Abbreviated Title: Phase II M7824 in UC

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Title: A Phase II Study of Bintrafusp alfa (M7824) in Checkpoint Inhibitor Naïve and Refractory Subjects with Urothelial Carcinoma

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Investigational Agents:

Drug Name:	Bintrafusp alfa (M7824)
IND Number:	149968
Sponsor:	Center for Cancer Research (CCR), NCI, NIH
Manufacturer:	EMD Serono
Supplier	EMD Serono

Commercial Agents: None

PRÉCIS

Background:

- Metastatic urothelial carcinoma is lethal and incurable with a median overall survival of 14 months from diagnosis.
- Immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway have greatly changed clinical management of metastatic urothelial carcinoma (mUC) improving survival by 3 months in the second-line setting.
- Five PD-1/PD-L1 inhibitors are FDA-approved for for second-line mUC, two agents for first-line cisplatin-ineligible mUC. However, response rates are modest, ranging from15–20% in the second-line and 24% in the first-line cisplatin-ineligible.
- Therefore, novel strategies are needed to extend benefit of immunotherapy to the remaining ~75% of non-responders.
- Higher levels of transforming growth factor-beta (TGF-β) are associated with immune escape, therapy resistance and poor outcomes in advanced malignancies. Non-responders to anti-PD-1/PD-L1 antibodies have also been found to have increased TGF-β in the tumor microenvironment.
- Bintrafusp alfa (M7824) is a novel first-in-class bifunctional fusion protein composed of a monoclonal antibody against PD-L1 fused to the extracellular domain of human TGF-β receptor II (TGFβRII), which effectively functions to sequester or "trap" all three TGF-β isoforms. A phase I study of M7824 (NCT02517398) demonstrated a manageable safety profile and clinical efficacy among patients with heavily pre-treated advanced solid tumors.
- We hypothesize that M7824 is safe and improves outcomes in patients with checkpoint naïve or refractory urothelial carcinoma.

Objectives:

- To evaluate the activity of M7824 as determined by objective response rate (ORR) in two metastatic urothelial carcinoma cohorts:
    - Cohort 1: Checkpoint inhibitor naïve
        - Cohort 1A: cisplatin ineligible
        - Cohort 1B: refractory post-platinum therapy
    - Cohort 2: Checkpoint inhibitor previously treated patients
        - Cohort 2A: previously achieved a CR/PR
        - Cohort 2B: previously had SD/PD

Eligibility:

- Patients must have a histologically confirmed diagnosis of metastatic urothelial cancer.
- Patients may have been previously treated with prior cytotoxic chemotherapy regimen or targeted agent. Patients may have received any number of prior cytotoxic agents.
- 18 years of age or older

Design:

- This is an open label, non-randomized, single arm phase II trial of M7824 in checkpoint inhibitor naïve and previously treated patients with urothelial carcinoma of the bladder.
- M7824 (intravenous 1200 mg fixed dose) will be delivered every 2 weeks
- Patients will receive treatment in cycles consisting of 4 weeks.
- A maximum of 75 subjects will be enrolled in this trial.

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STATEMENT OF COMPLIANCE

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

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

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

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

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

To evaluate the activity of Bintrafusp alfa (M7824) as determined by objective response rate (ORR) in two metastatic urothelial carcinoma cohorts:

- Cohort 1: Checkpoint inhibitor naïve
  - Cohort 1A: cisplatin ineligible
  - Cohort 1B: refractory post-platinum therapy
- Cohort 2: Checkpoint inhibitor previously treated patients
  - Cohort 2A: previously achieved a CR/PR
  - Cohort 2B: previously had SD/PD

1.1.2 Secondary Objective(s)

- To evaluate the activity of Bintrafusp alfa (M7824) in metastatic urothelial carcinoma patients as determined by progression free survival (PFS) and overall survival (OS).
- To evaluate the safety of bintrafusp alfa (M7824) in metastatic urothelial carcinoma patients.

1.1.3 Exploratory Objective(s)

(1) Determine peripheral immune modulation using:

- Multi-parametric flow cytometry – immune subset analysis
- Cytokine/chemokine analysis (i.e TNFα, IFN-γ, IL-6, IL-8, etc.)
- Blood-based immune parameters (neutrophil-to-lymphocyte ratio)
- C-Met analysis
- Circulating Tumor Cells (CTCs), and circulating cell-free tumor DNA (cfDNA)

(2) Determine immune status of the tumor microenvironment using:
- Multiplex IHC for PD-L1 expression, CD8, CD4, FoxP3 T-cell infiltration
- RNA-Seq transcriptome sequencing for tumor/immune gene expression profile (and peripheral blood mononuclear cells)
- T-cell receptor (TCR) sequencing for T-cell clonality and MET protein levels
- Next generation sequencing (TSO-500) for tumor biomarkers in tumor samples

(3) Determine the diagnostic accuracy of diffusion weighted whole body MRI in this patient population, compared to standard imaging

1.2 BACKGROUND

1.2.1 Immunotherapy and Targeting the PD-1/PD-L1 axis in urothelial carcinoma

For decades, the standard-of-care for advanced urothelial carcinoma (UC) has almost exclusively revolved around cisplatin-based chemotherapy [1]. However, approximately 50% of patients are deemed cisplatin-ineligible and patients with adverse prognostic features derive marginal benefit from cisplatin-based chemotherapy [2-4]. Cisplatin-ineligible and platinum-refractory patients have limited options with an unclear impact on prolonging survival [5]. As such, less toxic and more efficacious therapies are needed. Immunotherapy in the form of Bacillus Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis* has been a long-standing pillar in the management of non-muscle invasive bladder cancer (NMIBC) and has provided some insights that immune-based approaches may be effective in more advanced and lethal forms of urothelial carcinoma [6].

The advent of immune checkpoint blockade has revolutionized cancer immunotherapy leading to an explosion of FDA approvals in UC. This class of agents disrupts immune-inhibitory molecular pathways, termed immune checkpoints, via blockade of ligand-receptor interactions thereby promoting T-cell activation and effector function [7]. At present, monoclonal antibodies targeting immune checkpoints, cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) or cognate ligand, programmed cell death-ligand 1 (PD-L1), are the only FDA-approved immune checkpoint blocking agents. There are currently five PD-1/PD-L1 inhibitors approved for second-line advanced UC [8-13], two additional indications in the first-line setting [14, 15].

Despite altering the prognostic landscape for a subset of patients with limited and toxic treatment options, many patients fail to respond to upfront therapy with immune checkpoint blockade and additional subsets of patients ultimately develop acquired resistance to this approach. To underscore this point, objective response rates in the second-line UC setting range from 15-21% with modestly higher response rates in the upfront UC setting (23.5-24%). As shown by Keynote-45 PD-1/PD-L1 check point blockade can have a durable response in metastatic urothelial patients. When compared to chemotherapy in the second line setting patients receiving pembrolizumab had an overall survival of 10.3 months (95% confidence interval [CI], 8.0 to 11.8) compared with 7.4 months (95% CI, 6.1 to 8.3). Novel combination strategies are needed to extend the benefit of immunotherapy to the remaining 75-85% of non-responders with metastatic non-prostate genitourinary cancer [8]. This population represents a clear unmet clinical need as these patients continue to succumb to rapid and lethal progression of disease despite currently approved therapies.

1.2.2 Rationale to Target Dual Immunosuppressive Pathways: PD-L1 and TGF-β blockade

Given that only a subset of patients (~20%) derive clinical benefit from anti-PD-1/PD-L1 immune checkpoint blockade, there is growing interest in combination approaches to improve outcomes. The concurrent targeting of additional immune suppressive pathways to enhance responses to anti-PD-1/PD-L1 blockade is emerging as an attractive therapeutic strategy.

TGF-β is pleotropic cytokine with potent immunosuppressive activity within the tumor microenvironment (TME) in advanced cancer.

Several recent preclinical reports have demonstrated that TGF-β signaling can directly and indirectly restrain effector T-cell function which promotes tumor immune evasion and therapeutic resistance to immunotherapy [16-18]. Poor response to anti-PDL-1 agent was associated with TGF- β signaling in fibroblasts, particularly in patients with CD8+ T cells that were excluded from the tumor parenchyma and instead found in the fibroblasts and collagen-rich peritumoral stroma, which is common in patients with metastatic UC [19]. Blockade of surface-bound TGF-β on regulatory T-cells (Tregs) has been shown to abrogate suppression of effector T-cell function within the TME [20]. Recent clinical investigation in metastatic urothelial cancer patients has demonstrated that TGF-β attenuates response to PD-L1 blockade (atezolizumab) via T-cell exclusion and reduced T-cell tumor infiltration. *TGFBR2* gene expression was significantly associated with clinical non-response and with reduced overall survival (see Figure 1).

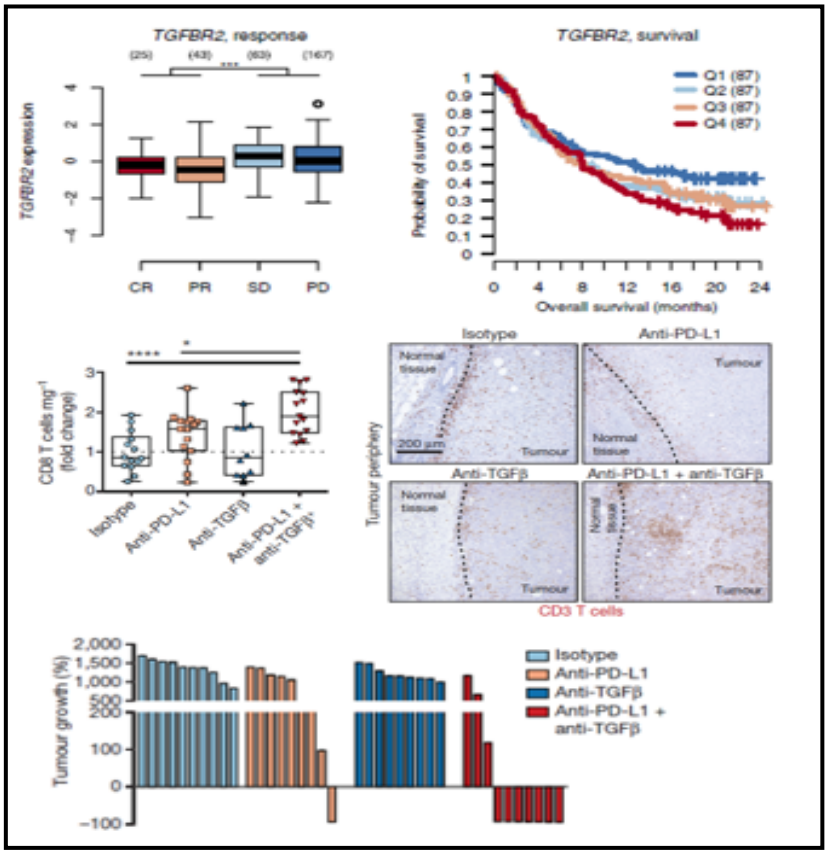


Figure 1*: TGF-β expression adversely correlates with clinical outcomes. TGFBR2 gene expression in urothelial carcinoma patients is significantly associated with non-response (P=0.0001) and reduced*

overall survival when split by quartiles (P=0.022) [top panel]. EMT6 and MC38 murine models demonstrate tumor regression and TME changes following therapeutic anti-TGF-β and anti PD-L1 treatment [middle and lower panel] [21].

Preclinical murine models demonstrated that stromal TGF-β signaling recapitulates an immune-excluded phenotype common to anti-PD-L1 non-responders. Further, the combination of PD-L1 blockade and TGF-β blockade facilitated T cell penetration into the center of the tumor, causing immune-mediated tumor regression and led to superior survival to either monotherapy in EMT6 and MC38 tumor models (see **Figure 1**). Additional, preclinical studies modeling human microsatellite-stable colorectal cancers which traditionally are characterized by low mutational load and poor response rates to anti-PD-1/PD-L1 checkpoint blockade have demonstrated that TGF-β inhibition promotes effector T-cell driven anti-tumor immune response and renders tumors susceptible to anti-PD-1/PD-L1 immunotherapy [22]. Other groups have independently confirmed the correlation of high mRNA expression of TGF-β1-3 with adverse prognosis and with reduced disease-free survival. Taken together, TGF-β (stromal and non-stromal) signaling within the TME appears to be a dominant mechanism of immune evasion and therapeutic targeting of this immunosuppressive axis may enrich the proportion of patients responding to anti-PD-1/PD-L1 immunotherapy and/or re-invigorate responses to PD-1/PD-L1 blockade.

1.2.3 Preclinical Activity with M7284, a bifunctional molecule targeting PD-L1 and TGF-β

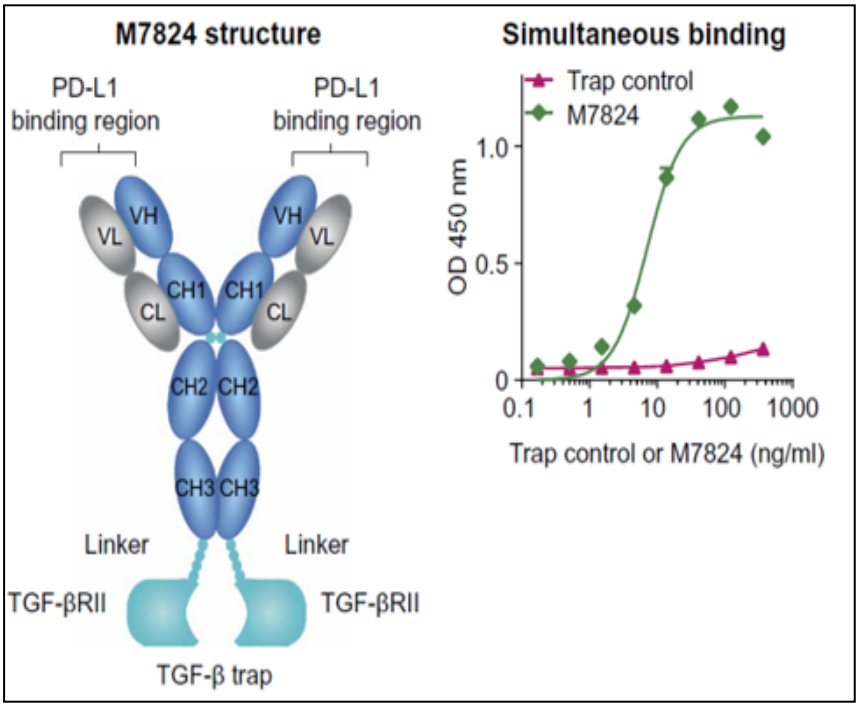


Figure 2: *M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. M7824 Structure (Left panel): Structure of M7824 composed of a fully human PD-L1 (IgG1) monoclonal antibody and TGF-β neutralizing trap moiety, fused to the CH3-C terminus of the FCγ1 domain of IgG via a flexible (Gl4-Ser)4 Gly linker. Simultaneous binding (Right Panel):*

M7824 (MSB0011359C) is a novel first-in-class bifunctional fusion protein composed of a monoclonal antibody against PD-L1 fused to the extracellular domain of human TGF-β receptor II (TGFβRII), which effectively functions to sequester or "trap" all three TGF-β isoforms [23].

Preclinical studies have shown that M7824 can simultaneously bind PD-L1 and TGF-β *in vitro* (**Figure 2**) and this bifunctional immunomodulatory capacity has translated to enhanced anti-tumor efficacy *in vivo* relative to blockade of either immune-inhibitory protein alone. Specifically, treatment with M7284 (IV 482µg) suppressed tumor growth, reduced lung metastases and prolonged survival in MC38 or EMT-6 tumor-bearing mice in comparison to treatment with isotype control, anti-PD-L1, Trap control and anti-PD-L1/Trap control. Furthermore, treatment with M7824 led to the development of long-term anti-tumor immunity in mice initially cured by M7284 that were subsequently re-challenged with EMT-6 or MC38 tumors – 100% and 80% of mice rejected tumor and survived re-challenge, respectively [23]. Quantitative and immunophenotypic analysis has shown that M7824 treatment is associated with robust CD8+ TME infiltration and a pro-inflammatory immune signature consistent with active innate and adaptive immunity (**Figure 3**). M7824 has also demonstrated encouraging efficacy across an array of human urothelial carcinoma cell lines (UMUC-3, UMUC-5, HTB-4, HTB-1, HTB-2, HTB-9 and HTB-5) [24]. Mechanistically, M7284 enhanced immune recognition of human urothelial cells via immunogenic modulation, increases in chemokine expression associated with T-cell trafficking, upregulation of TRAIL and antigen-specific CD8+ T-cell mediated lysis.

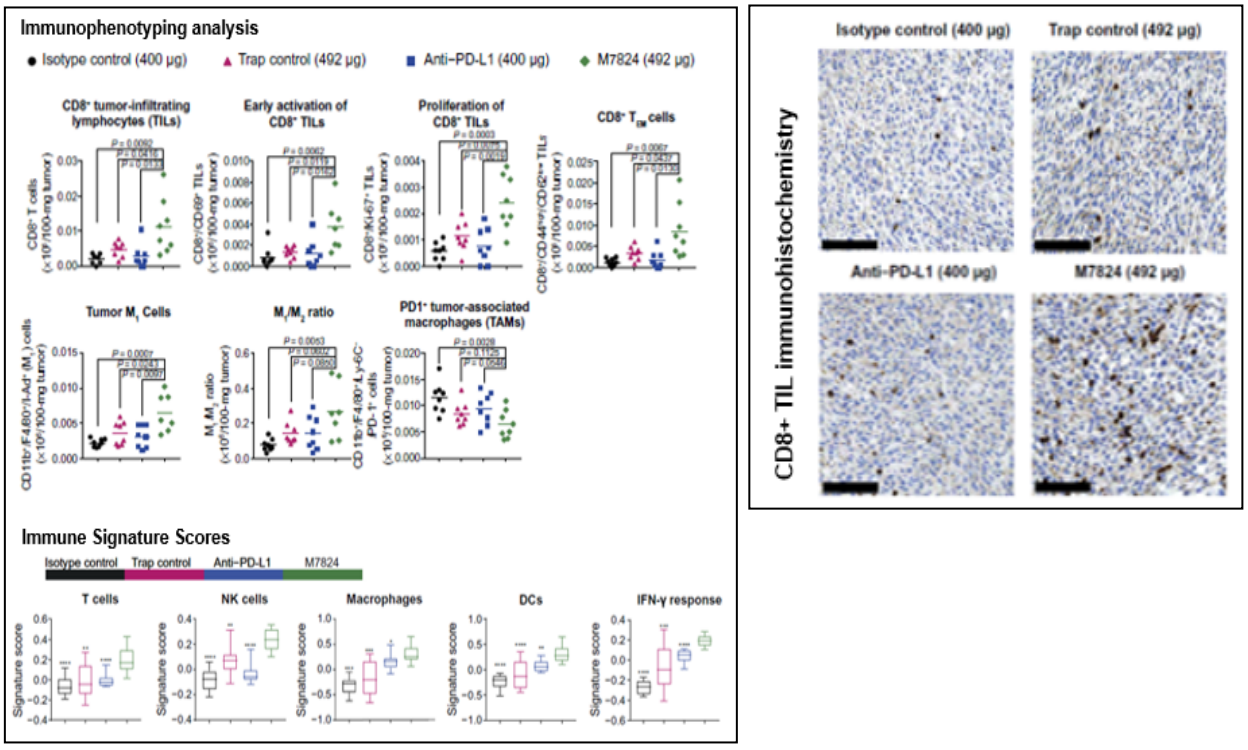


Figure 3: *M7824 promotes a distinct pro-immunogenic innate and adaptive immune cell signature in tumor-bearing mice. Representative anti-CD8a immunohistochemistry of treated EMT-6 tumors [right upper panels] and multi-parameter flow cytometric analysis of intratumoral immune subsets [left upper panels]. RNA-seq analysis of gene expression signatures associated with T -cell, NK cell, macrophages and dendritic cells tumor infiltrate and IFN-γ responses within intratumoral tissue [left lower panel]* [**23**].

The NCI led the first-in-human phase I trial of M7824 (NCT02517398) which demonstrated a manageable safety profile and nascent yet encouraging evidence of clinical activity in cohort of patients with heavily pre-treated advanced solid tumors [25]. In this standard 3+3 design phase I dose-escalation study, patients with tissue-confirmed metastatic or locally advanced solid tumors

that were refractory to or ineligible for standard therapy (ineligible if previously treated with prior immune checkpoint blockade) were treated with infusional M7824 doses of 1mg/kg, 3mg/kg, 10mg/kg and 20mg/kg once every two weeks (Q2W). A separate cohort of patients was initially treated with 0.3mg/kg to establish a pharmacokinetic/pharmacodynamic relationship followed by 10mg/kg dosing thereafter. A total of 19 patients were enrolled with maximum tolerated dose (MTD) not reached at the highest dose level of 20mg/kg Q2W. A total of 9 patients (47.4%) experienced any grade adverse event (AE) and four patients (21.1%) experienced ≥ grade 3 AE. Ultimately, three patients discontinued therapy due to treatment-related AEs (bullous pemphigoid, colitis, gastroparesis) leading the authors to conclude that M7824 had a manageable and acceptable toxicity/side effect profile across all evaluated dose levels. The side effect profile of M7824 is similar to other anti-PD-1/PD-L1 agents with the exception of increased incidence of keratocanthomas presumed to be related to TGF-β axis modulation which resolved following discontinuation of M7824 [26]. PK/PD studies demonstrated saturation of PD-L1 target occupancy and potent sequestration of all TGF-β isoforms at doses >1mg/kg.

Clinical activity was observed across all M7824 dose levels (**Figure 4**). One durable complete response (CR), two durable/confirmed partial responses (PR), one near PR, and two patients with stabilization of disease were observed. A single patient with progression of disease ultimately achieved a 45% decline in tumor burden on day 280 restaging suggestive of a treatment response with delayed kinetics [25]. As MTD was not reached, ongoing dose-expansion cohorts are evaluating several dose schedules including 1200mg Q2W and 10mg/kg Q2W.

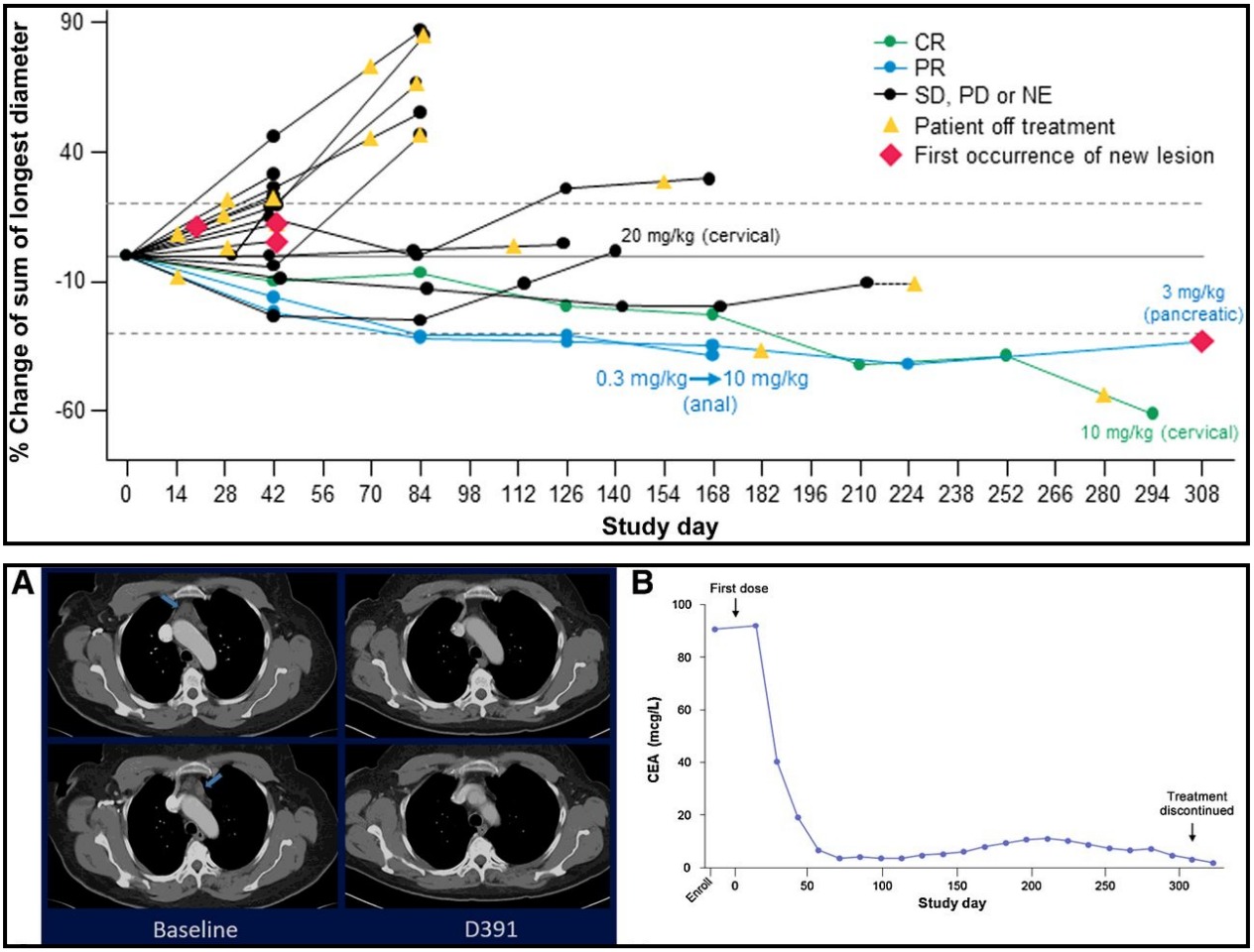


Figure 4: *Largest change from baseline in the sum of longest diameter per RECIST v1.1 and clinical responses with M7824. Dotted line at 30% indicates threshold for PR and at +20% indicates threshold for PD [upper panel]. Pre-treatment and post- M7824 (day 183) CT imaging and CEA biomarker trend in a 49-year-old patient with metastatic cervical cancer achieving a CR (per RECIST vl.l) [lower panels] A. This 49-year-old woman with metastatic cervical cancer after cisplatin/taxol followed by carboplatin/taxol plus bevacizumab was enrolled with two pathologically enlarging mediastinal lymph nodes (arrows in left side of figure). Restaging scan 7.5 months after enrollment showed reduction in lymph nodes to <1 cm by short-axis measurement, meeting RECIST v1.1 criteria for a CR. CR was durable as of her 13-month restaging scan (right side of figure). B. CEA curve for patient with ongoing durable confirmed CR. [25].*

1.2.4 M7824 dose levels and toxicity

In line with early clinical experience of similar anti-TGFβ agents, the toxicity profile of M7824 is predominantly manageable and highly comparable to that of checkpoint inhibitors. In the recently released results of the phase I study NCT02517398 (M7824) 'Phase 1 Trial of M7824, a Bifunctional Fusion Protein Targeting PD-L1 and TGFβ, in Advanced Solid Tumors' with dose escalation cohorts of 1, 3, 10, and 20mg/kg (exceeding the 1,200mg flat dose in this study), M7824 was given every two weeks and the MTD was not reached. Data suggested overall good tolerance. One out of 16 patients developed a keratoacanthoma which could be related to the TGFβ inhibition mechanism of M7824 [25]. There was no grade 4-5 TEAEs. The only dose-limiting toxicity (DLT)

observed was colitis. The highest dose for M7824 tested in EMR200647-001 was 30 mg/kg once every 2 weeks and the maximum tolerated dose was not reached. Three participants in EMR200647-001 dose escalation cohorts received the actual dose of at least 2400 mg q2w: 2 in 30 mg/kg cohort and 1 in 20 mg/kg. All 3 participants who received ≥ 2400 mg q2w had no DLTs. The overall safety profile of M7824 in Phase I was considered well tolerated, can be adequately managed and consistent across various tumor types. Refer to Investigator's Brochure for more details.

1.2.5 Hypothesis and Summary

Although PD-1/PD-L1 immune check-point inhibitors can lead to a durable response in advanced urothelial cancer patients, only a subset ranging from 15-20% have a clinical "response." Therefor enhancing PD-1/PD-L1 blockage to increase the responder rates is a logical strategy. Higher levels of transforming growth factor (TGF-β) are associated with immune escape, therapy resistance, and poor outcomes in advanced malignancies[27, 28]. Non-responders to anti-PD-L1 antibodies have been found to have increased TGF-β in the tumor microenvironment as well. [23, 29] Thus, TGF-β plays an important role in immune resistance to checkpoint inhibitors and other therapies.

M7824 is a bifunctional fusion protein composed of the extracellular domain of two TGF-β sequestering or trap molecule fused to a fully humanized monoclonal antibody against PD-L1 [30]. M7824 simultaneous blocks the PD-1 and TGF-β pathways of immune evasion which effects both the innate and adaptive immune systems. Preclinical studies show that M7824 enables immune-cell infiltration and can overcome resistance seen with other antibodies targeting the PD-1/PD-L1 interaction through phenotypic modifications[23, 31]. Early data from phase 1 trials demonstrated that M7824 has an acceptable safety profile and higher responses than expected in heavily pretreated patients with immunologically "cold" tumors [25, 31]. M7824-induced combined PD-L1 inhibition and TGF-β sequestration has the potential to overcome immune escape thereby increasing anti-tumor activity. This could potentially increase clinical response rates compared to standard PD-1/PD-L1 checkpoint blockage. There is currently no standard therapy for advanced urothelial cancer patients who have received prior PD-1/PD-L1 therapy that allows for challenging with a subsequent immunotherapy therapy. These patients are left with 2nd/3rd line chemotherapy such as taxanes and pemetrexed which have low response rates. Thus, M7824 could represent a potential treatment option for these patients. We hypothesize that M7824 is safe and improves outcomes in urothelial carcinoma of the bladder patients who are checkpoint naïve or refractory.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Ability to understand the purpose of the study, provide signed and dated informed consent, and able to comply with all procedures.

2.1.1.2 Male or female patients aged ≥ 18 years of age at time of consent.

2.1.1.3 Patients with histologically confirmed diagnosis of urothelial carcinoma of the urinary tract, including the renal pelvis, ureter, bladder, or urethra. Differentiation with variant

histologies (e.g. squamous cell differentiated) will be permitted. Mixed histologies are required to have a dominant urothelial/transitional cell pattern.

2.1.1.4 Patients must have metastatic disease defined as new or progressive lesions on cross-sectional imaging. Radiological evaluation should occur within 21 days prior to enrollment.

2.1.1.5 Patient must have evaluable and measurable disease, per RECIST 1.1. See Section **6.3** for the evaluation of measurable disease.

2.1.1.6 Patients may have been previously treated with prior cytotoxic chemotherapy regimen or targeted agent. Patients may have received any number of prior cytotoxic agents.

2.1.1.7 Patients may have had prior immunomodulating therapy including therapy targeting the PD-1/PDL-1 axis (cohort 2A and B) but excluding prior treatment with M7824.

2.1.1.8 Pre-treatment tissue biopsy and/or archival tissue availability for PD-L1 expression testing is mandatory for enrollment.

2.1.1.9 Eastern Cooperative Oncology Group (ECOG) performance status ≤2(see Section **16.2**)

2.1.1.10 Required laboratory values reflective of organ function are listed below:

- Absolute neutrophil count ≥ 1500/µL
- Platelets ≥ 100,000/µL
- Hemoglobin ≥ 9 g/dL (erythrocyte tranfusions are allowed to achieve acceptable Hgb)
- AST(SGOT)/ALT(SGPT) ≤ 1.5 × institutional upper limit of normal (ULN) with the following exception:
    - Patients with liver involvement who have AST and ALT ≤ 5 × ULN may be enrolled.
- Total bilirubin within normal limits with the following exceptions:
    - Patients with known Gilbert disease who have serum bilirubin level ≤ 3 ULN may be enrolled.
    - Patients with tumor liver involvement bilirubin with ≤ 3.0 × ULN.
- INR and aPTT ≤ 1.5 × ULN
    - This applies only to patients who are not receiving therapeutic anticoagulation; patients receiving therapeutic anticoagulation (such as low-molecular-weight heparin or warfarin) should be on a stable dose.
- Creatinine clearance (CrCl) ≥ 30 mL/min/1.73 m^2 (GFR may be used in place of CrCl. Creatinine clearance or eGFR should be calculated per institutional standard)

2.1.1.11 The effects of M7824 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use strict and effective contraception (hormonal or barrier method of birth control; abstinence) during treatment

and for at least 65 days for women and 125 days for men, after the last dose of M7824 administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.12 Human immunodeficiency virus (HIV) positive patients are eligible if on stable dose of highly active antiretroviral therapy (HAART), CD4 counts are greater than 350 cells/mm^3 and viral load is undetectable.

2.1.1.13 Patients with previously treated brain or central nervous system (CNS) metastases are eligible provided that the subjects have recovered from any acute effects of radiotherapy and is not requiring steroids, and any whole brain radiation therapy or any stereotactic radiosurgery was completed at least 2 weeks prior to M7824 administration.

2.1.1.14 Hepatitis B virus (HBV) positive patients are eligible-they must have been treated and on a stable dose of antivirals (eg, entecavir, tenofovir, or lamivudine; adefovir or interferon are not allowed) at study entry and with planned monitoring and management according to appropriate labeling guidance.

2.1.1.15 Hepatitis C virus (HCV) positive patients are eligible if participants are on active HCV therapy at study entry and on a stable dose without documented clinically significant impaired liver function test or hematologic abnormalities and with planned monitoring and management according to appropriate labeling guidance.

2.1.2 Cohort 1A Cisplatin Ineligible Specific Inclusion Criteria (first-line for metastatic cisplatin-ineligible)

- No prior chemotherapy for inoperable locally advanced or metastatic or recurrent UC
    - For patients who received prior adjuvant/neoadjuvant chemotherapy or chemoradiation for UC, a treatment-free interval > 12 months between the last treatment administration and the date of recurrence is required in order to be considered treatment naive in the metastatic setting. Prior local intravesical chemotherapy or immunotherapy is allowed if completed at least 4 weeks prior to the initiation of study treatment.
- Ineligible ("unfit") for chemotherapy or cisplatin-based chemotherapy as defined by any one of the following criteria [32]:
    - Impaired renal function (CrCl > 30 but < 60 mL/min); GFR should be calculated per institutional standard.
    - A hearing loss (measured by audiometry) of 25 dB at two contiguous frequencies
    - Grade ≥ 2 peripheral neuropathy (i.e., sensory alteration or paresthesias including tingling)
    - ECOG performance score of 2
    - Patient declines chemotherapy after informed discussion with the study doctor

2.1.3 Cohort 1B Refractory Post-platinum Therapy Specific Inclusion Criteria (second-line for metastatic disease)

- Disease progression during or following treatment with a platinum-containing regimen for inoperable locally advanced or metastatic urothelial carcinoma or disease recurrence. Examples of regimens include cisplatin + gemcitabine (GC), methotrexate + vinblastine sulfate + doxorubicin + cisplatin (MVAC), and carboplatin + gemcitabine (CarboGem).
    - Patients who received prior adjuvant/neoadjuvant chemotherapy and progressed within 12 months of treatment with a platinum-containing adjuvant/neoadjuvant regimen will be considered as second-line patients.

2.1.4 Cohort 2A Checkpoint Inhibitor Previously Treated Patients that Previously Achieved a Complete Response (CR) or Partial Respsonse (PR) Specific Inclusion Criteria

- Patients must have been treated with at least one treatment of a PD-1/PD-L1 checkpoint inhibitor for advance or metastatic UC and achieved a complete response or partial respsonse by RECIST 1.1 criteria.

2.1.5 Cohort 2B Checkpoint inhibitor previously treated patients that previously had stable disease (SD) or progressive disease (PD)

2.1.6 Specific Inclusion Criteria

- Patients must have been treated with at least one treatment of a PD-1/PD-L1 checkpoint inhibitor for advance or metastatic UC and had stable disease or a progressive disease by RECIST 1.1 criteria.

2.1.7 Exclusion Criteria

2.1.7.1 History of allergic reactions attributed to compounds of similar chemical or biologic composition to M7824 investigational agents used in the study.

2.1.7.2 Uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

2.1.7.3 Symptomatic central nervous system metastasis.

2.1.7.4 Subjects unwilling to accept blood products as medically indicated

2.1.7.5 Pregnant women are excluded from this study because M7824 is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with M7824, breastfeeding should be discontinued if the mother is treated with these agents.

2.1.7.6 Patients with any active or recent history of a known or suspected autoimmune disease (with the exception of diabetes type I, vitiligo, psoriasis, or hypo- or hyperthyroid diseases not requiring immunosuppressive treatment) or recent history of a syndrome that required treatment with either systemic corticosteroids (>10 mg daily prednisone equivalent) or

immunosuppressive medications. Inhaled steroids and adrenal replacement steroid doses up to 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.

2.1.7.7 Patients with a "currently active" second malignancy other than non-melanoma skin cancers or cervical carcinoma in situ or incidental organ-confined prostate cancer found on cystoprostatectomy (provided that the following criteria are met: Stage T2N0M0 or lower; Gleason score ≤ 3+4, PSA undetectable). Patients are not considered to have a "currently active" malignancy if they have completed therapy and are free of disease for ≥ 2 years and currently do not require systemic therapy.

2.1.7.8 Patients who have received or will receive a live vaccine within 30 days prior to the first administration of study intervention. Seasonal flu vaccines that do not contain a live virus are permitted. Locally approved COVID vaccines are permitted.

2.1.7.9 Patients having tumor lesion(s) in the liver or chest which are 10 cm or larger.

2.1.7.10 Patients previously treated with M7824.

2.1.7.11 Patients previously treated with PD-1/PD-L1 checkpoint inhibitors (for Cohorts 1A and 1B only)

2.1.8 Recruitment Strategies

Both men and women and members of all races and ethnic groups are eligible for this trial. This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media. This study will be listed on www.clinicaltrials.gov and participants will be recruited from the current patient population as well as referrals at NIH.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

2.2.2 Screening activities performed after a consent for screening has been signed

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

All screening tests and procedures must be performed within 21 days prior to initiation of study therapy except physical exam with skin assessment, ECOG, vital signs, blood tests and preganacy test (repeated if >7 days):

- Complete medical history and physical examination (including height, weight, vital signs, and ECOG performance status) and skin assessment.
- Electrocardiogram (ECG)
- CT scan of chest, abdomen and pelvis with IV and PO (as indicated) contrast, or CT chest and MRI abdomen/pelvis may be performed, and [18F]-Sodium Fluoride (NaF) PET/CT bone scan (if indicated).
- Laboratory assessments: CBC with differential, acute care panel (sodium, potassium, chloride, total CO2 (Bicarbonate), creatinine, glucose, urea nitrogen), mineral panel (albumin, calcium total, magnesium total, phosphorus), hepatic panel (alkaline phosphatase, ALT/GPT, AST/GOT, total bilirubin, direct bilirubin), ionized calcium, amylase, lipase, LDH, total protein, GGT, Hepatitis B and C, PT/INR, PTT, urine analysis.
- Hepatitis B and C antibody panel
- Thyroid function tests – TSH, reflex T3 and T4
- Pre-treatment tissue biopsy and/or archival tissue availability for PD-L1 expression retrospective testing is mandatory for enrollment. PD-L1 will be performed retrospectively by EMD Serono using a test determined by EMD Serono.
- Pregnancy test for females of childbearing-potential (within 3 days prior to first dose of study drug).
- Lymphocyte Phenotyping - CD4/CD8 will be performed on known HIV-positive patients.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Treatment Assignment Procedures

Cohorts

Patients will be assigned to the appropriate cohort depending on prior checkpoint inhibitor therapy and documented or reviewed radiologic responses to prior checkpoint inhibitor therapy. Whenever possible prior scans will be loaded to our PACS and reviewed for chort 2A and 2B.

Subjects from all cohorts will be treated with a flat dose of M7824 (1200 mg) every 2 weeks (+/- 3 days for scheduling issues).

Number	Name	Description
1	Cohort 1A	Checkpoint inhibitor naïve patients who are cisplatin-ineligible
2	Cohort 1B	Checkpoint inhibitor naïve patients who are refractory post-platinum therapy
3	Cohort 2A	Checkpoint inhibitor refractory patients with prior Complete Response (CR) or Partial Response (PR) as per RECIST 1.1 as best response
4	Cohort 2B	Checkpoint inhibitor refractory patients with Stable Disease (SD) or Progressive Disease (PD) as per RECIST 1.1 as best response

Arms

Number	Name	Description
1	Arm 1	Treatment with fixed dose of M7824 (1200 mg) every 2 weeks

Arm assignment

Subjects from all cohorts (1A, 1B, 2A and 2B) will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

Tests done at screening do not need to be repeated on baseline (C1D1) if performed within 21 days prior to initiation of study therapy except physical exam, ECOG, vital signs, blood tests (CBC with differential, acute care, mineral and hepatic panels, amylase, lipase, LDH, GGT, thyroid function tests) and pregnancy test (repeated if >7 days). Please refer to Section **2.2** for details.

Correlative Studies: Please refer to Section **5.2** for information regarding collection of blood, and tissue for correlative research studies.

3 STUDY IMPLEMENTATION

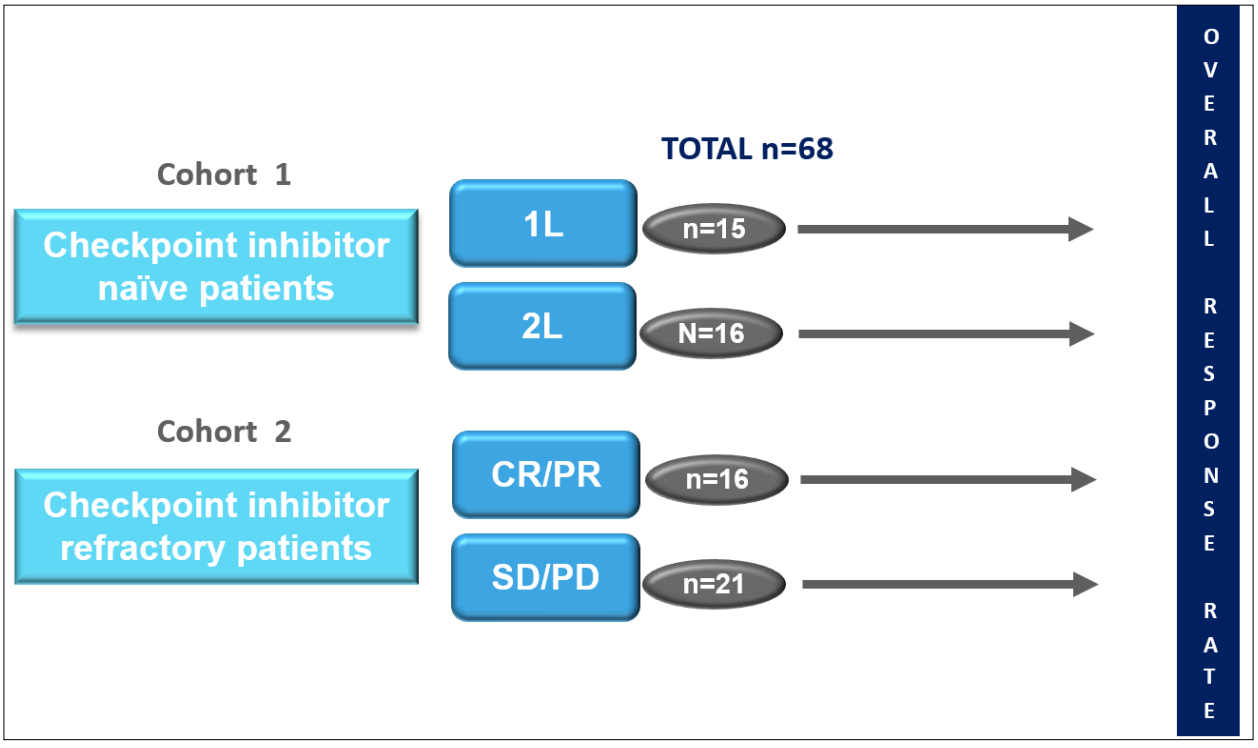
3.1 STUDY DESIGN

- This is an open label, non-randomized, single arm phase II trial of M7824 in checkpoint inhibitor naïve and refractory patients with urothelial carcinoma.
- We will plan to use a fixed dose of 1200 mg delivered IV every 2 weeks (Q2W) based on the recent NCI-led phase I dose-escalation trial of M7824 in advanced solid tumors (NCT02517398) [33]. M7284 appeared to have a manageable safety profile and early evidence of clinically efficacy. The MTD was not reached in this study at 20mg/kg and

PD-L1 receptor occupancy and sequestration of TGF-β isoforms 1-3 was achieved at M7284 dose levels >1mg/kg.

- A maximum of 68 evaluable subjects will be enrolled in checkpoint naïve [Cohort 1] and checkpoint inhibitor refractory [Cohort 2] patients with urothelial carcinoma of the bladder.
    - Cohort 1A: Checkpoint inhibitor naïve patients who are cisplatin-ineligible
    - Cohort 1B: Checkpoint inhibitor naïve patients who are refractory post-platinum therapy
    - Cohort 2A: Checkpoint inhibitor refractory patients with Complete Response (CR) or Partial Response (PR) as per RECIST 1.1
    - Cohort 2B: Checkpoint inhibitor refractory patients with Stable Disease (SD) or Progressive Disease (PD) as per RECIST 1.1
- Enrollment of 2-3 patients per month is expected on this trial; thus, 2 to 3 years is anticipated as the accrual period.
- Restaging will be performed every 2 cycles (8 weeks). Safety/tolerability analysis and secondary clinical efficacy analysis will be ongoing through cycle 4. Patients can continue study drug in all arms provided there is no confirmed evidence of progression, unacceptable toxicity or withdrawal from the study.

3.1.1 Schema



1L - First line; 2L - Second line; CR - complete response; PR - partial response; SD - stable disease; PD - progressive disease

Treatment Schema of M7824: 28-day cycle (D1/D15)

If appropriate screening imaging is done within 21 days of C1D1 repeat screening imaging is not required.

Cycles and Day	C1D1	C1D15	C2D1	C2D15	C3D1	C3D15	C4D1	C4D15
M7824 (1200mg IV Q2W)	X	X	X	X	X	X	X	X

3.2 DRUG ADMINISTRATION

3.2.1 M7824

See Section **14.1** for drug preparation, administration and infusion times.

3.3 DOSE MODIFICATIONS

3.3.1 M7824

M7824 is planned to be administered at 1200 mg (flat dose) every two weeks. Dosing may be delayed up to 12 weeks for toxicity or logistical reasons, including the inability of the patient to get to the clinic. Dose reductions are not allowed.

3.3.1.1 Premedications

Current experience revealed that infusion-related reactions (IRRs) to bintrafusp alfa occur seldomly and are generally mild to moderate in severity. Therefore, administration of a premedication is generally not required.

If an Investigator deems necessary to administer a premedication to a particular participant, an antihistamine (e.g. 25 to 50 mg diphenhydramine) and paracetamol (acetaminophen, 500 to 650 mg intravenously or equivalent oral dose) 30 to 60 minutes prior to bintrafusp alfa infusion is recommended. Premedication should be administered for subsequent bintrafusp alfa doses based upon clinical judgment and presence/severity of prior infusion reactions. This regimen may be modified based on local treatment standards and guidelines as appropriate, provided it does not include systemic corticosteroids. If Grade ≥ 2 infusion reactions are seen during the first two infusions, premedication should not be stopped. Steroids as premedication are not permitted.

3.3.1.2 Adverse Drug Reactions (ADRs) Requiring Treatment Discontinuation

Any Grade 4 ADRs require permanent treatment discontinuation except for single laboratory values out of normal range that do not have any clinical correlate, and resolve to Grade ≤ 1 or baseline grade within 7 days with adequate medical management. Grade 3 or 4 symptomatic endocrinopathies (e.g., thyroiditis or hypophysitis), treatment should be delayed and treatment started according to NCCN guidelines. If condition improves to Grade 1, treatment may be resumed.

Subjects with grade 4 amylase or lipase abnormalities must permanently discontinue treatment. If the participant develops any evidence of pancreatitis, it should be managed accordingly.

Any Grade 3 ADRs require treatment discontinuation <u>except</u> for any of the following:

- Transient (≤ 6 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management.
- Transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to ≤ Grade 1 or Baseline grade.
- Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor that resolves within 7 days with optimal medical management.
- Any single Grade ≥ 3 drug-related transaminase, alkaline phosphatase, or bilirubin abnormality with no other associated laboratory (or other) abnormality that is not associated with symptoms or clinical manifestations of hepatitis. If the liver function abnormality not associated with symptoms or clinical manifestations of hepatitis has not resolved to Grade ≤ 1 within the subsequent 2 cycles (28 days), the subject should permanently discontinue treatment with M7824.
- Subjects with asymptomatic Grade 3 drug-related amylase or lipase abnormalities may continue treatment.
- .
- Grade 3 Hgb decrease (< 8.0 g/dL) that is clinically manageable with blood transfusions or erythroid growth factor use.
- Increases in Eastern Cooperative Oncology Group performance status (ECOG PS) ≥ 3 that resolves to ≤ 2 by Day 1 of the next cycle (infusions should not be given if the ECOG PS is ≥ 3 on the day of M7824 administration and should be delayed until ECOG PS ≤ 2).
- Keratoacanthoma and squamous cell carcinoma of the skin. Any suspicious skin lesion should be biopsied for confirmation of diagnosis and be surgically removed as indicated by dermatologist and a dermatological consult obtained.
- Other immune-related ADRs, see NCCN guidelines for the management of immune related adverse events

3.3.1.3 Adverse Drug Reactions Requiring Management

Any Grade 2 ADR should be managed as follows:

- If a Grade 2 ADR resolves to Grade ≤ 1 by the last day of the current cycle, treatment may continue.
- If a Grade 2 ADR does not resolve to Grade ≤ 1 by the last day of the current cycle but it is manageable and / or not clinically relevant, it is possible the infusion will be given on the following cycle.

3.3.1.4 Infusion-related Reactions (IRR)

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. These possible IRR are identified based on a list of MedDRA PTs and divided into reactions versus signs and symptoms.

- An IRR should be considered when onset is on the day of infusion (during or after the infusion) or the day after the infusion (irrespective of resolution date) for any infusion related reaction, drug hypersensitivity, anaphylactic reaction, hypersensitivity and/or Type 1 hypersensitivity.
- Signs and symptoms of infusion related reactions and hypersensitivity/allergic reactions should be considered when onset is on the day of infusion (during or after the infusion) and resolved completely with the end date within 2 days after onset. Signs and symptoms may include but not limited to: fever, chills or shaking, rigors, flushing, hypotension, wheezing, pruritus, rash and hypoxemia, shortness of breath, back or neck pain, facial swelling, dizziness, feeling of passing out.
- Infusion-related reactions and hypersensitivity reactions (Grades 1 to 4) should be handled according to the Guidelines provided in **Table 1**.
- Management of immune-mediated reactions is described in **Table 2**.

Table 1: Treatment Modifications for Symptoms of Infusion-related Reactions including Immediate Hypersensitivity

NCI-CTCAE Grade	Treatment Modification
Grade 1 - mild Mild transient reaction; in general, infusion interruption not indicated; intervention not indicated	• Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • Hold infusion if deemed necessary by the investigator.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, nonsteroidal anti-inflammatory drugs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	• Stop the infusion of the study intervention. • Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • If symptoms resolve quickly, resume infusion at 50% of original rate with close monitoring of any worsening signs and symptoms, otherwise dosing held until resolution of symptoms with mandated premedication for the next scheduled visit. • If not improving, consider administration of glucocorticoids and stop the infusion for that day. • If the participant has a second IRR Grade ≥ 2 on the slower infusion rate despite premedication, the infusion should be stopped, and the investigator may consider withdrawal of this participant from the study.
Grade 3 or Grade 4 – severe or life-threatening ○ Grade 3: Prolonged (for example, not rapidly responsive to symptomatic	• Stop the infusion of study intervention immediately and disconnect infusion tubing from the participant with additional appropriate medical measures and closely monitor until

medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. ○ Grade 4: Life-threatening consequences; urgent intervention indicated.	deemed medically stable by the attending Investigator. Hospitalization and/or close monitoring is recommended Administration of glucocorticoids may be required • For Grade 3 or 4 IRRs, permanent discontinuation of study intervention is mandated.
Once the infusion is interrupted or rate reduced to 50% of previous infusion rate, it must remain decreased for all subsequent infusions. For all types and grades of infusion reactions, details about drug physical constitution, method of preparation, and infusion must be recorded. Participants should be instructed to report any delayed reaction immediately.	
1 IRR=infusion-related reactions, IV=intravenous, NCI-CTCAE=National Cancer Institute-Common Terminology Criteria for Adverse Event, NSAIDs=nonsteroidal anti-inflammatory drugs.	

Table 2: Management of immune- mediated adverse events

Immune-related AEs are specific to immunotherapies and vary by organ system. The following immune-related AEs are important identified risks for M7824:

- Immune-related pneumonitis
- Immune-related hepatitis
- Immune-related colitis
- Immune-related nephritis and renal dysfunction
- Immune-related endocrinopathies
- (thyroid disorders, adrenal insufficiency, type 1 diabetes mellitus, pituitary disorders)
- Immune related rash
- Other immune-related events (myositis, myocarditis, encephalitis)

The following immune-related AEs are important potential risks for M7824:

- Guillain-Barré syndrome
- Uveitis
- Pancreatitis
- Myasthenia gravis/myasthenic syndrome

Recommended guidance and management for specific irAEs are provided in the current National Comprehensive Cancer Network (NCCN) guideline available at http://www.nccn.org.

Requirements in addition to NCCN guidelines:

- Permanent treatment discontinuation is required in case of immune-related Grade 4 rash/inflammatory dermatitis, nephritis, autoimmune hemolytic anemia, hemolytic uremic

syndrome, aplastic anemia, immune thrombocytopenia, acquired thrombotic thrombocytopenic purpura inflammatory arthritis, myositis and polymyalgia-like syndrome.

- For Grade 4 immune-related lymphopenia, permanent treatment discontinuation will be required, if lymphopenia is considered immune-related in nature, no clear alternative explanation exists for the event, and it does not resolve within 14 days. Permanent treatment discontinuation is not required when the AE is manifested by a single laboratory value out of normal range without any clinical correlates. In this case, treatment should be held until the etiology is determined. If the event is not considered immune-related and resolves to Grade ≤ 1, restarting treatment may be considered.
- For Grade 1 immune-related pneumonitis: continue treatment. If clinically indicated, monitor participants weekly or more frequently as needed with history, physical examination and pulse oximetry. If symptoms appear and/or changes in the physical exam are noted, treat as Grade 2.
- For myositis: in case of management with rituximab, treatment should be discontinued.
- For Grade 3 or 4 endocrinopathies: withhold until clinically stable or permanently discontinue depending on severity.
- For hepatitis with no tumor involvement of the liver: withhold if total bilirubin increases to more than 1.5 and up to 3 times ULN, permanently discontinue if more than 3 times ULN
- Hepatitis with tumor involvement of the liver: permanently discontinue if total bilirubin increases to more than 3 times ULN.

3.3.1.5 Additional Modifications for Subjects with Grade 2 Infusion-related Reactions

If, in the event of a Grade 2 infusion-related reaction that does not improve or worsens after implementation of the modifications indicated in Table 1.

- Management of immune-mediated reactions is described in **Table 2.**

Table 1: Treatment Modifications for Symptoms of Infusion-related Reactions including Immediate Hypersensitivity

NCI-CTCAE Grade	Treatment Modification
Grade 1 - mild Mild transient reaction; in general, infusion interruption not indicated; intervention not indicated	• Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • Hold infusion if deemed necessary by the investigator.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, nonsteroidal	• Stop the infusion of the study intervention. • Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator.

anti-inflammatory drugs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	• If symptoms resolve quickly, resume infusion at 50% of original rate with close monitoring of any worsening signs and symptoms, otherwise dosing held until resolution of symptoms with mandated premedication for the next scheduled visit. • If not improving, consider administration of glucocorticoids and stop the infusion for that day. • If the participant has a second IRR Grade ≥ 2 on the slower infusion rate despite premedication, the infusion should be stopped, and the investigator may consider withdrawal of this participant from the study.
Grade 3 or Grade 4 – severe or life-threatening o Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. o Grade 4: Life-threatening consequences; urgent intervention indicated.	• Stop the infusion of study intervention immediately and disconnect infusion tubing from the participant with additional appropriate medical measures and closely monitor until deemed medically stable by the attending Investigator. Hospitalization and/or close monitoring is recommended Administration of glucocorticoids may be required • For Grade 3 or 4 IRRs, permanent discontinuation of study intervention is mandated.
Once the infusion is interrupted or rate reduced to 50% of previous infusion rate, it must remain decreased for all subsequent infusions. For all types and grades of infusion reactions, details about drug physical constitution, method of preparation, and infusion must be recorded. Participants should be instructed to report any delayed reaction immediately.	
2 IRR=infusion-related reactions, IV=intravenous, NCI-CTCAE=National Cancer Institute-Common Terminology Criteria for Adverse Event, NSAIDs=nonsteroidal anti-inflammatory drugs.	

(including reducing the infusion rate by 50%), the investigator may consider treatment with corticosteroids and the infusion of M7824 should be stopped for that day. At the next infusion, the investigator may consider the addition of H2-blocker antihistamines (for example, famotidine), in addition to premedication, for select subjects. However, prophylactic steroids are NOT permitted. If the subject has a second infusion-related reaction Grade ≥ 2 on the slower infusion rate, with or without the addition of further medication to premedication, the infusion should be stopped and the subject may be removed from M7824 treatment at the discretion of the investigator.

3.3.1.6 Immediate Hypersensitivity Reaction

Hypersensitivity reactions may require immediate intensive care. Bintrafusp alfa should be administered in a setting that allows immediate access to an intensive care unit or equivalent

environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Potent steroids (e.g. dexamethasone), catecholamines (e.g. epinephrine), allergy medications (IV antihistamines), bronchodilators, or equivalents and oxygen should be available for immediate access.

A complete guideline for the emergency treatment of anaphylactic reactions according to the Working Group of the Resuscitation Council United Kingdom and can be found at https://www.resus.org.uk/pages/reaction.pdf.

3.3.1.7 Severe Hypersensitivity Reactions and Flu-like Symptoms

If a hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice including ACLS guidelines.

Subjects should be instructed to report any delayed reactions to the investigator immediately.

A. Symptoms

- Impaired airway
- Decreased oxygen saturation (< 92%)
- Confusion
- Lethargy
- Hypotension
- Pale / clammy skin
- Cyanosis

B. Management

- Epinephrine injection and IV dexamethasone
- Patient should be placed on cardiac, blood pressure, heart rate, and oxygen saturation monitor immediately
- Alert intensive care unit for possible transfer if required

Treatment is based on clinical assessment and at the discretion of the Investigator. For prophylaxis of flu-like symptoms, a NSAID, for example, ibuprofen 400 mg or comparable NSAID dose, may be administered 2 hours before and 8 hours after the start of each dose of M7824 IV infusion.

3.3.1.8 Immune-Related Adverse Events

Immune-related adverse events (irAEs) are specific to immunotherapy. IrAEs are important identified risks for M7824. Immune-related adverse events should be documented as an 'Adverse Event of Special Interest (AESI),' and it is recommended to involve the principal investigator at first incidence and subsequently as needed for follow-up. Details of the diagnostic work-up will be requested by the study team. An immune related adverse event (irAE) is defined as off target side effects associated with exposure of immunogenic drug and is consistent with immune mechanism. In the process of identification of irAEs, any possible etiology of neoplastic, infectious, metabolic, toxin, or any other factor should be ruled out. Serologic, histologic (biopsy),

and/or immunologic results should be obtained to evaluate the differential diagnosis and/or support an immune-mediated cause.
The recommendations for irAE management, in accordance with the joint American Society of Clinical Oncology (ASCO) Clinical Practice Guidelines (Brahmer, 2018) and National Comprehensive Cancer Network (NCCN Guidelines®). General management by CTCAE v5.0 grading, as per ASCO, is listed below (ASCO organ/system specific toxicity management takes precident over below general guidelines):
Grade 1: study treatment should be continued with close monitoring, with the exception of some neurologic, hematologic, and cardiac toxicities.
Grade 2: study treatment may be suspended for most Grade 2 toxicities, with consideration of resuming when symptoms revert to Grade 1 or less. Corticosteroids may be administered (initial dose of 0.5 to 1 mg/kg/d of prednisone or equivalent).
Grade 3: study treatment is generally suspended and the high-dose corticosteroids (prednisone 1 to 2 mg/kg/d or methylprednisolone 1 to 2 mg/kg/d) treatment should initiated. Corticosteroids should be tapered over the course of at least 4 to 6 weeks. Some refractory cases may require infliximab or other immunosuppressive therapy.
Grade 4: in general, permanent discontinuation of study treatment is recommended, with the exception of endocrinopathies that have been controlled by hormone replacement.

3.3.1.9 Anemia

For anemia events assessed as treatment-related, items queried may include but are not limited to detailed relevant past medical and treatment history, bruising tendency, history of blood transfusions and/or dependency, details such as concomitant medications, all laboratory data, updated dosing information and recent tumor evaluation scans.

M7824 treatment-related anemia is an important risk (see Investigators' Brochure). Notably, there are many reasons for anemia in patients with advanced cancer, and Hb level of at least 9 g/dl is required for this study. A thorough investigation of new anemia cases of unspecified etiology is requested. Safety laboratory testing of relevant blood parameters is conducted per schedule of assessment.

General Guidance for anemia management and evaluation:

- Participants must enter the study with Hgb values at least 9 g/dL and baseline anemia evaluation is conducted per **Table 3.**
- All relevant hematologic testing for treatment related anemias should be done prior to blood transfusion, if clinically feasible.
- If a participant experiences significant anemia (<8g/dl), then the amount of blood to be drawn may be reduced by not taking blood at selected time points for -exploratory biomarkers. The decision to reduce the time points for these biomarkers will be taken by the investigator in consultation with the medical monitor. This will be documented.
- Transfusion should be performed at the discretion of the investigator, based on clinical assessment and considered when participant experiences significant anemia. Attempt should be made to initiate work-up (as specified below) for cause of anemia prior to transfusion if clinically feasible to not confound this workup.

- Guidance for evaluation of suspected treatment-related anemias is provided in **Table 3.**

Management of treatment-related anemias is provided in Table 4.

Table 3: Evaluation Guidance of Suspected Treatment-related Anemia AEs

Basic anemia evaluation (prior to transfusion, if feasible)	
CBC with differential (e.g. MCV, RDW, ANC, hemoglobin, hematocrit) Reticulocytes counts Peripheral blood smear for cell morphological assessment Complete metabolic panel including liver panel-LFTs, bilirubin, LDH, renal function, and serum folate, B12 values and other chemistries Coagulation factors (PT, PTT, INR) Urinalysis including culture Iron panel (TIBC, ferritin, Fe) TSH/thyroid panel Fecal-occult blood testing Erythropoietin Haptoglobin	
Further recommendation based on suspected etiology (in addition to baseline anemia testing)	
Unknown etiology, suspect possible hemolysis	Coombs test, fibrinogen, d-dimer Consider hematology consultation. Consider blood transfusion at clinical discretion.
Unknown etiology, suspect possible bleeding	Consider blood transfusion at clinical discretion. Consider surgical/interventional radiology consultation. Consider imaging, as clinically indicated (e.g. FAST scan, CT scan, MRI, angiography). Consider endoscopy (upper/lower)
Unknown etiology despite above work-up	Hematology consultation Consider bone marrow aspiration/morphologic evaluation

Table 4: Management of Treatment-Related Anemia

• Hematology assessment must be performed at baseline, prior to each bintrafusp alfa dose, at the end of treatment visit and at 28 (±5 days) days post-treatment safety follow-up. • Participants must enter the study with Hgb values at least 9g/dl • All relevant hematological testing for treatment-related anemias should be done prior to a blood transfusion, if clinically feasible
Diagnostic Work-up

• Baseline Anemia Evaluation
  • CBC with emphasis on red cell indices
• If indicated and at clinical discretion, the following should be considered:
  • Iron studies
  • Serum Folate and Vit B12 values
  • Coagulation factors
  • Fecal occult blood
  • Urinalysis
  • Hormone panel: TSH, Erythropoietin
  • Peripheral blood smear

Further recommendation based on suspected etiology (in addition to basic anemia testing)

• Suspected Hemolysis
  • bilirubin, LDH, Coombs test, haptoglobin
• Suspected bleeding:
  • Consider imaging/interventional radiology consultation as indicated
  • Consider imaging and/or endoscopy as clinically indicated
• Suspected aplastic anemia:
  • Hematology consultation
  • Consider bone marrow aspiration/morphologic evaluation

Additional consideration In general, blood transfusions and erythroid growth factors are permitted as clinically indicated.

3.3.1.10 Rash with Hyperkeratosis / Keratoacanthoma / Squamous Cell Carcinoma of the Skin

Monitoring will include skin assessments every 4 weeks with biopsy of suspicious lesions. Dermatological consults should be requested as needed. Treatment modifications for reactions caused by M7824 are addressed in **Table 5.**

Table 5: Management of M7824 mediated Skin Reactions

• Hyperkeratosis • Keratoacanthoma • Cutaneous squamous cell carcinoma (cSCC) • Basal cell carcinoma • Actinic keratosis
Management

• Discontinuation or termination not required in most cases. Continuation of treatment should be evaluated by the Investigator.
• Emollients may be used
• Develop diagnostic and treatment plan in collaboration with Investigator and dermatologist
• Treatment follow-up will depend on number and localization of lesions.
  o Single lesion: full excision may be recommended
  o Multiple lesion or location not suitable for full excision: Mohrs surgery, cryotherapy or other standard treatment options depending on pathology. Retinoids may be used per Investigator decision.
• Close clinical follow-up for re-evaluation, resolution and potential recurrence should be implemented
• In general, treatment of M7824 mediated skin lesions should be based on local guidelines/standard of care.

Additional consideration: Keratoacanthoma lesions may resolve spontaneously without surgical intervention within weeks after discontinuing bintrafusp alfa. Consult with Medical Monitor as needed for management of M7824 mediated skin lesions.

3.3.1.11 Impaired Wound Healing o

Management should be discussed on a case-by-case basis. Dermatological consults should be requested as needed.

- Impaired wound healing is considered an important potential risk for bintrafusp alfa
- Management should be discussed with Medical Monitor for participants requiring surgery on study.
- It is recommended to hold study intervention for approximately 4 weeks post major surgery for observation.
- Post-operative wound healing should be closely monitored

3.3.1.12 Dose Interruptions for Adverse Events not Related to Study Drug

In case of Grade 3 and Grade 4 AEs not study drug related, the study treatment may be interrupted based on the investigator assessment and the subject will be medically treated for the event.

If the AE reduces to a lower tolerable grade the study treatment might be resumed in the subsequent cycle. If the AE remains the same despite the medical treatment until the next treatment (second cycle after the AE occurred) a consideration of a possible extension of the dose interruption for up to 1 additional cycle or a permanent withdrawal from the study treatment should be considered.

If upon the resumed study treatment, the subject experiences the same AE, permanent withdrawal from the study treatment should be considered.

Grade 3 and 4 laboratory abnormalities that do not have clinical significance and are not related to study drugs do not require dose interruption.

3.3.1.13 Embryo-Fetal Toxicities

Embryo-fetal toxicities are a known risk of the PD-1/PD-L1 targeting class and are considered important potential risk for M7824. Animal models link the PD-1/PD-L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue [34-37]. Embryofetal toxicity is an important potential risk of bintrafusp alfa. Embryo-fetal and reproductive toxicities have also been investigated in animal models for a humanized mAb targeting TGFβ1. At doses as high as 30 mg/kg, no maternal reproductive toxicity or embryo-fetal lethality were observed in rabbits [38]. To mitigate these potential risks, pregnant participants are excluded from the study, and all participants of childbearing/conceiving potential must use highly effective contraception (See Section **2.1.1.11**).

3.3.1.14 Mild to Moderate Bleeding Events

Bleeding events of mild to moderate severity were observed in participants treated with M7824 in ongoing studies and are a potential risk for M7824. Events may include epistaxis, hemoptysis, gingival bleeding, or hematuria among others. In general, majority of these events were observed to be self-limiting, did not require intervention, and resolved without discontinuation of study treatment.

Management of bleeding is provided in **Table 6**.

Table 6: Management of Bleeding Adverse Events

Bleeding Adverse Events	
• Bleeding adverse events are considered important identified risk for bintrafusp alfa. • In general, mild and moderate mucosal bleedings resolve without discontinuation of treatment. • These events may include, but are not limited to the following: o Epistaxis o Hemoptysis o Gingival bleeding o Hematuria	
Non-tumor Bleeding	
Grading	Management
Grade 2	• If resolves to Grade ≤ 1 by the day before the next infusion, study intervention may be continued • If not resolved to Grade ≤ 1 by the day before the next infusion, but is manageable and /or not clinically relevant, consult Medical Monitor to assess if clinically reasonable to administer the following infusion.
Grade 3	• Permanently discontinue treatment unless an alternative explanation can be identified (such as concomitant use of antithrombotic agents, traumatic events, etc.) • In case of alternative explanations, hold study treatment until the event recovers to Grade ≤ 1
Grade 4	• Treatment must be permanently discontinued if no alternative explanation is identified.
Tumor Bleeding	
Grade ≥ 2	• Study treatment must be held till the event recovers to Grade ≤ 1 • Permanently discontinue treatment if the Investigator considers the participant to be at risk for additional severe bleeding.

3.4 STUDY CALENDAR

Please refer to **Appendix A: Study Calendar**.

3.5 COST AND COMPENSATION

3.5.1 Costs

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

3.5.2 Compensation

No compensation will be provided for study participation.

3.5.3 Reimbursement

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

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 28-30 days after the last dose of study therapy.

3.6.1 Criteria for removal from protocol therapy

- Completion of study therapy
- Progressive disease
- DLT if clincially indicated.
- Intercurrent illness or medical circumstances.
- Voluntary withdrawal from the treatment regimen
- PI determines further treatment on this study is not in patient's best interest
- Patients who are off-treatment but still on-study will be followed as per the schedule in **Appendix A: Study Calendar**, until they begin alternative therapy or voluntarily withdrawal from follow-up testing. Thereafter, they may be followed on-study for survival and other secondary endpoints.
- Positive pregnancy test

3.6.2 Off-Study Criteria

- Death
- Patient request to be withdrawn from study
- Non-compliance with study treatment and/or testing, or lost to follow-up, at discretion of PI

3.6.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 6 scheduled visits and is unable to be contacted by the study site staff.

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

- The site will attempt to contact the participant and reschedule the missed visit for 3 months and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PERMITTED MEDICINES

Any medications (other than those excluded by the clinical trial protocol) that are considered necessary to protect subject welfare and will not interfere with the trial medication may be given at the Investigator's discretion.

4.2 PROHIBITED MEDICINES

The following treatments must not be administered during the trial:

- Immunotherapy including interferon, immunosuppressive drugs (for example, systemic corticosteroids except for short term treatment of allergic reactions, endocrine replacement therapy at low dose prednisone [>10 mg daily] or equivalent, or for the treatment of irAEs or other appropriate short-term steroid use), or other experimental pharmaceutical products. Short term administration of systemic steroid or other immunosuppressant such as infliximab or mycophenolate (that is, for allergic reactions or the management of irAEs) is allowed. Steroids with no or minimal systemic effect (topical, inhalation) are allowed.
- Prophylactic use of corticosteroids for infusion related reactions is prohibited.
- Herbal remedies with immunostimulating properties (for example, mistletoe extract) or known to potentially interfere with major organ function (for example, hypericin).
- Any live vaccine therapies for the prevention of infectious disease. Administration of inactivated vaccines is allowed (for example, inactivated influenza or SARS-CoV-2 vaccines, and locally approved COVID vaccines).

If the administration of a non-permitted concomitant drug becomes necessary during the trial, the subject will be withdrawn from trial treatment.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Please refer to Section **5.2** for sample collection and timepoints.

5.1.1 Immune Monitoring and Peripheral Immune Subset Analysis

Peripheral Blood Mononuclear Cells (PBMCs) will be analyzed for changes in standard immune cell types (T-cell receptor clonality, CD4 and CD8 T cells, natural killer [NK] cells, regulatory T

cells [Tregs], myeloid-derived suppressor cells [MDSCs], and dendritic cells) as well as 123 immune cell subsets, as described elsewhere [39, 40].

PBMC analysis may include phenotypic and functional analysis of immune-cell subsets (such as CD4 and CD8 T cells, NK cells, Tregs, and MDSCs) including proliferation, cytokine production, lysis, and suppression using multicolor flow cytometry based assays.

Collection and handling:

For PBMCs collection, six 10mL sodium heparin (green top) tubes will be used.

Sample Transport/Storage:

Samples will be stored by Clinical Services Program – Leidos Biomedical Research, Inc. (CSP)..

Attn: Theresa Burks/Dr. Inglefield
1050 Boyles Street
Bldg. 496/Room 121
Frederick, MD 21702

On days samples are drawn, Jen Bangh at CSP (part of NCI Frederick Central Repositories) should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens. Samples can be picked up Monday through Friday, three times per day (~9, 11, 2).

Samples will be analyzed by the Jeffery Schlom, Laboratory of Tumor Immunology and Biology (LTIB)NCI.

5.1.2 Comprehensive Cytokine/Chemokine analysis

To evaluate for circulating systemic levels of cytokines, chemokines of patients on treatment and correlate levels and changes with clinical activity of the study drug, plasma will be collected per PI discretion:

1. Plasma may be analyzed pre- and post-therapy for the following soluble factors: sCD27, sCD40 ligand using assays adapted to ECL platform.
2. Plasma may be analyzed for changes in cytokines (IFN-γ, IL-6, IL-8, TNF-α , etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers using ELISA or multiplexed assays (e.g. Mesoscale, Luminex, cytokine bead array).

Collection and handling:

For plasma collection, one 4 ml lavender top EDTA tube will be used..

Sample Transport/Storage:

Blood samples will be stored by Dr. Figg's laboratory and processed and analyzed by Dr. Liang Cao's laboratory.

For Figg lab, please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964. For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov by e-mail or at 240-858-3191.

5.1.3 Circulating cell-free DNA (cfDNA) isolation

The primary purpose of these studies is to evaluate for circulating cell-free DNA (cfDNA) of patients on treatment and correlate levels and changes with clinical activity of the study drug combination.

- Blood draw and storage
    - Draw 2 X 10 mL blood samples to EDTA tubes (lavender tops) follow the manufacture's instruction.
    - Remove tube from adaptor and immediately mix by gentle inversion 8 to 10 times. **CRITICAL STEP**: Do not shake or vortex tubes as cellular lysis could occur.
    - Tubes should be stored and transport at room temperature for up to four (4) hrs. Do not freeze.

Sample Transport/Storage:

Blood/plasma samples will be stored by Dr. Figg's laboratory and processed and analyzed by Dr. Liang Cao's laboratory.

For Figg lab, please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964. For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number). For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov by e-mail or at 240-858-3191.

5.1.4 HGF and Met

Plasma will be collected to determine whether plasma HGF and Met levels are biomarkers for metastatic non-prostate genitourinary tumors and/or response to treatment.

Collection and handling

10mL of whole blood collected in one 10mL EDTA tube at room temperature will be collected at each time point. Plasma samples will be stored by Dr. Figg's laboratory and processed and analyzed by Dr. Cao's laboratory.

5.1.5 Circulating Tumor Cells (CTCs)

To evaluate and characterize changes in expression of immune-related markers on CTCs. All patients enrolled on study will have CTC's measured using Epic Science's "no cell left behind" platform [41].

Collection and handling

CTC Samples should be collected in the 10 mL Streck Cell-Free DNA BCT. These tubes are commercially available through Streck (Omaha, NE).

IMPORTANT: The first 5 mL of blood collected from the fresh venipuncture cannot be used for the collection into the Streck tubes due to possibility of contaminating epithelial cells during venipuncture. Please ensure that at least one blood tube of 5 mL or more is collected prior to collection of the CTC sample to avoid adversely affecting the test results.

Prevention of Backflow:

Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:

- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper uppermost.
- Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.
- Tube contents should not touch stopper or the end of the needle during the collection procedure.

Blood Collection Instructions:

**Arrange for the same day shipment!

- Confirm blood tube is not expired. Expired tubes should not be used for blood collection.
- Draw whole blood sample into 10 mL Streck Cell-Free DNA BCT tube (*see note regarding prevention of backflow). Fill tube until blood flow stops. NOTE: Epic requires a minimum of 4 mL blood per sample, but a full 10 mL tube of blood should be provided when possible.
- Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Tube inversion prevents clotting. Inadequate or delayed mixing may result in inaccurate test results.
- Label the tube with subject's identification and date and time of blood draw. Unlabeled blood tubes may not be processed.
- Keep sample at room temperature and ship on day of collection in shipper with ambient gel packs.

Specimen Shipment Logistics

All shipments must include requisition forms that contain Patient ID, Collection Date and time, Collection Site (including address), Time Point (if applicable), and the appropriate trial code (20C0012/ Epic Internal ID: NE-003). Clinical sites should provide email notification of sample shipment to Epic Sciences on the day of collection. The email should contain:

- Trial codes (20C0012/ Epic Internal ID: NE-003)
- Patient ID
- Collection date and time*
- Time point/Visit

- Tracking information

If possible, include a scanned copy of the completed sample requisition form. Partner will be responsible for all blood collection supplies, shipping materials, and shipping expenses.

* When collection time is not provided, Epic Sciences will assume the sample was collected at 8:00AM (local time) on the date of collection.

Send notification to: partners@epicsciences.com

Ship to: Epic Science

Attn: (20C0012/Epic internal ID: NE-003)

9381 Judicial Dr. Suite 200

Sand Diego, CA 92121

Contact phone number is: 1-858-356-6610

Epic Science will promptly notify the project stakeholders via email of any holiday closures.

5.1.6 c-Met analysis by RT-PCR

Peripheral blood for analysis of c-Met mRNA will be collected in a PAXgene tube (PreAnalytix; 2.5 cc peripheral blood per tube) per the manufacturer's instructions. After the blood is drawn, the tube should be inverted several times, placed at 4°C. RNA will be isolated using the PAXgene Blood RNA Kit according to the manufacturer's instructions. The first strand cDNA will be synthesized from total RNA by using a HC reverse transcription kit (Applied Biosystems). The reaction mixture is diluted to 100 µL with TE. PCR will be performed using the c-met primers).

Sample Transport/Storage:

Blood/plasma samples will be stored by Dr. Figg's laboratory and processed and analyzed by the Dr. Liang Cao's laboratory.

For Figg lab, please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964. For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number). For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov by e-mail or at 240-858-3191.

5.1.7 Tumor Studies

5.1.7.1 RNA-Seq, TCR sequencing, and MET Protein

- **Single Cell RNA-Seq**: from RNA extracted from fresh frozen tumor tissue for transcriptome sequencing (RNA-Seq) to evaluate if RNA gene expression profiling that measure responsive genes relative to antigen presentation, chemokine expression, cytotoxic activity and adaptive immune resistance are correlated with clinical response.
- **TCR Sequencing**: from RNA extracted from fresh frozen tumor tissue: To evaluate and characterize the clonality of the T-cell population as a way to infer the peripheral expansion of tumor-reactive T-cell clones in response to study drug combination.

- **MET protein**: MET protein evaluated from tumor biopsies will be correlated with clinical responses

Tumor tissue will be prioritized (highest to lowest):

1) Formalin fixed for diagnostic and DNA/RNA extraction from FFPE (20 slides total)

- 10 unstained FFPE slides (room temperature) will be sent to Laboratory of Pathology. Please contact Dr. Jun Wei (weij@mail.nih.gov), prior to transport. Telephone: 240-760-7428.
- 10 unstained FFPE slides (room temperature) will be stored in the Laboratory of Pathology for additional tumor testing

2) Three cores will be collected when possible and flash frozen for RNA extraction

- For frozen tumor, place 1 core in 1.5mL Eppendorf or 15mL conical tube or 1-2 blocks on dry ice. DNA and RNA extracted by Laboratory of Pathology and sent to Oncogenomics Section for exome sequencing, and TCR sequencing (contact Dr. Young Song, songyo@mail.nih.gov). Single Cell RNA Seq will be performed in the Single Cell Analysis Facility (SCAF) (contact Dr. Michael Kelly, michael.kelly3@nih.gov / 240-760-6301).
- 1-2 frozen cores will be stored by Dr. Bottaro's lab for additional testing. Please contact Rene Costello (rene.costello@nih.gov / 301-443-6915) to arrange for pick-up of sample)

FFPE:

In the case of exisiting (pre-treatment) FFPE slide/blocks already available for the consenting patients, the sample will be transferred to the Laboratory of Pathology (LP).

Collection and handling

For DNA and RNA to be extracted from FFPE tissues the Laboratory of Pathology in consultation with the surgical pathologist will evaluate specimen for adequacy and diagnosis. For the majority of cases the Laboratory of Pathology will perform both the extraction and quality control (QC) of nucleic acids. This will include DNA and RNA from tumor, and DNA from germline tissue (matched normal, blood,or other tissues). The DNA will be used for exome sequencing, and RNA sequencing will be performed in a research setting. Final approval to proceed to sequencing will be determined by passing all QCs according to the SOPs.

Fresh/Frozen Tissue:

If a pre-treatment biopsy is performed, the surgical sample will be obtained by the interventional radiology or surgical dissection/resection, and the sample collection will be processed as follows:

Interventional Radiologist: Recommend 19 gauge needle or larger in caliber after discussion and at the discretion of the intervention radiologist

Contact Research Nurse or Rene Costello to be present at time of procedure.

1. 1-2 cores snap frozen sent to OncoGenomics (contact Dr. Young Song, songyo@mail.nih.gov). Transport to OncoGenomics in 4°C wet ice.
2. 1-2 cores in container cooled in dry ice for flash freezing. Transport to Don Bottaro's lab in dry ice. (contact Rene Costello, rene.costello@nih.gov).

Sample Transport/Storage:

Laboratory of Pathology (LP) will arrange transportation of the DNA (and RNA sample) to the Genetics Branch. Building 37, Room 6144, Bethesda, MD 20892. Please contact Dr. Jun Wei (weij@mail.nih.gov), prior to transport. Telephone: 240-760-7428. Dr. Wei will ensure that the patient has been consented and that he has been emailed a copy of the consent form before the samples are processed for RNA sequencing

The primary purpose of the studies is to utilize Omics high throughput techniques to identify novel biomarkers and targets of response to this study drug.

- **Transcriptome sequencing (RNA-Seq)** from RNA extracted from blood and fresh frozen tumor tissue: To evaluate if RNA gene expression profiling that measure responsive genes relative to antigen presentation, chemokine expression, cytotoxic activity and adaptive immune resistance are correlated with clinical response.
- **T-cell receptor (TCR) sequencing** from RNA extracted from fresh frozen tumor tissue: To evaluate and characterize the clonality of the T-cell population as a way to infer the peripheral expansion of tumor-reactive T-cell clones in response to study drug.

5.1.7.2 Genomics studies– TrueSight Oncology

Pre-treatment tissue biopsy and/or tissue availability is mandatory. On-treatment biopsy is required unless obtaining biopsy is not technically or clinically feasible. Post-treatment biopsy is optional.

- 10 unstained FFPE slides (room temperature) from in-house surgery and outside materials will be brought directly to General Surgical Pathology office - 10/Room 2S262.

Paraffin-embedded tissue specimens from outside must be formalin fixed. They will be submitted either as a tissue block containing >20% tumor, or a minimum of 10 FFPE unstained slides for molecular pathology testing and surgical pathology review.

Alterations to the above requirements on sample submission will be at the discretion of the Laboratory of Pathology.

The TruSight Oncology 500 (TSO-500) is a next-generation sequencing (NGS) assay that analyzes cancer-relevant genes from both DNA and RNA in one integrated workflow. During library preparation, enrichment chemistry is optimized to capture nucleic acid targets from formalin-fixed, paraffin-embedded (FFPE) tissues. With simultaneous analysis of both DNA and RNA, various types of biomarkers relevant to a given tumor type (single nucleotide variants (SNVs), indels, fusions, splice variants, tumor mutation burden (TMB), and microsatellite instability (MSI)) can be assessed from the same sample in a single assay. The RNA panel uses a probe design that enables capture of both known fusions and novel fusion partners. The TSO500 panel includes 523 genes for DNA mutation detection and 55 genes for fusion and splice variant detection.

The new molecular pathology report is also incorporated with reporting software for clinical actionability as Tier levels of FDA-approved drug and clinical trials, and pathogenicity based on AMP/ASCO/CAP/ACMG guidelines.

NIH Clinical Record Interactive System (CRIS) orders will be placed to notify the laboratory of pathology.

Collection and handling:

Please contact Mark Raffeld, mraff@mail.nih.gov at 301-480-8927.

5.1.8 Multiplex Immunohistochemistry

To correlate baseline expression of PD-L1 (on tumor cells, tumor-infiltrating immune cells or both) with activity of study drug. To evaluate modulation of intratumoral immune contexture / immunophenotype (i.e. CD3, CD8, CD4, FoxP3) in response to study drug compared with baseline.

- In the case of exisiting (pre-treatment) FFPE slide/blocks already available for the consenting patients, the sample will be transferred to the Laboratory of Pathology (LP).
- For patients undergoing dedicated research biopsies (baseline or on-treatment C3D1), tissue will be sent to the Laboratory of Pathology (LP) for accessioning, processing overnight in formalin and embedding in paraffin (FFPE).

Plan for analysis and quantification:

Multiplex Immunohistochemistry: 4-5 micron thick sections of tissue (from FFPE blocks mentioned above) will be mounted on 25 x 75mm, charged glass slides (routinely used in Laboratory of Pathology). These unstained slides (US) will undergo several cycles of immunostaining using the Opal Method after initial deparaffinization and hydration. In brief, the tissue will be subject to heat induced epitope retrieval (HIER), followed by blocking agent, then primary and secondary (HRP) antibody incubation. This is followed by signal amplification using tyramide based fluorophores which will covalently bind to tissue at the site of primary antibody attachement (antigen of interest). This is followed by stripping of antibodies using HIER, to allow for 2nd round of new antibodies of intetest. These steps are repeated for total of 6-7 times to allow detection of 6-7 markers. This is followed by DAPI (4',6-diamidino-2-phenylindole) counterstain to allow for nuclear detection. Once all antigens of interest are stained, a multiplex scanning protocol will be created on Vectra Polaris scanner (Perkinelmer), to allow for whole slide imaging at 10-20X. This is followed by higher resolution of regions of interest (ROIs) picked by trained research pathologist (Dr. H. Sater). The scans genetrated at high resolution are called multispectral images (MSIs) and have the im3 format. These can only be analyzed using inform software (available in Dr. Sater's Lab). The software allows for spectral unmixing based on a spectral library built from single stains with each fluorophore used and scanned with multiplex protocol as MSI.

The initial step will be to create monoplex IHC using CD3 antibody on all available cases. These will be scanned and MSIs will be generated to achieve the primary endpoint analysis (CD3+ cell density). Quantification of cells expressing CD3 will be perfomed on inform using training sets of images to create an algorithm. The algorithm, will be used for quantifying the cell number per area. Representative ROIs (planned to be 5 in number and randomly selected) might vary based on tissue size. Once CD3+ staining is verified for accuracy and integrity of images, we will move to next step which is multiplexing. The antibodies of interest are based on preclinical data or

hypothesis driven. We plan to investigate a panel of multiple immune cell susbets such as T-helper cells (CD4+), T regulatory cells (CD4+FOXP3+), Cytotoxic T cells (CD3+CD8+), NK cells (CD56+), macrophages (CD68), B cells (CD19+) and other immune activation/immunsuppresive markers (IDO, PD-1, PD-L1, Ki67, ICOS, CD69, CD27, CD16 and other T cell activation markers) as well as tumor cell markers (Cytokeratin, Vimentin, etc) in a multiplex staining method. This method ensures simultaneous staining of different markers in the same tissue plane for colocaliztion and near neighbor analysis. The staining will be done in a panel like fashion where 6-7 immunohistochemical (IHC) markers plus DAPI are chosen in any single slide.

All images are analyzed and saved in a secure server, accessible and monitored by Dr. H. Sater.

Collection and handing:

The Sater Lab will request 4-5 micron thick recuts of 5-10 FFPE slides by submitting a Tissue Resource Committee (TRC) request for Human Biological Materials for Research form. These biopsies will be examined by a research pathologist (Dr. H Sater) for the presence of tumor and tissue integrity and at least 5 consecutive recuts of each biopsy is deemed optimal for analysis. Hematoxyln and Eosin staining will be first done on the deepest level cut to reassess integrity of tissue for further analysis.

Please contact:
Dr. Houssein A. Sater MD / Beatriz Walters (GMC, CCR/NCI)
10 Center Drive, Building 10, Room 6B12
Bethesda, MD 20892
E-mail: houssein.abdulsater@nih.gov

5.1.9 Imaging

Background

Magnetic resonance imaging (MRI) offers a high tissue contrast which allows direct imaging of the bones and soft tissues. Application of whole-body MRI approach has been shown to be clinically useful in certain diseases such as myeloma, lung cancer, lymphoma at both initial diagnosis/staging and treatment follow up [42-44]. In addition to anatomic pulse sequences, inclusion of diffusion weighted MRI (DW MRI) has also been documented to improve disease detection and monitoring steps. In this protocol the whole-body MR imaging protocol will include two different anatomic pulse sequences in coronal planes (T1W MRI and Short TI Inversion Recovery [STIR]) and a functional imaging pulse sequence (b800 diffusion weighted MRI). The DW MRI enables detection of lesions/foci with increased cellularity whereas the two anatomic pulse sequences will enable characterization of these lesions/foci detected on DW MRI. There will be no intravenous gadolinium injection-based imaging sequences, therefore it is a relatively less invasive novel imaging technique for patients with urothelial carcinoma especially considering the need for follow up imaging.

Methods

Whole body MRIs will be performed in B3 Molecular Imaging Clinic using a clinical 3 Tesla scanner. The patients will be imaged in supine position using surface coils and no intravenous contrast injection will be utilized. The imaging will be done in 5 stacks (head/neck, chest, abdomen, pelvis, thighs) for T1W MRI and STIR and in 4 stacks (head/neck, chest, abdomen, pelvis/thighs) for DW MRI. For chest, abdomen and pelvis stacks the patient will be asked to hold

breath for a few times. The active scan time is planned to be 18-25 minutes. The entire imaging session is planned to be 40 minutes.

Timepoints: Please refer to Section **5.2** and **Appendix A: Study Calendar** for timepoints.

MPI MRI WB Protocol Parameters

Sequence	Plane	FOV	Slice Thickness	TR/TE/TI	Voxel size	Matrix	Flip Angle	Scan Time
T1W	Coronal 5 stacks	450 L-R 300 H-F	6 mm	694/8/N/A	Scan 1.6x1.6 Recon1.17x1.17	188x247	90	4 minutes
STIR	Coronal 5 stacks	450 L-R 300 H-F	6 mm	8399/70/230	Scan 1.6x1.6 Recon 1.0x1.0	188x248	125	4 minutes
DWIBS (DWI b-value 800)	Axial 4 stacks	450 L-R 300 H-F	6 mm	7730/66/220	Scan 3.5x3.5 Recon 1.7x1.7	128x81	N/A	10 minutes

Coronal 5 stacks: Thighs, Pelvis, Abdomen, Chest, Head/neck

Axial DWIBS 4 stacks: Head/Neck, Chest, Abdomen, Pelvis/thighs.

5.2 SAMPLE COLLECTION SCHEDULE

Test/Location	Baseline (C1D1)	All cycles (28 days)						EOT visit
		C1D15	C2D1	C3D1	C4D1	C5D1	C6D1 and beyond	
Correlative Research Studies (Blood-based)								
Immune subsets 5.1.1 Peripheral Blood Mononuclear Cells (PBMCs) ***Frederick/Schlom Lab***	X	X	X	X	X	X	X	X
	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top	6x10 mL Sodium Heparin green top
Cytokine / Chemokine Plasma 5.1.2 ***Figg/Cao Lab***	X	X	X	X	X	X	X	X
	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)	1x4 mL EDTA-Vacutainer tube (lavender top)
cfDNA Plasma 5.1.3 ***Figg/Cao Lab***	X		X	X		X		X
	1x10 mL EDTA-Vacutainer tube (lavender top)		1x10 mL EDTA-Vacutainer tube (lavender top)	1x10 mL EDTA-Vacutainer tube (lavender top)		1x10 mL EDTA-Vacutainer tube (lavender top)		1x10 mL EDTA-Vacutainer tube (lavender top)
HGF and MET Plasma 5.1.4 ***Figg/Cao***	X	X	X	X	X	X	X	X
	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top)	1x10mL EDTA-Vacutainer tube (lavender top
c-Met analysis Blood 5.1.6 ***Figg/Cao Lab***	X			X		X		X
	2x 5ml PAXGene RNA			2x 5ml PAXGene RNA		2x 5ml PAXGene RNA		2x 5ml PAXGene RNA
Circulating Tumor Cells (CTCs) 5.1.5 Peripheral Blood ***EPIC***	X	X		X				X
	1x10mL Streck Brown-Black tube	1x10mL Streck Brown-Black tube		1x10mL Streck Brown-Black tube				1x10mL Streck Brown-Black tube

Test/Location	Baseline (C1D1)	All cycles (28 days)						EOT visit
		C1D15	C2D1	C3D1	C4D1	C5D1	C6D1 and beyond	
Total Research Blood Volume per Timepoint (mL)	**118**	**88**	**98**	**118**	**78**	**108**	**78**	**118**
Correlative Research Studies (Tumor-based)								
Single cell RNA-Seq **5.1.7.2** 3 cores snap frozen tissue ***Kelly Lab***	X		X*					
TCR-Seq **5.1.4** Fresh frozen 1 core biopsy tissue ***LP***	X		X*					
TSO **5.1.7.2** FFPE/Core biopsy fresh tissue ***Raffeld lab***	X**							
MET protein **5.1.7.1** 10 Slides and/or 1-2 cores ***Bottaro lab***	X		X*					
Multiplex IHC **5.1.8** FFPE tissue, at least 5 consecutive slides ***Sater lab***	X		X*					
Correlative Research Studies (Imaging-based)								
Whole body MRI* ** **5.1.9** ***MIP, Turkbey***	X			X				

*Between cycles 2 and 4
** Optional collection based on history of prior tumor sequencing or clinical indication at the PI's discretion.
*** Mandatory unless the participant is unable to lay flat or be in a confined space for the duration of the procedure.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

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

5.3.1 Laboratory of Pathology

All consenting patients with available tissue will undergo comprehensive Omics analysis on the tumor and normal DNA, RNA. Samples collected from patients for Omics studies will be labeled with the medical record number, patient name, accessioning with SoftPath, barcode generated by Labmatrix and an assigned unique identifier.

This information will not be released to patients or families as these results are considered exploratory and of no established clinical utility. Research samples will be labeled with the Study ID number, barcode from Labmatrix and samples ID which is sequentially determined (e.g. CL0001, CL0002, etc., which is automatically coded by the LIMS system utilized). Laboratory personnel will store the key to the code in a secure, password protected database accessible only to them.

The entire DNA and RNA sequence data will be deposited in secure OncoGenomics server which exists within the NIH firewall. All data is coded and stripped of identifiable information. The code is kept by the principal investigator and the companion protocol PI and/or enrolling investigator. The data is stored in the form of coded BAM files, and processed vcf files contacting all calls in the germline and tumor DNA. The investigator will have access only to the subdirectory that contains the data from his/her patients. The folder can only be accessed using the investigators NIH username and password. All data will be coded and stripped of identifiable information.

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissue that is not placed in paraffin blocks is stored in formalin for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.3.2 Bottaro Laboratory

Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality. Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked.

Please contact Dr. Bottaro to notify of biopsy date and time at 240-858-3967 or Rene Costello at 240-858-3889 and to arrange for pick-up of sample.

5.3.3 Figg lab

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

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer locations. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (e.g. delay in sample processing, storage conditions on the ward, etc.). Barcoded samples are stored in barcoded boxes in locked freezers at either -20 C or -80 C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per IRB approved protocol) and that any unused samples must be returned to the BPC. Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. If, at any time, a patient withdraws consent, the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

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

5.3.4 Clinical Services Program

All data associated with the patient samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80ºC freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

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

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance. The subcontractor's role limited to clinical research databases and repositories containing patient specimens. The subcontractor does not conduct or have any vested interest in research on human subjects but does provide services and support the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of the subcontractor to accept only coded, linked samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

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

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

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Description of the scope of genetic/genomic analysis

Using the tissue, and blood samples we will perform next research generation sequencing (NGS) of DNA (Exome or Panel).

5.4.2 Privacy and Confidentiality of medical information/biological specimens

Initially the samples of each patient will be barcoded. At no time will patient's names be used on the blood and tissue samples. Sometimes, because a group collaboration or journal policy requires it, a subject's genetic data will be deposited in a database such as dbGaP. Although there is controlled access to such a database, such a submission carries theoretical risks of revealing the identity of the subject. This is discussed in the consent.

5.4.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists).

Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory.

If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling with the NCI Genetics Branch to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.4.4 Genetic counseling

The costs of CLIA testing will be paid for by the Center for Cancer Research, the Branch, or the Principal Investigator. If the health history, family history, or tumor diagnosis from the Laboratory of Pathology at the NIH Clinical Center suggests that the participant might benefit from genetic testing, we will discuss this with him/her.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study drug administration, Study Day 1, through 30 days after the end of treatment. Beyond 30 days after the last dose of study drug, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

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

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

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

6.1.1 Tumor data

- The tumor disease information that will be documented and verified at the screening visit for each subject includes the following. All these details will be documented in patient's history and physical exam and also recorded in the C3D database.
- Detailed history of the tumor, including histopathological diagnosis, grading and staging in accordance with the Union Internationale Contre le Cancer Tumor Node Metastasis Classification at diagnosis (UICC TNM).
    - The T and M category (T1-3 vs T4 and M0 or M1) of the tumor at the time of study entry, based on screening assessments
- All therapy used for prior treatment of the tumor (including surgery, radiotherapy and chemotherapy, immunotherapy).
- Any other conditions that were treated with chemotherapy, radiation therapy, or immunotherapy.

6.1.2 Concomitant Medications and Therapies

All concomitant medications taken by the subject during the trial, from the date of signature of informed consent are to be recorded in the appropriate section of the eCRF, noting the name, dose, duration and indication of each drug. Nondrug interventions (other than vitamins) and any changes to a concomitant medication or other intervention should also be recorded in the eCRF.

6.1.3 DATA ELEMENTS

- Documentation of dosage and timing of drug administration
- Outside laboratory, radiologic, and pathology results will be sent to the PI and entered into NCI C3D at NIH

- NIH labs and tests will be downloaded into C3D

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

Human data generated in this research for future research will be shared as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in another public repository.
- Identified or coded, linked data in BTRIS (automatic for activities in the Clinical Center)

Data will be shared through:

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

Data will be shared:

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

6.2.2 Genomic Data Sharing Plan

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

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. After one year response evaluation will be done every 12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4-8 weeks (not less than 4 weeks) following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [45]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.3.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By CT scan:
    - Scan slice thickness 5 mm or under: as $\geq$10 mm
    - Scan slice thickness >5 mm: double the slice thickness

- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥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 patient, these are preferred for selection as 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.

6.3.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

6.3.3 Response Criteria

6.3.3.1 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 of 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 of diameters while on study.

6.3.3.2 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 patient 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, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.3.3 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 progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

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

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.5 Progression-Free Survival

PFS will be defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.3.6 Response Review

6.3.6.1 Best overall response (BOR)

The duration of best overall response is measured from the time measurement criteria are met for CR, PR or SD (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

6.3.6.2 Duration of stable disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements. Stable Disease (SD) when sum of all target lesions does not qualify for CR/PR/PD for Target Lesion Response and Persistence of non-target lesions on Non-Target Lesion Response.

6.4 TOXICITY CRITERIA

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

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

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

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

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

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

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

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

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

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

8.1.2 Serious Adverse Events (SAE)

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

- Death,
- A life-threatening adverse event (see Section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

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

8.1.3 Life-threatening

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

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.

8.1.5 Relationship to Study Product

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

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

8.1.6 Adverse Events of Special Interest (AESI)

Adverse events of special interest (AESIs) are serious or nonserious AEs that are of clinical interest and should be closely followed.

AESIs include following:

- Infusion-related reactions including immediate hypersensitivity.
- Immune-related adverse events.
- M7824 mediated skin reactions.
- Anemia.
- Bleeding AEs

8.2 ASSESSMENT OF SAFETY EVENTS

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

occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

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

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in Section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in Section **8.4**.

All SAE reporting must include the elements described in Section **8.1.6**.

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

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

8.4 Waiver of expedited reporting to CCR

As overall survival which includes death due to disease progression is part of the study objectives, and captured as an endpoint in this study, death due to disease progression will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a casual relationship between the study drug and the event, report the event in an expedited manner according to Section **8.3**.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement.

8.6 REPORTING PREGNANCY

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

8.6.1 Maternal Exposure

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

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

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.6.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 125 days after treatment.

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 125 days after the last dose should, if possible, be followed up and documented.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

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

8.8 SPONSOR PROTOCOL NON-ADHERENCE REPORTING

Protocol non-adherence is defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol non-adherence identified by the Staff or the site Monitor on the OSRO Site Protocol Non-Adherence Log. The protocol-specific, cumulative non-adherence log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each**

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

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights of the participants are protected, that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Monitoring based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. The intensity and frequency of monitoring will be based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. OSRO Monitoring visits and related activities will be conducted throughout the life cycle of each protocol, with the first activity being before study start to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO regulatory file.

If protocol non-adherence is identified by the Monitor (i.e., any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the site Staff) the Monitor will note the observation, review with site Staff and if unresolved, request that the Staff document the non-adherence on the protocol-specific OSRO Site Protocol Non-Adherence Log (see Section **8.8**).

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

10.1.1 Primary Endpoint(s)

The primary endpoint of this trial is to evaluate the activity of bintrafusp alfa (M7824) as determined by objective response rate (ORR) in two metastatic urothelial carcinoma cohorts:

- Cohort 1: Checkpoint inhibitor naïve
  - Cohort 1A: cisplatin ineligible
  - Cohort 1B: refractory post-platinum therapy

- Cohort 2: Checkpoint inhibitor previously treated patients
  - Cohort 2A: previously achieved a CR/PR
  - Cohort 2B: previously had SD/PD

10.1.2 Secondary Endpoint(s)

- To evaluate the activity of bintrafusp alfa (M7824) in urothelial carcinoma patients as determined by progression free survival (PFS) and overall survival (OS).
- To obtain additional data to evaluate the safety of bintrafusp alfa (M7824) in urothelial carcinoma

10.2 SAMPLE SIZE DETERMINATION

10.2.1 Cohort 1A: Checkpoint inhibitor naïve patients who are cisplatin-ineligible

In this cohort, it would be desirable to demonstrate that the treatment exceeds a 30% response rate. As such, in this cohort, this trial will be conducted using a Simon minimax two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) to rule out an unacceptably low PR+CR rate of 15% (p0=0.15) in favor of an improved response rate of 40% (p1=0.40). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll 9 evaluable patients, and if 0 to 1 of 9 have a clinical response, then no further patients will be accrued in this trial. If 2 or more of the first 9 patients have a response, then accrual would continue until a total of 16 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 2 to 4 patients with a response out of 16 patients, this would be an uninterestingly low response rate. If there were 5 or more of 16 (31.3%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (15% response rate), the probability of early termination is 60.0%.

10.2.2 Cohort 1B: Checkpoint inhibitor naïve patients who are refractory post-platinum therapy

In this cohort, it would be desirable to demonstrate that the treatment exceeds a 20% response rate. As such, in this cohort, this trial will be conducted using a Simon minimax two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) to rule out an unacceptably low PR+CR rate of 10% (p0=0.10) in favor of an improved response rate of 40% (p1=0.40). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), the first stage will enroll 8 evaluable patients, and if 0 of 8 have a clinical response, then no further patients will be accrued in this cohort. If 1 or more of the first 8 patients have a response, then accrual would continue until a total of 15 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 3 patients with a response out of 15 patients, this would be an uninterestingly low response rate. If there were 4 or more of 15 (26.7%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (10% response rate), the probability of early termination is 43.1%.

10.2.3 Cohort 2A: Checkpoint inhibitor refractory patients with Complete Response (CR) or Partial Response (PR) as per RECIST 1.1

In this cohort, it would be desirable to demonstrate that the treatment exceeds a 15% response rate. As such, in this cohort, this trial will be conducted using a Simon minimax two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) to rule out an unacceptably low PR+CR rate of 5% (p0=0.05) in favor of an improved response rate of 25% (p1=0.25). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll 12 evaluable patients, and if 0 of the 12 have a clinical response, then no further patients will be accrued in this trial. If 1 or more of the first 12 patients have a response, then accrual would continue until a total of 16 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 patients with a response out of 16 patients, this would be an uninterestingly low response rate. If there were 3 or more of 16 (18.8%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 54.0%.

10.2.4 Cohort 2B. Checkpoint inhibitor refractory patients with Stable Disease (SD) or Progressive Disease (PD) as per RECIST 1.1

In this cohort, it would be desirable to demonstrate that the treatment exceeds a 5% response rate. As such, in this cohort, this trial will be conducted using a Simon minimax two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) to rule out an unacceptably low PR+CR rate of 5% (p0=0.05) in favor of an improved response rate of 20% (p1=0.20). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll 12 evaluable patients, and if 0 of the 12 have a clinical response, then no further patients will be accrued in this trial. If 1 or more of the first 12 patients have a response, then accrual would continue until a total of 21 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 patients with a response out of 21 patients, this would be an uninterestingly low response rate. If there were 3 or more of 21 (14.3%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 54.0%.

These phase II cohorts treated with TGFB trap alone may require up to 15+16+16+21=68 evaluable patients. To allow for a small number of inevaluable patients, this trial of up to 4 different cohorts will have an accrual ceiling of 75 patients. It is expected that 2-3 patients per month may enroll onto the trial; thus, it is expected that this trial may complete accrual in 2 to 3 years.

10.3 POPULATIONS FOR ANALYSES

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

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

Response fractions, and time to event endpoints will be reported along with appropriate confidence intervals.

10.4.2 Analysis of the Primary Endpoints

Separately by cohort, the fraction of patients in phase II cohorts who experience a PR or CR using the study treatment will be determined by dividing the number responders by the total evaluable patients. The fraction will be reported along with 80% and 95% two-sided confidence intervals.

10.4.3 Analysis of the secondary efficacy endpoints

Progression-free survival (PFS) will be determined using the Kaplan-Meier method, considering those who progress or die without progression as failures, and censoring those who do not.

Overall survival (OS) will be determined using the Kaplan-Meier method.

Each of these will be calculated starting from the date the patient enrolled onto the trial. Appropriate confidence intervals will be reported for each of these measures.

10.4.4 Baseline Descriptive Statistics

Demographic and baseline clinical characteristics of all patients will be reported.

10.4.5 Planned Interim Analyses

As indicated above, for each phase II cohort, using a Simon two-stage design, after the required number of evaluable patients have been enrolled in the first stage, an analysis of the response rate will be undertaken to determine if there are sufficient responses to proceed to the second stage.

10.4.6 Sub-Group Analyses

Analyses will be performed by arm without further sub-analyses.

10.4.7 Tabulation of Individual Participant Data

None will be provided.

10.4.8 Exploratory Analyses

1. Determine peripheral immune modulation using:
    - Multi-parametric flow cytometry – immune subset analysis
    - Cytokine/chemokine analysis (i.e TGF-α, IFN-γ, IL-6, IL-8, etc.)
    - Blood-based immune parameters (neutrophil-to-lymphocyte ratio)
    - Circulating Tumor Cells (CTCs) and circulating cell-free tumor DNA (cfDNA)
2. Determine immune status of the tumor microenvironment using:
    - Multiplex IHC for PD-L1 expression, CD8, CD4, FoxP3 T-cell infiltration
    - RNA-Seq transcriptome sequencing for tumor/immune gene expression profile (and peripheral blood mononuclear cells
    - T-cell receptor (TCR) sequencing for T-cell clonality and MET protein levels
    - Next generation sequencing (TSO-500) for tumor biomarkers in tumor samples

Any of these exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 CRADA

This study will receive the investigational study agent under a Cooperative Research and Development Agreement (CRADA) with EMD Serono. CRADA Number: 02666.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Cisplatin ineligible or platinum refractory metastatic urothelial cancer patients may have received pior checkpoint inhibitor therapy or be checkpoint inhibitor naïve to enroll. Patients with metastatic urothelial cancer who received prior checkpoint inhibitors are left with limited options and the effect of subsequent immunotherapy treatment is unknown. While current PD-1/PD-L1 checkpoint inhibtors have shown efficacy in checkpoint naïve patients who are either cisplatin ineligible or refractory response rates response rates are about 20% [46]. Therefore, it is imperative to improve on this response rate with drugs like M7824 that have potential of increasing response rates through immunomodulation. These reasons express the need to determine M7824 activity in our patient population.

Participants who do not accept blood transfusions will be excluded. As there is a risk of severe bleeding with this study drug, participants must be willing to receive blood transfusions if medically necessary for their own safety. Participants must be able to receive blood transfusions in order to minimize the risks of receiving M7824. Another reason for this is that including these patients could compromise the scientific validity of the study. For example, death from blood loss could make it difficult to assess other aspects of the investigational immunotherapy's safety—a primary scientific goal in this early-phase immunotherapy trial, which are carried out in small numbers of participants.

12.2 PARTICIPATION OF CHILDREN

Because no dosing or adverse event data are currently available on the use of M7824 in patients less than 18 years of age, children are excluded from this study.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. In the event this occurs, the subjects will remain in the study because there is a prospect of direct benefit (Section **12.5.2**) from research participation. All subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care

in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **12.6.1** for consent procedure.

12.4 Evaluation of Benefits and Risks/Discomforts

Patients will receive evaluation of their disease at the National Cancer Institute's Clinical Center.

Potential adverse reactions attributable to the administration of the study drug utilized in this trial are discussed in Section **14**. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. Patients will be examined and evaluated prior to enrollment. All evaluations to monitor the treatment of patients will be recorded in the patient chart. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland.

The potential benefit to a patient that goes onto study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival.

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

12.5 Risks/Benefits Assessment

12.5.1 Known Potential Risks

The primary risk to patients participating in this research study is the potential toxicity of the M7824. Potential adverse reactions attributable to the administration of the study drug utilized in this trial are discussed in Section **14.1.2**. The protocol provides detailed and careful monitoring of all patients to assess for toxicity. Toxicity data will be collected and reviewed to ensure that there were no severe toxicities that would preclude further patient enrollment. Patients will be treated with therapeutic intent and response to the therapy will be closely monitored.

12.5.1.1 Blood Sampling

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

12.5.1.2 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

12.5.1.3 Tumor Biopsy

Needle biopsy is minimally invasive and is typically a very safe procedure. Depending upon the site being biopsied and the type of biopsy being performed, risks can include infection of the

biopsy site, development of a hematoma, and bleeding. Rarely more significant complications can occur when structures near the biopsy target are entered with the needle (e.g. puncture of lung or bowel). Surgical procedures for biopsy specimens will not be conducted for the sole purpose of research specimen collection, although when surgical biopsies are performed for clinical or NCI protocol requirements, samples may be requested as part of participation on this trial. In this instance, no added risk is incurred.

12.5.1.4 Imaging

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

12.5.1.5 MR Imaging

The risks of MR imaging are relatively small. Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage.MRIs include the additional risk of damage to hearing.

12.5.1.6 Risks of exposure to ionizing radiation

This research study involves up to 7 CT C/A/P scans and up to 3 CT-guided biopsies. Pre-treatment tissue biopsy and tissue availability is mandatory. On-treatment biopsy is required unless obtaining biopsy is not technically or clinically feasible. Post-treatment biopsy is optional. Subjects will be exposed to up to approximately 10.1 rem.

12.5.1.7 Non-Physical Risks of Genetic Research

12.5.1.7.1 ***Risk of receiving unwanted information***

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

12.5.1.7.2 ***Risk related to possibility that information may be released***

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

12.5.1.7.3 ***Risk to family or relatives***

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems. As previously noted, patients will be notified of any medically significant and actionable incidental findings. Study results will not be shared with patients.

12.5.2 Known Potential Benefits

Patients who previously received checkpoint therapy may benefit from receiving further checkpoint block with M7824 they would otherwise not obtain in a standard of care setting. Immediate potential benefits include potential response to tumor progression, palliation of symptoms, and improved outcomes. Long range potential benefits of this study will build on knowledge and the science used to develop therapies for future cancer patients

12.5.3 Assessment of Potential Risks and Benefits

A number of clinically appropriate strategies to minimize risk to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefits of M7824 for patients retaining the ability to consent and those who lose capacity to consent during the course of the trial outweigh the risks associated with the proposed entry into this trial.

12.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

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

Note: When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

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

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the Adobe platform (which is not 21 CFR Part 11 compliant) to obtain the required signatures.

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations.

Both the investigator and the subject will sign the document with a hand signature using a finger, stylus or mouse.

Electronic signature on electronic document

When permitted by the NIH Clinical Center, electronic signatures may be obtained using the iMedConsent platform once it is designated as 21 CFR Part 11 compliant.

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations.

The identity of the participant will be determined by a prompt which will require the provision of information from a form of government issued identification prior to obtaining the signature. If participant does not have such identification available, security questions will be used to confirm identity.

Both the investigator and the subject will sign the document electronically per system prompts.

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

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.6**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

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

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

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

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

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

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

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

13.3 CONFLICT OF INTEREST POLICY

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

13.4 CONFIDENTIALITY AND PRIVACY

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

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

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

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure

location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

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

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

14 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

14.1 M7824 (BINTRAFUSP ALFA, MSB0011359C) (IND # 149968)

14.1.1 Source/Acquisition and Accountability

M7824 is manufactured and supplied for the trial by EMD Serono Research and Development Institute. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the manufacturer and the site.

14.1.2 Toxicity

In a phase 1, open-label 3+3 dose-escalation study of M7824 in 16 patients, 3 patients experienced grade 3 drug-related adverse events including skin infection secondary to grade 2 cccc, lipase increased, and colitis with associated anemia. There were no grade 4 – 5 treatment related adverse events. Please see table below for details:

Treatment-related TEAE Leading to Permanent Treatment Discontinuation by System Organ Class and Preferred Term in ≥ 2 participants in the Pooled Analysis of Dose Expansion Cohorts (Source: Investigator Brochure v6)

Primary System Organ Class Dictionary-Derived Term	EMR200647-001 (N = 539) N (%)	MS200647-0008 (N = 91) N (%)	Total (N = 630) N (%)
Participants with any SAE	321 (59.6)	50 (54.9)	371 (58.9)
Blood and lymphatic system disorders	27 (5.0)	0	27 (4.3)
Anaemia	19 (3.5)	0	19 (3.0)
Endocrine disorders	11 (2.0)	2 (2.2)	13 (2.1)
Adrenal insufficiency	5 (0.9)	2 (2.2)	7 (1.1)
Gastrointestinal disorders	64 (11.9)	13 (14.3)	77 (12.2)
Abdominal pain	5 (0.9)	1 (1.1)	6 (1.0)
Gastrointestinal haemorrhage	7 (1.3)	1 (1.1)	8 (1.3)
Upper gastrointestinal haemorrhage	2 (0.4)	4 (4.4)	6 (1.0)
Vomiting	6 (1.1)	0	6 (1.0)
General disorders and administration site conditions	70 (13.0)	10 (11.0)	80 (12.7)
Disease progression	43 (8.0)	6 (6.6)	49 (7.8)
General physical health deterioration	6 (1.1)	0	6 (1.0)
Pyrexia	8 (1.5)	1 (1.1)	9 (1.4)
Hepatobiliary disorders	10 (1.9)	8 (8.8)	18 (2.9)
Cholangitis	2 (0.4)	5 (5.5)	7 (1.1)
Infections and infestations	54 (10.0)	9 (9.9)	63 (10.0)
Pneumonia	14 (2.6)	3 (3.3)	17 (2.7)
Sepsis	8 (1.5)	0	8 (1.3)
Metabolism and nutrition disorders	25 (4.6)	3 (3.3)	28 (4.4)
Decreased appetite	5 (0.9)	3 (3.3)	8 (1.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	61 (11.3)	8 (8.8)	69 (11.0)
Keratoacanthoma	9 (1.7)	0	9 (1.4)
Squamous cell carcinoma of skin	22 (4.1)	1 (1.1)	23 (3.7)
Tumour haemorrhage	8 (1.5)	2 (2.2)	10 (1.6)
Renal and urinary disorders	14 (2.6)	0	14 (2.2)
Acute kidney injury	10 (1.9)	0	10 (1.6)
Respiratory, thoracic and mediastinal disorders	68 (12.6)	6 (6.6)	74 (11.7)
Dyspnoea	26 (4.8)	0	26 (4.1)
Pneumothorax	6 (1.1)	1 (1.1)	7 (1.1)
Pulmonary embolism	9 (1.7)	0	9 (1.4)

As of the data cutoff of 24 August 2018, 7 deaths (1.1%) were reported as due to treatment-related TEAE, however an additional death (primary cause of intra-abdominal hemorrhage, assessed as treatment-related) started more than 30 days after the end of treatment and is therefore is not included in above count of treatment-related death. In Study EMR200647-001, 3 participants had a treatment-related death: 1 participant had dyspnea, hemolysis and thrombocytopenia, 1 participant had an intracranial tumor hemorrhage and 1 participant had pneumonia. In Study MS200647-0008, 4 participants had a treatment-related death: 2 participants died due to ILD, 1 participant due to sudden death and 1 participant due to septic shock. Please refer to IB v 6.0 Section 5.2.2.9 for a summary of the SAEs from the Sponsor's Global Drug Safety database from 25 August 2018 to 31 December 2019 for ongoing studies.

In dose escalation phase of M7824 studies, 2 DLTs were observed at 20mg/kg dose level . One (1) subject (20 mg/kg, with underlying cervical cancer and medical history of radiation therapy was diagnosed with colitis (with subsequent bleeding and anemia) during the 21 day DLT observation period of the escalation part of the study. Colitis and associated secondary events of Grade 3 anemia and Grade 3 rectal hemorrhage (which occurred in a previously radiated area) were considered dose limiting. Other subject (20 mg/kg) with past history of recurrence of parotid cancer in the left mastoid sinus after heavy particle therapy and multiple chemotherapies experienced fainting and fall, CT scan revealed Grade 3 intracranial tumor hemorrhage in left mastoid sinus. The event occurred after 24 hours from first dose of M7824 during the 21-day DLT observation period of the escalation part of the study and was assessed as related to M7824.

Highest dose tested was 30mg/kg , and no MTD observed for M7824.

Based on accrued safety profile of M7824 in phase I studies, and with scientific rationale and consistency with observed safety findings with similar class drugs (anti PD-1/PDL-1 and anti-TGF-β targeting therapies), important identified and potential risks for M7824 were determined.

Important identified Risks includes Infusion related reactions (IRR), immune related adverse events (irAE) & skin adverse events due to anti-TGF-β action of M7824 and are adverse drug reactions for M7824.

IRR is a composite term which includes Reactions: Anaphylactic reaction, Drug hypersensitivity, Hypersensitivity, Infusion related reaction, Type I hypersensitivity and Signs and Symptoms: Abdominal pain, Back pain, Chills, Dyspnoea, Flushing, Hypotension, Pyrexia, Urticaria, Wheezing.

irAEs identified risk for M7824 (based on PDL-1blockade) are Thyroiditis, autoimmune thyroiditis, hypothyroidism, hyperthyroidism, Diabetes mellitus, Type I diabetes, type I diabetes mellitus, adrenal insufficiency, adrenalitis, Colitis Enteritis, enterocolitis, Nephritis, acute kidney injury, Myositis, Retinal microvasculitis, retinal vasculitis, rash, rash generalized, maculo-papular rash, erythematous, rash, pruritic rash, pemphigoid, Pneumonitis Interstitial pneumonitis, Interstitial lung disease.

irAEs potential risks for M7824 are Guillain-Barré syndrome, uveitis, pancreatisis, myasthenia gravis/myasthenic syndrome.

Skin AE (TGF-β blockade mediated) include rash with hyperkeratosis, cutaneous squamous cell carcinoma, keratoacanthoma.

Important potential risks includes treatment related anemia, delay in wound healing and embryofetal toxicity.

As of October 2018, more than 670 patients have been treated with M7824 1200mg flat dose q2w across multiple solid tumor expansion cohorts.

The safety profile is consistent with other monotherapy checkpoint inhibitors, with the exception of keratoacanthomas and cutaneous squamous cell carcinomas (similar to anti-TGF-β targeting therapy Fresolimumab and TGF-β genetic mutation inheritance -Ferguson- Smith Syndrome) which have occurred in approximately 7% of M7824 treated patients, and are well managed with surgical excision. These lesions have not been a criterion for treatment discontinuation.

In addition, after discussion among NCI investigators on multiple protocols using M7824, multiple bleeding events ranging from low grade gingival bleeding and epistaxis to more serious hemoptysis, mild and moderate mucosal bleedings, GI bleeding and hematuria have been observed. Some of these events can be attributed to bleeding events related to cancer directly and others bleeding events can be attributed to colitis or cystitis which is a known toxicity of anti-PD-L1 agents including M7824. However, there remains the possibility that M7824 may increase the overall risk of bleeding in ways that may not be directly related to direct tumor bleeding or inflammatory bleeding events described with checkpoint inhibitors like M7824. It is hypothesized that this possible increased bleeding risk may be due to TGF beta inhibition which has an effect on angiogenesis; bleeding has also been observed in patients receiving M7824 and may be drug-related (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool). Accordingly, patients will be notified of the same possible risk in the informed consent document for this study (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool).

14.1.3 Formulation and preparation

M7824 drug product is provided as a sterile liquid formulation.

The Concentrate for Solution for Infusion (liquid formulation) is packaged at a 10 mg/mL concentration in USP / Ph. Eur. type I 50R or 20R vials which are filled with drug product solution to allow an extractable volume of 60 mL (600 mg/60 mL). The vials are closed with rubber stoppers with the same composition as used for freeze-dried formulation, but in serum format complying with USP and Ph. Eur. with an aluminum crimp seal closure.

14.1.4 Stability and Storage

M7824 drug product must be stored at 2°C to 8°C until use. The storage condition is based on data from ongoing long term stability studies with M7824.

M7824 drug product stored at room (23°C to 27°C) or higher temperatures for extended periods of time might be subject to degradation.

The liquid formulation is diluted directly with 0.9% saline solution. The chemical and physical in-use stability for the infusion solution of M7824 in 0.9% saline solution has been demonstrated for a total of 72 hours at 2°C to 8°C and 24 hours at room temperature. However, from a microbiological point of view, the diluted solution should be used immediately and is not intended to be stored unless dilution has taken place in controlled and validated aseptic conditions. If not

used immediately, in-use storage times and conditions prior to administration are the responsibility of the user. Do not freeze or shake the diluted solution.

No other drugs should be added to the infusion containers containing M7824.

14.1.5 Administration procedures

M7824 will be administered as a 1-hour an intravenous (IV) infusion once every two weeks of each cycle. M7824 is administered as IV infusion via a peripheral or central vascular access device (VAD). Confirm patient has a port with titanium as the main material before accessing the Central VAD. A 0.2 micron polyethersulfone (PES) in-line filter is mandatory for administration.Premedications may be administered per Section **3.3.1.1**.

As with all monoclonal antibody therapies, there is a risk of allergic reaction including anaphylactic shock. M7824 should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.

Patients must be observed for 2 hours after the first M7824 dose. If no reactions are observed, the patients need to be monitored for only 30 minutes after subsequent doses.

If an allergic reaction occurs, the subject must be treated according to the best available medical practice. Please see the guidelines for handling of infusion-related reaction in Section **3.3.1.4**.

Investigators should also monitor subjects closely for potential irAEs, which may become manifest after several weeks of treatment. Such events may consist of persistent rash, diarrhea and colitis, autoimmune hepatitis, arthritis, glomerulonephritis, cardiomyopathy, or uveitis and other inflammatory eye conditions.

Vital signs must be measured within 30 minutes before and 30 minutes following M7824 infusions. In addition, at least one time during the infusion of M7824.

14.1.6 Incompatibilities

None known

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16 APPENDICES

16.1 APPENDIX A: STUDY CALENDAR

	Screening[2]	All Cycles (28 days each)												EOT visit	Follow up[9,11]
		C1 D1	C1 D15	C2 D1	C2 D15	C3 D1	C3 D15	C4 D1	C4 D15	C5 D1	C5 D15	C6 D1	C6 D15& Beyond		
History/PE incl weight and skin assessment [12]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Confirmation of pathology/Biopsy[1]	X					X								X	
ECOG, Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Tumor evaluation/ measurements	X					X				X			X[8,9]		
M7824[3,4]		X	X	X	X	X	X	X	X	X	X	X	X		
Lab assessments[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test[6]	X	X		X		X		X		X		X			
Lymphocyte phenotyping[7]	X														
ECG	X														
CT C/A/P or CT C+MRI A/P[8,9,10]	X					X				X			X[8,9]	X	
Follow-up Phone calls/emails															X[9]
Correlative Studies	**Please refer to Section 5.2**														

1. Pre-treatment tissue biopsy and/or tissue availability is mandatory. On-treatment biopsy is required unless obtaining biopsy is not technically or clinically feasible. Post-treatment biopsy is optional.
2. Tests done at screening do not need to be repeated on baseline (C1D1) if performed within 21 days prior to initiation of study therapy.
3. M7824 will be delivered intravenously every 2 weeks (+/- 3 days).
4. Vital signs must be measured within 30 minutes before and 30 minutes following M7824 infusions. In addition, at least one time during the infusion of M7824.
5. Laboratory assessments: CBC with differential, acute care panel (sodium, potassium, chloride, total CO2 (Bicarbonate), creatinine, glucose, urea nitrogen), mineral panel (albumin, calcium total, magnesium total, phosphorus), hepatic panel (alkaline phosphatase, ALT/GPT, AST/GOT, total bilirubin, direct bilirubin), ionized calcium, amylase, lipase, TSH with reflex T3 and T4, LDH, total protein, GGT, PT/INR, PTT, and urine analysis. Hepatitis B and C will be done at screening only.
6. For females of childbearing-potential (within 3 days prior to initiation of study therapy).
7. Lymphocyte phenotyping tests will be done to check the eligibility of HIV positive patients. HIV-positive patients are eligible only if CD4 counts are greater than 350 cells/mm^3 and viral load is undetectable.
8. CT of chest, abdomen and pelvis with IV contrast (oral contrast as indicated) or CT of the chest without contrast and MRI abdomen/pelvis with tumor measurements. For this study patients should be re-evaluated for response every 8 weeks (+/- 10 days). If a scan identifies objective response or progressive disease, a confirmatory scan should be obtained 4-8 weeks following initial documentation of objective response or progression. In patients with clinical progression or unequivocal scan, confirmatory progression scan can be deferred. Scans will continue every 8 weeks until disease progression or start of a new anti-cancer treatment.
9. After disease progression or in those subjects who were removed from study therapy for reasons other than disease progression, subjects will be followed every 12 weeks in clinic or by telephone/email for assessment of survival status, related adverse events and documentation of initiation of new anti-cancer therapy.
10. If appropriate screening imaging is done within 21 days of C1D1 repeat screening imaging is not required.
11. All SAEs ongoing at the 30-day (+/- 10 days) safety follow-up visit must be monitored and followed up by the investigator until stabilization or until the outcome is known, unless the subject is documented as “lost to follow-up.” In addition, all trial drug-related SAEs occurring after 30 day safety follow up visit and ongoing at the Safety Follow-up visit have to be followed up in the same manner.
12. Skin assessment must be performed at baseline and at least every 6 weeks during treatment and at the end of treatment or 28 (±5 days) days post-treatment safety follow-up (if not performed in the previous 6 weeks).

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16.2 APPENDIX B: PERFORMANCE STATUS CRITERIA

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

Appendix C: Management of ir AEs

16.2.1 Table A1 Management of Skin irAEs in Patients Treated With ICPis

1.0 Skin Toxicities	
1.1 Rash/inflammatory dermatitis	
Definition: Erythema multiforme minor (a targetoid reaction in the skin and mucous membranes usually triggered by infections, such as herpes simplex viruses, but can be associated with an immune-related drug eruption and if progresses to erythema multiforme major, it and can be a harbinger of SCAR, such as SJS), lichenoid (resembling the flat-topped, polygonal, and sometimes scaly or hypertrophic lesions of lichen-planus), eczematous (inflammatory dermatitis characterized by pruritic, erythematous, scaly, or crusted papules or plaques on the skin, which is vulnerable to superinfection, psoriasiform [resembling the well-demarcated, erythematous, and scaly papules and plaques of psoriasis], morbilliform [a nonpustular, nonbullous measles-like exanthematous rash of the skin often referred to as “maculopapular” and without systemic symptoms or laboratory abnormalities, excluding occasional isolated peripheral eosinophilia, palmoplantar erythrodysesthesia [hand-foot syndrome; redness, numbness, burning, itching, and superficial desquamation of the palms and soles], neutrophilic dermatoses [eg, Sweet syndrome], and others)	
Diagnostic workshop	
Pertinent history and physical examination Rule out any other etiology of the skin problem, such as an infection, an effect of another drug, or a skin condition linked to another systemic disease or unrelated primary skin disorder If needed, a biologic checkup, including a blood cell count and liver and kidney tests Directed serologic studies if an autoimmune condition is suspected, such as lupus or dermatomyositis: a screening antinuclear antibody test, SS-A/Anti-Ro, SS-B/Anti-La if predominantly photodistributed/photosensitivity, antihistone, double-stranded DNA, and other relevant serologies. Consider expanding serologic studies or diagnostic work-up if other autoimmune conditions are considered based on signs, symptoms Skin biopsy Consider clinical monitoring with use of serial clinical photography Review full list of patient medications to rule out other drug-induced cause for photosensitivity	
Grading	Management
Grading according to CTCAE is a challenge for skin. Instead, severity may be based on BSA, tolerability, morbidity, and duration.	
G1: Symptoms do not affect the quality of life or controlled with topical regimen and/or oral antipruritic	Continue ICPi Treat with topical emollients and/or mild-moderate potency topical corticosteroids Counsel patients to avoid skin irritants and sun exposure
G2: Inflammatory reaction that affects quality of life and equires intervention based on diagnosis	Consider holding ICPi and monitor weekly for improvement. If not resolved, interrupt treatment until skin AE has reverted to Grade 1 Consider initiating prednisone (or equivalent) at dosing 1 mg/kg, tapering over at least 4 weeks In addition, treat with topical emollients, oral antihistamines, and medium- to high-potency topical corticosteroids
G3: As G2 but with failure to respond to indicated interventions for a G 2 dermatitis	Hold ICPi therapy and consult with dermatology to determine appropriateness of resuming Treat with topical emollients, oral antihistamines, and high-potency topical corticosteroids Initiate (methyl)prednisolone (or equivalent) 1-2 mg/kg, tapering over at least 4 weeks
G4: All severe rashes unmanageable with prior interventions and intolerable	Immediately hold ICPi and consult dermatology to determine appropriateness of resuming ICPi therapy

1.0 Skin Toxicities	
	upon resolution of skin toxicity and once corticosteroids are reduced to prednisone (or equivalent) ≤ 10 mg Systemic corticosteroids: IV (methyl)prednisolone (or equivalent) dosed at 1-2 mg/kg with slow tapering when the toxicity resolves Monitor closely for progression to severe cutaneous adverse reaction Should admit patient immediately with direct oncology involvement and with an urgent consult by dermatology Consider alternative antineoplastic therapy over resuming ICPis if the skin irAE does not resolve to G1 or less; if ICPIs are the patient's only option, consider restarting once these adverse effects have resolved to a G1 level
1.2 Bullous dermatoses	
Definition: Including bullous pemphigoid or other autoimmune bullous dermatoses, bullous drug reaction Diagnostic work-up Physical examination Rule out any other etiology of the skin problem, such as an infection, an effect of another drug, or a skin condition linked to another systemic disease If needed, a biologic checkup, including a blood cell count, liver, and kidney tests; consider serum antibody tests to rule out bullous pemphigoid or, under the guidance of dermatology, sending patient serum for indirect immunofluorescent testing to rule out other autoimmune blistering diseases Referral to dermatology for blisters that are not explained by infectious or transient other causes (eg, herpes simplex, herpes zoster, bullous impetigo, bullous insect bite, friction or pressure blister) Consider skin biopsy (both hematoxylin and eosin evaluation of lesional skin and direct immunofluorescence evaluation of perilesional skin)	
Grading	Management
G1: Asymptomatic, blisters covering < 10% BSA and no associated erythema	If blisters are < 10% BSA, asymptomatic, and noninflammatory (such as the case with friction blisters or pressure blisters), cessation of ICPi is not necessary, and only observation and/or local wound care is warranted. When symptomatic bullae or erosions, which are deroofed vesicles or bullae, are observed on the skin or mucosal surfaces, the cutaneous irAE is by definition considered at least G2 See G2 management recommendations
G2: Blistering that affects quality of life and requires intervention based on diagnosis not meeting criteria for Grade > 2 Blisters covering 10%-30% BSA	Hold ICPi therapy and consult with dermatology for work-up and to determine appropriateness of resuming Attention given to general local wound care, which includes plain petrolatum ointment and bandages or plain petrolatum ointment gauze and bandage over any open erosions, which are left over on the skin after the blister has popped or if the roof of the blister easily sloughs off Counsel patients to avoid skin irritants and overexposure to sun, wear protective clothing, use sunscreens Work-up for autoimmune bullous disease as above Initiate Class 1 high-potency topical corticosteroid (eg, clobetasol, betamethasone or equivalent) and reassess every 3 days for progression or improvement

1.0 Skin Toxicities	
	Low threshold to initiate treatment with prednisone (or equivalent) at 0.5-1 mg/kg dosing and taper over at least 4 weeks Monitor patients with G2 irAEs closely for progression to involvement of greater BSA and/or mucous membrane involvement. Consider following patients closely using serial photography Primer on monitoring for complicated cutaneous adverse drug reactions:
	Review of systems: Skin pain (like a sunburn), fevers, malaise, myalgias, arthralgias, abdominal pain, ocular discomfort or photophobia, sores or discomfort in the nares, sores or discomfort in the oropharynx, odynophagia, hoarseness, dysuria, sores or discomfort in the vaginal area for women or involving the meatus of the penis for men, sores in the perianal area, or pain with bowel movements
	Physical examination: Include vital signs and a full skin examination specifically evaluating all skin surfaces and mucous membranes (eyes, nares, oropharynx, genitals, and perianal area). Assess for lymphadenopathy, facial or distal extremity swelling (may be signs of DIHS/DRESS). Assess for pustules or blisters or erosions in addition to areas of “dusky erythema,” which may feel painful to palpation. To assess for a positive Nikolsky sign, place a gloved finger tangentially over erythematous skin and apply friction parallel to the skin surface. Nikolsky sign is positive if this results in detached or sloughing epidermis demonstrating poor attachment of the epidermis to the dermis, which is the case in some autoimmune disorders (eg, pemphigus) and SJS/TEN
G3: Skin sloughing covering > 30% BSA with associated pain and limiting self-care ADL	Hold ICPi therapy and consult with dermatology to determine appropriateness of resuming Administer IV (methyl)prednisolone (or equivalent) 1-2 mg/kg, tapering over at least 4 weeks If bullous pemphigoid is diagnosed, it may be possible to avoid long-term use of systemic corticosteroids and treat with rituximab, as an alternative approach to treating the irAE Seek infectious disease consultation if patient might have secondary cellulitis or if patient has other infection risk factors, such as neutropenia, etc.
G4: Blisters covering > 30% BSA with associated fluid or electrolyte abnormalities	Permanently discontinue ICPi Admit patient immediately and place under supervision of a dermatologist Administer IV (methyl)prednisolone (or equivalent) 1-2 mg/kg with tapering over at least 4 weeks when the toxicity resolves If bullous pemphigoid is diagnosed, it may be possible to avoid long-term use of systemic corticosteroids and treat with rituximab as an alternative approach to treating the irAE

1.0 Skin Toxicities	
	Seek infectious disease consultation if patient might have secondary cellulitis or if patient has other infection risk factors, such as neutropenia, etc
1.3 SCARs, including SJS, TEN, acute generalized exanthematous pustulosis, and DRESS/DIHS	
Definition: Severe changes in either structure or functions of skin, the appendages or the mucous membranes due to a drug Diagnostic work-up	
Total body skin examination with attention to examining all mucous membranes as well as complete review of systems Rule out any other etiology of the skin problem, such as an infection, an effect of another drug, or a skin condition linked to another systemic disease A biologic checkup, including a CBC with differential test, and liver and kidney function tests, including urinalysis, in addition to the blood work; if the patient is febrile, blood cultures should be considered as well Skin biopsies to assess for full-thickness epidermal necrosis, as is seen in SJS/TEN, as well as other possible etiologies like paraneoplastic pemphigus or other autoimmune blistering dermatoses or other drug reactions, such as acute generalized exanthematous pusulosis Consider following patients closely using serial clinical photography If mucous membrane involvement or blistering is observed on the skin, consider early admission to a burn center for further monitoring and management Primer on monitoring for complicated cutaneous adverse drug reactions: Review of systems: Skin pain (like a sunburn), fevers, malaise, myalgias, arthralgias, abdominal pain, ocular discomfort or photophobia, sores or discomfort in the nares, sores or discomfort in the oropharynx, odynophagia, hoarseness, dysuria, sores or discomfort in the vaginal area for women or involving the meatus of the penis for men, sores in the perianal area, or pain with bowel movements Physical examination: Include vital signs and a full skin examination specifically evaluating all skin surfaces and mucous membranes (eyes, nares, oropharynx, genitals, and perianal area). Assess for lymphadenopathy, facial or distal extremity swelling (may be signs of DIHS/DRESS). Assess for pustules or blisters or erosions in addition to areas of “dusky erythema,” which may feel painful to palpation. To assess for a positive Nikolsky sign, place a gloved finger tangentially over erythematous skin and apply friction parallel to the skin surface. Nikolsky sign is positive if this results in detached or sloughing epidermis demonstrating poor attachment of the epidermis to the dermis, which is the case in some autoimmune disorders (eg, pemphigus) and SJS/TEN	
Grading	Management
All Grades	In cases of suspected SJS or any mucous membrane involvement, discontinue ICPi treatment and monitor closely for improvement, regardless of grade
G1: NA	For SCARs, there is no G1 category; if lower BSA is involved with bullae or erosions, there should remain a high concern that this reaction will progress to G3 or G4
G2: Morbilliform (“maculopapular”) exanthem covering 10%-30% BSA with systemic symptoms, lymphadenopathy, or facial swelling	Hold ICPi and monitor patients closely every 3 days with G2 irAEs for progression to involvement of greater BSA and/or mucous membrane involvement Consider following patients closely using serial photography Initiate therapy with topical emollients, oral antihistamines, and medium- to high-strength topical corticosteroids Consider initiation of prednisone (or equivalent) 0.5-1 mg/kg tapered over at least 4 weeks
G3: Skin sloughing covering < 10% BSA with mucosal involvement associated signs (eg, erythema, purpura, epidermal detachment, mucous membrane detachment)	Hold ICPi therapy and consult with dermatology Treat skin with topical emollients and other petrolatum emollients, oral antihistamines, and high-strength topical corticosteroids; dimethicone may also be offered as an alternative to petrolatum

1.0 Skin Toxicities	
	Administer IV (methyl)prednisolone (or equivalent) 0.5-1 mg/kg and convert to oral corticosteroids on response, wean over at least 4 weeks Admit to burn and/or consult wound services with attention to supportive care, including fluid and electrolyte balance, minimizing insensible water losses, and preventing infection Given the immune mechanism of action of these medicines, use of immune suppression is warranted and should be offered For mucous membrane involvement of SJS or TEN, appropriate consulting services should be offered to guide management in preventing sequelae from scarring (eg, ophthalmology; ear, nose, and throat; urology; gynecology; etc, as appropriate)
G4: Skin erythema and blistering/sloughing covering ≥ 10% to > 30% BSA with associated signs (eg, erythema, purpura, epidermal detachment, mucous membrane detachment) and/or systemic symptoms and concerning associated blood work abnormalities (eg, liver function test elevations in the setting of DRESS/DIHS)	Permanently discontinue ICPi Admit patient immediately to a burn unit or ICU with consulted dermatology and wound care services Consider further consultations based on management of mucosal surfaces (eg, ophthalmology; urology; gynecology; ear, nose, and throat surgery; etc) Initiate IV (methyl)prednisolone (or equivalent) 1-2 mg/kg, tapering when toxicity resolves to normal IVIG or cyclosporine may also be considered in severe or corticosteroid-unresponsive cases Consider pain/palliative consultation and/or admission in patients presenting with DRESS manifestations
Additional considerations: The usual prohibition of corticosteroids for SJS is not relevant here, as the underlying mechanism is a T-cell immunodirected toxicity Adequate suppression is necessary with corticosteroids or other agents and may be prolonged in cases of DRESS/DIHS All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate Abbreviations: ADL, activities of daily living; AE, adverse event; BSA, body surface area; CBC, complete blood count; CTCAE, Common Terminology Criteria for Adverse Events; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug reaction with eosinophilia and systemic symptoms; G, Grade; ICPi, immune checkpoint inhibitor; ICU, intensive care unit; irAE, immune-related adverse event; IV, intravenous; IVIG, intravenous immunoglobulin; NA, not applicable; SCAR, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TENS, toxic epidermal necrolysis.	

16.2.2 Table A2 Management of GI irAEs in Patients Treated With ICPis

2.0 GI Toxicities	
2.1 Colitis	
Definition: A disorder characterized by inflammation of the colon Diagnostic work-up	
G2 Work-up of blood (CBC, comprehensive metabolic panel, TSH, ESR, CRP), stool (culture, Clostridium difficile, parasite, CMV or other viral etiology, ova and parasite) should be performed Consider testing for lactoferrin (for patient stratification to determine who needs more urgent endoscopy) and calprotectin (to follow-up on disease activity) Screening laboratories (HIV, hepatitis A and B, and blood quantiferon for TB) to prepare patients to start infliximab should be routinely done in patients at high risk for those infections and appropriately selected patients based on infectious disease expert's evaluation Imaging (eg, CT scan of abdomen and pelvis and GI endoscopy with biopsy) should be considered as there is evidence showing that the presence of ulceration in the colon can predict a corticosteroid-refractory course, which may require early infliximab Consider repeating endoscopy for patients who do not respond to immunosuppressive agents; repeating endoscopy for disease monitoring can be considered when clinically indicated and when planning to resume therapy	
G3-4 All the work-up listed for G2 (blood, stool, imaging, and scope with biopsy) should be completed immediately Consider repeating endoscopy for patients who do not respond to immunosuppressive agents; repeating endoscopy for disease monitoring should only be considered when clinically indicated and when planning to resume ICPi	
Grading (based on CTCAE for diarrhea, as most often used clinically)	Management
All patients	Counsel all patients to be aware of and inform their health care provider immediately if they experience: Abdominal pain, nausea, cramping, blood or mucus in stool or changes in bowel habits, fever, abdominal distention, obstipation, constipation For G2 or higher, consider permanently discontinuing CTLA-4 agents and may restart PD-1, PD-L1 agents if patient can recover to G1 or less; concurrent immunosuppressant maintenance therapy should be considered only if clinically indicated in individual cases
G1: Increase of fewer than four stools per day over baseline; mild increase in ostomy output compared with baseline	Continue ICPi; alternatively, ICPi may be held temporarily and resumed if toxicity does not exceed G1 Monitor for dehydration and recommend dietary changes Facilitate expedited phone contact with patient/caregiver May obtain gastroenterology consult for prolonged G1 cases
G2: Increase of four to six stools per day over baseline; moderate increase in ostomy output compared with baseline	Should hold ICPi temporarily until patient's symptoms recover to G1; can consider permanently discontinuing CTLA-4 agents and may restart PD-1, PD-L1 agents if patient can recover to G1 or less Concurrent immunosuppressant maintenance therapy (, 10 mg prednisone equivalent dose) may be offered only if clinically indicated in individual cases

2.0 GI Toxicities	
	May also include supportive care with medications such as Imodium if infection has been ruled out Should consult with gastroenterology for G2 or higher Administer corticosteroids, unless diarrhea is transient, starting with initial dose of 1 mg/kg/day prednisone or equivalent When symptoms improve to G1 or less, taper corticosteroids over at least 4-6 weeks before resuming treatment, although resuming treatment while on low-dose corticosteroid may also be an option after an evaluation of the risks and benefits EGD/colonoscopy, endoscopy evaluation should be highly recommended for cases Grade $ 2 to stratify patients for early treatment with infliximab based on the endoscopic findings and to determine the safety of resuming PD-1, PD-L1 therapy Stool inflammatory markers can be considered (lactoferrin and calprotectin) in cases of G2 or higher to differentiate functional versus inflammatory diarrhea, and use calprotectin to monitor treatment response if provider prefers Repeat colonoscopy is optional for cases of G2 or higher for disease activity monitoring to achieve complete remission, especially if there is a plan to resume ICPi
G3: Increase of seven or more stools per day over baseline, incontinence, hospitalization indicated, severe increase in ostomy output compared with baseline, limiting self-care ADL	Should consider permanently discontinuing CTLA-4 agents and may restart PD-1, PD-L1 agents if patient can recover to G1 or less. Administer corticosteroids (initial dose of 1-2 mg/kg/d prednisone or equivalent) Consider hospitalization or outpatient facility for patients with dehydration or electrolyte imbalance If symptoms persist $ 3-5 days or recur after improvement, consider administering IV corticosteroid or noncorticosteroid (eg, infliximab) Consider colonoscopy in cases where patients have been on immunosuppression and may be at risk for opportunistic infections as an independent cause for diarrhea (ie, CMV colitis) and for those who are anti-TNF or corticosteroid refractory
G4: Life-threatening consequences; urgent intervention indicated	Permanently discontinue treatment Should admit patient when clinically indicated; patients managed as outpatients should be very closely monitored Administer 1-2 mg/kg/d methylprednisolone or equivalent until symptoms improve to G1, and then start taper over 4-6 weeks Consider early infliximab 5-10 mg/kg if symptoms refractory to corticosteroid within 2-3 days Consider lower GI endoscopy if symptoms are refractory despite treatment or there is concern of new infections
Additional considerations The use of vedolizumab may be considered in patients refractory to infliximab and/or contraindicated to TNF-a blocker. The decision should be made on an individual basis from gastroenterology and oncology evaluation. This is based on case series showing promising results	

2.0 GI Toxicities	
Patients with hepatitis and irAE colitis are rare, and management should include permanently discontinuing ICPi and offering other immunosuppressant agents that work systemically for both conditions Currently, enteritis alone as the cause of diarrhea is uncommon and requires small bowel biopsy as the evaluation tool. It may be managed similar as colitis,including corticosteroid and/or infliximab, etc	
2.2 Hepatitis	
Definition: A disorder characterized by a viral pathologic process involving the liver parenchyma Diagnostic work-up Monitor patient for abnormal liver blood tests: AST, ALT, and bilirubin prior to each infusion and/or weekly if G1 liver function test elevations. No treatment is recommended for G1 liver function test abnormality For G2 or higher: Work-up for other causes of elevated liver enzymes should be tested, viral hepatitis, alcohol history, iron study, thromboembolic event, liver ultrasound, cross-sectional imaging for potential liver metastasis from primary malignancy. If suspicion for primary autoimmune hepatitis is high, can consider ANAs, antismooth muscle antibodies, antineutrophil cytoplasmic antibodies. If patients with elevated alkaline phosphatase alone, g-glutamyl transferase should be tested. For isolated elevation of transaminases, consider checking CK for other etiologies	
Grading	Management
All patients	Counsel all patients to be aware of and inform their health care provider immediately if they experience: Yellowing of skin or whites of the eyes Severe nausea or vomiting Pain on the right side of the abdomen Drowsiness Dark urine (tea colored) Bleeding or bruising more easily than normal Feeling less hungry than usual
G1: Asymptomatic (AST or ALT > ULN to 3.0 x ULN and/or total bilirubin > ULN to 1.5 x ULN)	Continue ICPi with close monitoring; consider alternate etiologies Monitor laboratories one to two times weekly Manage with supportive care for symptom control
G2: Asymptomatic (AST or ALT > 3.0 to ≤ 5 x ULN and/or total bilirubin > 1.5 to ≤ 3 x ULN)	Hold ICPi temporarily and resume if recover to G1 or less on prednisone ≤ 10 mg/d For Grade 2 hepatic toxicity with symptoms, may administer corticosteroid 0.5-1 mg/kg/d prednisone or equivalent if the abnormal elevation persists with significant clinical symptoms in 3-5 days Increase frequency of monitoring to every 3 days Infliximab might not be the most appropriate treatment option in the situation of immune-mediated hepatitis given the potential risk of idiosyncratic liver failure (Note: No clear evidence shows the liver toxicity from infliximab from other studies) In follow-up, may resume ICPi treatment followed by taper only when symptoms improve to G1 or less and corticosteroid ≤ 10 mg/d; taper over at least 1 month Patients should be advised to stop unnecessary medications and any known hepatotoxic drugs
G3: Symptomatic liver dysfunction, fibrosis by biopsy, compensated cirrhosis, reactivation of chronic hepatitis (AST or ALT 5-20 x ULN and/or total bilirubin 3-10x3 ULN)	Permanently discontinue ICPi Immediately start corticosteroid 1-2 mg/kg methylprednisolone or equivalent If corticosteroid refractory or no improvement after 3 days, consider mycophenolate mofetil or azathioprine (if using azathioprine should test for thiopurine methyltransferase deficiency)

2.0 GI Toxicities	
	Laboratories at daily or every other day; consider inpatient monitoring for patients with AST/ALT > 8 x ULN and/or elevated TB 3 x ULN Increase frequency of monitoring to every 1-2 days Infliximab might not be the most appropriate treatment option in the situation of immune-mediated hepatitis given the potential risk of liver failure (Note: No clear evidence shows that the liver toxicity from infliximab from other studies); alternatives include non–TNF-a agents as systemic immunosuppressants If no improvement is achieved with corticosteroids or for patients on combination therapy with a novel agent, with standard chemotherapy, or with targeted therapy, refer to hepatologist for further pathologic evaluation of hepatitis Corticosteroid taper can be attempted around 4-6 weeks; re-escalate if needed; optimal duration unclear
G4: Decompensated liver function (eg, ascites, coagulopathy, encephalopathy, coma; AST or ALT > 20 x ULN and/or total bilirubin > 10 x ULN)	Permanently discontinue ICPi Administer 2 mg/kg/d methylprednisolone equivalents If corticosteroid refractory or no improvement after 3 days, consider mycophenolate mofetil Monitor laboratories daily; consider inpatient monitoring Avoid the use of infliximab in the situation of immune-mediated hepatitis Hepatology consult if no improvement was achieved with corticosteroid Corticosteroid taper can be attempted around 4-6 weeks when symptoms improve to G1 or less; re-escalate if needed; optimal duration unclear Consider transfer to tertiary care facility if necessary
All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations is moderate. Abbreviations: ADL, activities of daily living; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; CBC, complete blood count, CK, creatine kinase; CMV, cytomegalovirus; CRP, C-reactive protein; CT, computed tomography; CTCAE, Common Terminology Criteria for Adverse Events; CTLA-4, cytotoxic T-cell lymphocyte-4; EGD, esophagogastroduodenoscopy; ESR, erythrocyte sedimentation rate; G, Grade; GI, gastrointestinal; HIV, human immunodeficiency virus; ICPi, immune checkpoint inhibitor; irAE, immune-related adverse event; IV, intravenous; PD-1; programmed death 1; PD-L1, programmed death ligand 1; TB, tuberculosis; TNF, tumor necrosis factor; TSH, thyroid-stimulating hormone; ULN, upper limit of normal.	

16.2.3 Table A3 Management of Lung irAEs in Patients Treated With ICPis

3.0 Lung Toxicities	
3.1 Pneumonitis	
Definition: Focal or diffuse inflammation of the lung parenchyma (typically identified on CT imaging) No symptomatic, pathologic, or radiographic features are pathognomonic for pneumonitis Diagnostic work-up Should include the following: CXR, CT, pulse oximetry For G2 or higher, may include the following infectious work-up: nasal swab, sputum culture and sensitivity, blood culture and sensitivity, urine culture and sensitivity	
Grading	Management
G1: Asymptomatic, confined to one lobe of the lung or < 25% of lung parenchyma, clinical or diagnostic observations only	Hold ICPi with radiographic evidence of pneumonitis progression May offer one repeat CT in 3-4 weeks; in patients who have had baseline testing, may offer a repeat spirometry/DLCO in 3-4 weeks May resume ICPi with radiographic evidence of improvement or resolution. If no improvement, should treat as G2 Monitor patients weekly with history and physical examination and pulse oximetry; may also offer CXR
G2: Symptomatic, involves more than one lobe of the lung or 25%-50% of lung parenchyma, medical intervention indicated, limiting instrumental ADL	Hold ICPi until resolution to G1 or less Prednisone 1-2 mg/kg/d and taper by 5-10 mg/wk over 4-6 weeks Consider bronchoscopy with BAL Consider empirical antibiotics Monitor every 3 days with history and physical examination and pulse oximetry, consider CXR; no clinical improvement after 48-72 hours of prednisone, treat as G3
G3: Severe symptoms, hospitalization required, involves all lung lobes or 50% of lung parenchyma, limiting self-care ADL, oxygen indicated G4: Life-threatening respiratory compromise, urgent intervention indicated (intubation)	Permanently discontinue ICPi Empirical antibiotics; (methyl)prednisolone IV 1-2 mg/kg/d; no improvement after 48 hours, may add infliximab 5 mg/kg or mycophenolate mofetil IV 1 g twice a day or IVIG for 5 days or cyclophosphamide; taper corticosteroids over 4-6 weeks Pulmonary and infectious disease consults if necessary Bronchoscopy with BAL 6 transbronchial biopsy Patients should be hospitalized for further management
Additional considerations GI and Pneumocystis prophylaxis with PPI and Bactrim may be offered to patients on prolonged corticosteroid use (> 12 weeks), according to institutional guidelines Consider calcium and vitamin D supplementation with prolonged corticosteroid use The role of prophylactic fluconazole with prolonged corticosteroid use (> 12 weeks) remains unclear, and physicians should proceed according to institutional guidelines Bronchoscopy + biopsy; if clinical picture is consistent with pneumonitis, no need for biopsy All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: ADL, activities of daily living; BAL, bronchoalveolar lavage; CT, computed tomography; CXR, chest x-ray; DLCO, diffusing capacity of lung for carbon monoxide; G, Grade; GI, gastrointestinal; ICPi, immune checkpoint inhibitor; irAE, immune-related adverse event; IV, intravenous; IVIG, intravenous immunoglobulin; PPI, proton pump inhibitor.	

16.2.4 Table A4 Management of Endocrine irAEs in Patients Treated With ICPis

4.0 Endocrine Toxicity	
Counsel patients to inform their health care provider immediately if they experience any changes in their health since their last visit, especially any of the following:	
Headaches that will not go away or unusual headache patterns Vision changes Rapid heartbeat Increased sweating Extreme tiredness or weakness Muscle aches Weight gain or weight loss Dizziness or fainting Feeling more hungry or thirsty than usual Hair loss Changes in mood or behavior, such as decreased sex drive, irritability, or forgetfulness Feeling cold Constipation Voice gets deeper Urinating more often than usual Nausea or vomiting Abdominal pain	
4.1 Thyroid	
4.1.1 Primary hypothyroidism	
Definition: Elevated TSH, normal or low FT4 Diagnostic work-up TSH and FT4 every 4-6 weeks as part of routine clinical monitoring on therapy or for case detection in symptomatic patients	
Grading	Management
G1: TSH < 10 mIU/L and asymptomatic G2: Moderate symptoms; able to perform ADL; TSH persistently > 10 mIU/L	Should continue ICPi with close follow-up and monitoring of TSH, FT4 May hold ICPi until symptoms resolve to baseline Consider endocrine consultation Prescribe thyroid hormone supplementation in symptomatic patients with any degree of TSH elevation or in asymptomatic patients with TSH levels that persist > 10 mIU/L (measured 4 weeks apart) Monitor TSH every 6-8 weeks while titrating hormone replacement to normal TSH FT4 can be used in the short term (2 weeks) to ensure adequacy of therapy in those with frank hypothyroidism where the FT4 was initially low Once adequately treated, should monitor thyroid function (at least TSH) every 6 weeks while on active ICPi therapy or as needed for symptoms to ensure appropriate replacement; repeat testing annually or as indicated by symptoms once stable
G3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Hold ICPi until symptoms resolve to baseline with appropriate supplementation Endocrine consultation

4.0 Endocrine Toxicity	
	May admit for IV therapy if signs of myxedema (bradycardia, hypothermia) Thyroid supplementation and reassessment as in G2
Additional considerations For patients without risk factors, full replacement can be estimated with an ideal body weight–based dose of approximately 1.6 µg/kg/d For elderly or fragile patients with multiple comorbidities, consider titrating up from low dose, starting at 25-50 mg Extreme elevations of TSH can be seen in the recovery phase of thyroiditis and can be watched in asymptomatic patients to determine whether there is recovery to normal within 3-4 weeks Under guidance of endocrinology, consider tapering hormone replacement and retesting in patients with a history of thyroiditis (initial thyrotoxic phase) Adrenal dysfunction, if present, must always be replaced before thyroid hormone therapy is initiated	
4.1.2 Hyperthyroidism	
Definition: Suppressed TSH and high normal or elevated FT4 and/or triiodothyronine Diagnostic work-up Monitor TSH, FT4 every 4-6 weeks from the start of therapy or as needed for case detection in symptomatic patients Consider TSH receptor antibodies if there are clinical features and suspicion of Grave disease (eg, ophthalmopathy) Close monitoring of thyroid function every 2-3 weeks after diagnosis to catch transition to hypothyroidism in patients with thyroiditis and hyperthyroidism	
Grading	**Management**
G1: Asymptomatic or mild symptoms	Can continue ICPi with close follow-up and monitoring of TSH, FT4 every 2-3 weeks until it is clear whether there will be persistent hyperthyroidism (see below) or hypothyroidism (see 4.1.1) Consider holding ICPi until symptoms return to baseline Consider endocrine consultation b-Blocker (eg, atenolol, propranolol) for symptomatic relief Hydration and supportive care Corticosteroids are not usually required to shorten duration For persistent hyperthyroidism (> 6 weeks) or clinical suspicion, work-up for Graves disease (TSI or TRAb) and consider thionamide (methimazole or PTU) Refer to endocrinology for Graves disease
G3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Hold ICPi until symptoms resolve to baseline with appropriate therapy Endocrine consultation b-Blocker (eg, atenolol, propranolol) for symptomatic relief For severe symptoms or concern for thyroid storm, hospitalize patient and initiate prednisone 1-2 mg/kg/d or equivalent tapered over 1-2 weeks; consider also use of SSKI or thionamide (methimazole or PTU).
Additional considerations Thyroiditis is transient and resolves in a couple of weeks to primary hypothyroidism or normal. Hypothyroidism can be treated as above. Graves disease is generally persistent and is due to increased thyroid hormone production that can be treated with antithyroid medical therapy. Physical examination findings of ophthalmopathy or thyroid bruit are diagnostic of Graves and should prompt early endocrine referral.	
4.2 Adrenal – primary adrenal insufficiency	

4.0 Endocrine Toxicity	
Definition: Adrenal gland failure leading to low morning cortisol, high morning ACTH, as well as hyponatremia and hyperkalemia with orthostasis and volume depletion due to loss of aldosterone	
Diagnostic work-up for patients in whom adrenal insufficiency is suspected: Evaluate ACTH (AM), cortisol level (AM) Basic metabolic panel (Na, K, CO_2, glucose) Consider ACTH stimulation test for indeterminate results If primary adrenal insufficiency (high ACTH, low cortisol) is found biochemically: Evaluate for precipitating cause of crisis such as infection Perform an adrenal CT for metastasis/hemorrhage	
Grading	Management
G1: Asymptomatic or mild symptoms	Consider holding ICPi until patient is stabilized on replacement hormone Endocrine consultation Replacement therapy with prednisone (5-10 mg daily) or hydrocortisone (10-20 mg orally every morning, 5-10 mg orally in early afternoon) May require fludrocortisone (0.1 mg/d) for mineralocorticoid replacement in primary adrenal insufficiency Titrate dose up or down as symptoms dictate
G2: Moderate symptoms, able to perform ADL	Consider holding ICPi until patient is stabilized on replacement hormone Endocrine consultation Initiate outpatient treatment at two to three times maintenance (if prednisone, 20 mg daily; if hydrocortisone, 20-30 mg in the morning, and 10-20 mg in the afternoon) to manage acute symptoms. Taper stress-dose corticosteroids down to maintenance doses over 5-10 days Maintenance therapy as in G1.
G3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Hold ICPi until patient is stabilized on replacement hormone Endocrine consultation See in clinic or, for after hours, make an emergency department referral for normal saline (at least 2 L) and IV stress-dose corticosteroids on presentation (hydrocortisone 100 mg or dexamethasone 4 mg (if the diagnosis is not clear and stimulation testing will be needed) Taper stress-dose corticosteroids down to maintenance doses over 7-14 days after discharge Maintenance therapy as in G1
Additional considerations Primary and secondary adrenal insufficiency can be distinguished by the relationship between ACTH and cortisol. If the ACTH is low with low cortisol, then management is as per 4.3. Patients on corticosteroids for management of other conditions will have low morning cortisol as a result of iatrogenic, secondary adrenal insufficiency. ACTH will also be low in these patients. A diagnosis of adrenal insufficiency is challenging to make in these situations (see next section on hypophysitis).	

4.0 Endocrine Toxicity	
Emergent therapy for someone with suspected adrenal insufficiency is best done with dexamethasone as a stimulation test can still be performed. If the diagnosis is already confirmed, can use hydrocortisone 100 mg. All patients need education on stress dosing and a medical alert bracelet for adrenal insufficiency to trigger stress-dose corticosteroids by EMS. Endocrine consultation prior to surgery or any procedure for stress-dose planning.	
4.3 Pituitary - hypophysitis	
Definition: Inflammation of the pituitary with varying effects on hormone function. Most commonly presenting with central adrenal insufficiency. May also have central hypothyroidism, diabetes insipidus, and hypogonadism. Diagnostic work-up Diagnosis: Low ACTH with a low cortisol. Low or normal TSH with a low FT4. Hypernatremia and volume depletion with diabetes insipidus. Low testosterone or estradiol with low LH and FSH. Testing: Evaluate ACTH, cortisol (AM), TSH, FT4, electrolytes Consider evaluating LH, FSH, and testosterone levels in males or estrogen in premenopausal females with fatigue, loss of libido, and mood changes Consider MRI of the brain with or without contrast with pituitary/sellar cuts in patients with multiple endocrine abnormalities 6 new severe headaches or complaints of vision changes	
Grading	Management
G1: Asymptomatic or mild symptoms	Consider holding ICPi until patient is stabilized on replacement hormones Endocrine consultation Hormonal supplementation as in G1
G3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Hold ICPi until patient is stabilized on replacement hormones Endocrine consultation Hormonal supplementation as in G1 Consider initial pulse dose therapy with prednisone 1-2 mg/kg oral daily (or equivalent) tapered over at least 1-2 weeks
Additional considerations Be aware of the need to start corticosteroids first when planning hormone replacement therapy for multiple deficiencies All patients need instruction on doubling doses for illness (stress dosing) and a medical alert bracelet for adrenal insufficiency to trigger stress-dose corticosteroids by EMS Corticosteroid use can cause isolated central adrenal insufficiency Work-up cannot be done with a simple AM cortisol in a patient on corticosteroids for other conditions Laboratory confirmation of adrenal insufficiency should not be attempted until treatment with corticosteroids for other disease is ready to be discontinued. For long-term exposure, consult endocrinology for recovery and weaning protocol using hydrocortisone.	
4.4 Diabetes	
Definition: T2DM is a combination of insulin resistance and insufficiency that may require oral or insulin therapy. It may be new onset or exacerbated during therapy for nonimmunologic reasons, such as corticosteroid exposure. Autoimmune T1DM results from islet cell destruction and is often acute onset, with ketosis and an insulin requirement Diagnostic work-up Monitor patients for hyperglycemia or other signs and symptoms of new or worsening DM, including measuring glucose at baseline and with each treatment cycle during induction for 12 weeks, then every 3-6 weeks thereafter. To guide the work-up in new-onset hyperglycemia, clinicians should consider a patient's medical background, exposure history, and risk factors for each subtype of DM. Laboratory evaluation in suspected T1DM should include testing for ketosis in urine and an assessment of the anion gap on a metabolic panel. Anti–glutamic acid decarboxylase, anti–islet cell, or anti–insulin antibodies are highly specific for autoimmune diabetes. Insulin and C-peptide levels can also assist in the diagnosis.	

4.0 Endocrine Toxicity	
Grading	Management
G1: Asymptomatic or mild symptoms; fasting glucose value > ULN (160 mg/dL); fasting glucose value > ULN (8.9 mmol/L); no evidence of ketosis or laboratory evidence of T1DM	Can continue ICPi with close clinical follow-up and laboratory evaluation May initiate oral therapy for those with new-onset T2DM Screen for T1DM if appropriate, for example, acute onset with prior normal values or clinical concern for ketosis
G2: Moderate symptoms, able to perform ADL, fasting glucose value > 160-250 mg/dL; fasting glucose value> 8.9-13.9 mmol/L, ketosis or evidence of T1DM at any glucose level	May hold ICPi until glucose control is obtained Titrate oral therapy or add insulin for worsening control in T2DM Should administer insulin for T1DM (or as default therapy if there is confusion about type) Urgent endocrine consultation for any patient with T1DM; in the absence of endocrinology, internal medicine may suffice Consider admission for T1DM if early outpatient evaluation is not available or signs of ketoacidosis are present
G3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL G3: > 250-500 mg/dL (> 13.9-27.8 mmol/L) G4: > 500 mg/dL (> 27.8 mmol/L)	Hold ICPi until glucose control is obtained on therapy with reduction of toxicity to G1 or less Urgent endocrine consultation for all patients Initiate insulin therapy for all patients Admit for inpatient management: Concerns for developing DKA, Symptomatic patients regardless of diabetes type, New-onset T1DM unable to see endocrinology
Additional considerations Insulin therapy can be used as the default in any case with hyperglycemia Long-acting therapy alone is not usually sufficient for T1DM, where half of daily requirements are usually given in divided doses as prandial coverage and half as long acting. Insulin doses will be lower in T1DM because of preserved sensitivity (total daily requirement can be estimated at 0.3-0.4 units/kg/d). In T2DM, sliding-scale coverage with meals over a few days provides data to estimate a patient's daily requirements and can be used to more rapidly titrate basal needs. All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: ACTH, adrenocorticotropic hormone; ADL, activities of daily living; CT, computed tomography; DKA, diabetic ketoacidosis; DM, diabetes mellitus; EMS, emergency medical services; FSH, follicle-stimulating hormone; FT4, free thyroxine; G, Grade; ICPi, immune checkpoint inhibitor; irAE, immune-related adverse event; LH, luteinizing hormone; MRI, magnetic resonance imaging; PTU, propylthiouracil; 2L, second-line; SSKI, potassium iodide; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TRAb, thyroid-stimulating hormone receptor antibody; TSH, thyroid-stimulating hormone; TSI, thyroid-stimulating immunoglobulin; ULN, upper limit ofnormal.	

16.2.5 Table A5 Management of Musculoskeletal irAEs in Patients Treated With ICPis

5.0 Musculoskeletal Toxicities	
5.1 Inflammatory arthritis	
Definition: A disorder characterized by inflammation of the joints Clinical symptoms: Joint pain accompanied by joint swelling; inflammatory symptoms, such as stiffness after inactivity or in the morning, lasting > 30 minutes to 1 hour; improvement of symptoms with NSAIDs or corticosteroids but not with opioids or other pain medications may also be suggestive of inflammatory arthritis.	
Diagnostic work-up G1 Complete rheumatologic history and examination of all peripheral joints for tenderness, swelling, and range of motion; examination of the spine Consider plain x-ray/imaging to exclude metastases and evaluate joint damage (erosions), if appropriate Consider autoimmune blood panel including ANA, RF, and anti-CCP, and anti-inflammatory markers (ESR and CRP) if symptoms persist; if symptoms are suggestive of reactive arthritis or affect the spine, consider HLA B27 testing G2 Complete history and examination as above; laboratory tests as above Consider US 6 MRI of affected joints if clinically indicated (eg, persistent arthritis unresponsive to treatment, suspicion for differential diagnoses such as metastatic lesions or septic arthritis) Consider early referral to a rheumatologist, if there is joint swelling (synovitis) or if symptoms of arthralgia persist > 4 weeks G3-4 As for G2 Seek rheumatologist advice and review Monitoring: Patients with inflammatory arthritis should be monitored with serial rheumatologic examinations, including inflammatory markers, every 4-6 weeks after treatment is instituted.	
Grading	Management
All Grades	Clinicians should follow reports of new joint pain to determine whether inflammatory arthritis is present; question whether symptom new since receiving ICPi
G1: Mild pain with inflammation, erythema, or joint swelling	Continue ICPi Initiate analgesia with acetaminophen and/or NSAIDs
G2: Moderate pain associated with signs of inflammation, erythema, or joint swelling, limiting instrumental ADL	Hold ICPi and resume upon symptom control and on prednisone ≤ 10 mg/d Escalate analgesia and consider higher doses of NSAIDS as needed If inadequately controlled, initiate prednisone or prednisolone 10-20 mg/d or equivalent for 4-6 weeks If improvement, slow taper according to response during the next 4-6 weeks; if no improvement after initial 4-6 weeks, treat as G3 If unable to lower corticosteroid dose to < 10 mg/d after 3 months, consider DMARD Consider intra-articular corticosteroid injections for large joints Referral to rheumatology
G3-4: Severe pain associated with signs of inflammation, erythema, or joint swelling; irreversible joint damage; disabling; limiting self-care ADL	Hold ICPi temporarily and may resume in consultation with rheumatology, if recover to G1 or less Initiate oral prednisone 0.5-1 mg/kg If failure of improvement after 4 weeks or worsening in meantime, consider synthetic or biologic DMARD Synthetic: methotrexate, leflunomide

5.0 Musculoskeletal Toxicities	
	Biologic: consider anticytokine therapy such as TNF-a or IL-6 receptor inhibitors. (Note: As caution, IL-6 inhibition can cause intestinal perforation; while this is extremely rare, it should not be used in patients with colitis.) Test for viral hepatitis B, C, and latent/active TB test prior to DMARD treatment Referral to rheumatology.
Additional considerations Early recognition is critical to avoid erosive joint damage. Corticosteroids can be used as part of initial therapy in inflammatory arthritis, but due to likely prolonged treatment requirements, physicians should consider starting corticosteroid-sparing agents earlier than one would with other irAEs Oligoarthritis can be treated early on with intra-articular corticosteroids; consider early referral. Consider PCP prophylaxis for patients treated with high dose of corticosteroids for 12 weeks, as per local guidelines.	
5.2 Myositis	
Definition: A disorder characterized by muscle inflammation with weakness and elevated muscle enzymes (CK). Muscle pain can be present in severe cases. Can be life threatening if respiratory muscles or myocardium are involved Diagnostic work-up Complete rheumatologic and neurologic history regarding differential diagnosis; rheumatologic and neurologic examination, including muscle strength; and examination of the skin for findings suggestive of dermatomyositis. Muscle weakness is more typical of myositis than pain. Consider preexisting conditions that can cause similar symptoms. Blood testing to evaluate muscle inflammation CK, transaminases (AST, ALT), LDH, and aldolase can also be elevated Troponin to evaluate myocardial involvement and other cardiac testing, such as echocardiogram, as needed Inflammatory markers (ESR and CRP) Consider EMG, imaging (MRI), and/or biopsy on an individual basis when diagnosis is uncertain and overlap with neurologic syndromes, such as myasthenia gravis, is suspected Consider paraneoplastic autoantibody testing for myositis and neurologic conditions, such as myasthenia gravis Monitoring: CK, ESR, CRP	
G1: Complete examination and laboratory work-up as above G2: Complete history and examination as above; autoimmune myositis blood panel; EMG, MRI of affected joints Early referral to a rheumatologist or neurologist G3-4: As for G2 Urgent referral to a rheumatologist or neurologist	
Grading	Management
G1: Mild weakness with or without pain	Continue ICPi If CK is elevated and patient has muscle weakness, may offer oral corticosteroids, and treat as G2 Offer analgesia with acetaminophen or NSAIDs if there are no contraindications
G2: Moderate weakness with or without pain, limiting age-appropriate instrumental ADL	Hold ICPi temporarily and may resume upon symptom control, if CK is normal and prednisone dose 10 mg; if worsens, treat as per G3 NSAIDs as needed Referral to rheumatologist or neurologist If CK is elevated three times or more), initiate prednisone or equivalent at 0.5-1 mg/kg May require permanent discontinuation of ICPi in most patients with G2 symptoms and objective findings

5.0 Musculoskeletal Toxicities	
	(elevated enzymes, abnormal EMG, abnormal muscle MRI or biopsy)
G3-4: Severe weakness with or without pain, limiting self-care ADL	Hold ICPi until G1 or less while off immune suppression and permanently discontinue if any evidence of myocardial involvement Consider hospitalization for severe weakness Referral to rheumatologist or neurologist Initiate prednisone 1 mg/kg or equivalent. Consider 1-2 mg/kg of methylprednisolone IV or higher-dose bolus if severe compromise (weakness severely limiting mobility, cardiac, respiratory, dysphagia) Consider plasmapheresis Consider IVIG therapy Consider other immunosuppressant therapy, such as methotrexate, azathioprine, or mycophenolate mofetil, if symptoms and CK levels do not improve or worsen after 4-6 weeks; rituximab is used in primary myositis but caution is advised given its long biologic duration
Additional considerations: Caution is advised with rechallenging	
5.3 Polymyalgia-like syndrome	
Definition: Characterized by marked pain and stiffness in proximal upper and/or lower extremities and no signs of true muscle inflammation such as CK elevation or EMG findings of myositis. No true muscle weakness, difficulty in active motion related to pain Diagnostic work-up	
G1 Complete rheumatologic history regarding differential diagnosis and examination of all joints and skin Check for symptoms of temporal arteritis, such as headache or visual disturbances; refer to ophthalmologist if present, and consider temporal artery biopsy ANA, RF, anti-CCP CK to evaluate differential diagnosis of myositis Inflammatory markers (ESR, CRP) Monitoring: ESR, CRP	
G2: Complete history and examination as above; autoimmune tests as required for differential diagnosis; early referral to a rheumatologist G3-4: As for G2; see rheumatologist advice and review	
Grading	Management
G1: Mild stiffness and pain	Continue ICPi Initiate analgesia with acetaminophen and/or NSAIDs if there are no contraindications
G2: Moderate stiffness and pain, limiting age-appropriate instrumental ADL	Consider holding ICPi and resuming upon symptom control, prednisolone < 10 mg; if worsens, treat as per G3 Initiate prednisone 20 mg/d or equivalent; if symptoms improve, start to taper dose after 3-4 weeks If no improvement or need for higher dosages after 4 weeks, escalate to G3 Consider referral to rheumatology
G3-4: Severe stiffness and pain, limiting self-care ADL	Hold ICPi and may resume, in consultation with rheumatology, if recover toG1 or less; however, note that cases of toxicity returning upon rechallenge have been reported. Referral to rheumatology

5.0 Musculoskeletal Toxicities	
	Should initiate prednisone 20 mg/d or equivalent. If no improvement or need for higher dosages for prolonged time, may offer a corticosteroid-sparing agent such as methotrexate or IL-6 inhibition with tocilizumab (Note: As caution, IL-6 inhibition can cause intestinal perforation; while this is extremely rare, it should not be used in patients with colitis or GI metastases). Consider admission for pain control
All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: ADL, activities of daily living; ALT, alanine aminotransferase; ANA, antinuclear antibodies; AST, aspartate aminotransferase; CCP, citrullinated protein antibody; CK, creatine kinase; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; EMG, electromyography; ESR, erythrocyte sedimentation rate; G, Grade; HLA, human leukocyte antigen; ICPi, immune checkpoint inhibitor; IL, interleukin; irAE, immune-related adverse event; IV, intravenous; IVIG, intravenous immunoglobulin; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging, NSAID, nonsteroidal anti-inflammatory drug; PCP, Pneumocystis pneumonia; RF, rheumatoid factor; TB, tuberculosis; TNF, tumor necrosis factor.	

16.2.6 Table A6 Management of Renal irAEs in Patients Treated With ICPis

6.0 Renal Toxicities	
Nephritis and renal dysfunction: diagnosis and monitoring For any suspected immune-mediated adverse reactions, exclude other causes Monitor patients for elevated serum creatinine prior to every dose Routine urinalysis is not necessary, other than to rule out UTIs, etc; nephrology may consider further If no potential alternative cause of AKI identified, then one should forego biopsy and proceed directly with immunosuppressive therapy Swift treatment of autoimmune component important	
6.1 Nephritis	
Definition: Inflammation of the kidney affecting the structure	
Grading	Management
G1: Creatinine level increase of > 0.3 mg/dL; creatinine 1.5-2.0 3 over baseline	Consider temporarily holding ICPi, pending consideration of potential alternative etiologies (recent IV contrast, medications, fluid status) and baseline renal function. A change that is still < 1.5 ULN could be meaningful
G2: Creatinine 2-3 x above baseline	Hold ICPi temporarily Consult nephrology Evaluate for other causes (recent IV contrast, medications, fluid status, etc); if other etiologies ruled out, administer 0.5-1 mg/kg/d prednisone equivalents If worsening or no improvement: 1 to 2 mg/kg/d prednisone equivalents and permanently discontinue treatment If improved to G1 or less, taper corticosteroids over 4-6 weeks If no recurrence of chronic renal insufficiency, discuss resumption of ICPI with patient after taking into account the risks and benefits.
G3: Creatinine > 3 x baseline or > 4.0 mg/dL; hospitalization indicated	Permanently discontinue ICPi
G4: Life-threatening consequences; dialysis indicated	Consult nephrology Evaluate for other causes (recent IV contrast, medications, fluid status, etc) Administer corticosteroids (initial dose of 1-2 mg/kg/d prednisone or equivalent)
Additional considerations Monitor creatinine weekly Reflex kidney biopsy should be discouraged until corticosteroid treatment has been attempted	
6.2 Symptomatic nephritis: follow-up	
Grading	Management
G1	Improved to baseline, resume routine creatinine monitoring
G2	If improved to G1, taper corticosteroids over at least 3 weeks before resuming treatment with routine creatinine monitoring If elevations persist > 7 days or worsen and no other cause found, treat as G3
G3	If improved to G1, taper corticosteroids over at least 4 weeks If elevations persist 3-5 days or worsen, consider additional immunosuppression (eg, mycophenolate)

6.0 Renal Toxicities	
G4	If improved to G1, taper corticosteroids over at least 4 weeks If elevations persist 2-3 days or worsen, consider additional immunosuppression (eg, mycophenolate)
All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: AKI, acute kidney injury; G, Grade; ICPi, immune checkpoint inhibitor; irAE, immune-related adverse event; IV, intravenous; ULN, upper limit of normal; UTI, urinary tract infection.	

16.2.7 able A7 Management of Nervous System irAEs in Patients Treated With ICPis

7.0 Nervous System Toxicities	
7.1 Myasthenia gravis	
Definition: Fatigable or fluctuating muscle weakness, generally more proximal than distal. Frequently has ocular and/or bulbar involvement (ptosis, extraocular movement abnormalities resulting in double vision, dysphagia, dysarthria, facial muscle weakness). May have neck and/or respiratory muscle weakness. (Note: May occur with myositis and/or myocarditis. Respiratory symptoms may require evaluation to rule out pneumonitis, myocarditis. Miller Fisher variant of Guillain-Barré syndrome (ophthalmoparesis) and the oculobulbar myositis (ptosis, ophthalmoparesis, dysphagia, neck and respiratory weakness) with ICPi may have overlapping symptoms.	
Diagnostic work-up AChR and antistriated muscle antibodies in blood; if AChR antibodies are negative, consider muscle specific kinase and lipoprotein-related 4 antibodies in blood Pulmonary function assessment with NIF and VC CPK, aldolase, ESR, CRP for possible concurrent myositis Consider MRI of brain and/or spine, depending on symptoms to rule out CNS involvement by disease or alternate diagnosis If respiratory insufficiency or elevated CPK, troponin T, perform cardiac examination with ECG and TTE for possible concomitant myocarditis Neurologic consultation Electrodiagnositic studies, including neuromuscular junction testing with repetitive stimulation and/or jitter studies, NCS to exclude neuropathy, and needle EMG to evaluate for myositis	
Grading	Management
All grades	All grades warrant work-up and intervention given potential for progressive myasthenia gravis to lead to respiratory compromise
No G1	
G2: Some symptoms interfering with ADL MGFA severity class 1 (ocular symptoms and findings only) and MGFA severity class 2 (mild generalized weakness)	Hold ICPi and may resume in G2 patients (MGFA 1 and 2) only if symptoms resolve Should consult neurology Pyridostigmine starting at 30 mg orally three times a day and gradually increase to maximum of 120 mg orally four times a day as tolerated and based on symptoms Administer corticosteroids (prednisone, 1-1.5 mg/kg orally daily) if symptoms G2; wean based on symptom improvement
G3-4: Limiting self-care and aids warranted, weakness limiting walking, ANY dysphagia, facial weakness, respiratory muscle weakness, or rapidly progressive symptoms, or MGFA severity class 3-4 moderate to severe generalized weakness to myasthenic crisis	Permanently discontinue ICPi Admit patient, may need ICU-level monitoring Neurology consult Continue corticosteroids and initiate IVIG 2 g/kg IV over 5 days (0.4 g/kg/d) or plasmapheresis for 5 days Frequent pulmonary function assessment Daily neurologic review
Additional considerations Avoid medications that can worsen myasthenia: b-blockers, IV magnesium, fluoroquinolones, aminoglycosides, and macrolides Initially a 5-day course of plasmapheresis or a 2 g/kg course of IVIG over 5 days 1-2 mg/kg methylprednisolone daily, wean based on symptom improvement Pyridostigmine, wean based on improvement ICPi-associated myasthenia gravis may be monophasic, and additional corticosteroid-sparing agents may not be required	
7.2 Guillain-Barré syndrome	
Definition: Progressive, most often symmetrical muscle weakness with absent or reduced deep tendon reflexes. Often starts with sensory symptoms/neuropathic pain localized to lower back and thighs. May involve extremities	

<table>
<tr><th colspan="2">7.0 Nervous System Toxicities</th></tr>
<tr><td colspan="2">(typically ascending weakness but not always), facial, respiratory, and bulbar and oculomotor nerves. May have dysregulation of autonomic nerves.</td></tr>
<tr><td colspan="2">Diagnostic work-up
Neurologic consultation
MRI of spine with or without contrast (rule out compressive lesion and evaluate for nerve root enhancement/thickening)
Lumbar puncture: CSF typically has elevated protein and often elevated WBCs; even though this is not typically seen in classic Guillain-Barré syndrome, cytology should be sent with any CSF sample from a patient with cancer.
Serum antibody tests for Guillain-Barré syndrome variants (GQ1b for Miller Fisher variant a/w ataxia and ophthalmoplegia) Electrodiagnostic studies to evaluate polyneuropathy
Pulmonary function testing (NIF/VC)
Frequent neurochecks</td></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>All grades</td><td>Warrant work-up and intervention given potential for progressive Guillain-Barré syndrome to lead to respiratory compromise
Note: There is no G1 toxicity</td></tr>
<tr><td>G1: Mild, none</td><td>NA</td></tr>
<tr><td>G2: Moderate, some interference with ADL, symptoms concerning to patient</td><td>Discontinue ICPi</td></tr>
<tr><td>G3-4: Severe, limiting self-care and aids warranted, weakness, limiting walking, ANY dysphagia, facial weakness, respiratory muscle weakness, or rapidly progressive symptoms</td><td>Admission to inpatient unit with capability of rapid transfer to ICU-level monitoring Start IVIG (0.4 g/kg/d for 5 days for a total dose of 2 g/kg) or plasmapheresis. Corticosteroids are usually not recommended for idiopathic Guillain-Barré syndrome; however, in ICPi-related forms, a trial is reasonable (methylprednisolone 2-4 mg/kg/d), followed by slow corticosteroid taper
Pulse corticosteroid dosing (methylprednisolone 1 g/d for 5 days) may also be considered for G3-4 along with IVIG or plasmapheresis
Frequent neurochecks and pulmonary function monitoring
Monitor for concurrent autonomic dysfunction
Nonopioid management of neuropathic pain
Treatment of constipation/ileus</td></tr>
<tr><td colspan="2">Additional considerations
Slow prednisone taper after corticosteroid pulse plus IVIG or plasmapheresis May require repeat IVIG courses
Caution with rechallenging for severe cases</td></tr>
<tr><td colspan="2">7.3 Peripheral neuropathy</td></tr>
<tr><td colspan="2">Definition: Can present as asymmetric or symmetric sensory, motor, or sensory motor deficit. Focal mononeuropathies, including cranial neuropathies (eg, facial neuropathies/Bell palsy) may be present. Numbness and paresthesias may be painful or painless. Hypo- or areflexia or sensory ataxia may be present.</td></tr>
<tr><td colspan="2">Diagnostic work-up
G1
Screen for reversible neuropathy causes: diabetic screen, B12, folate, TSH, HIV, consider serum protein electrophoresis, and other vasculitic and autoimmune screen Neurologic consultation
Consider MRI of spine with or without contrast
G2: in addition to above
MRI spine advised/MRI of brain if cranial nerve Consider EMG/NCS
Consider neurology consultation
G3-4: go to Guillain-Barré syndrome algorithm</td></tr>
</table>

7.0 Nervous System Toxicities	
Grading	Management
G1: Mild, no interference with function and symptoms not concerning to patient. Note: Any cranial nerve problem should be managed as moderate	Low threshold to hold ICPi and monitor symptoms for a week If to continue, monitor very closely for any symptom progression
G2: Moderate, some interference with ADL, symptoms concerning to patient (ie, pain but no weakness or gait limitation)	Hold ICPi and resume once return to G1 Initial observation OR initiate prednisone 0.5-1 mg/kg (if progressing from mild) Neurontin, pregabalin, or duloxetine for pain
G3-4: Severe, limiting self-care and aids warranted, weakness limiting walking or respiratory problems (ie, leg weakness, foot drop, rapidly ascending sensory changes) Severe may be Guillain-Barré syndrome and should be managed as such	Permanently discontinue ICPi Admit patient Neurologic consultation Initiate IV methylprednisolone 2-4 mg/kg and proceed as per Guillain-Barré syndrome management
7.4 Autonomic neuropathy	
Definition: Nerves that control involuntary bodily functions are damaged. This may affect blood pressure, temperature control, digestion, bladder function, and sexual function. A case of severe enteric neuropathy with ICPi has been reported. Can present with GI difficulties such as new severe constipation, nausea, urinary problems, sexual difficulties, sweating abnormalities, sluggish pupil reaction, and orthostatic hypertension.	
Diagnostic work-up An evaluation by neurologist or relevant specialist, depending on organ system, with testing that may include Screening for other causes of autonomic dysfunction: diabetic screen, adrenal insufficiency, HIV, paraproteinemia, amyloidosis, botulism; consider chronic diseases such as Parkinson and other autoimmune screening AM orthostatic vitals Consider electrodiagnostic studies to evaluate for concurrent polyneuropathy Consider paraneoplastic Lambert-Eaton myasthenic syndrome, antineutrophil cytoplasmic antibodies, and ganglionic AChR antibody testing	
Grading	Management
G1: Mild, no interference with function and symptoms not concerning to patient	Low threshold to hold ICPi and monitor symptoms for a week; if to continue, monitor very closely for any symptom progression
G2: Moderate, some interference with ADL, symptoms concerning to patient	Hold ICPi and resume once return to G1 Initial observation OR initiate prednisone 0.5-1 mg/kg (if progressing from mild) Neurologic consultation
G3-4: Severe, limiting self-care and aids warranted	Permanently discontinue ICPi Admit patient Initiate methylprednisolone 1 g daily for 3 days followed by oral corticosteroid taper Neurologic consultation
7.5 Aseptic meningitis	
Definition: may present with headache, photophobia, and neck stiffness; often afebrile but may be febrile. There may be nausea/vomiting. Mental status should be normal (distinguishes from encephalitis). Diagnostic work-up MRI of brain with or without contrast + pituitary protocol AM cortisol, ACTH to rule out adrenal insufficiency Consider lumbar puncture: measure opening pressure; check cell count and protein glucose; and perform Gram stain, culture, PCR for HSV, and other viral PCRs depending on suspicion, cytology May see elevated WBC count with normal glucose, normal culture, and Gram stain; may see reactive lymphocytes or histiocytes on cytology	

<table>
<tr><th colspan="2">7.0 Nervous System Toxicities</th></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>G1: Mild, no interference with function and symptoms not concerning to patient. Note: Any cranial nerve problem should be managed as moderate.
G2: Moderate, some interference with ADL, symptoms concerning to patient (ie, pain but no weakness or gait limitation)
G3-4: Severe, limiting self-care and aids warranted</td><td>Hold ICPi and discuss resumption with patient only after taking into account the risks and benefits
Consider empirical antiviral (IV acyclovir) and antibacterial therapy until CSF results Once bacterial and viral infection are negative, may closely monitor off corticosteroids or consider oral prednisone 0.5-1 mg/kg or IV methylprednisolone 1 mg/kg if moderate/severe symptoms</td></tr>
<tr><td colspan="2">7.6 Encephalitis</td></tr>
<tr><td colspan="2">Definition: As for aseptic meningitis, need to exclude infectious causes, especially viral (ie, HSV).
Confusion, altered behavior, headaches, seizures, short-term memory loss, depressed level of consciousness, focal weakness, speech abnormality Diagnostic work-up</td></tr>
<tr><td colspan="2">Diagnostic work-up
Neurologic consultation
MRI of brain with or without contrast may reveal T2/fluid-attenuated inversion recovery changes typical of what is seen in autoimmune encephalopathies or limbic encephalitis or may be normal
Lumbar puncture: check cell count and protein glucose and perform Gram stain, culture, PCR for HSV and other viral PCRs depending on suspicion, cytology, oligoclonal bands, autoimmune encephalopathy, and paraneoplastic panels.
May see elevated WBC count with lymphocytic predominance and/or elevated protein
EEG to evaluate for subclinical seizures
Blood: metabolic, CBC, ESR, CRP, ANCA (if suspect vasculitic process), thyroid panel including TPO and thyroglobulin Rule out concurrent anemia/thrombocytopenia, which can present with severe headaches and confusion</td></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>G1: Mild, no interference with function and symptoms not concerning to patient. Note: Any cranial nerve problem should be managed as moderate.
G2: Moderate, some interference with ADL, symptoms concerning to patient (ie, pain but no weakness or gait limitation)
G3-4: Severe, limiting self-care and aids warranted</td><td>Hold ICPi and discuss resumption with patient only after taking into account the risks and benefits
As above for aseptic meningitis, suggest concurrent IV acyclovir until PCR results obtained and negative
Trial of methylprednisolone 1-2 mg/kg
If severe or progressing symptoms or oligoclonal bands present, consider pulse corticosteroids methylprednisolone 1 g IV daily for 3-5 days plus IVIG 2 g/kg over 5 days If positive for autoimmune encephalopathy antibody and limited or no improvement, consider rituximab or plasmapheresis in consultation with neurology</td></tr>
<tr><td colspan="2">7.7 Transverse myelitis</td></tr>
<tr><td colspan="2">Definition: Acute or subacute weakness or sensory changes bilateral, often with increased deep tendon reflexes</td></tr>
<tr><td colspan="2">Diagnostic work-up
Neurologic consultation
MRI of spine (with thin axial cuts through the region of suspected abnormality) and MRI of brain
Lumbar puncture: cell count, protein, glucose, oligoclonal bands, viral PCRs, cytology, onconeural antibodies Blood: B12, HIV, RPR, ANA, Ro/La, TSH, aquaporin-4 IgG
Evaluation for urinary retention, constipation</td></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>G1: Mild, no interference with function and symptoms not concerning to patient. Note: Any cranial nerve problem should be managed as moderate.</td><td>Permanently discontinue ICPi
Methylprednisolone 2 mg/kg
Strongly consider higher doses of 1 g/d for 3-5 days Strongly consider IVIG</td></tr>
</table>

7.0 Nervous System Toxicities	
G2: Moderate, some interference with ADL, symptoms concerning to patient (ie, pain but no weakness or gait limitation) G3-4: Severe, limiting self-care and aids warranted	
All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: AChR, acetylcholine receptor; ACTH, adrenocorticotropic hormone; ADL, activities of daily living; ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibodies; CBC, complete blood count; CNS, central nervous system; CPK, creatine phosphokinase; CRP, C-reactive protein; CSF, cerebrospinal fluid; ECG, electrocardiogram; EEG, electroencephalogram; EMG, electromyography; ESR, erythrocyte sedimentation rate; G, Grade; GI, gastrointestinal; HIV, human immunodeficiency virus; HSV, herpes simplex virus; ICPi, immune checkpoint inhibitor; ICU, intensive care unit; IgG, immunoglobulin G; IV, intravenous; IVIG, intravenous immunoglobulin; irAE, immune-related adverse event; MGFA, Myasthenia Gravis Foundation of America; MRI, magnetic resonance imaging; NA, not applicable; NCS, nerve conduction study; NIF, negative inspiratory force; PCR, polymerase chain reaction; RPR, rapid plasma reagin, TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone; TTE, transthoracic echocardiogram; VC, vital capacity; WBC, white blood cell count.	

16.2.8 Table A8 Management of Hematologic irAEs in Patients Treated With ICPis

8.0 Hematologic Toxicities	
8.1 Autoimmune hemolytic anemia	
Definition: A condition in which RBCs are destroyed and removed from the blood stream before their normal lifespan is over. Symptoms include weakness, paleness, jaundice, dark-colored urine, fever, inability to do physical activity, and heart murmur.	
Diagnostic work-up History and physical examination (with special consideration of history of new drugs and insect, spider, or snake bites) Blood chemistry, CBC with evidence of anemia, macrocytosis, evidence of hemolysis on peripheral smear; LDH, haptoglobin, bilirubin, reticulocyte count, free Hgb DIC panel, which could include PTNIR infectious causes Autoimmune serology Paroxysmal nocturnal hemoglobinuria screening Direct and indirect bilirubin; LDH; direct agglutinin test; and if no obvious cause, bone marrow analysis, cytogenetic analysis to evaluate for myelodysplastic syndromes Evaluation for viral/bacterial (mycoplasma, etc) causes of hemolysis studies Protein electrophoresis, cryoglobulin analysis Work-up for bone marrow failure syndrome if refractory, including B12, folate, copper, parvovirus, FE, thyroid, infection Glucose-6-phosphate dehydrogenase Evaluation of common drug causes (ribavirin, rifampin, dapsone, interferon, cephalosporins, penicillins, NSAIDs, quinine/quinidine, fludarabine, ciprofloxacin, lorazepam, diclofenac, etc) Assessment of methemoglobinemia	
Grading	Management
G1: Hgb < LLN to 10.0 g/dL; < LLN to 6.2 mmol/L; < LLN to 100 g/L	Continue ICPi with close clinical follow-up and laboratory evaluation
G2: Hgb < 10.0 to 8.0 g/dL; < 6.2to4.9mmol/L; < 100 to 80 g/L	Hold ICPi and strongly consider permanent discontinuation Administer 0.5-1 mg/kg/d prednisone equivalents
G3: Hgb < 8.0 g/dL; < 4.9 mmol/L; < 80 g/L; transfusion indicated	Permanently discontinue ICPi Should use clinical judgment and consider admitting the patient Hematology consult Prednisone 1-2 mg/kg/d (oral or IV depending on symptoms/speed of development) If worsening or no improvement, 1-2 mg/kg/d prednisone equivalents and permanently discontinue ICPi treatment Consider RBC transfusion per existing guidelines; do not transfuse more than the minimum number of RBC units necessary to relieve symptoms of anemia or to return a patient to a safe Hgb range (7-8 g/dL in stable, noncardiac inpatients) Should offer patients supplementation with folic acid 1 mg once daily
G4: Life-threatening consequences, urgent intervention indicated	Permanently discontinue ICPi Admit patient Hematology consult IV prednisone corticosteroids 1-2 mg/kg/d If no improvement or if worsening while on corticosteroids or severe symptoms on presentation, initiate other immunosuppressive drugs, such as

8.0 Hematologic Toxicities	
	rituximab, IVIG, cyclosporin A, and mycophenolate mofetil RBC transfusion per existing guidelines; discuss with blood bank team prior to transfusions that a patient with possible ICPi serious AE is in house.
Additional considerations: Monitor Hgb levels on a weekly basis until the corticosteroid tapering process is complete; thereafter, less-frequent testing is needed	
8.2 Acquired TTP	
Definition: A disorder characterized by the presence of microangiopathic hemolytic anemia, thrombocytopenic purpura, fever, renal abnormalities, and neurologic abnormalities, such as seizures, hemiplegia, and visual disturbances. It is an acute or subacute condition.	
Diagnostic work-up History with specific questions related to drug exposure (eg, chemotherapy, sirolimus, tacrolimus, opana ER antibiotics, quinine) Physical examination, peripheral smear ADAMTS13 activity level and inhibitor titer LDH, haptoglobin, reticulocyte count, bilirubin, urinalysis to rule out other causes PT, activated PTT, fibrinogen Blood group and antibody screen, direct antiglobulin test, CMV serology Consider CT/MRI brain, echocardiogram, ECG Viral studies Note: This disorder is usually associated with a severe drop in platelets and hemolysis/anemia precipitously	
Grading	Management
All grades	The first step in the management of TTP is a high index of suspicion for the diagnosis and timely recognition; hematology consult should immediately be called, as delay in identification is associated with increased mortality/morbidity. Initially, the patient should be stabilized and any critical organ dysfunction stabilized
G1: Evidence of RBC destruction (schistocytosis) without anemia, renal insufficiency, or thrombocytopenia clinically G2: Evidence of RBC destruction (schistocytosis) without clinical consequence with G2 anemia and thrombocytopenia	Hold ICPi and discuss resumption with patient only after taking into account the risks and benefits, noting that there are currently no data to recommend restarting ICPi therapy Hematology consult Administer 0.5-1 mg/kg/d prednisone
G3: Laboratory findings with clinical consequences (G3 thrombocytopenia, anemia, renal insufficiency > 2) G4: Life-threatening consequences (eg, CNS hemorrhage or thrombosis/embolism or renal failure)	Hold ICPi and discuss resumption with patient only after taking into account the risks and benefits, noting that there are currently no data to recommend restarting ICPi therapy Hematology consult In conjunction with hematology, initiate PEX according to existing guidelines with further PEX dependent on clinical progress Administer methylprednisolone 1 g IV daily for 3 days, with the first dose typically administered immediately after the first PEX May offer rituximab
8.3 Hemolytic uremic syndrome	
Definition: A disorder characterized by a form of thrombotic microangiopathy with renal failure, hemolytic anemia, and severe thrombocytopenia. Signs and symptoms of hemolytic uremic syndrome can include: Bloody diarrhea Decreased urination or blood in the urine	

<table>
<tr><th colspan="2">8.0 Hematologic Toxicities</th></tr>
<tr><td colspan="2">Abdominal pain, vomiting, and occasionally fever
Pallor
Small, unexplained bruises or bleeding from the nose and mouth Fatigue and irritability
Confusion or seizures
High blood pressure
Swelling of the face, hands, feet, or entire body</td></tr>
<tr><td colspan="2">Diagnostic work-up
History and physical examination (special consideration for new history of high-risk drugs, hypertension, or cardiac causes) CBC with indices
Blood smear morphology. Note that the presence of schistocytes on smear is critical for diagnosis.
Serum creatinine
ADAMTS13 (to rule out TTP)
Homocysteine/methylmalonic acid
Complement testing C3, C4, CH50 (complement inhibitory antibodies for suspected familial)
Evaluate reticulocyte count and mean corpuscular volume
Evaluation of infectious cause, including screening for EBV, CMV, HHV6
Evaluation for nutritional causes of macrocytosis (B12 and folate)
Pancreatic enzymes
Evaluation for diarrheal causes, shiga toxin, Escherichia coli 0157, etc
Direct antibody test (Coombs test), haptoglobin, LDH, and other etiologies of anemia
Evaluation for common drugs causing hemolysis (tacrolimus, cyclosporine, sirolimus, etc)
Evaluation for concurrent confusion</td></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>G1-2: Evidence of RBC destruction (schistocytosis) without clinical consequences of anemia, thrombocytopenia Grade 2
G3: Laboratory findings with clinical consequences (eg, renal insufficiency, petechiae)
G4: Life-threatening consequences (eg, CNS thrombosis/ embolism or renal failure)</td><td>Continue ICPi with close clinical follow-up and laboratory evaluation
Supportive care Permanently discontinue ICPi
Begin therapy with eculizumab therapy 900 mg weekly for four doses, 1,200 mg week 5, then 1,200 mg every 2 weeks
Red blood transfusion according to existing guidelines</td></tr>
<tr><td colspan="2">8.4 Aplastic anemia</td></tr>
<tr><td colspan="2">Definition: Condition in which the body stops producing enough new blood cells</td></tr>
<tr><td colspan="2">Diagnostic work-up
History and physical examination (close attention to medications, exposure to radiation, toxins, recent viral infections) CBC, smear, reticulocyte count
Viral studies, including CMV, HHV6, EBV, parvovirus
Nutritional assessments including B12, folate, iron, copper, ceruloplasmin, vitamin D
Serum LDH, renal function
Work-up for infectious causes
Identify marrow hypo/aplasia
Bone marrow biopsy and aspirate analysis
Peripheral blood analysis, including neutrophil count, proportion of GPI-negative cells by flow for PNH
Flow cytometry to evaluate loss of GPI-anchored proteins
Type and screen patient for transfusions and notify blood bank that all transfusions need to be irradiated and filtered</td></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>G1: Nonsevere, < 0.5 polymorphonuclear cells x 10^9/L hypocellular marrow, with marrow cellularity < 25%, peripheral platelet count . 20,000, reticulocyte count < 20,000</td><td>Hold ICPi and provide growth factor support and close clinical follow-up, and laboratory evaluation
Supportive transfusions as per local guidelines</td></tr>
</table>

8.0 Hematologic Toxicities	
G2: Severe, hypocellular marrow < 25% and two of the following: ANC < 500, peripheral platelet < 20,000, and reticulocyte < 20,000	Hold ICPi and provide growth factor support and close clinical laboratory evaluations daily Administer ATG + cyclosporine; HLA typing and evaluation for bone marrow transplantation if patient is candidate; all blood products should be irradiated and filtered Supportive care with granulocyte colony-stimulating factor may be added in addition
G3-4: Very severe, ANC > 200, platelet count > 20,000, reticulocyte count > 20,000, plus hypocellular marrow > 25%	Hold ICPi and monitor weekly for improvement; if not resolved, discontinue treatment until AE has reverted to G1 Hematology consult, growth factor support Horse ATG plus cyclosporine If no response, repeat immunosuppression with rabbit ATG plus cyclosporine, cyclophosphamide For refractory patients, consider eltrombopag plus supportive care
8.5 Lymphopenia	
Definition: An abnormally low level of lymphocytes in PB; for adults, counts of < 1,500/mm^3	
Diagnostic work-up History and physical examination (special attention for lymphocyte-depleting therapy such as fludarabine, ATG, corticosteroids, cytotoxic chemotherapy, radiation exposure, etc, as well as history of autoimmune disease, family history of autoimmune disease) Evaluation of nutritional state as cause Spleen size CBC with differential, peripheral smear and reticulocyte counts CXR for evaluation of presence of thymoma Bacterial cultures and evaluation for infection (fungal, viral, bacterial specifically CMV/HIV)	
Grading	Management
G1-2: 500-1,000 PB lymphocyte count G3: 250-499 PB lymphocyte count G4: < 250 PB lymphocyte count	Continue ICPi Continue ICPi, checking CBC weekly for monitoring, initiation of CMV screening Consider holding ICPi Initiate *Mycobacterium avium* complex prophylaxis and *Pneumocystis jirovecii* prophylaxis, CMV screening. HIV/hepatitis screening if not already done May consider EBV testing if evidence of lymphadenopathy/hepatitis, fevers, hemolysis consistent with lymphoproliferative disease
8.6 Immune thrombocytopenia	
Definition: An autoimmune disorder characterized by immunologic destruction of otherwise normal platelets	
Diagnostic work-up History and physical examination (special attention for lymphocyte-depleting therapy, such as fludarabine, ATG, corticosteroids, cytotoxic therapy) Family history of autoimmunity or personal history of autoimmune disease History of viral illness CBC Peripheral blood smear, reticulocyte count Bone marrow evaluation only if abnormalities in the above test results and further investigation is necessary for a diagnosis Patients with newly diagnosed immune thrombocytopenia should undergo testing for HIV, hepatitis C virus, hepatitis B virus, and Helicobacter pylori Direct antigen test should be checked to rule out concurrent Evan syndrome Nutritional evaluation	

8.0 Hematologic Toxicities	
Bone marrow evaluation if other cell lines affected and concern for aplastic anemia	
Grading	Management
G1: Platelet count < 100/μL G2: Platelet count < 75/μL	Continue ICPi with close clinical follow-up and laboratory evaluation Hold ICPi but monitor for improvement; if not resolved, interrupt treatment until AE has reverted to G1 Administer prednisone 1 mg/kg/d (dosage range, 0.5-2 mg/kg/d) orally for 2-4 weeks after which time this medication should be tapered over 4-6 weeks to the lowest effective dose IVIG may be used in conjunction with corticosteroids if a more-rapid increase in platelet count is required.
G3: Platelet count < 50/μL	Hold ICPi but monitor for improvement; if not resolved, interrupt treatment until AE has reverted to G1
G4: Platelet count < 25/μL	Hematology consult Prednisone 1-2 mg/kg/d (oral or IV depending on symptoms) If worsening or no improvement, 1-2 mg/kg/d prednisone equivalents and permanently discontinue treatment IVIG used with corticosteroids when a more-rapid increase in platelet count is required If IVIG is used, the dose should initially be 1 g/kg as a one-time dose. This dosage may be repeated if necessary If previous treatment with corticosteroids and/or IVIG unsuccessful, subsequent treatment may include rituximab, thrombopoietin receptor agonists, or more-potent immunosuppression (From American Society of Hematology guideline on immune thrombocytopenia97; consult for further details)
8.7 Acquired hemophilia	
Definition: Disorder caused by the development of autoantibodies (inhibitors) directed against plasma coagulation factors	
Diagnostic work-up Full blood count to assess platelet number, fibrinogen, PT, PTT, INR; the typical finding in patients with acquired hemophilia A is a prolonged activated PTT with a normal PT MRI, CT, and ultrasonography may be indicated to localize, quantify, and serially monitor the location and response of bleeding Medication review to assess for alternative causes Determination of Bethesda unit level of inhibitor	
Grading	Management
G1: Mild, 5%-40% of normal factor activity in blood, 0.05-0.4 IU/mL of whole blood	Hold ICPi and discuss resumption with patient only after taking into account the risks and benefits Administer 0.5-1 mg/kg/d prednisone Transfusion support as required Treatment of bleeding disorders with hematology consult
G2: Moderate, 1%-5% of normal factor activity in blood, 0.01- 0.05 IU/mL of whole blood	Hematology consult Administration of factor replacement (choice based on Bethesda unit of titer) Administer 1 mg/kg/d prednisone 6 rituximab (dose, 375 mg/m^2 weekly for 4 weeks) and/or cyclophosphamide

8.0 Hematologic Toxicities	
	(dose, 1-2 mg/kg/d); choice of rituximab v cyclophosphamide is patient specific and should be done with assistance of hematology consult; prednisone, rituximab, and cyclophosphamide should be given for at least 5 weeks Factors should be provided to increase level during bleeding episodes, with choice of factor based on presence or absence of inhibitor
G3-4: Severe, < 1% of normal factor activity in blood, < 0.01 IU/mL of whole blood	Permanently discontinue ICPi Admit patient Hematology consult Administration of factor replacement, choice based on Bethesda unit level of inhibitor Bypassing agents may be used (factor VII, factor VIII inhibitor bypass activity); caution should be taken in the elderly and those with coronary artery disease Prednisone 1-2 mg/kg/d (oral or IV depending on symptoms) 6 rituximab (dose, 375 mg/m^2 weekly for 4 weeks) and/or cyclophosphamide (dose, 1-2 mg/kg/d). Transfusion support as required for bleeding If worsening or no improvement add cyclosporine or immunosuppression/immunoadsorption
Additional considerations: The American Heart Association requires specialist clinical and laboratory expertise. Consult and/or transfer to a specialist center is often appropriate. If consultation with or transfer to a hemophilia center is not immediately possible, then investigation and treatment should be initiated while a liaison is being established. All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: AE, adverse event; ANC, absolute neutrophil count; ATG, antithymocyte globulin; CBC, complete blood count; CMV, cytomegalovirus; CNS, central nervous system; CT, computed tomography; CXR, chest x-ray; DIC, disseminated intravascular coagulation; EBV, Epstein-Barr virus; ECG, electrocardiogram; ER, extended release; FE, ferritin; G, Grade; GPI, glycosylphosphatidylinositol; Hgb, hemoglobin; HHV6, human herpesvirus 6; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; ICPi, immune checkpoint inhibitor; INR, international normalized ratio; irAE, immune-related adverse event; IV, intravenous; IVIG, intravenous immunoglobulin; LDH, lactate dehydrogenase; LLN, lower limit of normal; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; PB, peripheral blood; PEX, plasma ex-change; PNH, paroxusmal nocturnal hemoglobinuria; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cell count; TTP, thrombotic thrombocytopenic purpura.	

16.2.9 Table A9 Management of Cardiovascular irAEs in Patients Treated With ICPis

<table>
<tr><th colspan="2">9.0 Cardiovascular Toxicities</th></tr>
<tr><td colspan="2">9.1 Myocarditis, pericarditis, arrhythmias, impaired ventricular function with heart failure and vasculitis</td></tr>
<tr><td colspan="2">Definition: Signs and symptoms may include chest pain, arrhythmia, palpitations, peripheral edema, progressive or acute dyspnea, pleural effusion, fatigue</td></tr>
<tr><td colspan="2">Diagnostic work-up
At baseline
ECG
Consider troponin, especially in patient treated with combination immune therapies Upon signs/symptoms (consider cardiology consult)
ECG
Troponin
BNP Echocardiogram CXR
Additional testing to be guided by cardiology and may include
Stress test
Cardiac catherization Cardiac MRI</td></tr>
<tr><td>Grading</td><td>Management</td></tr>
<tr><td>G1: Abnormal cardiac biomarker testing, including abnormal ECG
G2: Abnormal screening tests with mild symptoms
G3: Moderately abnormal testing or symptoms with mild activity
G4: Moderate to severe decompensation, IV medication or intervention required, life-threatening conditions</td><td>All grades warrant work-up and intervention given potential for cardiac compromise
Consider the following:
Hold ICPi and permanently discontinue after G1
High-dose corticosteroids (1-2 mg/kg of prednisone) initiated rapidly (oral or IV depending on symptoms)
Admit patient, cardiology consultation
Management of cardiac symptoms according to ACC/AHA guidelines and with guidance from cardiology
Immediate transfer to a coronary care unit for patients with elevated troponin or conduction abnormalities
In patients without an immediate response to high-dose corticosteroids, consider early institution of cardiac transplant rejection doses of corticosteroids (methylprednisolone 1 g every day) and the addition of either mycophenolate, infliximab, or antithymocyte globulin</td></tr>
<tr><td colspan="2">Qualifying statement: Treatment recommendations are based on anecdotal evidence and the life-threatening nature of cardiovascular complications. Holding checkpoint inhibitor therapy is recommended for all grades of complications. The appropriateness of rechallenging remains unknown. Note that infliximab has been associated with heart failure and is contraindicated at high doses in patients with moderate-severe heart failure.</td></tr>
<tr><td colspan="2">9.2 Venous thromboembolism</td></tr>
<tr><td colspan="2">Definition: A disorder characterized by occlusion of a vessel by a thrombus that has migrated from a distal site via the blood stream. Clinical signs and symptoms are variable and may include pain, swelling, increased skin vein visibility, erythema, and cyanosis accompanied by unexplained fever for DVT and dyspnea, pleuritic pain, cough, wheezing, or hemoptysis for PE</td></tr>
<tr><td colspan="2">Diagnostic work-up
Evaluation of signs and symptoms of PE or DVT may include Clinical prediction rule to stratify patients with suspected venous thromboembolism Venous ultrasound for suspected DVT
CTPA for suspected PE
Can also consider D-dimer for low-risk patients based on risk stratification by clinical prediction rule for DVT/PE when CT or Doppler are not available or appropriate Ventilation/perfusion scan is also an option when CTPA is not appropriate
Consider other testing, including ECG, CXR, BNP and troponin levels, and arterial blood gas</td></tr>
</table>

9.0 Cardiovascular Toxicities	
Grading	Management
G1: Venous thrombosis (eg, superficial thrombosis)	Continue ICPi Warm compress Clinical surveillance
G2: Venous thrombosis (eg, uncomplicated DVT), medical intervention indicated G3: Thrombosis (eg, uncomplicated PE [venous], nonembolic cardiac mural [arterial] thrombus), medical intervention indicated	Continue ICPi Management according to CHEST, ACC, and/or AHA guidelines and consider consult from cardiology or other relevant specialties LMWH is suggested over VKA, dabigatran, rivaroxaban apixaban, or edoxaban for initial and long-term treatment IV heparin is an acceptable alternative for initial use, and oral anticoagulants are acceptable for the long term
G4: Life-threatening (eg, PE, cerebrovascular event, arterial insufficiency), hemodynamic or neurologic instability, urgent intervention indicated	Permanently discontinue ICPi Admit patient and management according to CHEST, ACC, and/or AHA guidelines and with guidance from cardiology Respiratory and hemodynamic support LMWH is suggested over VKA, dabigatran, rivaroxaban, apixaban, or edoxaban for initial and long-term treatment IV heparin is an acceptable alternative for initial use, and oral anticoagulants are acceptable for the long term Further clinical management as indicated based on symptoms
Additional considerations While it may be impossible to determine the etiology of thromboembolic disease in patients with advanced cancer and the role, if any, that ICPi treatment plays, it is reasonable to remove the potential inciting agents given the severity and life-threatening potential of G4 complications. Clinicians are to use clinical judgment and take into account the risks and benefits when deciding whether to discontinue ICPi treatment. Anticoagulant therapy duration should continue for a minimum of 9-12 months to indefinitely in the setting of active cancer unless patient is asymptomatic, doing well, or in remission. All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: ACC, American College of Cardiology; AHA, American Heart Association; BNP, brain natriuretic peptide; CT, computed tomography; CTPA, computed tomography pulmonary angiography; CXR, chest x-ray; DVT, deep vein thrombosis; ECG, electrocardiogram; G, Grade; ICPi, immune checkpoint inhibitor; irAE, immune-related adverse event; IV, intravenous; LMWH, low-molecular-weight heparin; MRI, magnetic resonance imaging; PE, pulmonary embolism; VKA, vitamin K agonist.	

16.2.10 Table A10 Management of Ocular irAEs in Patients Treated With ICPis

10.0 Ocular Toxicities	
Counsel all patients to inform their health care provider immediately if they experience any of the following ocular symptoms Blurred vision Change in color vision Photophobia Distortion Scotomas Visual field changes Double vision Tenderness Pain with eye movement Eyelid swelling Proptosis	
Evaluation, under the guidance of ophthalmology Check vision in each eye separately Color vision Red reflex Pupil size, shape, and reactivity Fundoscopic examination Inspection of anterior part of eye with penlight	
Prior conditions Exclude patients with history of active uveitis History of recurrent uveitis requiring systemic immunosuppression or continuous local therapy Additional considerations Ocular irAEs are many times seen in the context of other organ irAEs High level of clinical suspicion as symptoms may not always be associated with severity Best to treat after ophthalmologist eye examination	
10.1 Uveitis/iritis	
Definition: Inflammation of the middle layer of the eye Diagnostic work-up: as per above	
Grading	Management
G1: Asymptomatic	Continue ICPi Refer to ophthalmology within 1 week Artificial tears
G2: Medical intervention required, anterior uveitis	Hold ICPi temporarily until after ophthalmology consult Urgent ophthalmology referral Topical corticosteroids, cycloplegic agents, systemic corticosteroids May resume ICPi treatment once off systemic corticosteroids, which are purely indicated for ocular adverse effects or once corticosteroids for other concurrent systemic irAEs are reduced to # 10 mg; continued topical/ocular corticosteroids are permitted when resuming therapy to manage and minimize local toxicity Re-treat after return to G1 or less
G3: Posterior or panuveitis	Permanently discontinue ICPi Urgent ophthalmology referral. Systemic corticosteroids and intravitreal/periocular/topical corticosteroids
G4: 20/200 or worse	Permanently discontinue ICPi Emergent ophthalmology referral Systemic corticosteroids (IV prednisone 1-2 mg/kg or methylprednisolone 0.8-1.6 mg/kg) and

10.0 Ocular Toxicities	
	intravitreal/periocular/topical corticosteroids per ophthalmologist opinion
Additional considerations: Consider use of infliximab or other TNF-a blockers in cases that are severe and refractory to standard treatment	
10.2 Episcleritis	
Definition: Inflammatory condition affecting the episcleral tissue between the conjunctiva and the sclera that occurs in the absence of an infection Diagnostic work-up: As per 10.0	
Grading	Management
G1: Asymptomatic	Continue ICPi Refer to ophthalmology within 1 week Artificial tears
G2: Vision 20/40 or better	Hold ICPi therapy temporarily until after ophthalmology consult Urgent ophthalmology referral Topical corticosteroids, cycloplegic agents, systemic corticosteroids
G3: Symptomatic and vision worse than 2/40	Permanently discontinue ICPi Urgent ophthalmology referral. Systemic corticosteroids and topical corticosteroids with cycloplegic agents
G4: 20/200 or worse	Permanently discontinue ICPi Emergent ophthalmology referral. Systemic corticosteroids and topical corticosteroids with cycloplegic agents
Additional considerations: Consider use of infliximab or other TNF-α blockers in cases that are severe and refractory to standard treatment	
10.3 Blepharitis	
Definition: Inflammation of the eyelid that affects the eyelashes or tear production Diagnostic work-up: As per 10.0	
Grading	Management
No formal grading system	Warm compresses and lubrication drops Continue therapy unless persistent and serious
All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate. Abbreviations: ICPi, immune checkpoint inhibitor; G, Grade; irAE, immune-related adverse event; IV, intravenous, TNF, tumor necrosis factor.	