cancer (Table 1). Collectively, these trials demonstrate durable responses are achieved in approximately 15-25% of patients with metastatic urothelial cancer with grade 3-4 adverse events occurring in approximately 5-15% of patients. Based on these findings, as of 5/2017, five PD-1/PD-L1 inhibitors had been approved by the United States Food and Drug Administration for the treatment of patients with metastatic urothelial cancer and has been integrated into standard therapy.

Table 1. Major trials exploring PD-1/PD-L1 blockade in patients with metastatic urothelial cancer

Reference	Drug	Antibody	Target	Setting	Phase	N	Response Rate
Balar, Lancet, 2016[1]	Atezolizumab	Humanized IgG1	PDL-1	First line cis-ineligible	Phase II	119	24%
Balar, ESMO, 2016[2]	Pembrolizumab	Human IgG4	PD-1	First line cis-ineligible	Phase II	100	24%
Rosenberg, Lancet, 2016[3]	Atezolizumab	Humanized IgG1	PDL-1	Post platinum	Phase II	310	16%
Sharma, Lancet Oncology, 2016[4]	Nivolumab	Human IgG4	PD-1	Post platinum	Phase II	270	20%
Bellmunt, NEJM, 2017[5]	Pembrolizumab	Human IgG4	PD-1	Post platinum	Phase II	270	21%
Plimack, Lancet Oncology, 2017[6]	Pembrolizumab	Human IgG4	PD-1	Post platinum	Phase I basket	29	28%
Massard, JCO, 2016[7]	Durvalumab	Human IgG1	PDL-1	Post platinum	Phase I basket	42	31%
Apolo, JCO, 2017[8]	Avelumab	Human IgG1	PD-L1	Post platinum	Phase I basket	44	16%

1.2.2 CTLA-4 blockade in advanced urothelial cancer

Though extensive studies have rapidly established proof of concept for immune checkpoint blockade with PD-1/PD-L1 blockade in advanced urothelial cancer leading to integration into standard treatment, CTLA-4 blockade has been understudied in this disease. Blocking the interaction between CTLA-4 expressed on T lymphocytes, and B7 family molecules expressed on antigen-presenting cells, has been shown to augment T cell activation, proliferation, and anti-tumor immunity. In a syngeneic murine bladder cancer model, CTLA-4 blockade induced tumor regression, improved survival, and increased levels of tumor-reactive T cells.[9]

A "window of opportunity" study explored the pharmacodynamic effects of the CTLA-4 antibody, ipilimumab, administered prior to radical cystectomy in 12 patients with localized invasive urothelial cancer of the bladder.[10] This study demonstrated infiltration of T cells in the post-ipilimumab cystectomy specimens. A phase II trial has explored gemcitabine, cisplatin, plus ipilimumab in patients with metastatic urothelial cancer.[11] This study demonstrated pharmacodynamics effects and unusual response kinetics suggestive of benefit with the addition of CTLA-4 blockade.

Preliminary results of the first study to explore single-agent CTLA-4 blockade in patients with metastatic urothelial cancer were reported at the Society for Immunotherapy of Cancer 2017 meeting. Sharma et al reported the results of a phase 2 multicenter study exploring tremelimumab monotherapy in patients with advanced solid tumors, including patients with advanced urothelial cancer (NCT02527434).[12] Among 32 patients with metastatic urothelial cancer enrolled, the confirmed objective response rate was 18.8% (95% CI 7.2-36.4) with 2 complete responses and 4 partial responses; the median duration of response was not estimable and all patients with confirmed CR or PR had disease control at 12 months.

1.2.3 CLTA-4 blockade may be non-cross resistant with single agent PD-1/PD-L1 blockade in advanced urothelial cancer

Given non-overlapping mechanisms of action, and potential synergy, emerging data suggest that single agent CTLA-4 blockade may be active in patients with disease progression despite prior single-agent PD-1/PD-L1 blockade. Such findings are critical as only 15-25% of patients with metastatic urothelial cancer respond to single-agent PD-1/PD-L1 blockade and the development of additional regimens with the potential to induce durable responses in this disease is urgently needed.

Preliminary results of a phase 2 study of ipilimumab added to nivolumab, in patients with metastatic urothelial cancer progressing despite single-agent nivolumab, were presented at the 2017 ASCO Genitourinary Cancer Symposium.[13] Ten patients who were refractory to nivolumab monotherapy were treated with the combination of ipilimumab (1 mg/kg) and nivolumab (3 mg/kg). Among these 10 patients, 1 patient achieved a partial response and three additional patients achieved stable disease.

Taken together, the findings outlined above support the need to further dissect the potential role of CTLA-4 blockade alone in patients with metastatic urothelial cancer progressing despite prior treatment with a PD-1/PD-L1 inhibitor.

1.3 Tremelimumab

Tremelimumab, a CTLA-4 mAb of the immunoglobulin G 2 kappa isotype, is an immunomodulatory therapy that is being developed by AstraZeneca for use in the treatment of cancer. CTLA-4 is a critical regulatory signal for T-cell expansion and activation following an immune response, and it serves as a natural braking mechanism that maintains T-cell homeostasis. During T-cell activation, T-cells upregulate CTLA-4, which binds to B7 ligands on APCs, sending an inhibitory signal that limits T-cell activation. Tremelimumab blocks the inhibitory signal resulting from CTLA-4 binding to B7, leading to indirect prolongation and enhancement of T-cell activation and expansion. Thus, the mechanism of action of tremelimumab is indirect and is applied through enhancing T-cell-mediated immune response.

An extensive program of non-clinical and clinical studies has been conducted for tremelimumab both as monotherapy and combination therapy with conventional anticancer agents to support various cancer indications using different dose schedules. To date tremelimumab has been given to more than 1500 patients as part of ongoing studies either as monotherapy or in combination with other anticancer agents. Details on the safety profile of tremelimumab monotherapy are summarized in Section. Refer to the current tremelimumab Investigator's Brochure for a complete summary of non-clinical and clinical information including safety, efficacy and pharmacokinetics. Refer to the tremelimumab Investigator's Brochure for a complete summary of non-clinical and clinical information.

1.4 Rationale

A prospective trial to assess the activity of tremelimumab in patients with metastatic urothelial cancer progressive despite prior PD-1/PD-L1 blockade is supported by the following lines of evidence:

1. Immune checkpoint blockade has changed the landscape of treatment for metastatic urothelial cancer, a disease without prior treatment advances in decades.
2. Single-agent PD-1/PD-L1 blockade results in durable responses in ~15-20% of patients with metastatic urothelial cancer.
3. Single-agent tremelimumab has shown similar response proportions to PD-1/PD-L1 blockade in metastatic urothelial cancer in a small phase 2 study.
4. The mechanism of action of CTLA-4 blockade and PD-1/PD-L1 blockade are non-overlapping and at least additive activity has been observed with combination therapy across several malignancies.
5. Treatment of patients with metastatic urothelial cancer progressing despite prior PD-1/PD-L1 blockade is a major unmet need.

1.5 Rationale for dose/schedule

An extensive program of non-clinical and clinical studies has been conducted for tremelimumab both as monotherapy and combination therapy with conventional anticancer agents to support various cancer indications using different dose schedules. A total of 34 sponsored clinical studies have been conducted as part of the tremelimumab clinical development program. Of these, 13 studies have completed and 21 are ongoing. Eight tremelimumab monotherapy studies have been completed and 3 are ongoing. As of the data cutoff date of 24 January 2016, 962 patients received tremelimumab in completed monotherapy studies and 380 patients in the Phase IIb

monotherapy study (D4880C00003; DETERMINE). Study D4881C00024 is an ongoing rollover study, which provides continued access to treatment to 37 patients treated in prior completed studies. In the third ongoing monotherapy study (D4884C00001), 44 patients have been treated as of the data cutoff date. In addition, approximately 80 patients have been treated with tremelimumab in monotherapy arms of combination studies. Five studies of tremelimumab in combination with other anticancer agents have been completed and 18 are ongoing. In total, approximately 250 patients with a variety of tumor types have received tremelimumab in combination with other anticancer agents in these studies.

In patients, tremelimumab exhibits linear (dose-proportional) PK following IV infusion. The estimate of clearance (CL), volume of distribution at steady state (Vss), and terminal-phase half-life is 0.132 mL/h/kg, 81.2 mL/kg and 22.1 days, respectively. These values are consistent with those of natural IgG2.

Across the clinical development program for tremelimumab, a pattern of efficacy has emerged that is similar to that of the related anti-CTLA-4 antibody, ipilimumab. In patients who respond, the responses are generally durable, lasting several months even in patients with aggressive tumors such as refractory metastatic melanoma. Some patients may have what is perceived to be progression of their disease in advance of developing disease stabilization or a tumor response.

The target trough concentration of tremelimumab is estimated to be approximately 30 μg/mL, based on enhanced interleukin (IL)-2 release (in vitro) and antitumor activity (in vivo) in preclinical studies. PK simulations indicate that, following a dose of 10 mg/kg q4w for 6 months, approximately 90% of patients are expected to be above this target level of 30 μg/mL during the induction phase. Tremelimumab at a dose of 10 mg/kg q4w for 6 months followed by 10 mg/kg every 90 days is expected to yield PK exposures similar to those of the related anti-CTLA-4 mAb ipilimumab, given at a dose of 10 mg/kg every 3 weeks (q3w) for 4 cycles (12 weeks) followed by 10 mg/kg every 3 months (beginning on Week 24), the dosing regimen that was tested in the pivotal first-line melanoma trial.

Retrospective analyses of the Phase I and II tremelimumab melanoma studies show an improvement in OS for patients who were able to achieve a higher tremelimumab exposure, as measured by the area under the plasma drug concentration-time curve (AUC). The median OS was significantly longer in the high-AUC group (15.3 months; N=164) versus the low- AUC group (6.0 months; N=163) (based on the median value of AUC [103570 μ.h /mL]). This difference in OS corresponds to a hazard ratio (HR) of 0.41 ($p<0.001$), and the estimated survival rates in these 2 groups were 59% versus 29%, respectively, at 1 year. Additionally, a retrospective exposure and survival analysis of 293 patients treated with tremelimumab in a Phase III study in patients with melanoma showed better OS in patients with higher exposure. The median OS was 18.4 months for the high-AUC group (≥123665 μg.h /mL) compared to 9.0 months for the low-AUC group (<123665 μ.h/mL) (HR 0.5; $p<0.001$). Tremelimumab monotherapy at a dose of 750 mg q4w for 7 doses (cycles) followed by 750 mg q12w for 2 additional doses (cycles) is equivalent to 10 mg/kg tremelimumab monotherapy with the same dosing schedule. Therefore, the current study will utilize the following dosing regimen: Tremelimumab 750 mg once every 4 weeks for up to 7 doses.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

Estimate the objective response rate (RECIST 1.1) with tremelimumab in subjects with metastatic urothelial cancer previously treated with PD-1/PD-L1 blockade

2.1.2 Secondary Objectives

- Describe the safety of the study treatment
- Describe the disease control rate (objective response + stable disease)
- Describe the duration of response
- Describe the progression-free survival
- Describe the overall survival

2.1.3 Correlative/Exploratory Objectives

- Explore the relationship between PD-L1 expression and response to treatment
- Explore the relationship between gene expression signatures and response to treatment
- Explore the relationship between genomic alterations including mutational load and response to treatment
- Explore the impact of treatment on the peripheral blood immune cells and other circulating biomarkers
- Explore the relationship between the microbiome and adverse events and outcomes with treatment

2.2 Endpoints

2.2.1 Primary Endpoint

Objective Response rate as determined by RECIST 1.1

2.2.2 Secondary Endpoints

- Safety will be determined according to the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03
- The disease control rate is the rate of objective response and stable disease as determined by RECIST 1.1
- Duration of response will be the time from the first documentation of response to the time of progression
- Progression-free survival which is defined as the time from treatment initiation to death or progression, depending on which occurs first
- Overall survival is defined as the time from treatment initiation to death

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

1. Written informed consent and HIPAA authorization for release of personal health information prior to registration. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately.

2. Age ≥ 18 years at the time of consent.

3. ECOG Performance Status of 0 or 1 within 14 days prior to registration.

4. Histologically or cytologically documented urothelial cancer. Locally advanced (T4b, any N; or any T, N 2−3) or metastatic disease (M1, Stage IV) (also termed TCC or UCC of the urinary tract; including renal pelvis, ureters, urinary bladder, and urethra) Subjects with mixed histologies are eligible provided that the predominant component is urothelial cancer. Locally advanced bladder cancer must be inoperable on the basis of involvement of pelvic sidewall or adjacent viscera (clinical Stage T4b) or bulky nodal metastasis (N2−N3).

5. Representative formalin-fixed paraffin-embedded (FFPE) tumor specimens in paraffin blocks (blocks preferred) or at least 15 unstained slides. If archival tissue is not available and the subject is undergoing a standard of care biopsy, tissue from the biopsy is required to be submitted for correlative analyses. Subjects without adequate baseline tumor tissue may be considered for enrollment on a case by case basis after discussion with the sponsor-investigator.

6. Measurable disease according to RECIST 1.1 within 28 days prior to registration. At least 1 lesion, not previously irradiated, that can be accurately measured at baseline as ≥10 mm in the longest diameter (except lymph nodes, which must have short axis ≥15 mm) with computed tomography (CT) (preferred) or magnetic resonance imaging (MRI) scans, preferably with IV contrast, and that is suitable for accurate repeated measurements as per RECIST 1.1 guidelines; lesions in a previously irradiated field can be used as a measurable disease provided that there has been demonstrated progression in the lesion.

7. A subject with prior brain metastasis may be considered if they have completed their treatment for brain metastasis at least 4 weeks prior to study registration, have been off of corticosteroids for ≥ 2 weeks, and are asymptomatic

8. Subjects must have progressed despite prior treatment with anti-PD-1/PD-L1 antibody therapy. In addition, subjects must meet the following criteria:
    - Subjects must not have progressed within 2 months of starting prior anti-PD-1/PD-L1 antibody therapy.
    - Subjects must have received at least 1 line of prior systemic therapy.
    - Must not have experienced a toxicity that led to permanent discontinuation of prior immunotherapy.

- All AEs while receiving prior immunotherapy must have completely resolved or resolved to baseline prior to screening for this study with the exception of endocrine related AEs that are stable on replacement therapy (e.g., steroids, thyroid hormone) which may be considered eligible but must be discussed with the sponsor-investigator.
- Must not have experienced a ≥ Grade 3 immune related AE or an immune related neurologic (neuro-muscular) or ocular AE of any grade while receiving prior immunotherapy. **NOTE**: Subjects with endocrine AE of ≤ Grade 2 are permitted to enroll if they are stably maintained on appropriate replacement therapy and are asymptomatic. Must not have required the use of additional immunosuppression other than corticosteroids for the management of an AE, not have experienced recurrence of an AE if re-challenged, and not currently require maintenance doses of > 10 mg prednisone or equivalent per day.
- Patients with Gr 3 AST/ALT elevation < 8 fold that resolved with steroids without additional immunosuppression can be included (Patients who experienced Hy's law on PD-1/L1 therapy will be excluded)

9. Prior cancer treatment must be completed at least 28 days or 5 half-lives (whichever is shorter) prior to first dose of study drug. Subjects must have recovered from all reversible acute toxic effects of the regimen (other than alopecia) to ≤ Grade 1 or baseline.

10. Demonstrate adequate organ function as defined in the table below. All screening labs to be obtained within 14 days prior to registration.

System	Laboratory Value
Hematological	
Absolute Neutrophil Count (ANC)	≥ 1500/mm^3
Hemoglobin (Hgb)	≥ 9 g/dL
Renal	
Calculated creatinine clearance[1] or creatinine	≥ 30 cc/min or creatinine ≤ 1.5
Hepatic	
Bilirubin	≤ 1.5 × upper limit of normal (ULN) This will not apply to subjects with confirmed Gilbert's syndrome (persistent or recurrent hyperbilirubinemia [predominantly unconjugated bilirubin] in the absence of evidence of hemolysis or hepatic pathology), who may be considered for enrollment after discussion with the Sponsor Investigator
Aspartate aminotransferase (AST)	≤ 2.5 × ULN (≤ 5 x ULN for subjects with hepatic metastases
Alanine aminotransferase (ALT)	≤ 2.5 × ULN (≤ 5 x ULN for subjects with hepatic metastases

1 Cockcroft-Gault formula will be used to calculate creatinine clearance or 24 urine collection for creatinine can be utilized

11. Evidence of postmenopausal status or negative urinary or serum pregnancy test for female premenopausal subjects. Women will be considered postmenopausal if they have been amenorrheic for 12 months without an alternative medical cause. The following age-specific requirements apply:
    - Women < 50 years of age would be considered postmenopausal 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 postmenopausal range for the institution or underwent surgical sterilization (bilateral oophorectomy, bilateral salpingectomy, or hysterectomy).
    - Women ≥ 50 years of age would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments, had radiation-induced oophorectomy with last menses >1 year ago, had chemotherapy-induced menopause with >1 year interval since last menses, or underwent surgical sterilization (bilateral oophorectomy, bilateral salpingectomy, or hysterectomy)

12. Females of childbearing potential who are sexually active with a non-sterilized male partner must be willing to abstain from heterosexual activity or to use 1 highly of effective method of contraception from the time of informed consent until 90 days after the last dose of tremelimumab. Non-sterilized male partners of a female patient must use male condom plus spermicide throughout this period. See Table 2 for acceptable contraceptive methods.

13. Non-sterilized males who are sexually active with a female partner of childbearing potential must use a male condom plus spermicide from screening through 90 days after receipt of the final dose of tremelimumab. Female partners (of childbearing potential) of male subjects must also use a highly effective method of contraception throughout this period.

14. Must have life expectancy of ≥ 12 weeks.

3.2 Exclusion Criteria

1. Pregnant or breastfeeding (**NOTE:** breast milk cannot be stored for future use while the mother is being treated on study).

2. Known additional malignancy that is active and/or progressive requiring treatment. Patients with incidental histologic findings of prostate cancer (tumor/node/metastasis stage of T1a or T1b or prostate-specific antigen <10) who have not received hormonal treatment may be included, pending a discussion with the sponsor-investigator

3. Treatment with any investigational drug within 28 days prior to registration.

4. Prior treatment with an anti-CTLA-4 antibody, including tremelimumab

5. Any unresolved toxicity National Cancer Institute (NCI) CTCAE Version 4.03 Grade ≥ 2 from previous anticancer therapy with the exception of alopecia, vitiligo, and 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 Sponsor Investigator
- Subjects with irreversible toxicity not reasonably expected to be exacerbated by treatment with tremelimumab (e.g., hearing loss) may be included after consultation with the sponsor-investigator

6. Any concurrent chemotherapy, investigational product, biologic, or hormonal therapy for cancer treatment. Concurrent use of hormonal therapy for non-cancer related conditions (e.g., hormone replacement therapy) is acceptable. Note: Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable (e.g., local surgery or radiotherapy)

7. Radiation therapy within 14 days of first dose of study drug

8. Major surgical procedure within 28 days prior to first dose of study treatment

9. History of allogeneic organ transplantation that requires use of immunosuppressive agents

10. Active or prior documented autoimmune of inflammatory disorders (including but not limited to inflammatory bowel disease [eg, colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome [granulomatosis with polyangiitis], Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc). The following are exceptions to this criterion:
    - Subjects with vitiligo
    - Subjects with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement
    - Any chronic skin condition that does not require systemic therapy
    - Subjects without active disease in the last 5 years may be considered for enrollment after discussion with the sponsor-investigator
    - Subjects with celiac disease controlled by diet alone may be considered for enrollment after discussion with the sponsor-investigator

11. QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥470 ms calculated. Any clinically significant abnormalities detected require triplicate ECG results and a mean QTcF <470 ms calculated from 3 ECGs obtained over a brief period (eg, 30 minutes)

12. Past medical history of Interstitial Lung Disease (ILD), drug-induced ILD, radiation pneumonitis which required steroid treatment, or any evidence of clinically active interstitial lung disease.

13. History of active primary immunodeficiency

14. Active infection, including tuberculosis (clinical evaluation that includes clinical history, physical examination and radiographic findings, and tuberculosis testing in line with local practice), hepatitis B, hepatitis C, or human immunodeficiency virus (HIV, positive HIV 1 or 2 antibodies). Active hepatitis B virus (HBV) infection is defined by a positive HBV surface antigen (HBsAg) result. Subjects with a past or resolved HBV infection (defined as the presence of hepatitis B core IgG antibody and the absence of HBsAg, deoxyribonucleic acid [DNA] negative) are eligible. Subjects positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV ribonucleic acid (RNA).

15. Current or prior use of immunosuppressive medication within 14 days before the first dose of tremelimumab. The following are exceptions to this criterion:
    - Intranasal, inhaled, topical steroids, or local steroid injections (eg, intra-articular injection)
    - Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or its equivalent
    - Steroids as premedication for hypersensitivity reactions (eg, CT scan premedication)

16. Receipt of live attenuated vaccine within 30 days prior to the first dose of study treatment. **NOTE:** Subjects, if enrolled, should not receive live vaccine during the study and up to 30 days after the last dose of tremelimumab.

17. Known allergy or hypersensitivity to tremelimumab or any investigation product excipient, or to other humanized mAbs

18. Weight ≤ 35 kg

3.3 Re-Treatment with Tremelimumab Criteria

Inclusion Criteria

1. ECOG Performance Status of 0 or 1 within 14 days prior to re-treatment.

2. Demonstrate adequate organ function as defined below. All labs to be obtained within 14 days prior to re-treatment.
    - Absolute Neutrophil Count (ANC): ≥ 1500/mm^3
    - Hemoglobin (Hgb): ≥ 9 g/dL
    - CrCl or creatinine: ≥ 30 cc/min or creatinine ≤ 1.5
    - AST and ALT: ≤ 2.5 × ULN (≤ 5 x ULN for subjects with hepatic metastases

    [1]Cockcroft-Gault formula will be used to calculate creatinine clearance or 24 urine collection for creatinine can be utilized

3. Evidence of postmenopausal status or negative urinary or serum pregnancy test for female premenopausal subjects prior to re-treatment.

4. Females of childbearing potential who are sexually active with a non-sterilized male partner must be willing to abstain from heterosexual activity or to use 1 highly of effective method of

contraception from the time of informed consent until 90 days after the last dose of tremelimumab. Non-sterilized male partners of a female patient must use male condom plus spermicide throughout this period.

5. Non-sterilized males who are sexually active with a female partner of childbearing potential must use a male condom plus spermicide from screening through 90 days after receipt of the final dose of tremelimumab. Female partners (of childbearing potential) of male subjects must also use a highly effective method of contraception throughout this period.

6. Must have life expectancy of ≥ 12 weeks.

Exclusion Criteria

1. No significant, unacceptable, or irreversible toxicities that indicate re-treatment will not benefit the subject

2. Active autoimmune or inflammatory disorders (including but not limited to inflammatory bowel disease [eg, colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome [granulomatosis with polyangiitis], Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc). The following are exceptions to this criterion:
    - Subjects with vitiligo
    - Subjects with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement
    - Any chronic skin condition that does not require systemic therapy
    - Subjects without active disease in the last 5 years may be considered for enrollment after discussion with the sponsor-investigator
    - Subjects with celiac disease controlled by diet alone may be considered for enrollment after discussion with the sponsor-investigator

3. QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥470 ms calculated. Any clinically significant abnormalities detected require triplicate ECG results and a mean QTcF <470 ms calculated from 3 ECGs obtained over a brief period (eg, 30 minutes)

4. Active infection

5. Current or prior use of immunosuppressive medication within 14 days before re-treatment with tremelimumab. The following are exceptions to this criterion:
    - Intranasal, inhaled, topical steroids, or local steroid injections (eg, intra-articular injection)
    - Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or its equivalent
    - Steroids as premedication for hypersensitivity reactions (eg, CT scan premedication)

6. Weight ≤ 35 kg

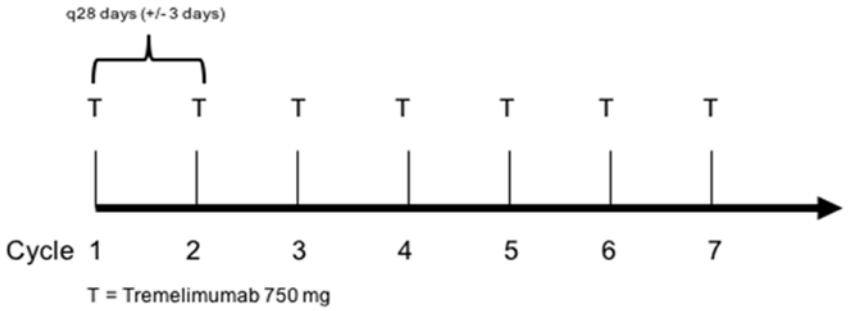
7. Has not received an intervening systemic anticancer therapy after initial treatment discontinuation

4. SUBJECT REGISTRATION

All subjects must be registered through HCRN's electronic data capture (EDC) system. A subject is considered registered when an "On Study" date is entered into the EDC system. Subjects must be registered prior to starting protocol therapy. Subjects must begin therapy **within 7 business days** of registration.

5. TREATMENT PLAN

This is a phase 2 study exploring single-agent tremelimumab in subjects with metastatic urothelial cancer progressive despite prior PD-1/PD-L1 blockade therapy. The study utilizes a Simon 2-stage design. Patients who do not receive drug or who do not complete 1 cycle of therapy for non-treatment related causes (e.g., traffic accidents, trauma etc.) will be replaced.



5.1 Study Drug Administration

There are no pre-medication or hydration requirements

Drug	Dose[1]	Route	Schedule	Cycle Length[2]	Number of cycles
Tremelimumab	750 mg	Intravenously (IV) over 1 hour	Day 1 Cycle 1-7	28 days (± 3 days)	Up to 7 cycles

[1] Fixed dosing will be utilized. However, if a patient's weight falls to 35kg or less, they should be discontinued from study treatment.
[2] A window of ± 5 minutes may be used for infusion of tremelimumab.

5.1.1 Monitoring During Study Drug Infusions

First Infusion: On the first infusion day, subjects will be monitored, and vital signs collected/recorded in eCRF prior to, during and after infusion as presented in the bulleted list below. BP and pulse will be collected from subjects before, during, and after each infusion at the following times (based on a 60-minute infusion):

- Prior to the beginning of the infusion (measured once from approximately 30 minutes before beginning of the infusion)
- Approximately 30 minutes during the infusion (halfway through infusion)
- At the end of the infusion (approximately 60 minutes ±5 minutes)

If the infusion takes longer than 60 minutes, then BP and pulse measurements should follow the principles as described above or be taken more frequently if clinically indicated. A 1-hour

observation period is recommended after the first infusion of tremelimumab.

Subsequent infusions: BP, pulse and other vital signs should be measured, collected/recorded in eCRF prior to the start of the infusion. Subjects should be carefully monitored, and BP and other vital signs should be measured during and post infusion as per institution standard and as clinically indicated. Any clinically significant changes in vital signs should be entered onto an unscheduled vital signs eCRF page.

5.1.2 Treatment Beyond RECIST 1.1 Progression

If there is evidence of RECIST 1.1 defined disease progression within the initial 7 cycles of tremelimumab, subjects may continue protocol therapy provided that none of the following apply:

- Absence of signs and symptoms indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

5.1.3 Re-treatment of Subjects Progressing within 1 Year of Completing Initial Tremelimumab

Patients that complete the initial 7 cycles of tremelimumab then have evidence of disease progression per RECIST 1.1 (with or without confirmation) during the 1-year follow-up period may be eligible for re-treatment with up to 7 additional cycles of tremelimumab. Such patients are required to meet the eligibility criteria outlined in Section 3.3. Testing done during progression may be used for screening prior to re-treatment. Subjects should follow the same schedule of events/procedures for the initial Cycles 1-7 of tremelimumab.

5.2 Concomitant Medications, Treatments, and Restrictions

5.2.1 Allowed Concomitant Medications/Treatments

Medication other than that described below that is considered necessary for the patient's safety and well-being may be given at the discretion of the site investigator and recorded in the eCRF.

Rescue/supportive medication/class of drug	Usage
Concomitant medications or treatments (eg, acetaminophen or diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care, except for those medications identified as "prohibited," as listed above	To be administered as prescribed by the site investigator
Basic Supportive Care (BSC) including antibiotics, nutritional support, correction of metabolic disorders, optimal symptom control, and pain management [including palliative radiotherapy to non-target lesions, etc])	Should be used, when necessary, for all subjects
Inactivated viruses, such as those in the influenza vaccine	Permitted

5.2.2 Prohibited Concomitant Medications/Treatments

Restricted and prohibited concomitant medications are described in the tables.

Prohibited medication/class of drug	Usage
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly while 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 while the patient is on study treatment
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment	Should not be given during the study. (Concurrent use of hormones for non-cancer-related conditions [eg, insulin for diabetes and hormone replacement therapy] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable [eg, by local surgery or radiotherapy])
Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-alpha blockers	Should not be given during the study. Short-term use of immunosuppressive medications, including corticosteroids for the acute management of non-IP emergencies (eg, COPD, asthma) or IP-related emergent AEs, is permitted. In addition, immunosuppressive medication for palliative treatment for oncologic emergencies or prior to imaging procedures in subjects with contrast allergies is acceptable. Use of inhaled, topical, and intranasal corticosteroids is permitted for all treatment arms.
Live attenuated vaccines	Should not be given through 30 days after the last dose of IP
Sunitinib	Should not be given to subjects within 3 months of a dose of tremelimumab, as acute renal failure has been reported with combination therapy of tremelimumab and sunitinib
Drugs with laxative properties	Should be used with caution through 90 days after the last dose of tremelimumab during the study. In case of strong medical need, should be used with caution.
Herbal and natural remedies	Should be avoided during the study.

AE adverse event; CTLA-4 cytotoxic T lymphocyte-associated antigen 4; IP investigational product; PD-1 programmed cell death 1; PD-L1 programmed cell death ligand 1

5.3 Contraception

5.3.1 Female patient of child-bearing potential

Females of childbearing potential are defined as those who are not surgically sterile (ie, bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or post-menopausal. Females of childbearing potential who are sexually active with a non-sterilized male partner must use at least 1 **highly** effective method of contraception (Table 2) from the time of screening and must agree to continue using such precautions for 90 days after the last dose of tremelimumab. Non-sterilized male partners of a female patient must use male condom plus spermicide throughout this period. Cessation of birth control after this point should be discussed with a responsible physician. Not engaging in sexual activity for the total duration of the drug treatment and the drug washout period is an acceptable practice; however, periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control.

Female subjects should also refrain from breastfeeding throughout this period.
Women will be considered post-menopausal if they have been amenorrheic for 12 months without an alternative medical cause. The following age-specific requirements apply:

- 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 or underwent surgical sterilization (bilateral oophorectomy or hysterectomy).
- 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, had chemotherapy-induced menopause with last menses >1 year ago, or underwent surgical sterilization (bilateral oophorectomy, bilateral salpingectomy or hysterectomy).

5.3.2 Male subjects with a female partner of childbearing potential

Non-sterilized males who are sexually active with a female partner of childbearing potential must use a male condom plus spermicide from screening through 90 days after receipt of the final dose of tremelimumab. Not engaging in sexual activity is an acceptable practice; however, occasional abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Male subjects should refrain from sperm donation throughout this period.

Female partners (of childbearing potential) of male subjects must also use a highly effective method of contraception throughout this period (Table 2). Highly effective methods of contraception, defined as one that results in a low failure rate (ie, less than 1% per year) when used consistently and correctly are described in Table 2. Note that some contraception methods are not considered highly effective (eg. male or female condom with or without spermicide; female cap, diaphragm, or sponge with or without spermicide; non-copper containing intrauterine device; progestogen-only oral hormonal contraceptive pills where inhibition of ovulation is not the primary mode of action [excluding Cerazette/desogestrel which is considered highly effective]; and triphasic combined oral contraceptive pills).

Table 2: Highly effective methods of contraception (<1% failure rate)

Barrier/Intrauterine methods	Hormonal Methods
• Copper T intrauterine device • Levonorgestrel-releasing intrauterine system (eg, Mirena®)[a]	• Etonogestrel implants: eg, Implanon or Norplan • Intravaginal device: eg, ethinylestradiol and etonogestrel • Medroxyprogesterone injection: eg, Depo-Provera • Normal and low dose combined oral contraceptive pill • Norelgestromin/ethinylestradiol transdermal system • Cerazette (desogestrel)

[a] This is also considered a hormonal method

5.4 Blood donation

Subjects should not donate blood while participating in this study, or for at least 90 days following the last infusion of tremelimumab.

6. TOXICITIES AND DOSE DELAYS/DOSE MODIFICATIONS

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 will be used to grade adverse events.

Subjects enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in Study Calendar & Evaluations.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation as specified in Study Calendar & Evaluations.

6.1 Infusion Reactions

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 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 (eg, diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the site investigator. If the infusion-related reaction is ≥ Grade 3 or higher in severity, study drug will be discontinued. For management of subjects who experience an infusion reaction, please refer to the toxicity and management guidelines in Appendix 1.

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.

6.2 Dose Delays/Dose Modifications

The following general guidance should be followed for management of tremelimumab-related toxicities.

- Treat each of the toxicities with maximum supportive care (including holding the agent suspected of causing the toxicity if required).
- If the symptoms promptly resolve with supportive care, consideration should be given to continuing study drug along with appropriate continuing supportive care.
- If medically appropriate, dose modifications are permitted (Appendix 1)
- All dose modifications should be documented with clear reasoning and documentation of the approach taken.

Guidelines for the management of immune-mediated reactions, infusion-related reactions, and non-immune-mediated reactions are provided in Appendix 1, Dosing Modification and Toxicity Management Guidelines. In addition, there are certain circumstances in which tremelimumab should be permanently discontinued (see Section 6.4 and Appendix 1).

Following the first dose of study drug, subsequent administration tremelimumab can be modified based on toxicities observed as described in Appendix 1, the Dosing Modification and Toxicity Management Guidelines. These guidelines apply to adverse events considered by the reporting site investigator to be causally related to tremelimumab. In case of doubt, the site investigator should consult with the sponsor-investigator.

6.3 Liver Function Test Abnormalities

If a patient shows an AST or ALT ≥ 3 x ULN together with total bilirubin ≥ 2 x ULN, refer to Appendix 1 for further instructions. These cases should be reported as SAEs if, after evaluation, they meet the criteria for a Hy's law case or if any of the individual liver test parameters fulfill any of the SAE criteria.

6.4 Protocol Therapy Discontinuation

In addition to discontinuation from therapy related to toxicities as outlined in Appendix 1, a subject will also be discontinued from protocol therapy and followed per protocol under the following circumstances outlined below. The reason for discontinuation of protocol therapy will be documented on the electronic case report form (eCRF)

- Confirmed disease progression per RECIST 1.1 prior to Cycle 7
- Completion of scheduled treatment
- Site investigator determines a change of therapy would be in the best interest of the subject
- Subject requests to discontinue protocol therapy, whether due to unacceptable toxicity or for other reasons
    - In a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if he or she may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.
- Female subject becomes pregnant.
- If subject weight falls to 35kg or less.

- Grade ≥ 3 infusion reaction.
- Study therapy is interrupted for ≥ 120 days.

Subjects who are permanently discontinued from receiving investigational product will be followed for safety per Section 7, including the collection of any protocol-specified blood specimens, unless consent is withdrawn or the subject is lost to follow-up or enrolled in another clinical study. All subjects will be followed for survival. Subjects who decline to return to the site for evaluations will be offered follow-up by phone every 3 months as an alternative.

6.5 Protocol Discontinuation

If a subject decides to discontinue from the protocol (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. A complete final evaluation at the time of the subject's protocol withdrawal should be made with an explanation of why the subject is withdrawing from the protocol. If the reason for removal of a subject from the study is an adverse event, it will be recorded on the eCRF.

7. STUDY CALENDAR & EVALUATIONS

Study Evaluations Cycle = 28 days	Screening	Cycles 1-7 (± 3 days)[16]	Cycles 1-3 (± 3 days)[16]	Safety Follow Up Visit[14]	Follow up[15]	Progression of Disease	Screening for Re-Treatment[17]
	-28 days	Day 1	Day 15	~30 days post last dose (+7 days)	Every 3 months (± 14 days)		
REQUIRED ASSESSMENTS							
Informed Consent	X						
Medical History[1]	X						
Physical Exam	X	X		X	X		X
Vital signs and ECOG Performance Status[2]	X	X[9]		X	X		X
ECG[3]	X						X
AEs & concomitant medications	X	X	X	X	X		X
LABORATORY ASSESSMENTS							
Complete Blood Cell Count with diff (CBC)	X	X		X	X		X
Comprehensive Metabolic Profile (CMP)	X	X		X	X		X
Amylase and Lipase	X	X					X
GGT and Magnesium level	X						X
PT/INR and aPTT	X						X
Thyroid Function (TSH, T4, free T3)[4]	X	X[4]					X
Pregnancy test (serum or urine) (WOCBP)[5]	X						X
Hepatitis B and C and HIV[6]	X						
Urinalysis[7]	X						X
DISEASE ASSESSMENT							
CT of chest[8]	X	X[8]		X[8]	Every 3 months (± 7 days)		X
CT or MRI of abdomen and pelvis[8]	X	X[8]		X[8]			X
Bone scan in subjects with bone metastases[8]	X	X[8]		X[8]			X
TREATMENT EXPOSURE							
Tremelimumab[9]		X[9] (750 mg)					
SPECIMEN COLLECTION							
Archival Tumor Tissue or Fresh Biopsy[10]	X[10]						
Tumor tissue obtained for clinical purposes[11]	X[11]	X[11]		X[11]	X[11]	X[11]	X[11]
Blood samples[12]	X[12]	X[12]				X[12]	X[12]
Stool sample for microbiome[13]		X[13]					
FOLLOW-UP							
Survival Status, Subsequent Therapy					X		

CBC with differential and platelet to include: Hgb, Hct, WBC, ANC, absolute lymphocyte count, platelets. CMP to include sodium, potassium, chloride, creatinine, blood urea nitrogen; liver function tests (LFTs) to include AST, ALT, total bilirubin, alkaline phosphatase

Key to Footnotes

1: Medical History includes: smoking history questionnaire and a question regarding how the patient heard about the study. Prior anti-cancer treatment should be documented including medications (chemotherapy, checkpoint inhibitors, etc) radiation or surgery. Antibiotic use for 6 months prior to stool collection will be documented. Diagnosis and staging to include pathology report and Tumor Node Metastasis (TNM) staging. Prior genomic or molecular testing results are required if available.

2: Vital signs to include: blood pressure, pulse, temperature, respiration rate, weight, height (screening only) and ECOG performance status. Please see Section 5 for guidelines regarding vital signs during study drug infusion.

3: Resting 12-lead ECGs will be recorded at screening and as clinically indicated throughout the study. ECGs should be obtained after the patient has been in a supine position for 5 minutes and 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 (eg, 30 minutes) to confirm.

4: Thyroid Function (TSH, T4 and T3) will be done prior to treatment C1D1 then C3D1, C5D1, and C7D1 or more often if clinically indicated per site investigator discretion. For T4 and T3, free versus total is at the discretion of the site investigator.

5: For women of childbearing potential (WOCBP): urine or serum βhCG if clinically appropriate. If a urine test is done and it is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

6: Active infection, including tuberculosis (clinical evaluation that includes clinical history, physical examination and radiographic findings, and tuberculosis testing in line with local practice), hepatitis B, hepatitis C, or human immunodeficiency virus (HIV, positive HIV 1 or 2 antibodies). Active hepatitis B virus (HBV) infection is defined by a positive HBV surface antigen (HBsAg) result. Subjects with a past or resolved HBV infection (defined as the presence of hepatitis B core IgG antibody and the absence of HBsAg, deoxyribonucleic acid [DNA] negative) are eligible. Subjects positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV ribonucleic acid (RNA).

7: Urinalysis for bilirubin, blood, glucose, ketones, pH, protein, specific gravity to be performed at screening and as clinically indicated throughout the study per site investigator's discretion.

8: Radiology imaging to include: CT of the chest, CT or MRI of the abdomen/pelvis and bone scan (in subjects with bone metastasis). Scans should be performed at screening then every 2 months (± 7 days) during Cycles 1-7 (e.g., after C2, C4, etc.) and then every 3 months (± 7 days) until the time of progression. Imaging for the D30 safety visit may be omitted if already performed within 2 past months.

9: Tremelimumab will be given approximately every month for up to 7 cycles in the absence of disease progression or prohibitive toxicity. Refer to Section 5 for information regarding monitoring of subjects during infusion.

10: Representative formalin-fixed paraffin-embedded (FFPE) tumor specimens in paraffin blocks (blocks preferred) or at least 15 unstained slides will be identified prior to subject registration and shipped prior to C1D1. If archival tissue is not available and the subject is undergoing a standard of care biopsy, tissue from the biopsy is required to be submitted for correlative analyses. Subjects without adequate baseline tumor tissue may be considered for enrollment on a case by case basis after discussion with the sponsor-investigator. See Correlative Laboratory Manual (CLM) for additional details.

11: Surplus tissue remaining after routine standard of care procedures (e.g., metastatic biopsies) during and after treatment will be collected during this study. Subjects may have tumor tissue obtained during the course of the study or at the time of disease progression for clinical purposes (e.g., confirmation of disease progression, management of complication of disease progression, etc.). Subjects will have the option of whether samples obtained during standard of care procedures may be used for research purposes. Samples for correlative analysis are required if a standard of care biopsy is performed at screening for the first 7 cycles of treatment. See CLM for additional details.

12: Blood samples for research will be drawn pre-treatment C1D1 (may be done during screening if subject is found eligible), C2D1, C3D1, C6D1 and at time of progression. See Table 3 and CLM for additional information. Blood samples for research will be collected for subjects receiving re-treatment after progression. If samples are collected at progression after the initial 7 cycles of tremelimumab, samples at screening prior to re-treatment C1D1 do not need to be repeated. Samples will also be done prior to treatment C2D1, C3D1, C6D1 and at progression after re-treatment.

13. Stool for microbiome analysis will be collected prior to treatment prior to C1D1 of initial treatment. Subjects will be provided a kit with detailed instructions regarding collection of the sample prior to the timepoint. Samples will not be collected during re-treatment. Please see the CLM for additional details.

14: The safety follow-up visit should only occur when subjects stop study treatment for whatever reasons (toxicity, progression, or at discretion of site investigator) and should be performed ~30 days (+7 days) after the last dose of treatment. Subjects who have an ongoing Grade ≥ 2 or serious AE (SAE) at this visit will continue to be followed until the AE resolves to $\leq$ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.

15: Subjects completing study treatment without evidence of disease progression will be followed every 3 months for progression. Subjects with disease progression within 1 year of completing initial treatment with tremelimumab may be eligible for re-treatment with tremelimumab. After progression for subjects who are not candidates for tremelimumab retreatment or who initiate other anticancer therapies, patients will be followed for survival which may be accomplished via clinic visit, phone call, or other avenues as appropriate. Subjects who decline to return to the site for evaluations will be offered follow-up by phone every 3 months as an alternative.

16: Results for LFTs, electrolytes and creatinine **MUST BE REVIEWED** by the treating physician or site investigator within 3 days prior to administration of study drug. For Day 15 Cycles 1-3, subjects will be contacted via phone call or other avenues as appropriate on Day 15 ($\pm$ 3 days) for AE assessment. If issues are identified during communication with the subject, appropriate assessment will be performed based on discretion of research staff. This may include a clinic visit, lab work or additional communication.

17**:** Patients that complete the initial 7 cycles of tremelimumab then have evidence of disease progression per RECIST 1.1 (with or without confirmation) during the 1-year follow-up period may be eligible for re-treatment with up to 7 additional cycles of tremelimumab. Such patients are required to meet the eligibility criteria outlined in Section 3.3. Testing done during progression may be used for screening prior to re-treatment. Subjects should follow the same schedule of events/procedures for the initial Cycles 1-7 of tremelimumab. Baseline tumor assessment will be within 28 days of starting retreatment. Subjects should follow the same schedule of events/procedures for the initial cycles 1-7 of tremelimumab. Re-treatment should start within 28 days of documented progression.

Table 3: Schedule for correlative blood sample collections

Timepoint	Correlative blood samples
Pre-Treatment Cycle 1 Day 1	5 purple top tubes (50 ml total) 2 ACD tubes (17 ml total) 1 PAXgene tube (8.5 ml total)
Pre-Treatment Cycle 2 Day 1	2 purple top tube (20 ml total) 2 ACD tubes (17 ml total) 1 PAXgene tube (8.5 ml total)
Pre-Treatment Cycle 3 Day 1	2 purple top tubes (20 ml total) 5 ACD tubes (42.5 ml total) 1 PAXgene tube (8.5 ml total)
Pre-Treatment Cycle 6 Day 1	2 purple top tubes (20 ml total) 5 ACD tubes (42.5 ml total) 1 PAXgene tube (8.5 ml total)
Time of progression	5 purple top tubes (50 ml total) 2 ACD tubes (17 ml total) 1 PAXgene tube (8.5 ml total)

8. BIOSPECIMEN STUDIES AND PROCEDURES

Tumor tissue, peripheral blood, and possibly normal urothelium will be used for biospecimen-based research in this study. Full details of specimen collection and processing can be found in the CLM.

Correlative studies may include genomic sequencing of tumor tissue and/or peripheral blood and immune monitoring studies including T cell receptor sequencing and flow cytometry and/or mass cytometry on tissue and/or peripheral blood, antigen-specific T cell assays, HLA typing, and gene expression.

8.1 Tissue

Analysis of tumor tissue will include assessment for changes in the composition of immune cells, tumor cells, and tumor microenvironment. Immune cell composition and changes in the tumor microenvironment will be analyzed by platforms including but not limited to immunohistochemistry, flow cytometry, and mass cytometry. Tumor cells will be analyzed by immunohistochemistry, DNA, and RNA sequencing. Normal tissue or blood will be used for the germline genome. An effort will be made to identify tumor antigens for each subject using strategies including, but not limited to, collating the “antigenome” using immunohistochemistry data as well as identifying mutation-derived tumor antigens through genome sequencing and computational biology approaches to predict epitope: MHC binding affinity. Subject HLA typing will be done by sequence specific oligonucleotide probing and sequence specific priming of genomic DNA using standard procedures. T-cell responses will be detected using standard ELISPOT and intracellular cytokine assays or other emerging technologies. Tumor tissue will also be utilized for PD-L1 expression as determined by immunohistochemistry.

8.1.1 Archival Tissue

Representative formalin-fixed paraffin-embedded (FFPE) tumor specimens in paraffin blocks (blocks preferred) or at least 15 unstained slides will be identified prior to subject registration and shipped after registration but prior to C1D1. Representative formalin-fixed paraffin-embedded (FFPE) tumor specimens in paraffin blocks (blocks preferred) or at least 15 unstained slides. If archival tissue is not available and the subject is undergoing a standard of care biopsy, tissue from the biopsy is required to be submitted for correlative analyses. Subjects without adequate baseline tumor tissue may be considered for enrollment on a case by case basis after discussion with the sponsor-investigator. Specimens will be utilized for cellular and molecular analyses including but not limited to DNA sequencing, RNA sequencing, and tissue imaging/multiplex staining.

8.1.2 Tissue collected during routine clinical procedures

Surplus tissue remaining after routine standard of care procedures (e.g., metastatic biopsies) will be collected during this study. Subjects may have tumor tissue obtained during the course of the study or at the time of disease progression for clinical purposes (e.g., confirmation of disease progression, management of complication of disease progression, etc.). Specimens obtained in these settings may be accessed by the research team to facilitate an understanding of the pharmacodynamic effects of treatment at the level of the tumor and microenvironment including mechanisms of treatment resistance. Subjects will have the option of whether samples obtained during standard of care procedures may be used for research purposes.

8.2 Blood Samples

For blood samples, the volume of blood and type of tube to be used for each collection is specified in Table 3 and CLM. Collection of whole blood samples is mandatory for participation in this study. Blood samples for research will also be collected for subjects receiving re-treatment after progression.

8.2.1 Blood for Immune cell subsets

The quantity and composition of immune cells in the peripheral blood will be analyzed by flow cytometry or mass cytometry. Antigen specific T cell responses will be detected as described above.

8.2.2 Peripheral blood for DNA and RNA

Plasma will be stored for future ctDNA isolation and quantitative expression using sequence specific primers and/or for exome sequencing. DNA from whole blood may be used to germline sequencing to facilitate analysis of somatic tumor DNA sequencing. Whole blood will be stored for gene expression profiling from RNA.

8.3 Stool for microbiome analysis

Prior to treatment, patients will provide a stool sample from a single bowel movement for microbiome analysis as per the CLM. Stool samples will be analyzed to assess microbiome diversity and composition. Exploratory analyses correlating clinical outcomes and diversity/composition as well as assessment of metabolomics and metagenomic whole genome sequencing may be performed. Stool samples will not be collected during re-treatment with tremelimumab.

8.4 Storage of Biospecimens

Excess biospecimens not completely utilized in these experiments will be stored indefinitely at HCRN for future use in research focused on GU malignant diseases that are yet to be determined. All specimens collected will maintain the assigned sequence ID number of the corresponding patient. Deidentified samples may be shared with other research institutions. We believe that allowing for storage and usage of the remaining samples for future research is ethically justified and a preferred option to discarding these materials given the potential impact on improving clinical outcomes for subjects with bladder cancer. Subjects will be given the option to store excess specimens during the informed consent process.

8.5 Confidentiality of Biospecimens

Samples that are collected will be identified by a subject's sequence ID assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's sequence ID.

9. CRITERIA FOR DISEASE EVALUATION

See section 5.1.3 and 5.1.4 for criteria for continuing treatment past RECIST 1.1 disease progression.

9.1 RECIST 1.1

9.1.1 Measurable disease

The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

9.1.1.1 Measurable lesions

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$20 mm by chest x-ray, as $\geq$10 mm with CT scan, or $\geq$10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

9.1.1.2 Non- Measurable lesions

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with $\geq$ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.

9.1.1.3 Malignant lymph nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

9.1.1.4 Baseline documentation of "Target" and "Non-Target" lesions

Target lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

9.2 Response Criteria

Evaluation of target lesions

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD)	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Evaluation of non-target lesions

Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis) **NOTE:** If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.
Non-CR/ Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

*Although a clear progression of "non target" lesions only is exceptional, in such circumstances, the opinion of the site physician should prevail.

9.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target lesion	New Lesion	Overall response
CR	CR	No	CR
CR	Non-CR/ Non-PD	No	PR
	Not evaluated	No	PR
PR	Non-CR/ Non-PD/ not evaluated	No	PR
SD	Non-CR/ Non-PD/ not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

*In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment. In some circumstances it may be difficult to

distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

9.4 Definitions for Response Evaluation – RECIST 1.1

9.4.1 First documentation of response

The time between initiation of therapy and first documentation of PR or CR.

9.4.2 Confirmation of Response

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed no less than four weeks after the criteria for response are first met.

9.4.3 Duration of Response

Duration of overall response—the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded since treatment started.

9.4.4 Duration of Overall Complete Response

The period measured from the time that measurement criteria are met for complete response until the first date that recurrent disease is objectively documented.

9.4.5 Objective response rate

The objective response rate is the proportion of all subjects with confirmed PR or CR according to RECIST, from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the start of treatment).

9.4.6 Progression Free Survival

A measurement from the start of the treatment until the criteria for disease progression is met or death occurs, taking as reference the smallest measurements recorded since the treatment started.

Progression free survival will be measured from the date of initial treatment to the earliest date of disease progression, resection of measurable tumor or death for subjects who fail; and to the date of last contact for subjects who remain at risk for failure.

10. DRUG INFORMATION

10.1 Tremelimumab

Tremelimumab is an anti-CTLA-4 antibody being explored as a single-agent, and in combination with durvalumab, for the treatment of several malignancies.

10.1.1 Supplier/How Supplied

Tremelimumab will be supplied by AstraZeneca. Tremelimumab is formulated at 20 mg/mL in 20 mM histidine/histidine-HCl, 22 mM trehalose dihydrate, 0.02% (w/v) polysorbate 80, and 0.27 mM disodium edetate dihydrate, pH 5.5. The investigational product is supplied as a sterile liquid solution containing either 400 mg (nominal) tremelimumab per vial or 25 mg (nominal) per vial. The nominal fill volume for the 400 mg presentation is 20.0 mL and the nominal fill volume for the 25 mg presentation is 1.25 mL. Investigational product vials are stored at 2°C to 8°C (36°F to 46°F) and must not be frozen. Drug product should be kept in secondary container until use to prevent excessive light exposure.

10.1.2 Preparation

Total time from needle puncture of the tremelimumab 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

Tremelimumab will be administered using an IV bag containing 0.9% (w/v) saline or 5% (w/v) dextrose, with a final tremelimumab concentration ranging from 0.10 to 10 mg/mL, and delivered through an IV administration set with a 0.2- or 0.22-μm in-line filter. The IV bag size should be selected such that the final concentration is within 0.10 to 10 mg/mL. Mix the bag by gently inverting to ensure homogeneity of the dose in the bag.

Standard infusion time is 1 hour. However, if there are interruptions during infusion, the total allowed time should not exceed 8 hours at room temperature. Do not co-administer other drugs through the same infusion line.

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

10.1.3 Dispensing

Tremelimumab must be dispensed only from official study sites and to eligible subjects under the supervision of the site investigator. Tremelimumab should be stored in a secure area according to local regulations. It is the responsibility of the site investigator to ensure that study drug is only dispensed to subjects.

10.1.4 Adverse Events

Risks with tremelimumab monotherapy include, but are not limited to, GI effects (colitis, diarrhea, enterocolitis and intestinal perforation), endocrine disorders (hypo and hyperthyroidism, hypophysitis and adrenal insufficiency), skin effects (rash, and pruritus), elevations in lipase and amylase and clinical manifestations of pancreatitis, other gastrointestinal

events e.g. ulcerative colitis, dehydration, nausea and vomiting; hepatic events including hepatitis, and liver enzyme elevations; pneumonitis and ILD; nervous system events including encephalitis, peripheral motor and sensory neuropathies, Guillain-Barre and proximal muscle weakness; cytopenias including thrombocytopenia, anemia and neutropenia; infusion-related reactions, anaphylaxis, and allergic reactions; renal events including renal failure, acute kidney injury, nephritis, nephrotic syndrome, autoimmune nephritis and electrolyte abnormalities such as hypokalemia; autoimmune diseases including autoimmune arthritis, Sjogren's syndrome and giant cell temporal arteritis; hyperglycemia and diabetes mellitus; and pyrexia. For information on all identified and potential risks with tremelimumab please always refer to the current version of the tremelimumab IB. Using pooled data from monotherapy clinical studies AEs (all grades) reported very commonly (≥ 10% of patients) were diarrhea, nausea, fatigue, pruritus, decreased appetite, rash, vomiting, dyspnea, constipation, cough, pyrexia, abdominal pain, decreased weight, headache, asthenia, and anemia. Approximately 16% of patients experienced an AE that resulted in permanent discontinuation of tremelimumab and approximately 45% of patients experienced an SAE.

11 ADVERSE EVENTS

The descriptions and grading scales found in the NCI CTCAE v4.03 will be utilized for AE assessment. A copy of the CTCAE v4.03 can be downloaded from the CTEP website at http://ctep.cancer.gov. All forms for AE/SAE recording and reporting can be found in the Study Procedure Manual or in the EDC system (Documents and Information Tab).

11.1 Definitions

11.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence whether or not considered related to the study drug that appears to change in intensity during the course of the study. The following are examples of AEs:

- Unintended or unfavorable sign or symptom
- A disease temporally associated with participation in the protocol
- An intercurrent illness or injury that impairs the well-being of the subject

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

11.1.2 Serious Adverse Event (SAE)

A SAE is an adverse event that:

- Results in death. **NOTE:** Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization for >24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

11.1.3 Adverse Events of Special Interest

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the study drug(s) and may require close monitoring and rapid communication by the site investigator to the sponsor-investigator. 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 the study drug(s). If any AESI occurs in the course of the study, then the site investigators or other site personnel should report to Hoosier Cancer Research Network (HCRN) **within 1 business day** of becoming aware of the event. HCRN will report to AstraZeneca **within 1 business day** of becoming aware of the event. AESIs for tremelimumab include, but are not limited to, events with a potential inflammatory or immune-mediated mechanism and that 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 tremelimumab therapy.

An imAE is defined as an AE 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 sponsor-investigator 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.

If the site investigator has any questions with regards to an AE being an imAE, the site investigator should immediately contact the sponsor-investigator.
AESIs observed with tremelimumab include the following:

- Diarrhea/Colitis and intestinal perforation
- Pneumonitis/Interstitial Lung Disease (ILD)
- Hepatitis/transaminase increases
- Neuropathy / neuromuscular toxicity (e.g. Guillain-Barré, and myasthenia gravis)
- Endocrinopathy (ie, events of hypophysitis, adrenal insufficiency, and hyperthyroidism and hypothyroidism, Type I diabetes)
- Rash/Dermatitis
- Nephritis/Blood creatinine increases
- Pancreatitis/ serum lipase and amylase increases
- Myocarditis
- Myositis/Polymyositis
- Other inflammatory responses that are rare / less frequent with a potential immune-mediated aetiology include, but are not limited to, pericarditis, sarcoidosis, uveitis and other events involving the eye, skin, haematological and rheumatological events.

In addition, infusion-related reactions and hypersensitivity/anaphylactic reactions with a different underlying pharmacological etiology are also considered AESIs. Further information on these risks (e.g. presenting symptoms) can be found in the current version of the tremelimumab Investigator's Brochure. More specific guidelines for their evaluation and treatment are described in detail in the Dosing Modification and Toxicity Management Guidelines (please see Appendix 1). These guidelines have been prepared by the Funder to assist the investigators 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)

11.1.4 Unexpected Adverse Event

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current IB, prescribing information or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

11.1.5 Relatedness

AEs will be categorized according to the likelihood that they are related to the study drug(s). Specifically, they will be categorized using the following terms:

Unrelated	Adverse Event is ***not related*** to the study drug(s)
Unlikely	Adverse Event is ***doubtfully related*** to the study drug(s)
Possible	Adverse Event ***may be related*** to the study drug(s)
Probable	Adverse Event is ***likely related*** to the study drug(s)
Definite	Adverse Event is ***clearly related*** to the study drug(s)

11.2 Reporting

11.2.1 Adverse Events

AEs will be recorded from time of signed informed consent until 30 days after discontinuation of study drug(s) or until a new anti-cancer treatment starts, whichever occurs first.

- AEs will be recorded regardless of whether or not they are considered related to the study drug(s).
- All AEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- AEs considered related to study drug(s) will be followed until resolution to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever occurs first.
- Asymptomatic laboratory abnormalities that do not require treatment will not be collected as adverse events.

11.2.2 Serious Adverse Events (SAEs)

11.2.2.1 Site Requirements for Reporting SAEs to HCRN

- SAEs will be reported from time of signed informed consent until 90 days after discontinuation of study drug(s) or until a new anti-cancer treatment starts, whichever occurs first.
- SAEs will be reported on the SAE Submission Form **within 1 business day** of discovery of the event.
- SAEs include events related and unrelated to the study drug(s).
- All SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- All SAEs regardless of relation to study drug will be followed until resolution to ≤ Grade 1 or baseline and/or deemed clinically insignificant and/or until a new anti-cancer treatment starts, whichever occurs first.

The site will submit the completed SAE Submission Form to HCRN **within 1 business day** of discovery of the event**.** The form may be submitted to HCRN electronically to safety@hoosiercancer.org. The site investigator is responsible for informing the IRB and/or other local regulatory bodies as per local requirements.

The original copy of the SAE Submission Form and the email correspondence must be kept within the study file at the study site.

Once the SAE has resolved (see resolution guidelines listed above), sites must submit a follow-up SAE Submission Form within a reasonable timeframe to HCRN electronically to safety@hoosiercancer.org.

11.2.2.2 HCRN Requirements for Reporting SAEs to AstraZeneca

HCRN will report all SAEs to AstraZeneca as stipulated by company **within 1 business day** of receipt of the SAE Submission Form from a site. Follow-up information will be provided to AstraZeneca as it is received from site. Send SAE report and accompanying cover page by way of email to AstraZeneca's designated mailbox: AEMailboxClinicalTrialTCS@astrazeneca.com

11.3 Sponsor-Investigator Responsibilities

HCRN will send a SAE summary to the sponsor-investigator **within 1 business day** of receipt of SAE Submission Form from a site. The sponsor-investigator will promptly review the SAE summary and assess for expectedness and relatedness.

11.4 HCRN Responsibilities to FDA

HCRN will manage the Investigational New Drug Application (IND) associated with this protocol on behalf of the sponsor-investigator. HCRN will cross-reference this submission to the AstraZeneca's parent IND at the time of submission. Additionally, HCRN will submit a copy of these documents to AstraZeneca at the time of submission to FDA.

For protocols conducted under an IND, HCRN will be responsible for all communication with the FDA in accordance with 21CFR312 including but not limited to the 7 and 15 Day Reports, as well as an Annual Progress Report. Additionally, HCRN will submit a copy of these reports to AstraZeneca at the time of submission to FDA.

11.5 IND Safety Reports Unrelated to this Trial

AstraZeneca will provide to HCRN IND safety reports from external studies that involve the study drug(s) per their guidelines. HCRN will forward safety reports to the sponsor-investigator who will review these reports and determine if revisions are needed to the protocol or consent. HCRN will forward these reports to participating sites **within 1 business day** of receiving the sponsor-investigator's review. Based on the sponsor-investigator's review, applicable changes will be made to the protocol and informed consent document (if required). All IND safety reports will also be made available to sites via the EDC system.

Upon receipt from HCRN, site investigators (or designees) are responsible for submitting these safety reports to their respective IRBs, as per their IRB policies.

11.6 Other events requiring reporting

11.6.1 Overdose

Use of tremelimumab in doses in excess of that specified in the protocol is considered to be an overdose. There is currently no specific treatment in the event of overdose of tremelimumab, and possible symptoms of overdose are not established. An overdose with associated AEs will be recorded as the AE diagnosis or symptoms in the relevant eCRF.

If an overdose of a study drug(s) occurs in the course of the study, then the site investigator or other site personnel will inform HCRN **within 1 business day** of becoming aware of the event. HCRN will report the event to AstraZeneca **within 1 business day** of becoming aware of the event.

11.6.2 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to AstraZeneca except for:

- Pregnancy discovered before the study subject has received any study drugs.
- Pregnancy of a female partner of male subject, providing there is no restriction of male subject fathering a child.

11.6.3 Maternal exposure

If a patient becomes pregnant during the course of the study, the study drug(s) should be discontinued immediately. Pregnancy itself is not regarded as an AE unless there is a suspicion that the study drug(s) may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented even if the patient was discontinued from the study.

If any pregnancy occurs in the course of the study, then the site investigator or other site personnel will inform HCRN **within 1 business day** of becoming aware of the event. HCRN will report the event to AstraZeneca **within 1 business day** of becoming aware of the event.

11.6.4 Paternal exposure

Male subjects should refrain from fathering a child or donating sperm during the study and for 90 days after the last dose of tremelimumab. Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 90 days after the last dose of tremelimumab therapy should, if possible, be followed up and documented.

11.6.5 Reporting of deaths to AstraZeneca

All deaths that occur during the study, or within the protocol-defined 90-day post-last dose of tremelimumab safety follow-up period must be reported to AstraZeneca as follows:

- Death that is clearly the result of disease progression should be documented but should not be reported as an SAE.
- Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported to HCRN **within 24 hours** and HCRN will report to AstraZeneca as a SAE **within 24 hours**. The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.
- Deaths with an unknown cause should always be reported as a SAE.

12 STATISTICAL METHODS

12.1 Study Design

This is a phase II trial designed to estimate the activity of single agent tremelimumab in subjects with metastatic urothelial cancer with disease progression despite prior treatment with PD-1/PD-L1 blockade. There is currently no standard treatment for such subjects. Therefore, given the durability of responses observed with immune checkpoint blockade, even a relatively low response proportion could be clinically meaningful. A Simon's 2-stage design will be applied.

12.2 Endpoints

12.2.1 Definition of Primary Endpoint

The primary endpoint is to determine the response rate by RECIST 1.1

12.2.2 Definition of Secondary Endpoints

- Safety will be determined according to the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03
- Duration of response will be the time from the first documentation of response to the time of progression as per RECIST 1.1 or death
- Progression-free survival which is defined as the time from treatment initiation to death or progression, depending on which occurs first
- Overall survival is defined as the time from treatment initiation to death

12.3 Sample Size and Accrual

A Simon's 2-stage design[14] will be applied. Patients who do not receive drug or who do not complete 1 cycle of therapy for non-treatment related causes (eg, traffic accidents, trauma etc) will be replaced.

The null hypothesis that the true response rate is 2% will be tested against a one-sided alternative. In the first stage, 18 subjects will be accrued. If there are 0 responses in these 18 subjects, enrollment will be stopped. Otherwise, 10 additional subjects will be accrued for a total of 28. The null hypothesis will be rejected if 3 or more responses are observed in 28 subjects. This design yields a type I error rate of 0.02 and power of 80% when the true response rate is 15% or larger.

12.4 Assessment of Safety

The safety population will be defined as any patients that receive at least one dose of tremelimumab and will be used in the assessment of toxicity. Safety will be determined according to the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03. All subjects will be evaluable for adverse event evaluation from the time of their first dose of study treatment.

12.5 Assessment of Efficacy

All subjects who have received at least one cycle of therapy and have had their disease re-evaluated will be considered evaluable for response (exceptions will be those who exhibit objective disease progression prior to the end of Cycle 1 who will also be considered evaluable).

Patients who do not receive drug or who do not complete 1 cycle of therapy for non-treatment related causes will be replaced.

12.6 Data Analysis Plans

12.6.1 Analysis Plans for Primary Objective

All subjects with measurable disease who have received at least one cycle of treatment and have their disease re-evaluated will be evaluable for assessment of objective response. The objective response rates as determined by RECIST 1.1 and their associated 95% confidence intervals will be constructed based on the exact binomial test.

12.6.2 Analysis Plans for Secondary Objectives

Any subject who receives at least one dose of treatment on this protocol is evaluable for toxicity. Toxicity rates will be summarized using frequency tables. Disease control rates will be analyzed based on the exact binomial test. Duration of response will be the time from the first documentation of response to the time of progression or death will be summarized using frequency tables. Progression free survival and overall survival curves will be based on the Kaplan-Meier method.

12.6.3 Analysis Plans for Exploratory Objectives

To explore the relationship between PD-L1 expression and response to treatment, Fisher's exact test will be used. To explore the relationship between molecular subtype of bladder cancer and response to treatment, frequency tables will be constructed and Fisher's exact test will be used. To explore the relationship between genomic alterations, including mutational load, and changes in circulating immune biomarkers and response to treatment both Fisher's exact test and T-test will be used. Exploratory analyses correlating clinical outcomes and adverse events with stool microbiome diversity/composition as well as assessment of metabolomics and metagenomic whole genome sequencing may also be performed.

12.7 Interim Analysis/Criteria for Stopping Study

Though there has been limited experience with single agent CTLA-4 blockade in metastatic urothelial cancer, prior studies exploring combination PD-1/PD-L1 plus CTLA-4 blockade in metastatic urothelial cancer have reported a treatment-related Grade ≥ 3 adverse event rate of approximately 30%.[15] A stopping rule for excessive toxicity will be employed after 6, 12, 18, and 24 subjects finish at least one cycle of treatment. This safety stopping rule will be performed with respect to all treatment-related Grade ≥ 3 adverse events. Two-sided 95% exact binomial confidence intervals (CIs) of these treatment-related Grade ≥ 3 adverse event rates will be constructed. If their lower bounds exceed 33%, the study drug will be considered unacceptably toxic for this patient population and enrollment will be halted. This corresponds to ≥ 5 out of 6 patients with grade ≥ 3 adverse events, ≥ 8 out of 12 patients with grade ≥ 3 adverse events, ≥ 10 out of 18 patients with grade ≥ 3 adverse events , and ≥ 13 out of 24 subjects with grade ≥ 3 adverse events.

There is a possibility of stopping at the 1st stage if no patients respond to treatment. In addition, the study accrual may be suspended in the case when none of the first 17 patients respond and

the decision to move onto the 2nd stage hinges on the response of the 18th patient. Otherwise, during these safety analyses, enrollment will continue.

13 TRIAL MANAGEMENT

13.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted with guidance from the Tisch Cancer Institute Cancer Center's DSMP.

HCRN oversight activities include:

- Review and process all adverse events requiring expedited reporting as defined in the protocol
- Provide trial accrual progress, safety information, and data summary reports to the sponsor-investigator
- Submit data summary reports at least every 6 months to the DSMC for review according to the Tisch Cancer Institute Cancer Center's DSMP

13.2 Tisch Cancer Institute Cancer Center Data Safety Monitoring Committee

HCRN will provide the following for the DSMC to review:

- Adverse event summary report
- Audit results, if applicable
- Data related to stopping/decision rules described in study design
- Study accrual patterns
- Protocol deviations

The DSMC will review study data at least every 6 months. Documentation of DSMC reviews will be provided to sponsor-investigator and HCRN. Issues of immediate concern by the DSMC will brought to the attention of the sponsor-investigator and other regulatory bodies as appropriate. The sponsor-investigator will work with HCRN to address the DSMC's concerns.

13.3 Data Quality Oversight Activities

Remote validation of EDC system data will be completed on a continual basis throughout the life cycle of the study. Automated edit check listings will be used to generate queries in the EDC system and transmitted to the site to address in a timely fashion. Corrections will be made by the study site personnel.

Participating sites may also be subject to quality assurance audits by AstraZeneca-MedImmune or its designee as well as inspection by appropriate regulatory agencies.

13.3.1 Onsite Monitoring

Monitoring visits to the trial sites may be made periodically during the trial to ensure key aspects of the protocol are followed. Selected source documents will be reviewed for verification of agreement with data entered into the EDC system. It is important for the site investigator and their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by HCRN or its designee.

13.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. All results of primary and secondary objectives must be posted to CT.gov within a year of completion. The sponsor-investigator has delegated responsibility to HCRN for registering the trial and posting the results on clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

14. DATA HANDLING AND RECORD KEEPING

14.1 Data Management

HCRN will serve as the Clinical Research Organization for this trial. Data will be collected through a web based clinical research platform, a system compliant with Good Clinical Practices and Federal Rules and Regulations. HCRN personnel will coordinate and manage data for quality control assurance and integrity. Select data will be collected and entered into the EDC system by study site personnel from participating institutions.

14.2 Case Report Forms and Submission

Generally, clinical data will be electronically captured in the EDC system and correlative results will be captured in the EDC system or other secure database(s). If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in the EDC system, according to study-specific objectives.

The completed dataset is the sole property of the sponsor-investigator's institution and should not be exported to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without permission from the sponsor-investigator and HCRN.

14.3 Record Retention

To enable evaluations and/or audits from Health Authorities/HCRN, the site investigator agrees to keep records, including the identity of all subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's file and retained by the site investigator in compliance with the site contract with HCRN. No records will be destroyed until HCRN confirms destruction is permitted.

14.4 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study site personnel. Subjects will

be informed in writing that some organizations including the sponsor-investigator and his/her research associates, HCRN, AstraZeneca, IRB, or government agencies, like the FDA, may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

15 ETHICS

15.1 Institutional Review Board (IRB) Approval

The final study protocol and the final version of the informed consent form must be approved in writing by an IRB. The site investigator must submit written approval by the IRB to HCRN before he or she can enroll subjects into the study.

The site investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB, as local regulations require.

Progress reports and notifications of serious and unexpected adverse events will be provided to the IRB according to local regulations and guidelines.

15.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki. Conduct of the study will be in compliance with ICH Good Clinical Practice, and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws.

15.3 Informed Consent Process

The site investigator will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The site investigator must store the original, signed informed consent form. A copy of the signed informed consent form must be given to the subject.

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APPENDIX 1

Dosing Modification and Toxicity Management Guidelines for Immune-Mediated, Infusion-Related, and Non-Immune–Mediated Reactions

General Considerations

Dose Modifications	Toxicity Management
Drug administration modifications of study drug/study regimen will be made to manage potential immune-related AEs based on severity of treatment-emergent toxicities graded per NCI CTCAE v4.03. In addition to the criteria for permanent discontinuation of study drug/study regimen based on CTC grade/severity (table below), permanently discontinue study drug/study regimen for the following conditions: • Inability to reduce corticosteroid to a dose of ≤10 mg of prednisone per day (or equivalent) **within 12 weeks** after last dose of study drug/study regimen • Recurrence of a previously experienced Grade 3 treatment-related AE following resumption of dosing **Grade 1** No dose modification **Grade 2** Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1. 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. Patients with endocrinopathies who may require prolonged or continued steroid replacement can be retreated with study drug/study regimen on the following conditions: 1. The event stabilizes and is controlled. 2. The patient is clinically stable as per Investigator or treating physician's clinical judgement. 3. Doses of prednisone are at ≤10 mg/day or equivalent.	It is recommended that management of immune-mediated adverse events (imAEs) follows the guidelines presented in this table: – It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs, some of them not noted specifically in these guidelines. – Whether specific immune-mediated events (and/or laboratory indicators of such events) are noted in these guidelines or not, patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, concomitant medications, and infections) to a possible immune-mediated event. In the absence of a clear alternative etiology, all such events should be managed as if they were immune related. General recommendations follow. – Symptomatic and topical therapy should be considered for low-grade (Grade 1 or 2, unless otherwise specified) events. – For persistent (>3 to 5 days) low-grade (Grade 2) or severe (Grade ≥3) events, promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – Some events with high likelihood for morbidity and/or mortality – e.g., myo-carditis, or other similar events even if they are not currently noted in the guidelines – should progress rapidly to high dose IV corticosteroids (methylprednisolone at 2 to 4 mg/kg/day) even if the event is Grade 2, and if clinical suspicion is high and/or there has been clinical confirmation. Consider, as necessary, discussing with the study physician, and promptly pursue specialist consultation. – If symptoms recur or worsen during corticosteroid tapering (28 days of taper), increase the corticosteroid dose (prednisone dose [e.g., up to 2 to 4 mg/kg/day PO or IV equivalent]) until stabilization or improvement of symptoms, then resume corticosteroid tapering at a slower rate (>28 days of taper). – More potent immunosuppressives such as TNF inhibitors (e.g., infliximab) (also refer to the individual sections of the imAEs for specific type of immunosuppressive) should be considered for events not responding to systemic steroids. Progression to use of more potent immunosuppressives should proceed more rapidly in events with high likelihood for morbidity and/or mortality – e.g., myocarditis, or other similar events even if they are not currently noted in the guidelines – when these events are not responding to systemic steroids. – With long-term steroid and other immunosuppressive use, consider need for *Pneumocystis jirovecii* pneumonia (PJP, formerly known as *Pneumocystis carinii* pneumonia) prophylaxis, gastrointestinal protection, and glucose monitoring.

Dosing Modification and Toxicity Management Guidelines for Immune-Mediated, Infusion-Related, and Non-Immune–Mediated Reactions

General Considerations

Dose Modifications		Toxicity Management
Grade 3	Depending on the individual toxicity, study drug/study regimen may be permanently discontinued. Please refer to guidelines below.	– Discontinuation of study drug/study regimen is not mandated for Grade 3/Grade 4 inflammatory reactions attributed to local tumor response (e.g., inflammatory reaction at sites of metastatic disease and lymph nodes). Continuation of study drug/study regimen in this situation should be based upon a benefit-risk analysis for that patient.
Grade 4	Permanently discontinue study drug/study regimen.	
Note: For Grade ≥3 asymptomatic amylase or lipase levels, hold study drug/study regimen, and if complete work up shows no evidence of pancreatitis, study drug/study regimen may be continued or resumed.		
Note: Study drug/study regimen should be permanently discontinued in Grade 3 events with high likelihood for morbidity and/or mortality – e.g., myocarditis, or other similar events even if they are not currently noted in the guidelines. Similarly, consider whether study drug/study regimen should be permanently discontinued in Grade 2 events with high likelihood for morbidity and/or mortality – e.g., myocarditis, or other similar events even if they are not currently noted in the guidelines – when they do not rapidly improve to Grade <1 upon treatment with systemic steroids and following full taper		
Note: There are some exceptions to permanent discontinuation of study drug for Grade 4 events (i.e., hyperthyroidism, hypothyroidism, Type 1 diabetes mellitus).		

AE Adverse event; CTC Common Toxicity Criteria; CTCAE Common Terminology Criteria for Adverse Events; imAE immune-mediated adverse event; IV intravenous; NCI National Cancer Institute; PO By mouth.

Specific Immune-Mediated Reactions

Adverse Events	Severity Grade of the Event (NCI CTCAE version 4.03)	Dose Modifications	Toxicity Management
Pneumonitis/Interstitial Lung Disease (ILD)	**Any Grade**	**General Guidance**	**For Any Grade:** – Monitor patients for signs and symptoms of pneumonitis or ILD (new onset or worsening shortness of breath or cough). Patients should be evaluated with imaging and pulmonary function tests, including other diagnostic procedures as described below. – Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up, and high- resolution CT scan.
	Grade 1 (asymptomatic, clinical or diagnostic observations only; intervention not indicated)	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 (radiographic changes only):** – 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. – Consider Pulmonary and Infectious disease consult.
	Grade 2 (symptomatic; medical intervention indicated; limiting instrumental ADL)	Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1. • If toxicity worsens, then treat as Grade 3 or Grade 4. • 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 completion of steroid taper.	**For Grade 2 (mild to moderate new symptoms):** – Monitor symptoms daily and consider hospitalization. – Promptly start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent). – Reimage as clinically indicated. – If no improvement within 3 to 5 days, additional workup should be considered and prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day started – If still no improvement within 3 to 5 days despite IV methylprednisolone at 2 to 4 mg/kg/day, promptly start immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, or

			anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])[a] – Consider pulmonary and infectious disease consult. – Consider, as necessary, discussing with study physician.
	Grade 3 or 4 (Grade 3: severe symptoms; limiting self-care ADL; oxygen indicated) (Grade 4: life-threatening respiratory compromise; urgent intervention indicated [e.g., tracheostomy or intubation])	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4 (severe or new symptoms, new/worsening hypoxia, life-threatening):** – Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent. – Obtain Pulmonary and Infectious disease consult; consider, as necessary, discussing with study physician. – Hospitalize the patient. – Supportive care (e.g., oxygen). – If no improvement within 3 to 5 days, additional workup should be considered and prompt treatment with additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks' dose) started. Caution: rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and, in particular, anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]
Diarrhea/Colitis	**Any Grade**	**General Guidance**	**For Any Grade:** – 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). – 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. – 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 event.

		– Use analgesics carefully; they can mask symptoms of perforation and peritonitis.
Grade 1 (Diarrhea: stool frequency of <4 over baseline per day) (Colitis: asymptomatic; clinical or diagnostic observations only)	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), and loperamide. Use probiotics as per treating physician's clinical judgment.
Grade 2 (Diarrhea: stool frequency of 4 to 6 over baseline per day) (Colitis: abdominal pain; mucus or blood in stool)	Hold study drug/study regimen until resolution to Grade ≤1 • If toxicity worsens, then treat as Grade 3 or Grade 4. • If toxicity improves to Grade ≤1, then study drug/study regimen can be resumed after completion of steroid taper.	**For Grade 2:** – Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), and loperamide and/or budesonide. – Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 3 to 5 days or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, GI consult should be obtained for consideration of further workup, such as imaging and/or colonoscopy, to confirm colitis and rule out perforation, and prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day started. – If still no improvement within 3 to 5 days despite 2 to 4 mg/kg IV methylprednisolone, promptly start immunosuppressives such as infliximab at 5 mg/kg once every 2 weeks[a]. **Caution:** it is important to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. – Consider, as necessary, discussing with study physician if no resolution to Grade ≤1 in 3 to 4 days. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]

	Grade 3 or 4 (Grade 3 diarrhea: stool frequency of ≥7 over baseline per day; Grade 4 diarrhea: life threatening consequences) (Grade 3 colitis: severe abdominal pain, change in bowel habits, medi-cal intervention indi-cated, peritoneal signs; Grade 4 colitis: life-threatening consequences, urgent intervention indicated)	**Grade 3** Permanently discontinue study drug/study regimen for Grade 3 if toxicity does not improve to Grade ≤1 within 14 days; study drug/study regimen can be resumed after completion of steroid taper. **Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4:** – Promptly initiate empiric IV methylprednisolone 2 to 4 mg/kg/day or equivalent. – Monitor stool frequency and volume and maintain hydration. – Urgent GI consult and imaging and/or colonoscopy as appropriate. – If still no improvement within 3 to 5 days of IV methylprednisolone 2 to 4 mg/kg/day or equivalent, promptly start further immunosuppressives (e.g., infliximab at 5 mg/kg once every 2 weeks). **Caution**: Ensure GI consult to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]
Hepatitis (elevated LFTs) Infliximab should not be used for management of immune-related hepatitis.	**Any Grade**	**General Guidance**	**For Any Grade:** – Monitor and evaluate liver function test: AST, ALT, ALP, and TB. – Evaluate for alternative etiologies (e.g., viral hepatitis, disease progression, concomitant medications).
	Grade 1 (AST or ALT >ULN and ≤3.0×ULN and/or TB > ULN and ≤1.5×ULN)	• No dose modifications. • If it worsens, then treat as Grade 2 event.	**For Grade 1:** – Continue LFT monitoring per protocol.
	Grade 2 (AST or ALT >3.0×ULN and ≤5.0×ULN and/or TB >1.5×ULN and ≤3.0×ULN)	• Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1. • If toxicity worsens, then treat as Grade 3 or Grade 4. • If toxicity improves to Grade ≤1 or baseline, resume study	**For Grade 2:** – Regular and frequent checking of LFTs (e.g., every 1 to 2 days) until elevations of these are improving or resolved. – If no resolution to Grade ≤1 in 1 to 2 days, consider, as necessary, discussing with study physician. – If event is persistent (>3 to 5 days) or worsens, promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent.

	drug/study regimen after completion of steroid taper.	– If still no improvement within 3 to 5 days despite 1 to 2 mg/kg/day of prednisone PO or IV equivalent, consider additional work up and start prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day. – If still no improvement within 3 to 5 days despite 2 to 4 mg/kg/day of IV methylprednisolone, promptly start immunosuppressives (i.e., mycophenolate mofetil).[a] Discuss with study physician if mycophenolate mofetil is not available. **Infliximab should NOT be used.** – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]
Grade 3 or 4 (Grade 3: AST or ALT >5.0×ULN and ≤20.0×ULN and/or TB >3.0×ULN and ≤10.0×ULN) (Grade 4: AST or ALT >20×ULN and/or TB >10×ULN)	**For Grade 3:** For elevations in transaminases ≤8 × ULN, or elevations in bilirubin ≤5 × ULN: • Hold study drug/study regimen dose until resolution to Grade ≤1 or baseline • Resume study drug/study regimen if elevations downgrade to Grade ≤1 or baseline within 14 days and after completion of steroid taper. • Permanently discontinue study drug/study regimen if the elevations do not downgrade to Grade ≤1 or baseline within 14 days For elevations in transaminases >8 × ULN or elevations in bilirubin >5 × ULN, discontinue study drug/study regimen. Permanently discontinue study drug/study regimen for any case	**For Grade 3 or 4:** – Promptly initiate empiric IV methylprednisolone at 1 to 4 mg/kg/day or equivalent. – If still no improvement within 3 to 5 days despite 1 to 4 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with immunosuppressive therapy (i.e., mycophenolate mofetil). Discuss with study physician if mycophenolate is not available. **Infliximab should NOT be used.** – Perform hepatology consult, abdominal workup, and imaging as appropriate. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]

		meeting Hy's law criteria (AST and/or ALT >3 × ULN + bilirubin >2 × ULN without initial findings of cholestasis (i.e., elevated alkaline P04) and in the absence of any alternative cause.[b] **For Grade 4:** Permanently discontinue study drug/study regimen.	
Nephritis or renal dysfunction (elevated serum creatinine)	**Any Grade**	**General Guidance**	**For Any Grade:** – Consult with nephrologist. – 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, decrease in urine output, or proteinuria). – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression or infections). – Steroids should be considered in the absence of clear alternative etiology even for low-grade events (Grade 2), in order to prevent potential progression to higher grade event.
	Grade 1 (Serum creatinine > 1 to 1.5 × baseline; > ULN to 1.5 × ULN)	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 symptomatic treatment, including hydration, electrolyte replacement, and diuretics.

Grade 2 (serum creatinine >1.5 to 3.0 × baseline; >1.5 to 3.0 × ULN)	Hold study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity worsens, then treat as Grade 3 or 4. • If toxicity improves to Grade ≤1 or baseline, then resume study drug/study regimen after completion of steroid taper.	**For Grade 2:** – Consider symptomatic treatment, including hydration, electrolyte replacement, and diuretics. – Carefully monitor serum creatinine every 2 to 3 days and as clinically warranted. – Consult nephrologist and consider renal biopsy if clinically indicated. – If event is persistent (>3 to 5 days) or worsens, promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 3 to 5 days or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, additional workup should be considered and prompt treatment with IV methylprednisolone at 2 to 4 mg/kg/day started. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a] – When event returns to baseline, resume study drug/study regimen and routine serum creatinine monitoring per study protocol.
Grade 3 or 4 (Grade 3: serum creatinine >3.0 × baseline; >3.0 to 6.0 × ULN; Grade 4: serum creatinine >6.0 × ULN)	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4:** – Carefully monitor serum creatinine on daily basis. – 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 or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, additional workup should be considered and prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day started. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]

Rash (excluding bullous skin formations)	**Any Grade** (refer to NCI CTCAE v 4.03 for definition of severity/grade depending on type of skin rash)	**General Guidance**	**For Any Grade:** – Monitor for signs and symptoms of dermatitis (rash and pruritus). – IF THERE IS ANY BULLOUS FORMATION, THE STUDY PHYSICIAN SHOULD BE CONTACTED **AND STUDY DRUG DISCONTINUED.**
	Grade 1	No dose modifications.	**For Grade 1:** – Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., urea cream).
	Grade 2	For persistent (>1 to 2 weeks) Grade 2 events, hold scheduled study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity worsens, then treat as Grade 3. • If toxicity improves to Grade ≤1 or baseline, then resume drug/study regimen after completion of steroid taper.	**For Grade 2:** – Obtain dermatology consult. – Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., urea cream). – Consider moderate-strength topical steroid. – If no improvement of rash/skin lesions occurs within 3 to 5 days or is worsening despite symptomatic treatment and/or use of moderate strength topical steroid, consider, as necessary, discussing with study physician and promptly start systemic steroids such as prednisone 1 to 2 mg/kg/day PO or IV equivalent. – Consider skin biopsy if the event is persistent for >1 to 2 weeks or recurs.
	Grade 3 or 4	**For Grade 3:** Hold study drug/study regimen until resolution to Grade ≤1 or baseline. If temporarily holding the study drug/study regimen does not provide improvement of the Grade 3 skin rash to Grade ≤1 or baseline within 30 days, then permanently	**For Grade 3 or 4:** – Consult dermatology. – Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent. – Consider hospitalization. – Monitor extent of rash [Rule of Nines]. – Consider skin biopsy (preferably more than 1) as clinically feasible. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for

		discontinue study drug/study regimen. **For Grade 4:** Permanently discontinue study drug/study regimen.	treatment of cancer-related infections [Category 2B recommendation]).[a] – Consider, as necessary, discussing with study physician.
Endocrinopathy (e.g., hyperthyroidism, hypothyroidism, Type 1 diabetes mellitus, hypophysitis, hypopituitarism, and adrenal insufficiency; exocrine event of amylase/lipase increased also included in this section)	**Any Grade** (depending on the type of endocrinopathy, refer to NCI CTCAE v4.03 for defining the CTC grade/severity)	**General Guidance**	**For Any Grade:** – Consider consulting an endocrinologist for endocrine events. – Consider, as necessary, discussing with study physician. – Monitor patients for signs and symptoms of endocrinopathies. Non-specific symptoms include headache, fatigue, behavior changes, changed mental status, vertigo, abdominal pain, unusual bowel habits, polydipsia, polyuria, hypotension, and weakness. – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression including brain metastases, or infections). – Depending on the suspected endocrinopathy, monitor and evaluate thyroid function tests: TSH, free T3 and free T4 and other relevant endocrine and related labs (e.g., blood glucose and ketone levels, HgA1c). – 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. – 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.
	Grade 1	No dose modifications.	**For Grade 1 (including those with asymptomatic TSH elevation):** – Monitor patient with appropriate endocrine function tests. – For suspected hypophysitis/hypopituitarism, consider consultation of an endocrinologist to guide assessment of early-morning 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	For Grade 2 endocrinopathy other than hypothyroidism and Type 1 diabetes mellitus, hold study drug/study regimen dose until patient is clinically stable. • If toxicity worsens, then treat as Grade 3 or Grade 4. Study drug/study regimen can be resumed once event stabilizes and after completion of steroid taper. Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen on the following conditions: 1. The event stabilizes and is controlled. 2. The patient is clinically stable as per investigator or treating physician's clinical judgement. 3. Doses of prednisone are ≤10 mg/day or equivalent.	**For Grade 2 (including those with symptomatic endocrinopathy):** – 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 Type 1 DM, 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 (e.g., hydrocortisone, sex hormones). – Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids. – Isolated Type 1 diabetes mellitus (DM) may be treated with appropriate diabetic therapy, without study drug/study regimen interruption, and without corticosteroids. – Once patients on steroids are improving, gradually taper immunosuppressive steroids (as appropriate and with guidance of endocrinologist) over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a] – For patients with normal endocrine workup (laboratory assessment or MRI scans), repeat laboratory assessments/MRI as clinically indicated.
Grade 3 or 4	For Grade 3 or 4 endocrinopathy other than hypothyroidism and Type 1 diabetes mellitus, hold	**For Grade 3 or 4:** – Consult endocrinologist to guide evaluation of endocrine function and, as indicated by suspected endocrinopathy and as

		study drug/study regimen dose until endocrinopathy symptom(s) are controlled. Study drug/study regimen can be resumed once event stabilizes and after completion of steroid taper. Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen on the following conditions: 1. The event stabilizes and is controlled. 2. The patient is clinically stable as per investigator or treating physician's clinical judgement. 3. Doses of prednisone are ≤10 mg/day or equivalent.	clinically indicated, consider pituitary scan. Hospitalization recommended. – For all patients with abnormal endocrine work up, except those with isolated hypothyroidism or Type 1 DM, and as guided by an endocrinologist, promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent, as well as relevant hormone replacement (e.g., hydrocortisone, sex hormones). – For adrenal crisis, severe dehydration, hypotension, or shock, immediately initiate IV corticosteroids with mineralocorticoid activity. – Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids. – Isolated Type 1 diabetes mellitus may be treated with appropriate diabetic therapy, without study drug/study regimen interruption, and without corticosteroids. – Once patients on steroids are improving, gradually taper immunosuppressive steroids (as appropriate and with guidance of endocrinologist) over ≥28 days and consider prophylactic antibiotics, antifungals, and anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]
Neurotoxicity (to include but not be limited to limbic encephalitis and autonomic neuropathy, excluding Myasthenia Gravis and Guillain-Barre)	**Any Grade** (depending on the type of neurotoxicity, refer to NCI CTCAE v4.03 for defining the CTC grade/severity)	**General Guidance**	**For Any Grade:** – Patients should be evaluated to rule out any alternative etiology (e.g., disease progression, infections, metabolic syndromes, or medications). – Monitor patient for general symptoms (headache, nausea, vertigo, behavior change, or weakness). – Consider appropriate diagnostic testing (e.g., electromyogram and nerve conduction investigations). – Perform symptomatic treatment with neurological consult as appropriate. –

Grade 1	No dose modifications.	**For Grade 1:** – See "Any Grade" recommendations above.
Grade 2	For acute motor neuropathies or neurotoxicity, hold study drug/study regimen dose until resolution to Grade ≤1. For sensory neuropathy/neuropathic pain, consider holding study drug/study regimen dose until resolution to Grade ≤1. If toxicity worsens, then treat as Grade 3 or 4. Study drug/study regimen can be resumed once event improves to Grade ≤1 and after completion of steroid taper.	**For Grade 2:** – Consider, as necessary, discussing with the study physician. – Obtain neurology consult. – Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin or duloxetine). – Promptly start systemic steroids prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If no improvement within 3 to 5 days despite 1 to 2 mg/kg/day prednisone PO or IV equivalent, consider additional workup and promptly treat with additional immunosuppressive therapy (e.g., IV IG).
Grade 3 or 4	**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. **For Grade 4:** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4:** – Consider, as necessary, discussing with study physician. – Obtain neurology consult. – Consider hospitalization. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – If no improvement within 3 to 5 days despite IV corticosteroids, consider additional workup and promptly treat with additional immunosuppressants (e.g., IV IG). – Once stable, gradually taper steroids over ≥28 days.

Peripheral neuromotor syndromes (such as Guillain-Barre and myasthenia gravis)	**Any Grade**	**General Guidance**	**For Any Grade:** − The prompt diagnosis of immune-mediated peripheral neuromotor syndromes is important, since certain patients may unpredictably experience acute decompensations that can result in substantial morbidity or in the worst case, death. Special care should be taken for certain sentinel symptoms that may predict a more severe outcome, such as prominent dysphagia, rapidly progressive weakness, and signs of respiratory insufficiency or autonomic instability. − Patients should be evaluated to rule out any alternative etiology (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, and "repetitive stimulation" if myasthenia is suspected) are routinely indicated upon suspicion of such conditions and may be best facilitated by means of a neurology consultation. − It is important to consider that the use of steroids as the primary treatment of Guillain-Barre is not typically considered effective. Patients requiring treatment should be started with IV IG and followed by plasmapheresis if not responsive to IV IG.
	Grade 1	No dose modifications.	**For Grade 1:** − Consider, as necessary, discussing with the study physician. − Care should be taken to monitor patients for sentinel symptoms of a potential decompensation as described above. − Obtain a neurology consult.
	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	**For Grade 2:** − Consider, as necessary, discussing with the study physician. − Care should be taken to monitor patients for sentinel symptoms of a potential decompensation as described above. − Obtain a neurology consult

	or if there are signs of respiratory insufficiency or autonomic instability.	– Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin or duloxetine). *MYASTHENIA GRAVIS:* o Steroids may be successfully used to treat myasthenia gravis. It is important to consider that steroid therapy (especially with high doses) may result in transient worsening of myasthenia and should typically be administered in a monitored setting under supervision of a consulting neurologist. o Patients unable to tolerate steroids may be candidates for treatment with plasmapheresis or IV IG. Such decisions are best made in consultation with a neurologist, taking into account the unique needs of each patient. o If myasthenia gravis-like neurotoxicity is present, consider starting AChE inhibitor therapy in addition to steroids. Such therapy, if successful, can also serve to reinforce the diagnosis. *GUILLAIN-BARRE:* o It is important to consider here that the use of steroids as the primary treatment of Guillain-Barre is not typically considered effective. o Patients requiring treatment should be started with IV IG and followed by plasmapheresis if not responsive to IV IG.
Grade 3 or 4	**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 or autonomic instability.	**For Grade 3 or 4 (severe or life-threatening events):** – Consider, as necessary, discussing with study physician. – Recommend hospitalization. – Monitor symptoms and obtain neurological consult. *MYASTHENIA GRAVIS:* o Steroids may be successfully used to treat myasthenia gravis. They should typically be administered in a monitored setting under supervision of a consulting neurologist. o Patients unable to tolerate steroids may be candidates for treatment with plasmapheresis or IV IG.

		For Grade 4: Permanently discontinue study drug/study regimen.	o If myasthenia gravis-like neurotoxicity present, consider starting AChE inhibitor therapy in addition to steroids. Such therapy, if successful, can also serve to reinforce the diagnosis. *GUILLAIN-BARRE:* o It is important to consider here that the use of steroids as the primary treatment of Guillain-Barre is not typically considered effective. o Patients requiring treatment should be started with IV IG and followed by plasmapheresis if not responsive to IV IG.
Myocarditis	**Any Grade**	**General Guidance** Discontinue drug permanently if biopsy-proven immune-mediated myocarditis.	**For Any Grade:** – The prompt diagnosis of immune-mediated myocarditis is important, particularly in patients with baseline cardiopulmonary disease and reduced cardiac function. – Consider, as necessary, discussing with the study physician. – 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). A Cardiology consultation should be obtained early, with prompt assessment of whether and when to complete a cardiac biopsy, including any other diagnostic procedures. – Initial work-up should include clinical evaluation, BNP, cardiac enzymes, 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)
	Grade 1	No dose modifications required unless clinical suspicion is high, in which case hold study drug/study regimen dose during diagnostic	**For Grade 1 (no definitive findings):** - Monitor and closely follow up in 2 to 4 days for clinical symptoms, BNP, cardiac enzymes, ECG, ECHO, pulse oximetry

	(asymptomatic with laboratory (e.g., BNP) or cardiac imaging abnormalities)	work-up for other etiologies. If study drug/study regimen is held, resume after complete resolution to Grade 0.	(resting and exertion), and laboratory work-up as clinically indicated. - Consider using steroids if clinical suspicion is high.
	Grade 2, 3 or 4 (Grade 2: Symptoms with mild to moderate activity or exertion) (Grade 3: Severe with symptoms at rest or with minimal activity or exertion; intervention indicated) (Grade 4: Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support))	- If Grade 2 -- Hold study drug/study regimen dose until resolution to Grade 0. If toxicity rapidly improves to Grade 0, then the decision to reinitiate study drug/study regimen will be based upon treating physician's clinical judgment and after completion of steroid taper. If toxicity does not rapidly improve, permanently. discontinue study drug/study regimen. If Grade 3-4, permanently discontinue study drug/study regimen.	**For Grade 2-4:** – Monitor symptoms daily, hospitalize. – Promptly start IV methylprednisolone 2 to 4 mg/kg/day or equivalent after Cardiology consultation has determined whether and when to complete diagnostic procedures including a cardiac biopsy. – Supportive care (e.g., oxygen). – If no improvement within 3 to 5 days despite IV methylprednisolone at 2 to 4 mg/kg/day, promptly start immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]
Myositis/Polymyositis ("Poly/myositis")	**Any Grade**	**General Guidance**	**For Any Grade:** – 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, but rarely affects the extremities including hands and fingers; 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 study physician. – Initial work-up should include clinical evaluation, creatine kinase, aldolase, LDH, BUN/creatinine, erythrocyte sedimentation rate or C-reactive protein level, urine myoglobin, and additional laboratory work-up as indicated, including a number of possible rheumatological/antibody tests (i.e., consider whether a rheumatologist consultation is indicated and could guide need for rheumatoid factor, antinuclear antibody, anti-smooth muscle, antisynthetase [such as anti-Jo-1], and/or signal-recognition particle antibodies). Confirmatory testing may include electromyography, nerve conduction studies, MRI of the muscles, and/or a muscle biopsy. Consider Barium swallow for evaluation of dysphagia or dysphonia. Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections).
Grade 1 (mild pain)	- 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 study physician.
Grade 2 (moderate pain associated with weakness; pain limiting instrumental activities of daily living [ADLs])	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. – Obtain Neurology consult, and initiate evaluation. – Consider, as necessary, discussing with the study physician. – 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 3 to 5 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 to 5 days, consider start of immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). Caution: It is

		important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]
Grade 3 or 4 (pain associated with severe weakness; limiting self-care ADLs)	**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 4:** - Permanently discontinue study drug/study regimen.	**For Grade 3 or 4 (severe or life-threatening events):** – Monitor symptoms closely; recommend hospitalization. – Obtain Neurology consult, and complete full evaluation. – Consider, as necessary, discussing with the study physician. – 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 3 to 5 days, consider start of immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). 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. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).[a]

[a]ASCO Educational Book 2015 "Managing Immune Checkpoint Blocking Antibody Side Effects" by Michael Postow MD.

[b]FDA Liver Guidance Document 2009 Guidance for Industry: Drug Induced Liver Injury – Premarketing Clinical Evaluation.

AChE Acetylcholine esterase; ADL Activities of daily living; AE Adverse event; ALP Alkaline phosphatase test; ALT Alanine aminotransferase; AST Aspartate aminotransferase; BUN Blood urea nitrogen; CT Computed tomography; CTCAE Common Terminology Criteria for Adverse Events; ILD Interstitial lung disease; imAE immune-mediated adverse event; IG Immunoglobulin; IV Intravenous; GI Gastrointestinal; LFT Liver function tests; LLN Lower limit of normal; MRI Magnetic resonance imaging; NCI National Cancer Institute; NCCN National Comprehensive Cancer Network; PJP *Pneumocystis jirovecii* pneumonia (formerly known as *Pneumocystis carinii* pneumonia); PO By mouth; T3 Triiodothyronine; T4 Thyroxine; TB Total bilirubin; TNF Tumor necrosis factor; TSH Thyroid-stimulating hormone; ULN Upper limit of normal.