ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

ALLIANCE A031501

PHASE III RANDOMIZED ADJUVANT STUDY OF PEMBROLIZUMAB IN MUSCLE INVASIVE AND LOCALLY ADVANCED UROTHELIAL CARCINOMA (AMBASSADOR) VERSUS OBSERVATION

NCI-supplied agent(s): MK-3475 (pembrolizumab) (NSC #776864, IND #132331); IND holder: CTEP
Commercial agent(s): NONE

ClinicalTrials.gov Identifier: NCT03244384

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Study Resources:

Expedited Adverse Event Reporting https://ctepcore.nci.nih.gov/ctepaers	**Medidata Rave® iMedidata portal** https://login.imedidata.com
OPEN (Oncology Patient Enrollment Network) https://open.ctsu.org	**Biospecimen Management System** http://bioms.allianceforclinicaltrialsinoncology.org

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Protocol-related questions may be directed as follows:

Questions	Contact (via email)
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair, Nursing Contact, Protocol Coordinator, and (where applicable) Data Manager
Questions related to data submission, RAVE or patient follow-up:	Data Manager
Questions regarding the protocol document and model informed consent:	Protocol Coordinator
Questions related to IRB review	Alliance Regulatory Inbox regulatory@allianceNCTN.org
Questions regarding CTEP-AERS reporting:	Pharmacovigilance Inbox pharmacovigilance@allianceNCTN.org
Questions regarding specimens/specimen submissions:	appropriate Alliance Biorepository

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

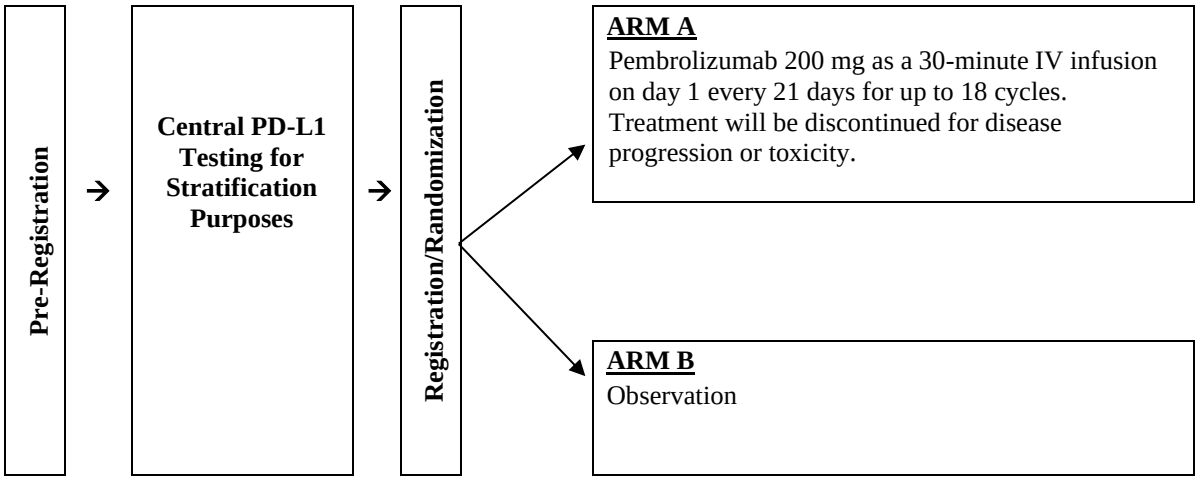
For regulatory requirements:	For patient enrollments:	For data submission:

Regulatory documentation must be submitted to the Cancer Trials Support Unit (CTSU) via the Regulatory Submission Portal. (Sign in at https://www.ctsu.org, and select the Regulatory > Regulatory Submission.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org for regulatory assistance.	Refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN). OPEN is accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions by phone or email : 1-888-823-5923, or ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Refer to the data submission section of the protocol for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific page located on the CTSU members' website (https://www.ctsu.org). Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the Roster Maintenance application and in most cases viewable and manageable via the Roster Update Management System (RUMS) on the CTSU members' website. requires logging a		
For clinical questions (i.e. patient eligibility or treatment-related) see Protocol Contacts, Page 2.		
For non-clinical questions (i.e., unrelated to patient eligibility, treatment, or clinical data submission) contact the CTSU Help Desk by phone or email: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		

Phase III randomized "Adjuvant study of Pembrolizumab in muScle invaSive and locAlly aDvanced urOthelial caRcinoma" (AMBASSADOR) versus observation

Pre-registration Eligibility Criteria	**Required Pre-registration Lab Values**	
Histologically confirmed muscle-invasive urothelial carcinoma of the bladder or upper tract	Absolute Neutrophil Count (ANC)	≥ 1,200/mm^3
Paraffin tissue available for PD-L1 analysis	Leukocytes	≥ 3,000/ mm^3
Disease status per Section 3.2.3	Platelet Count	≥ 75,000/mm^3
Radical resection of bladder cancer ≤16 weeks prior to pre-registration	Hemoglobin	≥ 9 g/dL or ≥5.6 mmol/L
No evidence of residual cancer or mets after surgery	Calc. Creatinine Clearance	≥ 30 ml/min
No measurable disease on cross-sectional imaging		
No active autoimmune disease or history of autoimmune disease that may recur	Total Bilirubin Bilirubin for pts w/ Gilbert's	≤ 1.5 x ULN ≤ 3.0 x ULN
No current or history of pneumonitis	AST/ALT	≤ 3.0 x ULN
No known active Hepatitis B or C	Serum Albumin	≥ 2.8 g/dL
No postoperative/adjuvant systemic therapy		
No prior treatment with any therapy on the PD-1 Or PD-L1 axis		
No, treatment with an investigational agent, major surgery, radiation therapy or neoadjuvant chemotherapy ≤4 weeks prior to pre-registration		
Age ≥18 years; Non-pregnant and non-nursing; ECOG PS 0-2		
Registration Eligibility Criteria		
Central PD-L1 results available		

Schema
1 Cycle = 21 Days



Treatment is to continue until metastatic recurrence or unacceptable toxicity for up to 18 cycles. Metastatic recurrence is defined by a new lesion on CT scan. Patients will be followed for a total of 5 years from the date of registration or until death, whichever comes first.

Please refer to the full protocol text for a complete description of the eligibility criteria and treatment plan.

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1.0 BACKGROUND

1.1 Pharmaceutical and Therapeutic Background

Pembrolizumab (previously known as SCH 9000475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [1]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [2; 3; 4; 5; 6]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [7; 8]. The structure of murine PD-1 has been resolved [9]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [7; 10; 11; 12]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [13; 14]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [15; 16]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [18; 19; 20; 13]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [13]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) [21]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pre-clinical and Clinical Trials:

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and leads ultimately to tumor rejection, either as a mono-therapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a mono-therapy in models of squamous cell carcinoma, pancreatic carcinoma, MEL and colorectal carcinoma. Blockade of the PD-1 pathway effectively promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-γ, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo [22; 23; 24; 25; 26; 27]. Experiments have confirmed the in vivo efficacy of PD-1 blockade as a mono-therapy as well as in combination with chemotherapy in syngeneic mouse tumor models (see the Investigator's Brochure [IB]).

1.2 Perioperative treatment strategies for muscle-invasive bladder cancer

Approximately 25% of patients with bladder cancer present with a tumor invading the muscle layer of the bladder wall (T2–T4) [1]. Muscle-invasive bladder cancer (MIBC) is associated with a high rate of recurrence and poor overall prognosis despite aggressive local and systemic therapies. Even after radical cystectomy, the 5-year mortality rate for patients with MIBC is about 50%–70% [2, 3]. The number of lymph nodes removed at the time of radical cystectomy has been found to correlate with survival [4-8]. MIBC is a systemic disease, and the cause of relapse in patients undergoing radical cystectomy is often attributable to micrometastases at the time of surgery. It is important to administer systemic therapy early in the disease process to eradicate micrometastases outside the surgical field [9]. In phase III clinical trials, neoadjuvant cisplatin-based chemotherapy has demonstrated a survival benefit in patients with MIBC [2, 10, 11] and is strongly associated with down-staging muscle-invasive tumor (stage T2–T4a) to non-muscle-invasive bladder cancer (NMIBC) (< T2) [2, 12-14]. In the phase III SWOG 8710 trial, the likelihood of long-term survival was > 85% at 5 years in patients who achieved a pT0 status and < 40% at 5 years in patients with residual muscle-invasive disease (≥ pT2 at time of cystectomy), regardless of treatment [2]. Therefore, patients who do not down-stage to < pT2 after neoadjuvant chemotherapy have a poor prognosis. However, no studies have demonstrated that additional adjuvant chemotherapy after 12 weeks of cisplatin-based neoadjuvant chemotherapy improves survival. Because of this unmet medical need, patients with high-risk MIBC with post-neoadjuvant chemotherapy residual disease will be one of the populations we will focus on in our study.

Although it is underused, neoadjuvant cisplatin-based chemotherapy is the appropriate approach in MIBC. Multiple retrospective studies have reported minimal use of perioperative chemotherapy (11%–12%), with neoadjuvant chemotherapy used in < 2% of MIBC patients [15, 16]. However, even when neoadjuvant chemotherapy was given, cisplatin was not used [17]. A more recent report of MIBC patients found that only 34% received perioperative chemotherapy, only 14% of which was neoadjuvant and only 11% of which was cisplatin-based [18].

MIBC patients predominantly receive adjuvant rather than neoadjuvant chemotherapy. Adjuvant chemotherapy cannot be administered in up to 30% of cases when needed due to surgical complications. Patients with bladder cancer are usually elderly and tend to have multiple comorbidities, making adjuvant chemotherapy after radical cystectomy challenging. The impact of post-operative complications on the timing of adjuvant chemotherapy was described in a study of 1,142 consecutive patients undergoing radical cystectomy [19]. A reported 30% of patients experienced grade 2–5 complications that could potentially interfere with administration of chemotherapy. Thus, less toxic therapies are urgently needed in this setting, especially since the data for adjuvant chemotherapy are less compelling than for neoadjuvant chemotherapy. Multiple studies have been underpowered and have not definitively shown an improvement in OS. Meta-analyses have demonstrated a survival benefit, though such analyses are limited by

the quality of the trial data. The most recent study reported by the EORTC demonstrated an improvement in DFS in patients receiving cisplatin-based adjuvant chemotherapy [20]. Standard practice is to offer cisplatin-eligible patients who have not received neoadjuvant chemotherapy adjuvant cisplatin-based combination therapy.

Up to 40% of bladder cancer patients cannot receive cisplatin, and non-cisplatin regimens have never been demonstrated to improve DFS or OS in this disease state. Cisplatin-based chemotherapy is contraindicated in patients with hearing loss/dysfunction, cardiac dysfunction, poor performance status, and renal insufficiency. A large proportion of patients with urothelial cancer have impaired renal function due to multiple factors, including medical comorbidities, age-related decline in glomerular filtration rate, and ureteral obstruction. The degree to which impaired renal function limits the widespread use of cisplatin in the perioperative setting was explored at MSKCC in a series of over 500 patients who underwent cystectomy without neoadjuvant chemotherapy [21]. Overall, using the Cockcroft-Gault equation, > 40% of patients ≥ 70 years old were ineligible for cisplatin-based chemotherapy.

This study also addresses the dilemma of cisplatin-ineligible patients who have no proven beneficial treatment options. An approach used in the management of patients with renal insufficiency is to replace cisplatin with carboplatin, despite the lack of any definitive data on clinical benefit. Carboplatin-based chemotherapy has not been satisfactorily compared with cisplatin-based therapy in phase III trials in patients with metastatic disease or MIBC. In fact, randomized phase II trials in patients with advanced disease have demonstrated that carboplatin-based chemotherapy is inferior in terms of complete response and overall response [22-24]. In these studies, median survival for carboplatin-treated patients is frequently 8–9 months, whereas median survival for cisplatin-treated patients is typically 12–15 months, suggesting that, for patients with metastatic disease, survival is compromised by carboplatin therapy. The phase II SWOG study (S0219) used carboplatin, gemcitabine, and paclitaxel in the neoadjuvant setting and demonstrated poor median OS and a very high rate of persistent cancer at cystectomy [25]. Cisplatin-ineligible patients with MIBC should proceed directly to cystectomy or be considered for trimodality therapy aimed at bladder preservation. Checkpoint inhibitor monotherapy has not shown significant renal toxicity compared to chemotherapy and is therefore a good therapeutic option to investigate in cisplatin-ineligible patients with high-risk MIBC.

1.3 Clinical Studies of PD-1/PD-L1 Inhibitors in Patients with Muscle-invasive Urothelial Carcimona

In March 12, 2021 in the issue of Lancet Oncology (25a), results of the IMvigor010 study, a multicentre, open-label, randomized, phase 3 trial done in 192 hospitals, academic centres, and community oncology practices across 24 countries or regions in patients aged 18 years and older with histologically confirmed muscle-invasive urothelial carcinoma and an Eastern Cooperative Oncology Group performance status of 0, 1, or 2 were enrolled within 14 weeks after radical cystectomy or nephroureterectomy with lymph node dissection. Patients had ypT2-4a or ypN+ tumours following neoadjuvant chemotherapy or pT3-4a or pN+ tumors if no neoadjuvant chemotherapy was received. Patients not treated with neoadjuvant chemotherapy must have been ineligible for or declined cisplatin-based adjuvant chemotherapy. No post-surgical radiotherapy or previous adjuvant chemotherapy was allowed. Patients were randomly assigned (1:1) to receive 1200 mg atezolizumab given intravenously every 3 weeks for 16 cycles or up to 1 year, whichever occurred first, or to observation. Randomization was stratified by previous neoadjuvant chemotherapy use, number of lymph nodes resected, pathological nodal status, tumour stage, and PD-L1 expression on tumour-infiltrating immune cells. The primary endpoint was disease-free survival in the intention-to-treat population. Between Oct 5, 2015, and July 30, 2018, 809 patients enrolled, of whom 406 were assigned to the atezolizumab group and 403 were assigned to the observation group. Median follow-up was 21.9 months (IQR 13.2-29.8).

Median disease-free survival was 19.4 months (95% CI 15·9-24·8) with atezolizumab and 16.6 months (11·2-24·8) with observation (stratified hazard ratio 0·89 [95% CI 0·74-1·08]; p=0·24). The most common grade 3 or 4 adverse events were urinary tract infection (31 [8%] of 390 patients in the atezolizumab group vs 20 [5%] of 397 patients in the observation group), pyelonephritis (12 [3%]) vs 14 [4%]), and anaemia (eight [2%] vs seven [2%]). Serious adverse events occurred in 122 (31%) patients who received atezolizumab and 71 (18%) who underwent observation. 63 (16%) patients who received atezolizumab had a treatment-related grade 3 or 4 adverse event. One treatment-related death, due to acute respiratory distress syndrome, occurred in the atezolizumab group.

In the June 3, 2021 the Checkmate 274 trial (Adjuvant Nivolumab versus Placebo in Muscle-Invasive Urothelial Cancer) was reported (25b). This was a phase 3, multicenter, double-blind, randomized, controlled trial. The study assigned patients with muscle-invasive urothelial carcinoma who had undergone radical surgery to receive, in a 1:1 ratio, either nivolumab (240 mg intravenously) or placebo every 2 weeks for up to 1 year. Neoadjuvant cisplatin-based chemotherapy before trial entry was allowed. The primary end points were disease-free survival among all the patients (intention-to-treat population) and among patients with a tumor programmed death ligand 1 (PD-L1) expression level of 1% or more. Survival free from recurrence outside the urothelial tract was a secondary end point. A total of 353 patients were assigned to receive nivolumab and 356 to receive placebo. The median disease-free survival in the intention-to-treat population was 20.8 months (95% confidence interval [CI], 16.5 to 27.6) with nivolumab and 10.8 months (95% CI, 8.3 to 13.9) with placebo. The percentage of patients who were alive and disease-free at 6 months was 74.9% with nivolumab and 60.3% with placebo (hazard ratio for disease recurrence or death, 0.70; 98.22% CI, 0.55 to 0.90; P<0.001). Among patients with a PD-L1 expression level of 1% or more, the percentage of patients was 74.5% and 55.7%, respectively (hazard ratio, 0.55; 98.72% CI, 0.35 to 0.85; P<0.001). The median survival free from recurrence outside the urothelial tract in the intention-to-treat population was 22.9 months (95% CI, 19.2 to 33.4) with nivolumab and 13.7 months (95% CI, 8.4 to 20.3) with placebo. The percentage of patients who were alive and free from recurrence outside the urothelial tract at 6 months was 77.0% with nivolumab and 62.7% with placebo (hazard ratio for recurrence outside the urothelial tract or death, 0.72; 95% CI, 0.59 to 0.89). Among patients with a PD-L1 expression level of 1% or more, the percentage of patients was 75.3% and 56.7%, respectively (hazard ratio, 0.55; 95% CI, 0.39 to 0.79). Treatment-related adverse events of grade 3 or higher occurred in 17.9% of the nivolumab group and 7.2% of the placebo group. Two treatment-related deaths due to pneumonitis were noted in the nivolumab group.

1.4 Muscle-invasive upper-tract urothelial carcinoma (MIUTUC)

Muscle-invasive urothelial carcinoma can develop anywhere within the urothelium (the epithelium lining the surface of the urinary bladder and tract) including the upper tract (renal pelvis and ureter). Muscle-invasive upper-tract urothelial carcinoma (MIUTUC) is treated with nephrectomy and make up about 10-15% of all muscle-invasive urothelial carcinomas. Metastatic upper-tract urothelial carcinoma has the same natural history as metastatic bladder cancer and is therefore managed identically. MIBC clinical trials have excluded patients with MIUTUC. This trial included patients with MIUTUC an important patient population.

1.5 Targeting immune checkpoints

Immune checkpoint blockade with monoclonal antibodies directed against CTLA-4, PD-1, and PD-L1 are revolutionizing treatment paradigms across multiple tumor types. These therapies have shown striking antitumor activity in an increasing number of solid tumors and hematologic malignancies, including tumors previously not considered to be immune-responsive. Bladder cancer, however, has long been known to be immune-responsive [26]. Intravesical instillation

of bacillus Calmette-Guerin (BCG) induces infiltration of cytotoxic T lymphocytes (CTLs) and mediates cell-mediated cytotoxicity against bladder tumors in patients with NMIBC. The emergence of BCG-refractory disease in a subset of patients suggests that BCG resistance may be mediated by a complex mechanism of immune escape [27]. An effective antitumor immune response involves a series of events: (a) cancer cells release cancer antigens; (b) dendritic cells and antigen-presenting cells (APCs) present these antigens; (c) APCs and T cells are primed and activated; (d) CTLs traffic to and infiltrate tumors; and (e) CTLs recognize and kill cancer cells. This process provides a framework for understanding the mechanisms of response and resistance to cancer therapy. Divergence from any of these steps facilitates immune escape, while optimizing each of these steps provides new therapeutic opportunities. For instance, while intravesical chemotherapies work by direct cytotoxic effects and release of cancer-cell antigens, intravesical BCG works by causing T cells to infiltrate tumors. While therapeutic cancer vaccines and anti-CTLA-4 antibodies work by priming, activating, and expanding T cells, immune checkpoint inhibitors such as anti-PD-1/PD-L1 antibodies restore effector T-cell function against cancer cells at the tumor site [28, 29].

Clinical studies of PD-1/PD-L1 inhibitors reported in urothelial carcinoma (Table 1)

	Metastatic second-line								**Metastatic first-line Cisplatin-ineligible**	
	Pembrolizumab		Atezolizumab		Nivolumab		Durvalumab	Avelumab	Atezolizumab	Pembrolizumab
Authors	Plimack, et al (30)	Bellmunt, et al. (83)	Powles, et al. (70)	Rosenberg, et al. (72)	Sharma, el al. (84)	Galsky, et al. (85)	Massard, et al. (86)	Apolo, et al. (34)	Balar, et al. (87)	Balar, et al. (88)
FDA approval				May 2016		February 2017				
Phase	Ib	III	I	II	I/II	II	I/II	I	II	II
N	27	542	100	331	78	265	42	44	119	100
ORR (%)	26 ($PD\text{-}L1^{+}$)	Pembro: 21.1 Chemo: 11.4	21	15	24.4	19.6	31	18.2	23	24
ORR $PD\text{-}L1^{+}$ (%)	26	Pembro: 21.6 Chemo: 6.7	43.3	26	24.0	28.4	46.4	53.8	28	37
PR (%)	15	Pembro:14.1 Chemo:8.1	NR	10	18	17.4	NR	6.8	13	18
CR (%)	11	Pembro:7 Chemo:3.3	7 ($PD\text{-}L1^{+}$)	5	6.4	2.3	NR	11.4	9	6
$PD\text{-}L1^{+}$ prevalence (%)	53	Pembro: 27.4 Chemo:33.1	27	32.2	32	30.5	65.6	29.5	27	30
PFS (months)	2	Pembro:2.1 Chemo:3.3	6 ($PD\text{-}L1^{+}$) 1 ($PD\text{-}L1^{-}$)	2.1	2.8	2.0	NR	2.9	2.7	NR
OS (months)	13	Pembro:10.3 Chemo:7.4	NR ($PD\text{-}L1^{+}$) 8 ($PD\text{-}L1^{-}$)	11.4	9.7	8.7	NR	13.7	15.9	NR
Grade 3/4 AEs (%)	15	Pembro: 15 Chemo:49.4	4.4	16	22	17.8	4.9	6.8	16	16

Abbreviations: AEs, adverse events; CR, complete response; FDA, Food & Drug Administration; IHC, expression; NR, not reported; ORR, objective response rate; OS, overall survival; $PD{-}L1^{+}$, programmed cell death ligand 1-positive; PFS, progression-free survival; PR, partial response

In a phase I study of pembrolizumab 10 mg/kg every 2 weeks in 33 patients with advanced urothelial carcinoma expressing PD-L1 in ≥ 1% of tumor cells by immunohistochemistry (IHC) (presented at ESMO 2014 and updated at ASCO 2015 [30]), 8 of 29 (28%) evaluable patients had a response: 3 (10.3%) achieved a complete response (CR) and 5 (17.2%) had a partial response (PR). Additionally, 64% of patients experienced a decrease in target lesions. With a

median follow-up of 15 months, 3 patients have ongoing responses (response duration 8–64+ weeks), median DFS is 2 months, and median OS is 13 months. PD-L1 IHC staining was assessed in both tumor cells and tumor-associated immune cells to assess the association between response and PD-L1 staining. Tumor-cell PD-L1 staining was assessed in 29 patients. The 18 patients who were positive had an overall response rate (ORR) of 33%; the 11 who were negative had an ORR of 9%. Tumor cells and tumor-associated inflammatory cells were assessed in 28 patients. The 24 patients who were positive had an ORR of 29%; the 4 who were negative had an ORR of 0%. In this heavily pretreated patient population, pembrolizumab showed a good response rate and a favorable toxicity profile. Grade 3/4 adverse events were reported in 5% of patients, most commonly fatigue, liver function test increase, nausea, dry mouth, and rash. No nephrotoxic adverse events were reported.

A phase I expansion cohort in patients with advanced/refractory urothelial carcinoma was treated with atezolizumab (MPDL3280A), a PD-L1 inhibitor, given i.v. every 3 weeks for ≤ 16 cycles. Data from this study were reported in 2014 [31] and updated at ASCO 2015 [32]. The reported response rate in patients who were PD-L1 2/3+ by IHC in tumor-infiltrating immune cells was 50% vs. 17% in patients with PD-L1 0/1+ tumor-infiltrating immune cells, for an overall response rate of 34% in 87 evaluable patients. Nine patients (20%) achieved a CR. Tumor responses were also seen in patients with visceral metastasis. Twenty of 30 responding patients had ongoing responses at the time of data cutoff, and 10 patients have been treated for > 1 year. The median OS for patients with IHC 2/3+ was not reached vs. 8 months in patients with IHC 0/1+ (median survival follow-up: 14 months (IHC 2/3) and 12 months (IHC 0/1)). A phase II study of atezolizumab in patients with locally advanced or metastatic urothelial carcinoma (IMvigor 210) reported data from 311 evaluable patients at ESMO/ECCO 2015 [33]. The primary endpoint was objective response compared to historical controls with a null hypothesis of 10% objective response rate. PD-L1 expression was prospectively assessed centrally using the SP142 IHC assay. Visceral metastases were seen in 78% of patients, and 40% of patients had previously received > 2 lines of chemotherapy. The PDL-1 IHC status of immune cells for this population was 32% IHC 2/3, 35% IHC 1, and 33% IHC 0. Atezolizumab was administered at 1200 mg i.v. every 3 weeks. ORR by RECIST v1.1 was 15% (P = 0.0058) in all patients. Subgroup analysis showed ORRs of 18% (P = 0.0004) in IHC 1/2/3 (any PD-L1 expression), 27% (P = 0.0001) in IHC 2/3, 10% in IHC 1, and 9% in IHC 0. Overall, 12 patients achieved a CR and 35 achieved a partial response (PR). An additional 15 unconfirmed RECIST v1.1 CR/PRs were seen. Median OS was 7.9 month in the overall cohort, with patients having IHC 0/1 achieving OS of 6.7 months compared to not reached in patients expressing PD-L1 IHC 2/3. Survival data were not mature. Median DFS was 2.1 months. Atezolizumab was well tolerated with no treatment-related deaths. Grade 3/4 treatment-related adverse events were seen in 15% of patients. Atezolizumab was approved by the US FDA on May 19, 2016 for the treatment of patients with locally advanced or metastatic urothelial carcinoma previously treated with platinum-based chemotherapy for metastatic disease, or for disease recurrence within 1 year of perioperative platinum-based chemotherapy.

A phase Ib study of avelumab in patients with locally advanced or metastatic urothelial carcinoma was presented at ESMO 2015 [34] and updated at GU ASCO (Apolo et al., J Clin Oncol 34, 2016 (suppl 2S; abstr 367). The results showed that avelumab was active in patients with metastatic urothelial carcinoma, leading to tumor shrinkage in lymph nodes and in visceral metastases. Patients received avelumab 10 mg/kg as a 1-hour infusion every 2 weeks until confirmed progression, unacceptable toxicity, or any criterion for withdrawal occurred. Tumors were assessed every 6 weeks by RECIST v1.1. A prespecified analysis of response was performed at 13 weeks. Unconfirmed ORR and DFS were evaluated. Patients (n = 44) with metastatic urothelial carcinoma of the urinary bladder (52.3%), urethra (31.8%), renal pelvis (6.8%), or ureter (9.1%) were treated with avelumab and followed for a median 3.5 months

(range, 3–5). At data cutoff, median duration of treatment was 13 weeks (range, 2–28), and 16 patients remained on avelumab. ORR was 15.9% (7 patients; 95% CI: 6.6, 39.4), with 1 CR and 6 PRs; 6 responses were ongoing at data cutoff. Best responses by RECIST were in patients with soft-tissue/visceral (57%) and lymph node (43%) metastases. Stable disease was observed in an additional 19 patients (43.2%). Avelumab was well tolerated—only one patient experienced a grade 3 treatment-related adverse event. PD-L1 expression was evaluable in 32 patients. Using a ≥5% cutoff (10/32 [31.3%] were PD-L1+), ORR was 40.0% in PD-L1+ patients (4/10) vs 9.1% in PD-L1– patients (2/22; p=0.060). PFS at 12 weeks was 70.0% (95% CI: 32.9, 89.2) in PD-L1+ patients vs 45.5% (95% CI 22.7, 65.8) in PD-L1− patients.

These results show the significant activity of PD-L1 inhibition and support the use of immune checkpoint blockade in bladder cancer. Pembrolizumab, atezolizumab, and avelumab have similar clinical activity in patients with metastatic urothelial carcinoma. The low toxicity reported in these studies, especially the lack of nephrotoxicity, make these therapies ideal candidates for investigation in the adjuvant setting.

1.6 Rationale for adjuvant immune checkpoint blockade in urothelial carcinoma

Patients with high-risk MIBC have a poor prognosis. Radical cystectomy remains the standard treatment in the United States and much of Europe. Yet despite substantial improvements in surgical techniques, mortality from metastatic recurrence of MIBC remains high. Although cisplatin-based neoadjuvant chemotherapy has been shown to improve survival, a large number of patients are resistant to cisplatin-based chemotherapy and have persistent muscle-invasive disease despite aggressive chemotherapy. Patients who do not receive neoadjuvant chemotherapy should consider adjuvant cisplatin-based chemotherapy. However, almost half of MIBC patients are not cisplatin-eligible and thus need additional treatment options. These 2 patient populations will be enrolled in our clinical trial of adjuvant pembrolizumab. Patients who receive adjuvant chemotherapy will not be eligible for enrollment. Increased heterogeneity, the timing of randomization, and rapid relapse of high-risk disease in patients still on chemotherapy can lead to study dropout and may confound results.

MIBC patients who receive neoadjuvant or adjuvant cisplatin-based chemotherapy have a poor 5-year survival rate if they are not downstaged to non-muscle invasive disease (Table 2), and that rate is even worse for patients with high-risk MIBC. The goal of this trial is to determine whether adjuvant pembrolizumab improves DFS and OS in patients with chemotherapy-nonresponsive/resistant muscle-invasive or node-positive urothelial carcinoma after radical cystectomy, or in patients ineligible for cisplatin-based chemotherapy. The rationale for this trial is based on the significant activity of immune checkpoint inhibitors seen in early-phase clinical trials in patients with advanced/chemotherapy-refractory metastatic urothelial carcinoma. In addition, the use of adjuvant checkpoint blockade with ipilimumab in melanoma leads to clinically significant improvements in disease-free survival, providing precedent for the potential benefits of adjuvant immune checkpoint blockade [35]. We hypothesize that immunotherapeutic strategies may be more effective in patients with low tumor burden, such as patients with MIBC or upper-tract muscle-invasive urothelial carcinoma who are likely to harbor micrometastases.

Table 2. Summary of neoadjuvant and adjuvant clinic trials in muscle-invasive bladder cancer.

	Cognetti	Paz-Ares	Sternberg	Grossman	MRC/ EORTC
Chemotherapy	Adjuvant GC x4	Adjuvant PGC x4	Adjuvant ddMVAC/GC/ MVAC x4	Neoadjuvant MVAC	Neoadjuvant CMV

Patients	T2G3, T3-T4, N0-2	T3-T4, N0-2	T3-T4 and/or pTxN1-3	T2-4aN0	T2-4aN0
Design					
α error	5%	5%	5%	5%	5%
Power	80%	80%	80%	80%	90%
Endpoint	OS	OS	OS	OS	OS
	50%->60% At 2yrs (10%)	50% -> 65% At 2 years (15%)	35% -> 42% At 5 years (7%)	35% -> 42% Median OS (50%)	50% -> 60% At 2 years (10%)
Hazard Ratio	0.75	0.77	0.826		
Sample Size	610	340	660 (originally 1344)	298	915
RESULTS					
Patients (n) Randomized	192	142	284	307	976
Years to accrue	6	7	6	11	6
5-year Recurrence Observation vs. chemo	DFS 42.3% vs. 37.2% *P=0.70 HR 1.08* All: 40%	3 years 44% vs.73% *P<0.0001 HR 0.36* All: 54%	DFS 31.8% vs 47.6% *P= <0.0001 HR 0.54*		5 year DFS 32% vs.39% 10 year DFS 20% vs 27% *p= 0.008 HR 0.82*
5-year OS Observation vs. chemo	53.7% vs 43.4% *P=0.24 HR 1.29* All pts: 48.5%	31% vs.60% *P<0.0009 HR 0.44* All pts: 49%	47.7% vs 53.6% *P=0.13 HR 0.78* All pts: 38.6%	43% vs.57% *P=0.06*	5 year OS 43% vs.49% 10 year 30% vs. 36% *p= 0.037 HR 0.84*
Median f/u	35 months	30 months	7 years	8.7 years	8 years

1.7 Renal Insufficiency for Muscle-invasive Bladder Cancer

A major factor limiting treatment options for patients with MIBC is that up to 40% cannot receive cisplatin, and non-cisplatin regimens have never been demonstrated to improve PFS or OS in this disease state. Cisplatin-based chemotherapy is contraindicated in patients with hearing loss/dysfunction, cardiac dysfunction, poor performance status, and renal insufficiency. A large proportion of patients with UC have impaired renal function due to multiple factors, including medical comorbidities, age-related decline in glomerular filtration rate, and ureteral obstruction (89). Memorial Sloan Kettering Cancer Center explored the degree to which impaired renal function limits widespread use of cisplatin in the perioperative setting in a series of over 500 patients who underwent cystectomy without neoadjuvant chemotherapy (90). Overall, using the Cockcroft-Gault equation, > 40% of patients ≥ 70 years old were ineligible for cisplatin-based chemotherapy.

Carboplatin is commonly substituted for cisplatin in combination chemotherapies for cisplatin-ineligible patients, despite a dearth of survival benefit data. Carboplatin-based chemotherapy combinations have not been compared with cisplatin-based combination therapies in phase III randomized trials in patients with metastatic disease or MIBC. In fact, randomized phase II trials in patients with advanced disease have demonstrated that carboplatin-based chemotherapy is

inferior in terms of either complete or overall response (91-93). In these studies, median OS for carboplatin-based combination treatment is 8–9 months, whereas median OS for cisplatin-based combination treatment is 12–15 months, suggesting that, for patients with metastatic disease, survival is compromised by carboplatin therapy. The phase II SWOG study (S0219) used carboplatin, gemcitabine, and paclitaxel in the neoadjuvant setting and demonstrated poor median OS and a very high rate of persistent invasive disease at cystectomy (94). Therefore, based on current data, cisplatin-ineligible patients with MIBC should proceed directly to cystectomy or be considered for trimodality therapy aimed at bladder preservation.

Immunotherapy with immune checkpoint blockers such as avelumab, atezolizumab, durvalumab, pembrolizumab, and nivolumab has been found to be safe in patients with renal impairment. According to pharmacokinetic studies, renal function does not affect drug clearance. Therefore, package inserts for these drugs do not recommend dose adjustments for chronic kidney disease (95-99). Two large trials of the anti–PD-1/PD-L1 agents atezolizumab and pembrolizumab for the first-line treatment of cisplatin-ineligible metastatic UC patients found these agents to be safe in this patient population. There have also been several case reports of checkpoint blockers being safely administered to patients with end-stage renal disease on dialysis (Level 4) (100). A major consideration in treating dialysis patients is the potential of anticancer drug ultrafiltration (101). Since these monoclonal antibodies have large molecular weights, they are likely not dialyzable and may possibly be given without regard to the timing of dialysis. However, prospective trials are needed to understand the safety profile of anti–PD-1/PD-L1 therapies in patients with significant renal impairment and end-stage renal disease.

Summary rationale:

- ~40% MIBC have renal insufficiency
- Avelumab, atezolizumab, durvalumab, pembrolizumab, and nivolumab has been found to be safe in patients with renal impairment
- Pharmacokinetic studies have found that renal function does not affect drug clearance; package inserts for these drugs do not recommend dose adjustments for chronic kidney disease.
- Two large trials of the anti–PD-1/PD-L1 agents atezolizumab and pembrolizumab for the first-line treatment of cisplatin-ineligible metastatic UC patients with renal insufficiency (creatinine clearance 30–60 mL/min) found these agents to be safe in this patient population
- Case reports of checkpoint blockers (nivolumab, pembrolizumab, and ipilimumab) being safely administered to patients with end-stage renal disease on dialysis
- These monoclonal antibodies have large molecular weights, they are not dialyzable and may be given without regard to the timing of dialysis

1.8 Registration Quality of Life (QOL) Measurements

QOL measurements of fatigue and overall perception of QOL are routinely included in Alliance studies and will be assessed upon registration in this study. Evidence has arisen indicating that baseline single-item assessments of fatigue and overall QOL are strong prognostic indicators for survival in cancer patients, independent of performance status. This evidence was derived from two separate meta-analyses recently presented at ASCO, the first involving 23 NCCTG and Mayo Clinic Cancer Center oncology clinical trials, the second involving 43 clinical trials. Routine inclusion of these measures should be considered similar to that of including performance status, either as stratification or prognostic covariates. It will take approximately one minute to complete this measure.[36, 37, 38]

2.0 OBJECTIVES

2.1 Dual primary objectives

To determine DFS and OS in all patients with muscle-invasive bladder and upper-tract urothelial carcinoma treated with adjuvant pembrolizumab vs. observation.

2.2 Secondary objectives

2.2.1 To determine DFS and OS in PD-L1 positive and negative patients with muscle-invasive bladder and upper-tract urothelial carcinoma treated with adjuvant pembrolizumab vs. observation.

2.2.2 To characterize the safety and tolerability of pembrolizumab when administered in the adjuvant setting in patients with muscle-invasive bladder and upper-tract urothelial carcinoma.

2.3 Other objective(s)

Results of the primary analysis will be examined for consistency, while taking into account the stratification factors and/or covariates of baseline QOL and fatigue.

2.4 Correlative science objective(s)

2.4.1 To determine if the 12 immune gene signatures are associated with OS and DFS.

2.4.2 To determine if tumor molecular subtype is associated with OS and DFS.

2.4.3 To investigate whether the diversity of T-cell receptor (TCR) clonotypes is associated with OS and DFS.

2.4.4 To investigate whether persistence of TCR clonotypes is associated with OS and DFS.

2.4.5 To determine if tumor burden and neoantigen burden are associated with OS and DFS.

2.4.6 To determine if HLA subtypes are associated with OS and DFS.

2.4.7 To conduct exploratory analyses regarding the association of plasma HGF and VEGF levels with IL-10 and IL-17 and OS and DFS and between treated and untreated patients.

2.5 Pharmacogenomic study objectives

2.5.1 To investigate the effect of PDCD1 SNP rs11568821 on severe (grade 3 or higher) immune-related toxicity in the pembrolizumab-treated cohort.

2.5.2 To investigate whether other SNPs commonly polymorphic within or near PDCD1 associate with development of pembrolizumab toxicity in the treated cohort.

2.5.3 To identify novel germline genetic markers of treatment-related toxicity through genome-wide association analysis of pembrolizumab-treated patients.

2.5.4 To identify novel germline genetic markers that are associated with DFS and OS through genome-wide association analysis.

2.6 Quality of Life Correlative Study objective

2.6.1 To compare health-related quality of life (HRQL) as assessed by the EORTC QLQ-C30 between patients randomized to pembrolizumab vs. observation.

2.6.2 To compare urinary symptoms as assessed by EORTC QLQ-BLM30 between patients randomized to pembrolizumab vs. observation.

2.6.3 To compare patient-reported fatigue, diarrhea, and pain between patients randomized to pembrolizumab vs. observation.

2.6.4 To compare health utilities and QALYs between patients randomized to pembrolizumab vs. observation.

2.6.5 To compare other scale scores of the EORTC QLQ-C30, EORTC QLQ-BLM30, and EQ5D-5L between patients randomized to pembrolizumab vs. observation.

2.6.6 To compare global quality of life, symptoms, health utilities, QALYs, and other scale scores of the three questionnaires between patients randomized to pembrolizumab vs. observation within subgroups defined by each of the stratification factors.

3.0 PATIENT SELECTION

For questions regarding eligibility criteria, see the Study Resources page. Please note that the Study Chair cannot grant waivers to eligibility requirements.

3.1 On-Study Guidelines

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate.

Physicians should consider whether any of the following may render the patient inappropriate for this protocol:

- Psychiatric illness which would prevent the patient from giving informed consent.
- Medical condition such as uncontrolled infection, uncontrolled diabetes mellitus or cardiac disease which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient.
- 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 $\leq$ 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 $\geq$ 3 years and currently do not require systemic therapy.
- Has received systemic chemotherapy in the adjuvant setting following cystectomy/nephrectomy/ureterectomy.

In addition:

- Women and men of reproductive potential should agree to use an appropriate method of birth control throughout their participation in this study due to the teratogenic potential of the therapy utilized in this trial. Appropriate methods of birth control include

abstinence, oral contraceptives, implantable hormonal contraceptives or double barrier method (diaphragm plus condom).

3.2 Pre-Registration Eligibility Criteria

Use the spaces provided to confirm a patient's eligibility for pre-registration by indicating Yes or No as appropriate. It is not required to complete or submit the following page(s).

When calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test were done on a Monday, the Monday one week later would be considered Day 7.

A female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

3.2.1 Documentation of Disease:

___ Histologically confirmed muscle-invasive urothelial carcinoma of the bladder, urethra, upper tract, or LN+ disease. Variant histology allowed as long as urothelial carcinoma is predominant (any amount of squamous differentiation is allowed). Any component of neuroendocrine carcinoma is excluded.

3.2.2 Tissue available for Central PD-L1 Testing

Paraffin tissue samples obtained by transurethral resection of muscle-invasive bladder tumor, upper tract resection, or radical cystectomy/nephrectomy/ureterectomy/ nephroureterectomy/cystoprostatectomy or urethrectomy must be available. This specimen submission is mandatory prior to registration as results will be used for stratification. Specimens from radical/definitive surgery (radical cystectomy/nephrectomy/ureterectomy /nephroureterectomy/cystoprostatectomy and LN dissection) are preferred over transuretheral resection, if available. See Section 6.2 for details on specimen submission.

3.2.3 Disease Status

___ Patient must fit into <u>one</u> of the following three categories:

- Patients who received neoadjuvant chemotherapy and pathologic stage at surgical resection is ≥ pT2 and/or N+

OR

- Patients who are not cisplatin-eligible (according to ≥ 1 of the following criteria: ECOG performance status of 2, creatinine clearance < 60 mL/min, grade ≥ 2 hearing loss, grade ≥ 2 neuropathy, or New York Heart Association Class III heart failure [38]) and pathologic stage at surgical resection is ≥ pT3 or pN+)

OR

- Patients that decline adjuvant cisplatin-based or other systemic chemotherapy based on an informed discussion with the physician and pathologic stage at surgical resection is ≥ pT3 or pN+

3.2.4 Surgical History

The 7th edition of AJCC staging will be utilized.

___ Patient must have had radical cystectomy (cystoprostatectomy for men) and lymph node dissection (for bladder primary), or nephrectomy, nephroureterectomy or ureterectomy (for uppertract tumors) or urethrectomy (in addition to a radical cystectomy-either simultaneously or in the past) ≥4 weeks but ≤ 16 weeks prior to pre-registration. Patients who have had a partial cystectomy as definitive therapy are not eligible.

___ No gross cancer at the surgical margins. Microscopic invasive urothelial carcinoma positive margins are allowed. CIS at margins is considered negative margins.

___ No evidence of residual cancer or metastasis after surgery. Patients with uppertract urothelial carcinoma must have a negative cystoscopy within 3 months prior to pre-registration. If the bladder has been removed a cystoscopy is not required.

3.2.5 No metastatic disease (or radiologic findings "concerning" for metastatic disease) on cross-sectional imaging (according to RECIST v1.1 criteria).

3.2.6 Patient History

___ No active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome because of the risk of recurrence or exacerbation of disease. HIV-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible.

___ No current pneumonitis or prior history of non-infectious pneumonitis that required steroids within the previous 5 years.

___ Patients with vitiligo, endocrine deficiencies including type I diabetes mellitus, thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible.

___ Patients with rheumatoid arthritis and other arthropathies, Sjögren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

___ No known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected)

___ No live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed

3.2.7 Prior Treatment

___ No postoperative/adjuvant systemic therapy.

___ No prior treatment with any therapy on the PD-1/PD-L1 axis.
___ No treatment with any other type of investigational agent ≤ 4 weeks before pre-registration
___ No major surgery ≤ 4 weeks before pre-registration
___ No radiation therapy ≤ 4 weeks before pre-registration
___ No neoadjuvant chemotherapy ≤ 4 weeks before pre-registration
___ Not currently requiring hemodialysis

3.2.8 Age ≥ 18 years

3.2.9 Not pregnant and not nursing, because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown.

3.2.10 ECOG Performance Status ≤ 2

3.2.11 Required Pre-registration Laboratory Values:

Absolute Neutrophil Count (ANC)	≥ 1,200/mm^3
Leukocytes	≥ 3,000/ mm^3
Platelet Count	≥ 75,000/mm^3
Hemoglobin	≥ 9 g/dL or ≥ 5.6 mmol/L
Total Bilirubin Bilirubin for patients with Gilbert's	≤ 1.5 x upper limit of normal (ULN) ≤ 3.0 x ULN
Calc. Creatinine Clearance	≥ 30 mL/min (using either CKD-EPI equation or Cockroft-Gault formula)
AST / ALT	≤ 3.0 x ULN
Serum Albumin	≥ 2.8 g/dL
For women of childbearing potential only: a negative urine or serum pregnancy test done ≤ 7 days prior to pre-registration is required.	

3.3 Registration Eligibility Criteria

3.3.1 Results of central PD-L1 testing available

Q2 Solutions will forward the PD-L1 results to the statistical center and the statistical center will notify the site that the result is available. Since the results will be blinded to the site the notification from the Alliance registration/randomization office will serve as a confirmation of this eligibility criteria; after sites receive the confirmation e-mail from Alliance they can register the patient.

4.0 PATIENT REGISTRATION

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR). The RCR is a self-service online person registration application with electronic signature and document submission capability.

RCR utilizes five person registration types.

- Investigator (IVR) — MD, DO, or international equivalent;
- Non Physician Investigator (NPIVR) — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- Associate Plus (AP) — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System [RUMS], OPEN, Rave, acting as a primary site contact, or with consenting privileges;
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	**IVR**	**NPIVR**	**AP**	**A**	**AB**
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
Agent Shipment Form (if applicable)	✔				
CV (optional)	✔	✔	✔		

IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster;
- Selection as the treating, credit, or drug shipment investigator or consenting person in OPEN;
- Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval; and
- Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

In addition, all investigators act as the Site-Protocol PI (investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the CI on the DTL must be rostered at the enrolling site with a participating organization (i.e., Alliance).

Refer to the NCI RCR page on the CTEP website for additional information. For questions, please contact the RCR Help Desk by email at RCRHelpDesk@nih.gov.

4.2 Cancer Trials Support Unit Registration Procedures

Permission to view and download this protocol and its supporting documents is restricted and is based on the person and site roster assignment housed in the Roster Maintenance application and in most cases viewable and manageable via the Roster Update Management System (RUMS) on the Cancer Trials Support Unit (CTSU) members' website.

This study is supported by the NCI CTSU.

IRB Approval:

As of March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB) in order to participate in Cancer Therapy Evaluation Program (CTEP) and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating with the NCI CIRB must submit the Study Specific Worksheet (SSW)for Local Context to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by emailor calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB, must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

- Have an active statusHave an active status at the site(s) on the IRB/REB approval (applies to US and Canadian sites only) on at least one participating organization's roster;
- If using NCI CIRB, be active on the NCI CIRB roster under the applicable Signatory Institution(s) record;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile;
- Lists all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and

- Have the appropriate CTEP registration type for the protocol.

4.2.1 Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO);
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
- Compliance with all applicable protocol-specific requirements (PSRs).

4.2.2 Downloading Site Registration Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a PO on the protocol.

- Log in to the CTSU members' website (https://www.ctsu.org)
- Click on *Protocols* in the upper left of your screen
  - Enter the protocol number in the search field at the top of the protocol tree, or
  - Click on the By Lead Organization folder to expand, the select *Alliance* and protocol number A031501
- Click on
- Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select Site Registration to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal log onto the CTSU members' website, go to the Regulatory section and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support.

4.2.4 Delegation of Tasks Log

Each site must complete a protocol-specific Delegation of Tasks Log (DTL) using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and to activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are

available in the Help Topics button in the DTL application and describe DTL task assignments, CI signature, and CTEP registration requirements, as well as include a Master Task List.

4.2.5 Checking Site's Registration Status

Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration; and*
- Enter the sites 5-character CTEP Institution Code and click on Go
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Pre-Registration Requirements

Eligibility of patients as outlined in Section 3.2 of the protocol should be confirmed prior to preregistration.

- **Informed consent:** the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Current human protection committee approval of this protocol and a consent form is required prior to patient consent and registration.

4.4 Patient Registration/Randomization Procedures (Step 1)

All patients pre-registered will submit tissue for central PD-L1 testing. PD-L1 results are required for ALL patients to be registered for stratification purposes. Patients should be registered to A031501 within 14 days of notification of the PD-L1 results.

- Patient questionnaire booklets: Patient questionnaire booklets are to be ordered prior to the registration of any patients. Patient completed booklets can be ordered by downloading and completing the CTSU supply request form (located under the site registration documents section of the A031501 CTSU site) and following the instructions for submission on the regulatory submission portal. Samples of the booklets are found in Appendix II, which is to be used for reference and IRB submission only. They are not to be used for patient completion.
- Protected Health Information: Tissue collected for this study will be sent directly to Q2 Solutions. These samples will be labeled with patient initials, study ID, and collection date/time.

4.5 Patient Enrollment

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN Corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrara must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for a protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.6 Registration to Correlative and Companion Studies

4.6.1 Registration to Substudies described in Section 14.0

There are three optional substudies within Alliance A031501. These correlative science studies must be offered to all patients enrolled on Alliance A031501 (although patients may opt to not participate). These substudies do not require separate IRB approval. The substudies included within Alliance A031501 are:

- Blood Correlative Science Studies in A031501 (named Alliance A031501-ST1)
- Pharmacogenomic Studies in A031501 (named Alliance A031501-PP1)
- Quality of Life Studies in A031501, (named Alliance A031501-HO1)

If a patient answers "yes" to "I agree to have my specimen collected and I agree that my specimen sample(s) and related information may be used for the laboratory study(ies) described above." Question #2 in the model consent, they have consented to participate in the correlative science and pharmacogenomics substudies. The patient should be registered to Alliance A031501-ST1 and A031501-PP1 at the same time they are registered to the treatment trial (A031501). Samples should be submitted per Section 6.1.

If a patient answers "yes" to "I choose to take part in the Quality of Life study and will fill out the forms," Question #1 in the model consent, they have consented to participate in the QOL substudy. The patient should be registered to Alliance A031501-HO1 at the same time they are registered to the treatment trial (A031501). Questionnaires should be submitted per Section 6.4.

4.7 Stratification (or Grouping) Factors and Treatment Assignments

- Neoadjuvant chemotherapy: yes vs. no
- Pathologic Stage:
  - pT2/3N0 or NX
  - pT4N0 or NX
  - pT-any N+ (any)
  - Positive invasive microscopic surgical margins (CIS at margins is considered negative margins)
- PD-L1 status: positive vs. negative (results will be sent from Q2 Solutions to the statistical center, sites will be blinded)

5.0 STUDY CALENDAR

The pre-study testing intervals are guidelines only. Laboratory and clinical parameters during treatment are to be followed using individual institutional guidelines and the best clinical judgment of the responsible physician. It is expected that patients on this study will be cared for by physicians experienced in the treatment and supportive care of patients on this trial.

Pre-Study Testing Intervals

- To be completed ≤ 7 days before pre-registration: Pregnancy test
- To be completed ≤ 28 DAYS before pre-registration: All laboratory studies, history and physical.
- To be completed ≤ 42 DAYS before pre-registration: Any X-ray or scan of any type which is utilized for tumor measurement per protocol
- To be completed ≤ 60 DAYS before registration: Any baseline exams used for screening, or any X-ray, or scan of any type of uninvolved organs which is not utilized for tumor measurement.

	Prior to Pre-Reg*	Prior to Reg*	Arm A Pembrolizumab: Day 1 each cycle	Arm B (Observation)**	Post treatment follow up***
Tests & Observations					
History & physical, weight, PS	X		X	X	X
Height	X				
Pulse, Blood Pressure	X		X	X	X
Adverse Event Assessment			X	X	X
Registration Fatigue/ Uniscale Assessment		X(2)			
Laboratory Studies[¥]					
Complete Blood Count, Differential, Platelets	X		X	PRN	
Serum Creatinine	X		X	q3 months for 1 yr	
Albumin, glucose	X		X	q3 months for 1 yr	
AST, ALT, Alk. Phos., Bili	X		X	q3 months for 1 yr	
Na, K, CO2, Cl, Cr, BUN, Ca	X		X	q3 months for 1 yr	
Lipase and Amylase	X		X	PRN	
Serum or Urine HCG	X(3)				
TSH w/ reflex T4			X		
PD-L1 Tissue Specimen		X(1)			
2 H&E slides		A			
Staging					
CT chest/abd/pelvis or CT chest and CT urography or MRI abd/pelvis and Chest	X(4)		B(4)	B(4)	B(4)

CT					
Urine cytology			C	C	C
Cystoscopy (upper tract patients)	D		D	D	
Correlative studies: For patients who consent to participate					
QOL assessment –QLQ-C30, QLQ-BLM30, EQ5D-5L (A031501-HO1)	*≤ 21 days prior to treatment, then 6 12 and 24 months after registration (See Section 6.4)*				
Whole blood sample (A031501-PP1)	*Prior to Treatment (preferred) or anytime during treatment (See Section 6.2)*				
Tissue Specimen (A031501-ST1)	*Prior to treatment (See Section 6.2)*				
Blood and Plasma samples (A031501-ST1)	*Prior to treatment, Day 1 of Cycle 5, 9, 13 and 17 (See Section 6.2)*				

* Labs completed prior to registration may be used for day 1 of cycle 1 tests if obtained ≤ 7 days prior to treatment. For subsequent cycles, labs, tests and observations may be obtained ≤ 48 hours prior to day of treatment.

¥ All tests should be performed prior to treatment. Tests performed on day 1 of cycle 1 should be recorded in the database as baseline. Tests prior to Cycle 2 and all subsequent cycles should be recorded in the database for the PREVIOUS cycle (i.e., day 1 cycle 2 should be recorded with cycle 1 in the database).

** Patients in the observation arm will be followed every 3 weeks +/- 7 days from registration with a phone-call made by the clinical team to discuss and document any new symptoms or issues. Patients will need to be seen every 6 weeks (this visit can be with an RN who will obtain vitals and a complete review of systems) +/- 7 days; the patient must be seen by an MD every 12 weeks +/- 14 days. This will mirror the treatment arm more closely without adding a travel burden to the patient. Labs and scans will be obtained every 12 weeks +/-1 14 days for 1 year. No further imaging is necessary once a patient has disease recurrence, the start of new anti-cancer treatment or withdrawl of consent. After 1 year patients will be followed according to the post-treatment follow-up schedule. Patients on the observation arm will have days counted using the definition of 1 cycle (1 cycle = 21 days), even though they are not receiving treatment.

*** An off-treatment assessment should be performed 21 days +/- 7 days from the last infusion (note: this may be extended for patients where treatment is held for toxicity). Once a patient ends therapy, tests and scans are required every 12 weeks +/- 14 days for 2 years following registration, then yearly +/- 28 days for years 3, 4 and 5 or until evidence of disease recurrence. Confirmatory scans should also be obtained (if biopsy is not feasible) at least 4-6 weeks following documentation of recurrence. If the new disease i is unequivocal on CT, MRI, FDG PET/CT or bone scan, a confirmatory scan is not needed. No further imaging is necessary once a patient has disease recurrence, the start of new anti-cancer treatment or withdrawal of consent. After progression, patients will be followed every 12 weeks +/- 14 days with a phone call for a total of 5 years from registration or until death.

1 Collect five (4-5 micron) unstained slides from the diagnostic TURBT/ureteroscopy or cystectomy/nephrectomy/ureterectomy for submission to Q2 Solutions for PD-L1 testing.

2 To be completed after registration and ≤ 21 days prior to treatment. See Section 1.6 and Appendix I.

3 For women of childbearing potential. Must be done ≤ 7 days prior to pre-registration.

4 CTs are to be performed with both IV and oral contrast (water may be used instead of omnipaque for oral contrast and patients may decline oral contrast but it is strongly encouraged) unless contraindicated, and the CT acquired with 5 mm or less slice thickness. MRI is to be performed with IV contrast. Supporting documentation is to be submitted. Modality of scans should be maintained consistent throughout the study. Imaging should follow calendar days and should not be adjusted for changes in treatment cycles.

A Two H&E slides from the diagnostic TURBT/ureteroscopy biopsy or cystectomy/nephrectomy/ureterectomy will be submitted prior to protocol treatment for histopathology review.

B Restaging scans are to be performed every 12 weeks (+/- 14 days) for 2 years following registration, then yearly (+/- 28 days) for years 3, 4 and 5 or until evidence of disease recurrence. Confirmatory scans for recurrence should also be obtained (if biopsy is not feasible) 4-6 weeks following documentation of recurrence. No further imaging is necessary once a patient has disease recurrence, the start of new anti-cancer treatment or withdrawal of consent.

C Urine cytology is to be performed every 12 weeks for patients with uppertract urothelial carcinoma and every 24 weeks for patients with bladder urothelial carcinoma for 2 years following registration. Urine cytology for both bladder and uppertract urothelial carcinoma will be performed yearly for years 3, 4 and 5 or until evidence of disease recurrence. Urine cytology may be done up to 14 days prior to the required timepoint.

D For patients with upper tract disease and native bladder in place a cystoscopy is required at baseline (within 3 months prior to registration) and then every 6 months (+/- 30 days) for 2 years, then annually for an additional 3 years (for a total of 5 years of follow-up or until disease progression).

6.0 DATA AND SPECIMEN SUBMISSION

6.1 Data Collection and Submission

6.1.1 Data Submission Schedule

A Schedule of Forms is available on the Alliance study webpage, within the Case Report Forms section. The Schedule of Forms is also available on the CTSU site within the study-specific Education and Promotion folder, and is named Time & Events.

6.1.2 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status, and timeliness reports. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website. Staff who have Rave study access can access the Rave study data via direct links available in the DQP modules.

CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms for each Rave study that a site

is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members' website.

To learn more about DQP use and access, click on the Help Topics button displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

6.1.3 Data Submission/Data Reporting

Medidata Rave is the clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems;
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

RAVE role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR); and
- Rave Read Only or Rave SLA must have an Associates (A) registration type.

Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

This study has a Delegation of Tasks Log (DTL). Therefore, those requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in the Regulatory application, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management > Rave Home* and click to *accept* the invitation in the *Tasks* pane located in the upper right corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study-specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

Site staff who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the *Data Management* section under the Rave resource materials (*Medidata Account Activation and Study Invitation Acceptance*). Additional information on iMedidata/Rave is available on the CTSU members' website in the *Data Management > Rave* section or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

6.2 Specimen Collection and Submission Overview

Required: There are 2 tissue submissions **required** for all patients:

1) Sites will submit tissue (5 unstained slides) to Q2 Solutions for PD-L1 testing. **The submission of these samples to Q2 Solutions for central testing is required for all patients pre-registered to this study for stratification purposes.** PD-L1 results will be blinded to the physicians and patients. The results will be sent from Q2 to the Alliance registration/randomization system and an e-mail will be generated to notify sites that there is a PD-L1 result available. **Sites should expect this e-mail for patient registration in 5-7 business days**

   and

2) Sites will submit two H&E slides to the Mayo Biorepository for histopathologic review.

Optional: For patients registered to the **optional** correlative substudies:

All participating sites must ask patients for their consent to participate in the correlative substudies A031501-ST1 and A031501-PP1, although patient participation is optional. **Kits for these studies can be requested using BioMS.** Biomarker and pharmacogenetic studies will be performed. Rationale and methods for the scientific components of these studies are described in Sections 14.1 and 14.2. Tissue and blood will be collected at the following time points for all studies (both required and optional):

	After Pre-reg	**After Consent. Before First Treatment**	**Day 1 of Cycles 5, 9, 13 and 17****	**Storage/ Shipping conditions**	**Submit to:**	**Kits available***
Mandatory for <u>all</u> patients pre-registered to A031501 (see Sections 6.3.1 and 6.3.2):						
Slides for Central PD-L1 Testing	X[1]			Cool pack/ship over night	Q2 Solutions	Yes (order from Q2)
2 H&E slides from diagnostic TURBT/ ureteroscopy biopsy or cystectomy/nephrectomy/ureterectomy		X		Cool pack/ship over night	AB Mayo	No
For patients registered to the optional substudy A031501-ST1, submit the following (see Section 6.3.3):						
	Number and volume of tubes to draw					
FFPE tumor[2]		X			AB Mayo	No
Whole Blood (PAXgene DNA tube)		1 x 8.5 mL	1 x 8.5 mL	Dry ice/ship over night	AB Mayo	Yes (order from BioMS)

Whole Blood (Paxgene RNA tube)		2 x 2.5 mL	2 x 2.5 mL	Dry ice/ship over night	AB Mayo	Yes (order from BioMS)
Peripheral Blood Plasma (Lavender top EDTA tube)		1 x 10 mL	1 x 10 mL	Dry ice/ship over night	AB Mayo	Yes (order from BioMS)
For patients registered to the optional substudy A031501-PP1, submit the following (see Section 6.3.3):						
Whole Blood[3] (Lavender top EDTA tube)		1 x 10 mL		Cool pack/ship over night	AB Mayo	Yes (order from BioMS)

* Collection/submission kits for the required PD-L1 testing will be ordered from Q2 solutions using the kit ordering form on the A031501 study page. Collection/submission kits for the optional blood substudies can be ordered through BioMS.

** Blood will continue to be collected in patients who discontinue treatment due to disease progression or other reasons. For patients not receiving treatment these collections would align with the MD visits in cycles 4, 8, 12 and 16.

1 Five (4-5 micron, charged) unstained slides from the diagnostic TURBT/ureteroscopy biopsy or cystectomy/nephrectomy/ureterectomy surgery are required for central testing to determine PD-L1 status, which will be used for stratification purposes. Slides will be submitted to Q2 Solutions after the patient is pre-registered.

2 1-2 blocks or (20) 10 micron unstained slides (or as many as possible, up to 20, no coverslip) from the diagnostic TURBT/ ureteroscopy biopsy and/or cystectomy/nephrectomy/ ureterectomy surgery (preferred) will be submitted to the Alliance Biorepository at Mayo Clinic.

3 Whole blood to be used for pharmacogenomic analyses. This tube of blood should be collected prior to the initiation of treatment but can be collected at any time while a patient is on study.

6.3 Specimen Submission using the Alliance Biospecimen Management System

USE OF THE ALLIANCE BIOSPECIMEN MANAGEMENT SYSTEM (BioMS) IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: http://bioms.allianceforclinicaltrialsinoncology.org using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS webpage to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or Bioms@email.wustl.edu.

After logging collected specimens in BioMS, the system will create a shipping manifest. This shipping manifest must be printed and placed in the shipment container with the specimens.

All submitted non-tissue specimens must be labeled with the protocol number (A031501), Alliance patient number, patient's initials, date and type of specimen collected (e.g., serum, whole blood). There are different labeling requirements for tissue submission. Please see the sections below for more information.

A copy of the Shipment Packing Slip produced by BioMS must be printed and placed in the shipment with the specimens. Please also include a hard copy of the pathology report.

Instructions for the collection of samples are included below. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary.

Ship specimens on Monday through Thursday only. Shipping by overnight service to assure receipt is encouraged. Do not ship specimens on Fridays or Saturdays.

6.3.1 Required PD-L1 Tissue Submission

A kit (including positively charge slides) is provided by Q2 Solutions and will be ordered from Q2. **Please note, the PD-L1 testing kit is ordered from Q2 Solutions, not BioMS. Complete the kit ordering form on the A031501 study page and e-mail to Q2 to request an initial supply of kits.**

Ship the five unstained slides from the resected tumor surgery specimens (preferred) and/or the diagnostic biopsies for PD-L1 testing to:

Q2 Solutions
27027 Tourney Road, Suite 2E
Valencia, CA 91355
1-800-877-7004
ctcrc@q2labsolutions.com

The submission of slides to Q2 must be logged into BioMS. Slides must be submitted to Q2 solutions **within 60 days** of cutting by the site. Q2 Solutions has provided manuals for collection, preparation and shipping, which can be found on the Alliance and CTSU A031501 study pages.

A de-identified surgical pathology report should be sent with the specimens. Usually, this is generated by obscuring all PHI (names and dates) with white-out or a black magic marker, labeling each page of the report with the Alliance patient ID, and photocopying the report. The surgical pathology case number and block identifier should be maintained on the report so that it can be matched with the physical labeling on the slides.

Ship specimens with Priority Overnight Monday through Friday for next day delivery. Do not send specimens on Saturday or the day before a holiday. When shipping tissues, it is important to avoid extreme heat. Please see the *A031501 PD-L1 tissue collection procedure* for detailed instructions for shipment to Q2. **Q2 Solutions will send the PD-L1 results to the Alliance registration/randomization office within 5-7 business days of receipt. Sites will be notified by the Alliance registration/randomization office when the results have been entered.**

Inadequate Submission/Results

Sites that have an inadequate submission will be contacted by Q2 Solutions and asked to submit additional specimens.

PD-L1 Device Description

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an IHC staining procedure for urothelial carcinoma specimens using Dako Autostainer Link 48. Wash buffer and hematoxylin are required but not included in the kit. Deparaffinization is performed in the PT Link. Coverslipping can be manual or automated, so capabilities for this are required, but not supplied. The kit contains the reagents necessary to perform 50 tests in up to 15 individual runs. An overview of the kit components is shown in Table 1 below.

Following incubation with the primary monoclonal antibody to PD-L1 or the Negative Control Reagent, specimens are incubated with a linker antibody specific to the host species of the primary antibody, and then are incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added chromogen results in precipitation of a visible reaction product at the site of antigen.

The color of the chromogenic reaction is modified by a chromogen enhancement reagent. The specimen may then be counterstained and coverslipped.

Table 1: Overview of PD-L1 IHC 22C3 pharmDx Components

Reagent	Description	Qty x Vol
Peroxidase-Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015mol/L sodium azide.	1 x 34.5mL
Monoclonal Mouse anti–PDL1, Clone 22C3	Monoclonal mouse anti-PD-L1 antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.	1 x 19.5mL
Negative Control Reagent	Monoclonal mouse control IgG antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.	1 x 15mL
Linker, Anti-Mouse	Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015mol/L sodium azide. 0.015mol/L sodium azide.	1x 34.5mL
Visualization Reagent-HRP	Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins in a buffered solution containing stabilizing protein and an antimicrobial agent.	1 x 34.5mL
DAB+ Buffered Substrate	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.	15 x 7.2mL
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride in an organic solvent.	1 x 5mL
DAB Enhancer	Cupric sulfate in water.	1 x 34.5mL
Target Retrieval Solution Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.	6 x 30mL
Cell Line Control Slides	Each slide contains sections of two pelleted, formalin-fixed paraffin embedded cell lines: NCI-H226 with moderate PD-L1 protein expression, and MCF-7 with negative PD-L1 protein expression.	3 x 5 slides

6.3.2 Required Tissue Submission for Histopathology Review

Histopathology review is required for all patient registered to this study. Submit 2 H&E slides from the surgical specimen (preferred) and/or diagnostic TURBT/ ureteroscopy biopsy for central histopathology review. While institutional staging will be used to determine patient eligibility, a retrospective review of the histologic slides used for diagnosis of muscle-invasive urothelial carcinoma of the bladder or upper tract bladder on these patients will be performed. Central pathology review is not required prior to registration.

H&E Slides should be labeled with the Alliance patient study ID number, specimen surgical pathology number and block number either via your institution's standard method for labeling clinical slides or using a permanent marker. **Labeling with sticky labels on slides are not acceptable**

A de-identified surgical pathology report should be sent with all blocks. Usually, this is generated by obscuring all PHI (names and dates) with white-out or a black magic marker,

labeling each page of the report with the Alliance patient ID, and photocopying the report. The surgical pathology case number and block identifier should be maintained on the report so that is can be matched with the physical labeling on the slides.

Ship tissue samples to the following address:

Alliance Biorepository at Mayo Clinic
Attn: PC Office (Study A031501)
RO-FF-03-24-CC/NW Clinic
200 First Street Southwest
Rochester, MN 55905
Tel: 507-284-3559
Email: sand.amanda@mayo.edu

Ship specimens with Priority Overnight on Monday through Thursday for next day delivery. Do not send specimens on Saturday or the day before a holiday. When shipping tissues, it is important to avoid extreme heat. If environmental conditions indicate, blocks may be shipped in containers containing cold packs. It is also important that blocks are shipped in appropriately padded and secure containers to avoid physical damage.

6.3.3 Optional Tissue Submission (A031501-STI)

Tissue for ST1 : For patients who answer "yes" to consent question #2, submit one block OR (20) 10 micron unstained slides (no coverslip) from the surgical specimen (preferred) and/or diagnostic TURBT/ ureteroscopy biopsy to the Alliance Biorepository at Mayo.

Blocks should be labeled with the following information: 1) Maintained Pathology accession number 2) Alliance patient ID (legible on the side of the block). All other information, include the procurement date/time, patient initials, Alliance study number and specimen type should be clearly noted/visible on the corresponding pathology report.

All other information including the procurement date/time, patient initials, Alliance study number, specimen type should be clearly noted/visible on the corresponding pathology report. All slides should be labeled with specimen surgical pathology number and block number either via your institution's standard method for labeling clinical slides or using a permanent marker. **Labeling with sticky labels on slides are not acceptable**. A de-identified surgical pathology report should be sent with all blocks. Usually, this is generated by obscuring all PHI (names and dates) with white-out or a black magic marker, labeling each page of the report with the Alliance patient ID, and photocopying the report. The surgical pathology case number and block identifier should be maintained on the report so that is can be matched with the physical labeling on the slides.

When shipping FFPE tumor tissues, it is important to avoid extreme heat. If environmental conditions indicate, blocks may be shipped in containers containing cold packs. It is also important that blocks are shipped in appropriately padded and secure containers to avoid physical damage. All blocks will be stored by the appropriate Alliance biorepository but will be returned within 30 days of a written request.

Ship tissue samples to:

Alliance Biorepository at Mayo Clinic
Attn: PC Office (Study A031501)
RO-FF-03-24-CC/NW Clinic
200 First Street Southwest

Rochester, MN 55905

Tel: 507-284-3559

Email: sand.amanda@mayo.edu

Ship specimens with Priority Overnight on Monday through Thursday for next day delivery. Do not send specimens on Saturday or the day before a holiday. When shipping tissues, it is important to avoid extreme heat. If environmental conditions indicate, blocks may be shipped in containers containing cold packs. It is also important that blocks are shipped in appropriately padded and secure containers to avoid physical damage.

6.3.4 Optional Blood Submission (A031501-STI and A031501-PP1)

Blood collection kits for the optional substudies will be ordered through BioMS.

Blood for ST1 and PP1: For patients who answer "yes" to consent question #2 submit the following:

Whole blood in PAXgene DNA tubes (for Germline DNA, Section 14.1)

Collect approximately 8.5 mL peripheral venous blood into one 8.5 mL PAXgene DNA tube. Gently invert the tube 8-10 times, let stand upright at room temperature for 2-72 hours, then transfer to -20º degree Celsius freezer. After 24 hours at -20º degree Celsius freezer, tubes can be transferred to a -80º Celsius or colder freezer. Once frozen, the PAXgene tubes should be shipped within 30 days of collection on dry ice by overnight express courier. If a -80º C or colder freezer is not available, temporary storage at -20º C prior to shipment is acceptable for up to 72 hours.

Whole blood in PAXgene RNA tubes (for PBMC RNA, Section 14.1)

Collect approximately 2.5 mL peripheral venous blood into each of two 2.5 mL PAXgene Blood RNA tubes. Gently invert the tube 8-10 times, let stand upright at room temperature for 2-72 hours, then transfer to -20º degree Celsius freezer. After 24 hours at -20º degree Celsius freezer, tubes can be transferred to a -80º Celsius or colder freezer. Once frozen, the PAXgene tubes should be shipped within 30 days of collection on dry ice by overnight express courier. If a -80º C or colder freezer is not available, temporary storage at -20º C prior to shipment is acceptable for up to 72 hours.

Peripheral Blood Plasma (for Peripheral Blood Plasma, Section 14.1)

Collect whole blood into one 10 mL lavender top (EDTA) tube. Keep tube at 4º C until blood is ready to be processed. Within 2 hours of blood collection, spin at 1300 RCF (RPM vary per centrifuge) for 10 minutes at room temperature; do not brake. This will yield plasma above a thin buffy coat with RBCs at the bottom. Remove plasma from the top without removing buffy coat and aliquot evenly (~1.0 mL) into three or four 2 mL cryovials. Label cryovials and store plasma at 4º C for a maximum of 2 hours before storage at -80º C or freeze immediately at -80ºC. Once frozen, ship frozen specimens within 30 days of collection on dry ice by overnight express courier to Alliance Biorepository at Mayo per Section 6.2.1.

Pharmacogenomic Whole Blood Sample Submission

For patients who consent to participate, whole blood samples will be used for the pharmacogenomic studies. It is preferable that this sample be collected prior to the initiation of protocol treatment, but it may be collected at any time while a patient is on protocol therapy.

Collect 10 mL of peripheral venous blood in an EDTA (lavender) tube. The tubes should be inverted several times to mix the EDTA and refrigerated until shipped. The sample should be placed in a biohazard bag and shipped the same day as the blood is drawn on

cool pack by overnight courier service to the Alliance Biorepository per Section 6.2.1. **Please do NOT freeze this whole blood sample.**

Label all samples with the following identification:

1) Procurement date and time
2) Alliance patient study ID number
3) Patient initials
4) Alliance study number (A031501-ST1 or A031501-PP1)
5) Specimen Type (Whole Blood, PBMC, etc.)

Ship blood samples to the following address:

Alliance Biorepository at Mayo Clinic

BAP Freezer

ST-SL-16

150 Third Street SW

Rochester, MN 55902

For questions about blood submission contact: ncctgpathology@mayo.edu.

Ship specimens with Priority Overnight on Monday through Thursday for next day delivery. Do not send specimens on Saturday or the day before a holiday.

6.4 Quality of Life Evaluation (for A031501-HO1, Section 14.3)

All participating institutions must ask patients for their consent to participate in the correlative study planned for Alliance A031501-HO1, although patient participation is optional. For patients registered to correlative study A031501-HO1 (model consent question, "I choose to take part in the Quality of Life study and will fill out these forms") the quality of life evaluation studies will be performed. For patients who consent to participate the EORTC QLQ C30, EORTC QLQ-BLM 30 and EQ5D-5L modules will be completed at the following time points (quality of life evaluations should continue in patients who have experienced disease progression, it may be mailed or e-mailed to patients who do not have a planned clinic visit at the specified QOL timepoint):

	≤ 21 days prior to treatment	**6 months after registration (+/- 14 days)**	**12 months after registration (+/- 21 days)**	**24 months after registration (+/- 28 days)**
For patients registered to A031501-HO1, submit the following:				
EORTC QLQ-C30	X	X	X	X
EORTC QLQ-BLM30	X	X	X	X
EQ5D-5L	X	X	X	X

* See Appendix II for EORTC QLQ-C30, QLQ-BLM30 and EQ5D-5L questionnaires for IRB submission and review only. Patients must complete QOL in booklet format. See Section 4.5 for ordering instructions.

6.5 Submission Instructions for Banking of Images

Collection of all CT and/or MRI images is required. Images will be collected digitally for archival and retrospective purposes. Images will be collected digitally at the following time points:

- **Baseline (within 42 days prior to patient pre-registration)**
- **Every 12 weeks for years 1 and 2**
- **Yearly for years 3, 4 and 5**
- **At progression: Confirmatory scan obtained within 4 weeks following progression**
- **Any scan performed outside the schedule documenting disease recurrence**

The complete CT and MRI scan data in digital **DICOM** format will be submitted electronically to the Imaging and Radiation Oncology Core at Ohio (IROC Ohio) within no more than **5 business days** upon patient enrollment (at baseline) or upon the image acquisition completeness (at follow-up visits). BMP files, JPG files, or hard copies (films) are not acceptable. The raw data of the entire study should be saved until the imaging data is accepted by IROC Ohio.

Sites need to de-identify the patient data using institutional procedures to remove patient name and medical record number while preserving the Alliance patient ID number (e.g., 112136) and protocol number (e.g., A031501), respectively.

Imaging data should be submitted **electronically** to IROC Ohio via TRIAD, Web transfer or FTP transfer:

6.5.1 Digital Radiation Therapy Data Submission Using Transfer of Images and Data

Transfer of Images and Data (TRIAD) is the American College of Radiology's (ACR) image exchange application. TRIAD provides sites participating in clinical trials a secure method to transmit images. TRIAD anonymizes and validates the images as they are transferred.

TRIAD Access Requirements

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems;
- Registration type of: Associate (A), Associate Plus (AP), Non-Physician Investigator (NPIVR), or Investigator (IVR). Refer to the CTEP Registration Procedures section for instructions on how to request a CTEP-IAM account and complete registration in RCR; and
- TRIAD Site User role on an NCTN or ETCTN roster.

All individuals on the Imaging and Radiation Oncology Core provider roster have access to TRIAD and may submit images for credentialing purposes, or for enrollments to which the provider is linked in OPEN.

TRIAD Installation:

To submit images, the individual holding the TRIAD Site User role will need to install the TRIAD application on their workstation. TRIAD installation documentation is available at https://triadinstall.acr.org/triadclient/.

This process can be done in parallel to obtaining your CTEP-IAM account and RCR registration.

For questions, contact TRIAD Technical Support staff via email TRIAD-Support@acr.org or 1-703-390-9858.

6.5.2 Web transfer http://upload.imagingcorelab.com)

Any PCs with internet access and web browser (e.g., Internet Explorer, Mozilla Firefox) can be used to web transfer DICOM images and other required files to IROC Ohio. The standard Web Transfer information will be provided separately through the specific trial e-

mail (alliance031501@irocohio.org), per the request by participating sites before their first data submission.

6.5.3 FTP Transfer

Any FTP software can be used to initiate access to the secure FTP Server of IROC Ohio. The standard FTP access information will be provided separately through the specific trial e-mail (alliance031501@irocohio.com), per the request by participating sites before their first data submission.

6.5.4 Mail/CD Shipment

Only if electronic data transfer approaches cannot be achieved at sites, the de-identified images in digital DICOM format can be burned to a CD and mailed to IROC Ohio. Submit only one patient's images per CD, and include the Alliance patient ID number (e.g., 112136), study type, date of scans and name of submitting institution. Submit this to:

IROC Ohio
Attn: Alliance A031501
The Ohio State University
395 W. 12th Ave, Rm #428
Columbus, Ohio, 43210
Phone: (614) 293-2929
Fax: (614) 293-9275

Once the imaging data submission is completed send an e-mail notification to IROC Ohio at the specific trial e-mail **alliance031501@irocohio.org** to notify IROC Ohio that the imaging has been submitted. IROC Ohio will notify the site and the Alliance A031501 imaging committee within **2 business days** of the data receipt. A quality check report with be sent **within 3 business** days following the data receipt.

Any questions or problems regarding the image submission to IROC Ohio should be directed to the IROC Ohio IT group at 614-293-2630 or 614-293-2929.

7.0 TREATMENT PLAN/INTERVENTION

7.1 Treatment Logistics

Protocol treatment is to begin ≤ 14 days of registration. For questions regarding treatment, please see the study contacts page. All trial treatments will be administered on an outpatient basis.

It is acceptable for treatment doses to be delivered up to 48-hours before and after the protocol-defined date for Day 1 of a new cycle. For example, if the treatment due date is a Friday, the window for treatment includes the preceding Wednesday through the following Tuesday. In addition, patients are permitted to have a new cycle of chemotherapy delayed up to 7 days for major life events (e.g., serious illness in a family member, major holiday, vacation that cannot be rescheduled) without this being considered a protocol violation. Documentation to justify this delay should be provided.

7.2 Treatment

This is a randomized trial. Patients will be randomized to pembrolizumab vs. observation.

Patients randomized to Arm A will receive treatment with pembrolizumab 200 mg as a 30 minute IV infusion on day 1 every 21 days on an outpatient basis. Infusion timing should be as close to 30 minutes as possible; however, a window of -5 minutes and +10 minutes is permitted (*i.e.*, infusion time is 25-40 minutes). Treatment will continue until metastatic recurrence or unacceptable adverse event for a maximum period of 18 cycles. Please refer to Section 10.1 for pembrolizumab compatible infusion set materials, including in-line filter.

Patients randomized to Arm B, Observation, will be followed per the schedule found in Section 5.0.

8.0 DOSE AND TREATMENT MODIFICATIONS

8.1 Ancillary therapy, concomitant medications, and supportive care

8.1.1 Patients should not receive any other agent which would be considered treatment for the primary neoplasm or impact the primary endpoint.

8.1.2 Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

8.1.3 Treatment with hormones or other chemotherapeutic agents may not be administered except for steroids given for adrenal failure; hormones administered for non-disease-related conditions (e.g., insulin for diabetes).

8.1.4 Antiemetics may be used at the discretion of the attending physician, with the exception of steroids above.

8.1.5 Alliance Policy Concerning the Use of Growth Factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the American Society of Clinical Oncology 2006 Update of Recommendations for the Use of White Blood Cell Growth Factors: An Evidence-Based, Clinical Practice Guideline. J Clin Oncol 24(19): 3187-3205, 2006.

Epoetin (EPO): Use of epoetin in this protocol is permitted at the discretion of the treating physician.

Filgrastim (G-CSF) tbo-filgrastim, and sargramostim (GM-CSF):

- Use of Filgrastim (G-CSF)/pegfilgrastim, tbo-filgrastim and sargramostim (GM-CSF) is prohibited.

8.2 Dose Management and Supportive Care

- If study drug is held for >12 consecutive weeks, pembrolizumab will be discontinued (with the exception if the drug is held for treatment of local recurrence).
- Any patients with autoimmune toxicity who require additional immune suppressive treatment beyond steroids should go off treatment.
- Patients may be dose-delayed for toxicity evaluation and restarted depending on results.
- Any patient started on corticosteroids initially for a presumed autoimmune adverse event, and subsequently found to not have an autoimmune etiology of their adverse event, may resume therapy after a 2 week observation period without recurrence of symptoms.
- Study drug should not be restarted until steroids have been tapered down to 10mg of prednisone equivalent.
- No dose reductions will be made during this study, only dose delays. Therefore there is only 1 dose level: 200 mg of pembrolizumab.

8.2.1 Dose Management Guidelines for Pembrolizumab

Adverse events (both nonserious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as described in Section 8.2.1.

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (*e.g.*, elective surgery, unrelated medical events, patient vacation, and/or holidays). Patients should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines for irAEs and infusion reactions associated with pembrolizumab are provided in the table below.

Note that non-irAEs will be managed as appropriate, following clinical practice recommendations.

Table Dose Modification and Toxicity Management Guidelines for Immune-related AEs and Infusion Reactions Associated with Pembrolizumab

General instructions:

1. For non-endocrine-related severe and life-threatening irAEs, investigators should consider the use of IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids. Some non-endocrine irAEs do not require steroids. For example, celiac disease induced by pembrolizumab can be controlled by diet alone.
2. For non-endocrine-related toxicities, pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last pembrolizumab-treatment.
3. Generally, when corticosteroids are used, investigators should begin a taper when the irAE is ≤Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld due to a non-endocrine irAE, pembrolizumab may generally resume after the irAE has decreased to ≤Grade 1 after a corticosteroid taper.

irAEs	**Toxicity grade (CTCAE V5.0)**	**Action with pembrolizumab**	**Corticosteroid and/or other therapies**	**Monitoring and follow-up**
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Add prophylactic antibiotics for opportunistic infections	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or	Monitor participants for signs and symptoms of enterocolitis (*i.e.*, diarrhea, abdominal pain, blood or

	Recurrent Grade 3 or Grade 4	Permanently discontinue	equivalent) followed by taper Patients who do not respond to corticosteroids should be seen by a gastroenterologist for confirmation of the diagnosis and consideration of secondary immune suppression	mucus in stool with or without fever) and of bowel perforation (*i.e.* peritoneal signs and ileus) Specifically assess for celiac disease serologically, and exclude *Clostridium difficile* infection Participants with ≥Grade 2 diarrhea suspecting enterocolitis should consider GI consultation and performing endoscopy to rule out enterocolitis and assess mucosal severity Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Grade 1 or 2	Continue		Investigate for diabetes. In the absence of corticosteroids or diabetes medication non-adherence, any grade hyperglycemia may be an indication of beta-cell destruction and pembrolizumab-induced diabetes akin to type 1 diabetes. This should be treated as a Grade 3 event. Given this risk, exercise caution in utilizing non-insulin hypoglycemic agents in this setting. After a thorough investigation of other potential causes, which may involve a referral to an endocrinologist, follow institutional guidelines. Monitor glucose control.
	New onset T1DM (evidence of β-cell failure) or Grade 3 or 4 hyperglycemia	Withhold [d] Resume pembrolizumab when symptoms resolve and glucose levels are stable	Initiate treatment with insulin If patient is found to have diabetic ketoacidosis or hyperglycemic hyperosmolar syndrome, treat as per institutional guidelines with appropriate management and laboratory values (*e.g*. anion gap, ketones, blood pH, *etc*.) reported	Monitor for glucose control Strongly consider referral to endocrinologist Obtain C-peptide level paired with glucose, autoantibody levels (*e.g*. GAD65, islet cell autoantibodies), and hemoglobin A1C level
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and	Monitor for signs and symptoms of

	Grade 3 or 4	Withhold or permanently discontinue [d]	initiate hormonal replacements as clinically indicated	hypophysitis (including hypopituitarism and adrenal insufficiency) Provide adrenal insufficiency precautions including indications for stress dose steroids and medical alert jewelry Strongly consider referral to endocrinologist
Hyperthyroidism	Grade 2	Consider withholding. Resume pembrolizumab when symptoms are controlled, and thyroid function is improving	Treat with nonselective beta-blockers (*e.g.*, propranolol) or thionamides as appropriate Initiate treatment with anti-thyroid drug such as methimazole or carbimazole as needed	Monitor for signs and symptoms of thyroid disorders Strongly consider referral to endocrinologist
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	Initiate thyroid replacement hormones (*e.g.*, levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function Strongly consider referral to nephrologist
	Grade 3 or 4	Permanently discontinue		

Cardiac Events (including myocarditis, pericarditis, arrhythmias, impaired ventricular function, vasculitis)	Asymptomatic cardiac enzyme elevation with clinical suspicion of myocarditis (previously CTCAE v4.0 Grade 1), or Grade 1	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes Strongly consider referral to cardiologist and cardiac MRI Consider endomyocardial biopsy If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month
	Grade 2, 3 or 4	Permanently discontinue	Initiate treatment with corticosteroids equivalent to 1-2 mg/kg/day IV methylprednisolone and convert to 1-2 mg/kg/day oral prednisone or equivalent upon improvement If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent Initiate treatment per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, extracorporeal membrane oxygenation (ECMO), ventricular assist device (VAD), or pericardiocentesis as appropriate	Ensure adequate evaluation to confirm etiology and/or exclude other causes Strongly consider referral to cardiologist and cardiac MRI Consider endomyocardial biopsy If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month

Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes Strongly consider referral to dermatologist Consider skin biopsy for evaluation of etiology
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

Infusion-Related Reactions

Infusion Reactions	**NCI CTCAE Grade**	**Treatment**	**Premedication at subsequent dosing**
Mild reaction; infusion interruption not indicated; intervention not indicated	Grade 1	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None

Infusion Reactions	NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Requires therapy or infusion interruption but responds promptly to symptomatic treatment (*e.g.*, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs.	Grade 2	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (*e.g.* from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of study intervention with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of analgesic).

Infusion Reactions	NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Prolonged (*i.e.*, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Grade 3	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids (*e.g.* methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours) • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing.
Life-threatening; pressor or ventilator support indicated	Grade 4	Admit participant to intensive care unit (ICU) and initiate hemodynamic monitoring, mechanical ventilation, and/or IV fluids and vasopressors as needed. Monitor other organ function closely. Manage constitutional symptoms and organ toxicities as per institutional practice. Follow Grade 3 recommendations as applicable.	No subsequent dosing.

Infusion Reactions	NCI CTCAE Grade	Treatment	Premedication at subsequent dosing

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; ECMO=extracorporeal membrane oxygenation; GI=gastrointestinal; ICU=intensive care unit; IO=immuno-oncology; ir=immune related; IV=intravenous; MRI=magnetic resonance imaging; PO=per os; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal; VAD=ventricular assist device.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to 5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include but are not limited to: encephalitis and other clinically important irAEs (*e.g*. vasculitis and sclerosing cholangitis).

Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov.

Neurological Toxicities

Event	Management
Immune-mediated neuropathy, Grade 1	• Continue pembrolizumab. • Investigate etiology. • Any cranial nerve disorder (including facial paresis) should be managed as per Grade 2 management guidelines below.

Immune-mediated neuropathy, including facial paresis, Grade 2	• Withhold pembrolizumab for up to 12 weeks after event onset. [a]
	• Investigate etiology and refer patient to neurologist.
	• Initiate treatment as per institutional guidelines.
	• For general immune-mediated neuropathy:
	  ○ If event resolves to Grade 1 or better, resume pembrolizumab. [b]
	  ○ If event does not resolve to Grade 1 or better while withholding pembrolizumab, permanently discontinue pembrolizumab. [c]
	• For facial paresis:
	  ○ If event resolves fully, resume pembrolizumab. [b]
	  ○ If event does not resolve fully while withholding pembrolizumab, permanently discontinue pembrolizumab. [c]

Immune-mediated neuropathy, including facial paresis, Grade 3 or 4	• Permanently discontinue pembrolizumab. [c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines.

Myasthenia gravis and Guillain-Barré syndrome (any grade)	• Permanently discontinue pembrolizumab. [c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines. • Consider initiation of corticosteroids equivalent to 1–2 mg/kg/day oral or IV prednisone.

[a] Pembrolizumab may be withheld for a longer period of time (*i.e.*, >12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be based on an assessment of benefit–risk by the investigator and in alignment with the protocol requirements for the duration of treatment and documented by the investigator.
[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before pembrolizumab can be resumed.
[c] Resumption of pembrolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-mediated event. The decision to re-challenge patients with pembrolizumab should be based on investigator's assessment of benefit–risk and documented by the investigator (or an appropriate delegate).

Event	Management
Immune-mediated myelitis, Grade 1	• Continue pembrolizumab unless symptoms worsen or do not improve. • Investigate etiology and refer patient to a neurologist.
Immune-mediated myelitis, Grade 2	• Permanently discontinue pembrolizumab. • Investigate etiology and refer patient to a neurologist. • Rule out infection. • Initiate treatment with corticosteroids equivalent to 1-2 mg/kg/day oral prednisone.
Immune-mediated myelitis, Grade 3 or 4	• Permanently discontinue pembrolizumab. • Refer patient to a neurologist. • Initiate treatment as per institutional guidelines.

Event	Management
Immune-mediated meningoencephalitis, all grades	• Permanently discontinue pembrolizumab. [a] • Refer patient to neurologist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

[a] Resumption of pembrolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-mediated event. The decision to re-challenge patients with pembrolizumab should be based on investigator's assessment of benefit–risk and documented by the investigator (or an appropriate delegate).

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

8.2.2 Supportive Care

Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are also outlined in the table in Section 8.2.1. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below).

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of the evaluation of the event.

- **Pneumonitis:**
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - **For Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis:**

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For Grade 2 diarrhea/colitis that persists greater than 3 days, administer oral corticosteroids.
  - For Grade 3 or 4 diarrhea/colitis that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

- **For T1DM or Grade 3-4 Hyperglycemia**
  - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
  - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis:**
  - For Grade 2 events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For Grade 3-4 events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism:**

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
  - **Grade 2 hyperthyroidism events (and Grade 2-4 hypothyroidism):**
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4 hyperthyroidism**
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic:**
  - **For Grade 2 events**, monitor liver function tests more frequently until returned to baseline values (consider weekly).
  - Treat with IV or oral corticosteroids
  - **For Grade 3-4 events**, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis with relationship to study therapy:**
  - For Grade 2 events, treat with corticosteroids.
  - For Grade 3-4 events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions:** Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  The table below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

9.0 ADVERSE EVENTS

The prompt reporting of adverse events is the responsibility of each investigator engaged in clinical research, as required by Federal Regulations. Adverse events must be described and graded using the terminology and grading categories defined in the NCI's Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0. The CTCAE is available at ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. Attribution to protocol treatment for each adverse event must be determined by the investigator and reported on the required

forms. Please refer the NCI Guidelines: Adverse Event Reporting Requirements for further details on AE reporting procedures.

9.1 Routine adverse event reporting

Adverse event data collection and reporting, which are required as part of every clinical trial are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times according to the study calendar in Section 5.0. For this trial, the Form "Adverse Events" will be used for routine AE reporting in Rave.

This trial will be utilizing the Rave-CTEP-AERS integration. All routine post-baseline adverse events reported in Rave will be evaluated for expedited reporting using protocol-specific rules. If an expedited report is recommended, a deep link within Rave can be used to automatically launch and log into CTEP-AERS to complete the expedited report. Adverse event data in Rave that is required for expedited reporting will be pushed to CTEP-AERS.

Note: Modifications to AE data must begin in Rave since it is the data source. Any Adverse Event data modified in Rave must be re-submitted for rule evaluation and to push to CTEP-AERs.

9.1.1 Rave-CTEP-AERS integration

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) Integration enables evaluation of Adverse Events (AE) entered in Rave to determine whether they require expedited reporting and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting**Sites must initiate all AEs for this study in Medidata Rave**.

Pre-existing medical conditions (formerly referred to as baseline AEs) identified during baseline assessment are not considered AEs and therefore should not be reported on the Pre-treatment Adverse Event form. If these pre-existing conditions worsen in severity, the investigator must reassess the event to determine if an expedited report is required. Whether or not an expedited report is required, the worsened condition should be reported in Rave as a routine AE.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 days after the last administration of the investigational study agent/intervention are collected using the Late Adverse Event form.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct; and
- AEs are recorded and complete (no missing fields) and the form is query

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via

a direct link on the Medidata Rave Expedited Reporting Evaluation form. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

In the rare occurrence, that Internet connectivity is lost; a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU website:

- Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting;* and
- Additional resources: *Resources > CTSU Operations Information> User Guides & Help Topics.*

NCI requirements for SAE reporting are available on the CTEP website:

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf

9.1.2 Solicited Adverse Events: The following adverse events are considered "expected" and their presence/absence should be solicited, and severity graded, at baseline and for each cycle of treatment. **Note: Baseline number of stools per day is required on the CRF.**

• **CTCAE v5.0 Term**	• **CTCAE v5.0 System Organ Class (SOC)**
• Hypothyroidism	• Endocrine disorders
• Abdominal Pain	• Gastrointestinal disorders
• Diarrhea	• Gastrointestinal disorders
• Nausea Gastrointestinal disorders	• Gastrointestinal disorders
• Fatigue	• General disorders and administration site conditions
• Blood bilirubin increased	• Investigations
• Anorexia	• Metabolism and nutrition disorders
• Arthralgia	• Musculoskeletal and connective tissue disorders
• Cough	• Respiratory, thoracic, and mediastinal disorders
• Dyspnea	• Respiratory, thoracic, and mediastinal disorders
• Peripheral sensory neuropathy	• Nervous system disorders

• Acute kidney injury	• Renal and urinary disorders

9.2 CTCAE Routine Reporting Requirements

In addition to the solicited adverse events listed in Section 9.1, the following table outlines the combinations of time points, grades and attributions of AEs that require routine reporting to the Alliance Statistics and Data Center. Questions about routine reporting should be directed to the Data Manager.

***Combinations of CTCAE Grade & Attribution Required for Routine AE Data Submission on Case Report Forms (CRFs)**

Attribution	Grade 1c	Grade 2c	Grade 3	Grade 4	Grade 5
Unrelated			a	a	a
Unlikely			a	a	a
Possible	a	a	a, b	a, b	a, b
Probable	a	a	a, b	a, b	a, b
Definite	a	a	a, b	a, b	a, b

a) Adverse Events: Other CRF - Applies to AEs occurring between registration and within 30 days of the patient's last treatment date, or as part of the Clinical Follow-Up Phase.
b) Adverse Events: Late CRF - Applies to AEs occurring greater than 30 days after the patient's last treatment date.
c) Patients on observation arm should only report "other adverse events" grade ≥3

9.3 Expedited Adverse Event Reporting (CTEP-AERS)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. Alliance investigators are required to notify the Alliance Central Protocol Operations Program, the Study Chair, and their Institutional Review Board if a patient has a reportable serious adverse event. The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5 will be utilized for AE reporting. The CTCAE is identified and located on the CTEP website at: ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTCAE. All reactions must first be reported in Rave on the "Adverse Events" form and sent for rule evaluation at the time of the event. Adverse event data should not be entered in CTEP-AERS prior to entry in Rave for rules evaluation. Additional information about Rave-CTEP-AERS is available at: https://www.ctsu.org, under the "Resources" tab for each protocol.

For further information on the NCI requirements for SAE reporting, please refer to the 'NCI Guidelines for Investigators: Adverse Event Reporting Requirements' document published by the NCI.

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided. Deaths due to progressive disease should be reported as Grade 5 "Disease progression" in the system organ class (SOC) **"**General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Patients who are on the observation arm of this study are not receiving any study treatment, thus do not fall under FDA reporting requirements for serious adverse events. Therefore, CTEP-AERS is not applicable, but the serious adverse events must be reporting in the study's adverse events case report form.

9.3.1 Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE ≤ 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death, including any death occurring within 30 days of the last dose, regardless of attribution to the investigational agent/intervention.
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>• Grade 1 Timeframes</th><th>• Grade 2 Timeframes</th><th>• Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour;
5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS ≤ 24 hours of learning of the AE, followed by a complete expedited report ≤ 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted ≤ 10 calendar days of learning of the AE.

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report ≤ 5 calendar days for:

- All Grade 4, and Grade 5 AEs (see above for use of legacy NCCTG AE notification form)

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

- Expedited AE reporting timelines defined:
  - ➢ "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS ≤ 24 hours of learning of the event followed by a complete CTEP-AERS report ≤ 5 calendar days of the initial 24-hour report.
  - ➢ "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted ≤ 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions (see below).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Additional Instructions or Exclusion to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or non-CTEP IND:

- All adverse events reported via CTEP-AERS (i.e., serious adverse events) should also be forwarded to your local IRB.
- Alliance A031501 uses a drug under a CTEP IND. The reporting requirements for investigational agents under a CTEP IND should be followed for Arm A only.
- Grade 3/4 hematosuppression and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results. All other grade 3, 4, or 5 adverse events that precipitate hospitalization or prolong an existing hospitalization must be reported via CTEP-AERS.
- All new malignancies must be reported via CTEP-AERS whether or not they are thought to be related to either previous or current treatment. All new malignancies should be reported, i.e. solid tumors (including non-melanoma skin malignancies), hematologic malignancies, myelodysplastic syndrome/acute myelogenous leukemia, and in situ tumors. In CTCAE version 5.0, the events may be reported as either: (1) Leukemia secondary to oncology chemotherapy, (2) Myelodysplastic syndrome, or (3) Treatment-related secondary malignancy. Whenever possible, the CTEP-AERS reports for new malignancies should include tumor pathology, history or prior tumors, prior treatment/current treatment including duration, any associated risk factors

or evidence regarding how long the new malignancy may have been present, when and how the new malignancy was detected, molecular characterization or cytogenetics of the original tumor (if available) and of any new tumor, and new malignancy treatment and outcome, if available.

- Treatment expected adverse events include those listed in Section 10.0 and in the package insert.
- Grade 1-3 nausea or vomiting and hospitalization resulting from such do not require AERS reporting, but should be reported via routine AE reporting
- CTEP-AERS reports should be submitted electronically.
- Pregnancy loss
  - Pregnancy loss is defined in CTCAE as "Death in utero."
  - Any Pregnancy loss should be reported expeditiously, as Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC.
  - A Pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP-AERS recognizes this event as a patient death.
- A neonatal death should be reported expeditiously as Grade 4, "Death neonatal" under the General disorders and administration SOC.

9.4 Comprehensive Adverse Events and Potential Risks List (CAEPR) for pembrolizumab, NSC 776864, IND #132331

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3793 patients.* Below is the CAEPR for Pembrolizumab (MK-3475).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.7, December 13, 2022[1]

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia[2]		
		Blood and lymphatic system disorders - Other (immune thrombocytopenic purpura)[2]	
	Lymph node pain[2]		
CARDIAC DISORDERS			
		Myocarditis[2]	

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
		Endocrine disorders - Other (hypoparathyroidism)	
	Endocrine disorders - Other (thyroiditis)[2]		
	Hyperthyroidism[2]		
	Hypophysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
EYE DISORDERS			
		Uveitis[2]	
		Eye disorders - Other (Vogt-Koyanagi-Harada syndrome)	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[2]		
	Diarrhea[2]		***Diarrhea[2] (Gr 2)***
	Mucositis oral[2]		
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[2]		
	Small intestinal mucositis[2]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills[2]		
Fatigue			***Fatigue (Gr 2)***
	Fever[2]		
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (autoimmune hepatitis)[2]		
		Hepatobiliary disorders - Other (sclerosing cholangitis)	
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis[2]	
		Cytokine release syndrome[2]	
		Immune system disorders - Other (acute graft-versus-host-disease)[2,3]	
		Immune system disorders - Other (hemophagocytic lymphohistiocytosis)[2]	
	Immune system disorders - Other (sarcoidosis)[2]		
		Serum sickness[2]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Infusion related reaction	

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased		
		GGT increased	
		Serum amylase increased	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Hyponatremia		
		Metabolism and nutrition disorders - Other (diabetic ketoacidosis)[2]	
		Metabolism and nutrition disorders - Other (type 1 diabetes mellitus)[2]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia[2]		***Arthralgia[2] (Gr 2)***
	Arthritis[2]		
	Back pain		
	Joint range of motion decreased		
	Myalgia[2]		
	Myositis[2]		
NERVOUS SYSTEM DISORDERS			
		Guillain-Barre syndrome[2]	
		Nervous system disorders - Other (myasthenic syndrome)[2]	
		Nervous system disorders - Other (neuromyopathy)[2]	
		Nervous system disorders - Other (non-infectious encephalitis)[2]	
		Nervous system disorders - Other (non-infectious meningitis)[2]	
		Nervous system disorders - Other (non-infectious myelitis)	
		Nervous system disorders - Other (optic neuritis)	
		Nervous system disorders - Other (polyneuropathy)[2]	
		Paresthesia	
		Peripheral motor neuropathy[2]	
RENAL AND URINARY DISORDERS			

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Renal and urinary disorders - Other (autoimmune nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
	Pneumonitis[2]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Bullous dermatitis[2]		
		Erythema multiforme[2]	
	Erythroderma		
		Palmar-plantar erythrodysesthesia syndrome	
	Pruritus[2]		***Pruritus[2] (Gr 2)***
	Rash acneiform[2]		
	Rash maculo-papular[2]		***Rash maculo-papular[2] (Gr 2)***
	Skin and subcutaneous tissue disorders - Other (dermatitis)[2]		
	Skin hypopigmentation[2]		
		Stevens-Johnson syndrome[2]	
		Toxic epidermal necrolysis	
	Urticaria[2]		
VASCULAR DISORDERS			
		Vasculitis[2]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Immune-mediated adverse reactions have been reported in patients receiving Pembrolizumab (MK-3475). Adverse events potentially related to Pembrolizumab (MK-3475) may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of Pembrolizumab (MK-3475), administration of corticosteroids and supportive care.

[3]Acute graft-versus-host disease has been observed in patients treated with Pembrolizumab (MK-3475) who received hematopoeitic stem cell transplants.

Adverse events reported on Pembrolizumab (MK-3475) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Pembrolizumab (MK-3475) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation; Hemolysis

CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Chest pain - cardiac; Heart failure; Myocardial infarction; Pericardial effusion; Pericardial tamponade; Ventricular arrhythmia

EYE DISORDERS - Eye pain

GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Constipation; Duodenal hemorrhage; Dysphagia; Gastritis; Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intussusception); Oral pain; Rectal hemorrhage; Small intestinal perforation; Upper gastrointestinal hemorrhage; Vomiting

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema limbs; Facial pain; Gait disturbance; General disorders and administration site conditions - Other (general physical health deterioration); Generalized edema; Malaise; Non-cardiac chest pain; Pain

INVESTIGATIONS - CPK increased; Cholesterol high; Creatinine increased; Fibrinogen decreased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Tumor lysis syndrome

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Generalized muscle weakness; Joint effusion[2]; Musculoskeletal and connective tissue disorder - Other (groin pain); Pain in extremity

NERVOUS SYSTEM DISORDERS - Aphonia; Depressed level of consciousness; Dysarthria; Edema cerebral; Encephalopathy; Headache; Hydrocephalus; Lethargy; Meningismus; Nervous system disorders - Other (brainstem herniation); Seizure; Syncope; Tremor

PSYCHIATRIC DISORDERS - Agitation; Confusion

RENAL AND URINARY DISORDERS - Acute kidney injury; Nephrotic syndrome; Proteinuria; Renal and urinary disorders - Other (hydronephrosis); Urinary incontinence; Urinary tract pain

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Dyspnea; Hypoxia; Laryngeal inflammation; Pleural effusion; Pleuritic pain[2]; Pneumothorax; Respiratory failure

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Skin and subcutaneous tissue disorders - Other (drug eruption)

VASCULAR DISORDERS - Hypertension; Peripheral ischemia; Thromboembolic even

Note: Pembrolizumab (MK-3475) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.0 DRUG INFORMATION

10.1 Pembrolizumab (MK-3475, IND 132331, NSC 776864, IND Holder: CTEP)

Investigator Brochure Availability

The current version of the Investigator Brochure (IB) will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status, a "current" password, and active person registration status. Questions about IB access may be directed to the PMB IB coordinator via email.

Procurement and Availability

Pembrolizumab (MK-3475) is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Pembrolizumab (MK-3475) is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI.

NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Study specific supplies will be provided to sites once a patient has been randomized. Starter supplies will not be provided.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

Supply

Pembrolizumab (MK-3475) is supplied by Merck & Co., Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI. Pembrolizumab (MK-3475) injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution for intravenous use. Each vial contains 100 mg of pembrolizumab (MK-3475) in 4 mL of solution. Each 1 mL of solution contains 25 mg pembrolizumab (MK-3475) and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

Preparation

Pembrolizumab (MK-3475) solution for infusion must be diluted prior to administration. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of pembrolizumab (MK-3475) to an infusion bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between 1 mg/mL to 10 mg/mL.

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin

Storage

Store intact vials between 2°C - 8°C (36°F - 46°F). Do not freeze. Protect from light by storing in the original box.

If a storage temperature excursion is identified, promptly return MK-3475 to between 2-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability

Refer to the package label for expiration.

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for up to 24 hours. Pembrolizumab (MK-3475) solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the IV bag, and the duration of infusion.

Route/Method of Administration

IV infusion only. Do not administer as an IV push or bolus injection.

Infuse over approximately 30 minutes (range: 25 - 40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required; however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer other drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

Compatible infusion set materials: PVC plasticized with DEHP or DEHT, PVC and tri-(2-ethylhexyl) trimellitate, polyethylene lined PVC, polyurethane, or polybutadiene

Drug Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP/
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

Adverse Events

See the CAEPR in Section 9.4.1.

Nursing Guidelines

Pembrolizumab (MK-3475) side effects vary greatly from those of traditional chemotherapy and can vary in severity from mild to life threatening. Instruct patients to report any side effects to the study team immediately. Side effects may be immediate or delayed up to months after discontinuation of therapy. Most side effects are reversible with prompt intervention of corticosteroids.

Diarrhea can be seen however is less common than that seen with anti-CTLA-4 agents. However it can be severe, leading to colonic perforation. Instruct patients to report ANY increase in the number of stools and/or change in baseline, blood in the stool, abdominal pain to the study team immediately.

Rash/pruirits/dermatitis is seen. Patients should report any rash to the study team. Treat per section 9.0 and monitor for effectiveness.

Monitor LFT's closely as elevations in these levels could indicate early onset autoimmune hepatitis. Patients should also be instructed to report any jaundice, or right upper quadrant pain to the study team immediately.

Pneumonitis can be seen and may be mild (only seen on imaging) to severe. Patients should be instructed to report any SOB, dyspnea, cough, chest pain, etc. to the study team immediately. Patients reporting these symptoms should have a pulse ox checked and consider immediate imaging per the treating MD.

Endocrinopathies (including hypopituitarism, hypothyroidism, hypophysistis, and adrenal insufficiency) are seen with this agent. Patients may present only with the vague sense of fatigue and "not feeling well" . Additional symptoms may be that of nausea, sweating and decreased activity tolerance. Instruct patients to report these signs or symptoms immediately and obtain appropriate labs as ordered by MD.

Patients who are started on steroid therapy for any side effects of pembrolizimab toxicity should be instructed to take the steroids as ordered, and not to discontinue abruptly as symptoms may return and be severe. Patients may be on steroid therapy for weeks. Instruct patients to report any increase or change in side effects with any dosage decrease as patients may need a slower taper.

Fatigue is common and may or may not be associated with immune related side effects. Assess patient's fatigue level prior to each cycle of therapy and report any changes to the study team.

Patients should avoid receiving live vaccines within 30 days of study drug administration or per other study guidelines.

11.0 MEASUREMENT OF EFFECT

Recurrence will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1) [39].

11.1 Schedule of Evaluations

For the purposes of this study, patients should be reevaluated every 12 weeks (+/- 14 days) for two years, then yearly for years 3, 4 and 5 (+/- 28 days). In addition to a baseline scan and follow-up scans, disease recurrence confirmatory scans should also be obtained 4-6 weeks following initial documentation of recurrence.

Supporting documentation of response should be submitted, per Section 6.5.

11.2 Definitions of Measurable and Non-Measurable Disease

11.2.1 Measurable Disease

A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥ 2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan or MRI.

A superficial non-nodal lesion is measurable if its longest diameter is ≥ 1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

A malignant lymph node is considered measurable if its short axis is ≥1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

11.2.2 Non-Measurable Disease

All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥ 1.0 to < 1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: '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. In addition, lymph nodes that have a short axis < 1.0 cm are considered non-pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.3.1 Measurement Methods

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at recurrence and for the confirmatory scans.
- Imaging-based evaluation is preferred to evaluation by clinical examination when evaluating for recurrent disease.

11.3.2 Acceptable Modalities for Measurable Disease

- **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. [to be confirmed with Imaging Committee] If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

  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. The lesions should be measured on the same pulse sequence. 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.

  Bone scans are NOT an acceptable modality for disease measurement. Bone scans can have a high false-positivity rate for tumor detection (bone injury may look like tumor), therefore, patients must have at least 2 new bone lesions with associated CT

sclerotic bone changes or unequivocal confirmation of a bone metastasis on MRI or biopsy in order to be considered to have disease progression.

11.4 Definition of Progression/Relapse/Recurrence

Progression/relapse/recurrence will be defined as any newly detectable metastatic lesion, measurable by RECIST or pathologically confirmed, whose presence can be confirmed as increasing in size on a repeat scan 4-6 weeks after initial detection. However, if the recurrence is unequivocal on CT, MRI, FDG PET/CT, or bone scan, a confirmatory scan is not needed. The first imaging date identifying progression/relapse/recurrence will define the date of recurrence. Pathological or cytological confirmation of recurrent metastatic urothelial carcinoma (now metastatic) should be performed when safe and feasible, and if results show recurrent metastatic cancer, it can replace the confirmatory 2nd scan performed 4-6 weeks later.

Patients with recurrent muscle-invasive disease (remaining bladder after nephrectomy/uretectomy or in the upper tract after cystectomy) will be considered to have "recurrent disease."

Local recurrence is defined as newly detectable non-muscle invasive urothelial carcinoma. These local recurrences will not be counted as progression events. Patients with recurrent non-muscle-invasive urothelial carcinoma will be treated with standard therapy (TURBT/ ureteroscopy or TURBT/ureteroscopy followed by BCG induction or intravesical chemotherapy). If BCG or intravesical chemotherapy is initiated, pembrolizumab will be held during the therapy course, 4 weeks before and 4 weeks afterwards.

12.0 END OF TREATMENT/INTERVENTION

12.1 Duration of Treatment

12.1.1 Disease Recurrence

Remove from protocol therapy any patient with confirmed metastatic or muscle-invasive disease recurrence. Document details, including tumor measurements, on data forms. When completing data forms the recurrence date would be the initial scan date.

After confirmed disease recurrence patients should be followed for survival per the study calendar (Section 5.0).

12.1.2 Treatment after Initial Radiologic Recurrence

Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

If radiologic imaging by local/site assessment shows disease recurrence (presence of any disease measurable by RECIST 1.1), regardless of arm, tumor assessment will be repeated by the site ≥ 4 weeks later in order to confirm recurrence, with the option of continuing treatment per below while awaiting radiologic confirmation of progression. However, if the recurrence is unequivocal on CT, MRI, FDG PET/CT, or bone scan, a confirmatory scan is not needed. In all cases, if RECIST-measurable disease is restricted to a solitary lesion, its neoplastic nature will be confirmed either by cytology/histology or by lesion progression on the next imaging examination. If repeat imaging shows no evidence of disease that is measurable by RECIST 1.1, treatment may be continued as per treatment calendar. If repeat imaging confirms recurrent disease, patients will be discontinued from study therapy.

The decision to continue study treatment after the 1st evidence of disease recurrence is at the Investigator's discretion based on the clinical status of the subject as described in Table 3. Confirmatory imaging will be performed as early as 28 days later. Subjects may receive study treatment while waiting for confirmation of disease recurrence if they are clinically stable as defined by the following criteria:

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

Table 3: Imaging and Treatment After 1st Radiologic Evidence of Disease Recurrence

	Clinically Stable		**Clinically Unstable**	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of disease recurrence (RECIST 1.1-measurable disease)	Repeat imaging at ≥ 4 weeks at site to confirm disease recurrence	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan by site	Repeat imaging at ≥ 4 weeks to confirm disease recurrence per physician discretion only	Discontinue treatment
Repeat scan confirms disease recurrence (any RECIST 1.1-measurable disease)	No additional imaging required	Discontinue treatment	No additional imaging required	N/A
Repeat scan shows no evidence of RECIST 1.1-measurable disease	Continue regularly scheduled imaging assessments	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion

NOTE: If a subject with confirmed radiographic recurrence (i.e. 2 scans at least 28 days apart demonstrating recurrence) is clinically stable or clinically improved, and there is no further increase in the tumor dimensions at the confirmatory scan (as assessed by the investigator and site radiologist), an exception may be considered to continue treatment upon consultation with the Study Chair.

12.1.3 Discontinuation of study agent:

- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- The subject has a confirmed positive serum pregnancy test

- Noncompliance with trial treatment or procedure requirements
- Completed 12 months of treatment with pembrolizumab
- Participants may discontinue study intervention at any time for any reason or be dropped from the study intervention at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Alliance if the study treatment is inappropriate, the study plan is violated, or for administrative and/or other safety reasons
- Any study intervention-related toxicity specified as a reason for permanent discontinuation as defined in the guidelines for dose modification due to AEs as described in Section 8.2.1.

If the patient discontinues pembrolizumab, patients should be followed for clinical disease status and/or survival per the study calendar (Section 5.0).

12.2 Managing ineligible patients and registered patients who never receive protocol intervention

Definition of ineligible patient

A study participant who is registered to the trial but does not meet all of the eligibility criteria is deemed to be ineligible.

Follow-up for ineligible patients who continue with protocol treatment

Patients who are deemed ineligible after registering may continue protocol treatment, provided the treating physician, study chair, and executive officer agree there are no safety concerns if the patient continues protocol treatment. All scans, tests, and data submission are to continue as if the patient were eligible. Notification of the local IRB may be necessary per local IRB policies.

Follow-up for ineligible patients who discontinue protocol treatment

For patients who are deemed ineligible after registering to the trial, who start treatment, but then discontinue study treatment, the same data submission requirements are to be followed as for those patients who are eligible and who discontinue study treatment.

Follow-up for patients who are registered, but who never start study treatment

For all study participants who are registered to the trial but who never receive study intervention (regardless of eligibility), the follow-up requirements are specified below.

Baseline, off treatment, and post-treatment follow up (i.e., relapse, progression, and survival) data submission are required. See the Data Submission Schedule accompanying the All Forms Packet.

12.3 Extraordinary Medical Circumstances

If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Document the reason(s) for discontinuation of therapy on data forms.
- Follow the patient for protocol endpoints as required by the Study Calendar.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Overview

This is a randomized phase III clinical trial that is designed to test the efficacy of adjuvant PD-1 blockade in patients with muscle-invasive bladder and upper-tract urothelial carcinoma. Patients will be randomized in 1:1 allocation ratio to either pembrolizumab or observation. Randomization will be stratified on: neoadjuvant chemotherapy (yes, no), pathologic stage (pT2/3N0, pT4N0 or NX, pT any N+ (any), positive margins) and PD-L1 status (positive, negative). Assignment to treatment arm will be performed centrally at the Alliance Statistics and Data Center following determination of eligibility. The randomization will be implemented using the permuted block design within each stratum of the stratification factors. This study is planned for dual-primary endpoints of overall survival (OS) and disease-free survival (DFS, defined in section 13.2). The study is designed to test the efficacy of pembrolizumab using the dual endpoints of OS and DFS in all patients, as well as OS and DFS in PD-L1 positive patients and negative patients. "PD-L1 positive patients" denotes "patients with tumor or tumor infiltrating PD-L1 expression", whereas "PD-L1 negative patients" denotes "patients with no detectable PD-L1 expression." PD-L1 expression is determined using Combined Positive Score (CPS), defined as the percentage of PD-L1 positive tumor and mononuclear inflammatory cells divided by the total number of tumor cells. The threshold for PD-L1 positivity is CPS ≥10%. and clarified in Update #12 in response to Merck's request (after completion of accrual and the first interim analysis),. The sample size is selected so that the log-rank test has at least 85% of power for each of the two primary hypotheses, i.e., OS and DFS in the overall patient population.

13.2 Endpoints

13.2.1 Primary Endpoints

Overall Survival (OS) in all subjects

Overall survival is defined as the time from randomization to the date of death from any cause. Patients who are alive will be censored at the date of last known contact. All dates in this study representing a date of patient contact will be used in the determination of the patient's last known survival status.

Disease-free Survival (DFS) in all subjects

Disease-free survival is defined as the time from randomization to the first metastatic recurrence (presence of any recurrent disease), or death, whichever occurs first. For patients who died without any recurrence, they will be considered as DFS event and the date of death will be the date of DFS. For patients without documented recurrence and are still alive, they will be censored at the last evaluable tumor assessment (or, if no tumor assessments were performed after the baseline visit at the time of randomization plus day 1). For patients who are lost to follow-up they will be censored at the last evaluable tumor assessment (MRI/CT scan). For patients who started any subsequent anti-cancer therapy without a prior progression will be censored at the last evaluable tumor assessment prior to receiving the non-protocol specified therapy.

13.2.2 Secondary Endpoints

OS and DFS in PD-L1 positive patients and PD-L1 negative patients.

13.3 Study design

13.3.1 Overall Type-I error rate and multiplicity

The multiplicity strategy specified in this section will be applied to the two parallel primary hypotheses (superiority of pembrolizumab on DFS and OS in all patients) and the secondary hypotheses (superiority of pembrolizumab on DFS in PD-L1 positive and negative patients, and OS in PD-L1 positive and negative patients).

The overall Type-I error is strongly controlled at the 0.025 level (one-sided) by the Bonferroni procedure, with 0.01 allocated to DFS in all patients (denoted as hypothesis H1), 0.005 allocated to OS in all patients (denoted as hypothesis H2); and 0.01 allocated to the secondary hypotheses: DFS in PD-L1 positive patients (denoted as hypothesis H3), OS in PD-L1 positive patients (denoted as hypothesis H4), DFS in PD-L1 negative patients (denoted as hypothesis H5), and OS in PD-L1 negative patients (denoted as hypothesis H6).

The two primary hypotheses will be tested using an iterative approach [40, 41] (Figure 1). Establishing both an overall OS and DFS benefit would provide the most convincing evidence for the clinical benefit of pembrolizumab in this setting, and sharing the alpha between these two primary hypotheses provides the greatest likelihood of that.

In the first step, H1 and H2 will be tested using the initially allocated alpha levels (0.01 for H1, 0.005 for H2). If one of the hypotheses (H1 and H2) is rejected, then the alpha allocated to that test will be shifted to the other hypothesis (between H1 and H2). For example, if H1 is rejected with alpha level of 0.01 but H2 is not, then the alpha level of H1 will be shifted to H2, and H2 will then be re-tested using an alpha level of 0.015.

In the second step, there are several scenarios to consider depending on the decision from the first step.

Scenario 1, H1 and H2 are rejected by using the initially allocated alpha levels

H5 will be tested with an alpha level of 0.025. If H5 is rejected, then H6 will be tested with an alpha level of 0.025.

Scenario 2, H1 is rejected but H2 is not by using the initially allocated alpha levels

Case 1: H2 is rejected after the shift of alpha from H1

H5 will be tested with an alpha level of 0.025. If H5 is rejected, then H6 will be tested with an alpha level of 0.025.

Case 2: H2 is not rejected after the shift of alpha from H1

H4 will be tested with an alpha level of 0.01. If H4 is rejected, then H5 will be tested with an alpha level of 0.01. (The test of H6 is not needed since H2 is negative and H4 is positive.)

Scenario 3, H2 is rejected but H1 is not by using the initially allocated alpha levels

Case 1: H1 is rejected after the shift of alpha from H2

H5 will be tested with an alpha level of 0.025. If H5 is rejected, then H6 will be tested with an alpha level of 0.025.

Case 2: H1 is not rejected after the shift of alpha from H2

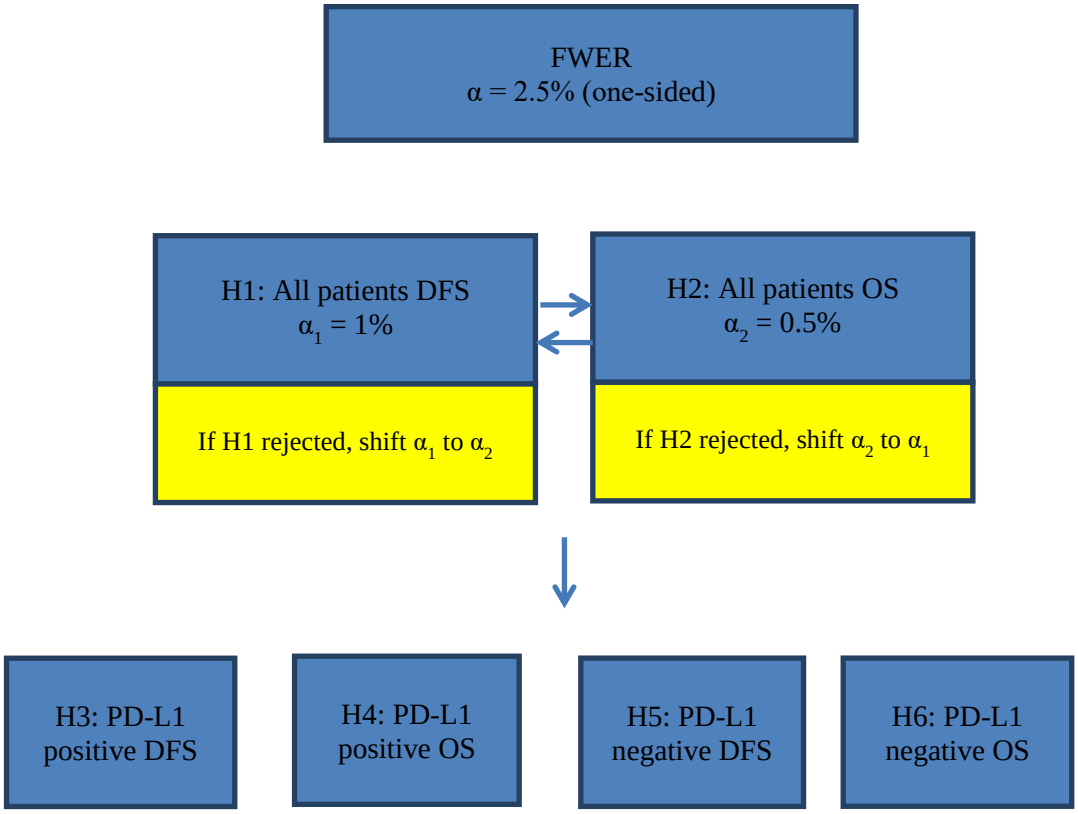
H6 will be tested with an alpha level of 0.01.

Scenario 4, neither H1 nor H2 is rejected by using initially allocated alpha levels

H3 will be tested with an alpha level of 0.01. If H3 is rejected, then H4 will be tested with an alpha level of 0.01. (The tests of H5 and H6 are not needed, since H1 and H2 are negative, and H3 and H4 are positive.)

For each hypothesis, the Type-I error rate for the interim analysis and final analysis is controlled through the alpha-spending functions as described in the interim analysis section.

Figure 1.



13.3.2 Interim Analysis

Efficacy (for both DFS and OS) and futility analysis (for both DFS and OS within PD-L1 negative patients) will be conducted during the trial. Under the alternative hypothesis for DFS, 387 events are expected at the end of the follow-up period. Analyses for harm for the DFS endpoint will be performed at 50% and PD-L1 negative (86 events) patients. If the observed hazard ratio for DFS is 1.25 or greater, the DSMB may recommend closing the enrollment for the trial depending on the futility analyses based on the OS endpoints.

In addition, analyses for harm will be performed on the OS endpoint. The target number of deaths is 320. Analyses will be performed at 50% of the expected information for the PD-L1 negative (56 deaths) patients. If the observed hazard ratio for OS is 1.0 or greater within the PD-L1 negative patients, this would trigger a futility analysis on the PD-L1 positive group when 50 deaths have been observed. If the observed hazard ratio for OS is 1.0 or greater within the PD-L1 positive patients, the DSMB may consider closing the trial. If the

cutoff is crossed only in the PD-L1 negative subgroup then only that subgroup is closed. These rules have a negligible impact on the type I and II error rates of this trial.

The Lan-DeMets α spending function with O'Brien and Fleming type of boundary will be used for monitoring the DFS and OS endpoints. The first superiority interim analysis will be performed on the DFS and OS endpoint once all patients have completed treatment. This is expected to take place approximately 49 months after study activation. The second and final analyses will be performed once the required number of DFS and OS events has been observed. The boundaries for superiority for DFS and OS are presented in Tables 1 and 2, respectively.

Table 1: Boundaries for DFS superiority analysis

Interim Analysis (Approximate calendar days)	**Percent Information (number of DFS events)**	**Boundaries for DFS Superiority Analysis (Z values, hazard ratios, and nominal significance levels)**
1 (49 months)	63% (244)	3.0092 0.6802 0.0013
2 (64 months)	82% (319)	2.6312 0.7448 0.0043
Final (81 months)	100% (387)	2.2885 0.7844 0.0085

Table 2. Boundaries for OS superiority analysis

Interim Analysis (Approximate Calendar time)	**Percent Information (number of deaths)**	**Boundaries for Superiority Analysis (Z values, hazard ratios, and nominal significance levels)**
1 (49 months)	61% (196)	3.3564 0.618 0.00039
2 (64 months)	80% (257)	2.9308 0.6933 0.00169
Final (81 months)	100% (320)	2.62140 0.7459 0.00438

If only the DFS boundary is crossed at the second interim analysis, the Alliance DSMB will determine whether the DFS and interim OS data can be released to the study team. Alternatively, if only the OS boundary is crossed at the second interim analysis, the Alliance DSMB will determine whether the OS and interim DFS data can be released to the study team.

13.4 Sample Size and Power Considerations

13.4.1 Sample Size and Power Computation for the DFS endpoint

Data from the adjuvant chemotherapy EORTC 30994 (21) study of pT3-4Nx/pTxN+ patients reported a 5-year DFS rate of 31.8% in the non-chemotherapy arm and the neoadjuvant CMV MRC BA06 30894 Trial (3, 11, 12) in MIBC patients with cT2-4aN0 (lymph node status at cystectomy not reported) had reported 5-year DFS rate of 32% in the no chemotherapy arm. We anticipate that 70% of these patients will make up this population in our study. The remaining 30% of MIBC patients will have received neoadjuvant chemotherapy without a pathologic response.

In the neoadjuvant CMV MRC BA06 30894 Trial (3, 11, 12) in which patients received chemotherapy, the 5year DFS rate is 39% for all patients regardless of their response to chemotherapy (we do not have the specific DFS for patients that have residual disease). We anticipate that 50% of patients receiving chemotherapy will have residual disease in the LN or >= pT2 (did not respond to neoadjuvant chemotherapy based on data from the neoadjuvant MVAC SWOG 8710 Trial) (3). The 5-year DFS rate for the 15% of patients remaining will be somewhere between 31.8% and 39% for a mean of 35.2%. Based on these rates and the expected proportion of each patient group in the study population, the anticipated 5-year DFS rate for the observation arm of the trial in our patient population will be 33.4%.

The null and alternative hypothesis for the primary DFS endpoint can be written as H0: $\Delta=1$ or Ha: $\Delta=0.65$, where $\Delta=$ is the hazard ratio defined as λ_1/λ_2 where they represent the hazard of disease recurrence or death for Arm 1 (pembrolizumab) and Arm 2 (observation), respectively. Under the alternative hypothesis, the expected number of DFS events is 387 in the two arms. Assuming a one-sided marginal Type-I error rate of 0.01 and an exponential DFS failure distribution, the log-rank test had at least 96.7% power to detect a hazard ratio=0.65. The target sample size is 739 patients. Assuming an accrual rate of 14 patients per month, accrual period is about 50 months, a 31 months minimum follow-up period is needed to achieve the target number of 387 DFS events.

We will also test the efficacy of pembrolizumab in prolonging DFS in the PD-L1 positive patients. Assuming PD-L1 positivity prevalence of 0.65, a one-sided marginal Type-I error rate of 0.01, an exponential DFS distribution, a sample size of 739 patients, and an accrual rate of 14 patients per month, a minimum of 31 months follow-up period is needed to achieve the target number of 252 DFS events in the PD-L1 positive patients. With 252 DFS event, the log-rank test has 85% power to detect a hazard ratio of 0.65 for the DFS endpoint in this subgroup of patients.

13.4.2 Sample Size and Power Computation for the OS Endpoint

With the OS endpoint, neoadjuvant cisplatin-based chemotherapy is underutilized in patients with MIBC. We assume that 30% of patients entering the study will have received neoadjuvant chemotherapy and had residual >T2 disease. Therefore their 5-year OS rate is approximately 40%. Combined with the fact that 70% of patients who did not receive

chemotherapy previously will have 5-year OS rate of 45%, it will be assumed that the 5-year OS rate for observation arm is 43.5%.

The null and alternative hypothesis for the primary OS endpoint can be written as H0: $\Delta=1$ or Ha: $\Delta=0.65$, where $\Delta=$ is the hazard ratio defined as λ_1 / λ_2 where they represent the hazard of death for Arm 1 (pembrolizumab) and Arm 2 (observation), respectively. Under the alternative hypothesis, the expected number of OS events is 320 in the two arms. Assuming a one-sided marginal Type-I error rate of 0.005 and an exponential OS failure distribution, the log-rank test had at least 89% power to detect a hazard ratio=0.65. Assuming an accrual rate of 14 patients per month, an accrual period of about 50 months, a minimum of 31 months follow-up period is needed to achieve the target number of 320 OS events.

We will also test the efficacy of the pembrolizumab in prolonging OS in the PD-L1 positive patients. Assuming PD-L1 positivity prevalence of 0.65, a one-sided marginal Type-I error rate of 0.01, an exponential OS distribution, a sample size of 739 patients, and an accrual rate of 14 patients per month, a minimum of 31 months follow-up period is needed to reach the target number of 208 OS events in the PD-L1 positive patients. With 208 OS, the log-rank test has 78% power to detect a hazard ratio of 0.65 for the OS endpoint in the PD-L1 positive patients.

13.4.3 Potential Effect of Non-proportional Hazard Ratio

The current design assumes an exponential distribution with constant hazard ratio for the OS and DFS endpoints. Recent studies, though, have suggested a non-constant hazard ratio with a more significant benefit from treatment in the first two years after randomization. The impact of the non-constant hazard ratio has been investigated by a simulation study, with results shown in Table 5.

According to the result in this simulation study, assuming that we follow the primary study design, if the hazard ratio is 0.65 within the first two years and changes to 0.70 (0.80, 0.9) after the end of the second year, then the design will achieve 83% (68%, 51%) marginal power for the OS endpoint and 95% (85%, 73%) marginal power for the DFS endpoint. Similarly, if the hazard ratio is 0.60 within the first two years and changes to 0.70 (0.80, 0.9) after the end of the second year, then the design will achieve 91% (79%, 65%) marginal power for the OS endpoint and 98% (93%, 85%) marginal power for the DFS endpoint.

In conclusion, to guarantee approximate 80% marginal power for the OS endpoint and 85% marginal power for the DFS endpoint in the event of a non-constant HR, it would not be necessary to increase the sample size as planned in the original study design in either of the following two scenarios:

1. HR of 0.65 within the first two years and less than 0.72 after the second year.
2. HR of 0.60 within the first two years and less than 0.79 after the second year.

Table 5 Statistical Power for Non-Constant Hazard Ratio

HR within 2 years	HR after 2 years	Endpoint	
		OS	DFS
0.60	0.70	0.91	0.98
	0.80	0.79	0.93
	0.90	0.65	0.85
0.65	0.70	0.83	0.95

	0.80	0.68	0.85
	0.90	0.51	0.73
0.70	0.70	0.74	0.87
	0.80	0.56	0.75
	0.90	0.38	0.59

13.4.4 Accrual time and study duration

In the recently completed CALGB/Alliance 90601 trial, the average patient accrual rate was 8 patients per month. As the currently proposed study is in the adjuvant setting, we estimate that our accrual rate will be 1.5- 2 times faster (≥ 12 patients per month. We anticipate the accrual rate will be 14 patients per month. This accrual rate would allow us to complete accrual in approximately 4.4 years.

13.4.5 Data Analysis plan

An intent-to-treat approach will be used in this phase III study to analyze all the clinical outcomes (DFS and OS) except toxicity. Patients who withdraw consent for treatment or withdraw from the study due to toxicity will continue to be followed for overall survival, even if they begin another therapy. The stratified proportional hazards model will be the primary analysis to compare the two arms on DFS and OS endpoints adjusting for the stratification factor (neoadjuvant chemotherapy, pathologic stage, and PD-L1 status).

The hazard ratio and its 95% confidence interval from the stratified Cox model with a single treatment covariate will be reported. The unstratified hazard ratio will also be presented.

If the treatment effect in the overall population is found to be significant, the impact of PD-L1 status on the efficacy of pembrolizumab will be evaluated by the proportional hazards model with an interaction term for PD-L1 status and treatment arm. In addition, the Kaplan-Meier product-limit method will be used to estimate the DFS and OS distributions. DFS and OS rates at 6, 12 and 18 months will also be estimated using KM estimates on the OS curve for each randomized arm. Furthermore, the Cochran-Mantel-Haenszel test will be used to compare the two arms on the proportion of patients who experience grade 3 or higher adjusting on the stratification factors.

13.4.6 Sensitivity and Exploratory Efficacy Analyses

The robustness of the treatment benefit on DFS will be demonstrated by performing sensitivity analyses. The number of tumor assessments by arm will be tabulated and the extent of missing tumor assessments between the arms will be evaluated by three types of analyses. First, a series of analyses will assesses DFS based only on radiographic assessments, reassigning DFS events to fixed assessment dates (next nearest, next earliest, or next latest date).

Second, DFS and OS rates at 6, 12, and 18 months will also be estimated using the KM product-limit approach on the DFS and OS distributions for each randomized arm.

Thirdly, the trial was closed to accrual slightly before the target accrual (after 702 patients were enrolled) due to the approval of nivolumab as adjuvant treatmeint in this patient population. Patients still on treatment were offereted the option to start nivolumab, which means that patient on the observation arm could start nivolumab and patients on the pembrolizumab arm had the option to switch to treatment with nivolumab. For the primary

DFS analysis, patients who start a subsequent anti-cancer therapy prior to a disease event are censored at the time at the last evaluable tumor assessment prior to receiving the non-protocol specified treatment. They also will no longer be followed on protocol for diease events. Hence the DFS analysis will not change.

For the overall survival endpoint, we will also perform two sensivity analyses. The first analysis will censor patients on the observation arm at the time they receive nivolumab. Patients who switch from pembrolizumab to nivolumab will not be censored since it is believed the potential difference in efficacy between these drugs is minimal or a least considerably smaller than the difference in efficacy between observation versus adjuvant nivolumab treatment. The second analysis will use a Cox model to compare the two treatment arms with respect to OS. The model will contain the stratification variables, treatment arm (the variable of interest), and the receipt of nivolumab as a time-dependent variable, regardless of treatment arm.

Finally, the effects of demographic and baseline prognostic characteristics on OS and DFS will be examined as exploratory analyses. It is expected that accrual in subgroups defined by these characteristics will not be large enough for definitive treatment comparisons to be made between these subgroups. The following demographic and baseline characteristics will be considered: site of disease, neoadjuvant chemotherapy,pathologic stage, and PD-L1 status.

Descriptive summaries of DFS and OS will be produced for each level of the categorical variables listed above for each treatment arm. These descriptive summaries will consist of the unstratified hazard ratio and the Kaplan-Meier estimates of median and 95% CI for time to event endpoint and DFS and OS rates as discussed above. The effect of each of the baseline variables on DFS and OS will be assessed using the proportional hazards model. For each variable, the proportional hazards model will include factors for treatment and the individual variable.

13.4.7 Study monitoring

This study will be monitored by the NCI-approved Alliance Data Safety Monitoring Board (DSMB). Reports on efficacy, adverse events, and administrative information will be provided to the DSMB every 6 months as per NCI guidelines.

Additionally, this study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis by FTP burst of data. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

13.4.8 Protocol-Specific Monitoring Plan

Source Data Verification: Source data verification will be completed for all patients at each enrolling site. For these patients, source documentation for the following items will be verified and tracked: 1) informed consent (deidentified signature page(s) with patient name redacted and signature date(s) retained), 2) key protocol eligibility criteria, 3) drug administration for the first two cycles of treatment.

13.5 Descriptive Factors

Patients in the two treatment arms will be compared by various descriptive factors, including but not limited to: age by decade; gender; and stratification factors (site of disease; neoadjuvant chemo status; pathologic stage; and PD-L1 status).

13.6 Inclusion of Women and Minorities

Note that based on the accrual of n=506 urothelial carcinoma patients to study CALGB 90601, the Alliance does not anticipate accrual from some subgroups. However, to comply with CTEP requirements that these tables contain no zero counts, these projections have been modified to include two accruals where the Alliance expects none.

Additionally, all projected accruals of Native Americans have been restricted to North American accruals, with none expected in Europe.

Total Accrual Targets					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	2	2	2	2	8
Asian	2	7	2	2	13
Native Hawaiian or Other Pacific Islander	2	2	2	2	8
Black or African American	7	25	2	2	36
White	127	523	4	12	666
More Than One Race	2	2	2	2	8
Total	142	561	14	22	739

14.0 CORRELATIVE AND COMPANION STUDIES

14.1 Correlative Science Studies in Alliance A031501

14.1.1 Background

The randomized phase III trial of adjuvant pembrolizumab in high risk bladder cancer patients s/p radical cystectomy is a superb opportunity to answer a number of unanswered scientific questions that are relevant to bladder cancer and specifically the role of adjuvant immunecheckpoint blockade. Specifically, we hypothesize that adjuvant pembrolizumab will prolong overall survival (OS) in a subset of this population and that a detailed characterization of the intratumoral immune microenvironment as well as peripheral blood correlates will further refine our understanding of the immune microenvironment of high-grade bladder cancer with the hope of defining potential predictive biomarkers of benefit from adjuvant PD1 axis blockade. Finally, given the global nature of NextGen Sequencing (NGS), the data generated from these correlative studies will provide a significant amount of data for future investigation.

The successful completion of the correlatives in this protocol will result in a comprehensive immune portrait of the high-grade, muscle invasive bladder cancer as well as evaluate potential biomarkers that predict enhanced OS in response to pembrolizumab therapy. The studies will broadly consist of (1) characterization of the intratumoral immune microenvironment. (2) longitudinal assessment of peripheral blood T-cell receptor (TCR) clonal restriction and peripheral blood mononuclear cell (PBMC) RNA profiling as well as (3) direct HLA haplotype sequencing.

<u>Intratumoral Immune Characterization</u>

Prior work suggests a correlation between specific intratumoral features such as predicted neoantigen burden and PD-L1 expression by IHC and response to PD1 axis blockade in other cancers (i.e. melanoma and lung) [42-46] We hypothesize that these characteristics

may also correlate with benefit from adjuvant pembrolizumab in bladder cancer and will evaluate them as well as a number of other intratumoral immune parameters.

Intratumoral immune characterization will take place through immunogenomic analyses based on data generated by (1) whole exome sequencing (WES) of both tumor and germline DNA, (2) global transcriptome assessment by RNAseq and (3) direct TCR sequencing of tumor infiltrating lymphocytes [TILs]. Sequencing data will be used to determine (1) somatic mutations and burden, (2) predicted neoantigen burden, (3) expression of immune and cancer-related RNA gene signatures and RNA subtype [Luminal, Basal, etc], and (4) intratumoral TCR repertoire expression and clonal restriction. Please note that PD-L1 expression by IHC is an integral biomarker in the study and while the data will be available, it will not be repeated as a correlative study.

Peripheral Blood Immune Monitoring

Adaptive immune responses are characterized by clonal expansion of antigen-specific T cells. While the work above will determine the TCR diversity and expression of TILs, this study presents an opportunity to query the TCR diversity, expression, and persistence in the peripheral blood. We speculate that patients most likely to benefit from adjuvant pembrolizumab will have tumors with clonal restriction in T cell receptor (TCR) repertoires, that these tumor specific clonotypes can be detected in the peripheral blood, and that persistence or diminution of these tumor specific clonotypes in the peripheral blood may correlate with overall survival.

The peripheral blood will be monitored at baseline (pre-pembrolizumab treatment) as well as at specified timepoints during therapy. Specifically, we will perform (1) direct TCR sequencing to assess the peripheral blood TCR diversity, (2) NanoString nCounter® Immune profiling on PBMCs to determine the gene expression changes in immune-effector cells, and (3) ELISA based assays on plasma to assess the levels of circulating HGF, VEGFA, IL10, IL17. All parameters will be correlated to outcome based on their baseline levels as well as their dynamic changes over time.

Direct HLA Sequencing

Given the critical role of HLA haplotype on mutant peptide display, this study will also perform direct sequencing of the polymorphic regions of the MHC Class I alleles (HLA-A, HLA-B, HLA-C) to assess their correlation to OS in response to pembrolizumab. We hypothesize that HLA haplotypes will correlate with clinical benefit from pembrolizumab.

Summary

In summary, the proposed correlative studies will generate comprehensive intratumoral and circulating immune portraits of high-grade, muscle-invasive bladder cancers. The adjuvant design of the study will present unique opportunities to examine the value of peripheral blood immune monitoring as a correlate to clinical benefit (prolonged OS) from adjuvant pembrolizumab. In addition, the wealth of data generated from these correlative studies will provide data for future investigation.

14.1.2 Objectives

Overall Objectives for Correlative Studies:

1. To determine if mutational rate and predicted neoantigen burden are associated with OS
2. To determine if tumor molecular subtype or immune gene expression signature expression is associated with OS

3. To determine whether intratumoral T-cell receptor (TCR) diversity is associated with OS
4. To determine whether persistence of specific T-cell receptor (TCR) clonotypes in the peripheral blood is associated with OS
5. To determine whether HLA subtype is associated with OS
6. To conduct exploratory analyses regarding the association of plasma HGF, VEGF, IL-10, and IL-17 levels with OS and between treated and untreated patients.

Specific Objectives for Specific Studies:

Study 1: "Neoantigen and HLA mapping by whole exome sequencing and expression profiling of peripheral blood immune-effector cells to determine predictors of response to immune-checkpoint inhibition in bladder cancer"

Bishoy Faltas et. al.

1. To determine if the immune gene signatures in peripheral blood monocytes are associated with DFS and OS.

Study 2: "Identifying Immunomodulation by HGF and VEGF Pathways in Bladder Cancer Patients"

Donald P Bottaro

1. To conduct exploratory analyses regarding the association of plasma HGF and VEGF levels with IL-10 and IL-17.
2. To compare the changes in levels of plasma measures between patients with treatment and without treatment.

Study 3: "Tumor immune microenvironment profiling and molecular subtype determination by RNA-seq."

William Kim and Ben Vincent

1. To determine if the 12 immune gene signature expression are associated with disease free survival (DFS) and overall survival (OS).
2. To determine if the tumor molecular subtype is associated with DFS and OS.

Study 4: "Immune monitoring of tumor specific TCR clonotype persistence in PBMCs"

William Kim and Ben Vincent

1. To investigate if the diversity of T cell receptor (TCR) clonotypes is associated with DFS and OS.
2. To investigate if the persistence in TCR clonotypes is associated with DFS and OS.

Study 5: "HLA and Immunogenetics component"

Michael Dean and Mary Carrington

1. To determine if the HLA subtypes are associated with DFS and OS.

Study 6: "Whole exome sequencing will identify mutations and potential neo-antigens present in tumors of patients predictive of response to MK-3475 (pembrolizumab)."

Parkih et al.

1. To determine if the somatic mutation rate and neo-antigen burden are associated with DFS and OS.

14.1.3 Methods

A multidisciplinary team will utilize the data derived from the NextGen sequencing (whole exome sequencing [WES] and RNAseq) of tumor, germline, and peripheral blood mononuclear cells (PBMCs) to examine the objectives outlined above. FASTQ files will be made available to all interested co-investigators. Teleconferences will be held quarterly until data production. Once data production and data analysis begins teleconferences will become monthly with each group expected to present progress on each call.

Components/Teams

Study 1: Immune profiling of PBMCs

- Bishoy Faltas (Cornell)

Study 2: Identifying Immunomodulation by HGF and VEGF Pathways

- Don Bottaro (NCI)

Study 3: Tumor immune microenvironment profiling and molecular subtype determination with RNAseq and TCR repertoire sequencing.

- William Kim (UNC)
- Ben Vincent (UNC)

Study 4: Immune monitoring of tumor specific TCR diversity and clonotype persistence in PBMCs.

- William Kim (UNC)
- Ben Vincent (UNC)

Study 5: Germline HLA gene sequencing

- Michael Dean (NCI)
- Mary Carrington (NCI)

Study 6: Neoantigen Prediction

- Rahul Parkih (UPitt)
- William LaFramboise (UPitt)
- Bishoy Faltas (Cornell)
- Ben Vincent (UNC)
- William Kim (UNC)
- Michael Dean (NCI)

Sample Processing and Collection

Tumor DNA

- Tumor DNA will be extracted from formalin-fixed paraffin-embedded (FFPE) sections of tumor.

Tumor RNA

- Tumor RNA will be extracted from FFPE sections of tumor. RNA will be used for both RNAseq as well as TCR repertoire sequencing from TILs.

Germline DNA

- Germline DNA will be collected in PAXgene Blood DNA tubes (Qiagen), preferably at baseline but may be collected at any time during the study. DNA will be extracted per manufacturer's instructions and stored at -80 degrees Celsius.

PBMC RNA

- PBMC RNA will be collected in PAXgene Blood RNA tubes (Qiagen) at the specified timepoints and used for both peripheral blood TCR repertoire monitoring as well as for NanoString nCounter Immune Panel analysis. RNA will be extracted per manufacturer's instructions and stored at -80 degrees Celsius.

Peripheral Blood Plasma

- Plasma will be derived from a lavender top (EDTA) tube. Process tube for plasma within 2 h of draw and kept at 4C from draw to plasma. Separate and store plasma at -80C.

Techniques

Whole Exome Sequencing (WES)

- The WES data will be generated by Merck and FASTQ files or lossless BAM files will be deposited onto a central, password protected, FTP server. In the event that Merck does not perform the WES, UNC or other partner will serve as the Genome Sequencing Center (GSC). Whole exome capture will be done using the Nextera Rapid Capture Exome protocol. Exome libraries will be sequenced on two lanes of an Illumina HiSeq2500 sequencer run in high-output mode using 2x100 paired-end chemistry. Exome sequence data will be processed using the following pipeline: sequence reads will aligned to the genome using the BWA MEM algorithm (v0.7.4) with re-alignment performed simultaneously for tumor and normal pairs using ABRA (v0.46)[45] (Mose et al., 2014). Variants will be called using FreeBayes and somatic mutations using Strelka [47].

RNAseq

- RNAseq will be performed at UNC, which is the RNAseq Genome Sequencing Center (GSC) for the TCGA. mRNA-Seq libraries will made according to the Illumina TruSeq RNA Access protocol and run as three samples per lane of an Illumina HiSeq2500 sequencer in high-output mode using 2x100 paired-end chemistry (which yields 60-80 million reads per sample). Sequence data will be processed, filtered, and aligned and transcript abundance quantitated as per TCGA protocols (RSEM).

Predicted Neoantigen Burden

- Class I HLA-type for each patient will be determined using PHLAT (Bai et al., 2014). The set of mutated peptides of length 8-11aa that may result from the discovered mutations and represented by minimum expression in the mRNA sequence data will be enumerated using custom software. Predicted neoantigens will be discovered using netMHCpan [48]. Peptides predicted to bind MHC at IC50 less than 500nm will be considered as putative neoantigens. Neoantigen burden will be calculated as the number of these per tumor.

TCR seq

- UNC has developed a method for TCR repertoire amplification and sequencing on the Illumina MiSeq platform that includes primer molecular tags to account for amplification bias (a critical step when working with low input material). This

approach is similar to that of the commercially available assay from Adaptive Biotechnologies (http://www.adaptivebiotech.com/immunoseq) but uses an alternative method of controlling for primer bias. Our informatics pipeline allows for resolution of the frequency of TCR clonotypes present (with a clonotype defined by variable gene + joining gene + CDR3 usage) and analysis of the following features: variable/joining gene usage, complementary-determining region 3 (CDR3) length, charge, hydrophobicity, and amino acid motifs, frequency of dominant clonotypes, shared clonotypes, and overall population diversity among samples.

NanoString nCounter Immune Profiling

- NanoString nCounter technology allows direct RNA counting without amplification and can be performed on very small amounts of starting RNA. A multiplexed panel of 770 genes is assayed in a single reaction, which includes a number of immune checkpoint molecules such as PDL1 (CD274).

ELISA

- IL10 and IL17 will be measured using immunoassay kits from Meso Scale Discovery (Gaithersburg, MD) as per manufacturer's instructions. HGF and soluble Met ELISA will be measured by immunoassays developed in house at the NCI.

HLA sequencing

- Germline DNA will be used to amplify all polymorphic regions of the HLA-A, B and C genes using Roche 454 sequencing chemistry.

14.1.4 Statistical Considerations

Study 1:

Power Computations

Power computation for study 1 is provided based on objective 1. It is expected that 628 patients (85% of the target 739 patients) to have specimen available. For the power computation, the two measures (i.e. somatic mutation rate and the number of the neo-antigens per sample) will be dichotomized at the median value although these measures will be analyzed as continuous covariates. With 272 OS events, the log-rank test has 80% and 85% to detect hazard ratios of 1.45 and 1.49 assuming death rate of 43% (which is similar to the treatment trial), a two-sided type I error rate of 0.025 (adjusting for the two measures), and that the OS endpoint follows an exponential distribution.

Data Analysis

The Kaplan-Meier product [50] limit method will be used to estimate the DFS and OS distributions. The log-rank [51] statistic will be used to compare the DFS and OS by high- and low- somatic mutation rate, neo-antigen burden, expressed immune gene levels, changes in somatic mutation rate and neo-antigen burden before and after treatment.

Furthermore, the proportional hazards model [52] with each measure (somatic mutation rate and neo-antigen burden), treatment and measure (somatic mutation rate and neo-antigen burden)-treatment interaction will be performed to test for the treatment-measure interaction term. The proportional hazards model will be used to assess the prognostic importance of each measure as continuous variable in predicting DFS and OS. Exploratory data analysis will be conducted to examine the association between somatic mutation rate and neo-antigen burden, as well as the association between the changes in somatic mutation rate and neo-antigen burden before and after treatment. The Spearman's rank correlation (Spearman's rho) will be used to evaluate the correlation between them. Penalized

regression models will be used to select 30-50 expressed immune gene signatures based on their ability to distinguish between progressors and non-progressors. Ten-fold cross validation and the bootstrapped methods will be utilized to obtain valid estimates of signature performance.

Study 2:

Statistical considerations

The Spearman's rank correlation (Spearman's rho) will be used to evaluate the correlation between HGF with IL-10, HGF with IL-17, VEGF with IL-10 and VEGF with IL-17. The association is considered to be strong if |rho|>0.7, moderately strong if 0.5<|rho|<0.7, weak to moderately strong if 0.3<|rho|<0.5, and weak if |rho|<0.3. Since the clinical trial from which these measures will be taken may enroll 739 patients, and the assays to obtain these results are not expensive or challenging to perform, there will not be any official upper limits on the number of specimens obtained. The precision of the estimates will be enhanced with larger numbers of specimens. Table 1 shows the sample sizes required to obtain 97.5% lower one-sided confidence interval with width of 0.1 based on the method of Spearman's rank correlation. Thus, 739 patients would be more than adequate to essentially ensure that the widths of any likely one-sided confidence intervals would be 0.10 or less.

Table 1 Required sample size to obtain 97.5% lower one-sided confidence interval with width of 0.1 based on the method of Spearman's rank correlation.

Sample correlation	Sample size
0.9	27
0.8	72
0.7	129
0.6	189
0.5	246
0.4	295
0.3	334
0.2	362
0.1	379

In addition, the levels and changes in levels in a well-defined time point from baseline can be compared between patients on the active vs. the observation arm. As an illustration, if we were to compare up to 10 measures or changes in measures between the two arms, 109 patients per arm would be required assuming a two-sided type 1 error rate of 0.005 (.0.5/10) and 80% power in order to detect a difference with an effect size of 0.5. The Bonferroni approach is conservative as it does not take into account the correlation between changes in levels of measures between patients. In practice, a Wilcoxon rank sum test may be used if any of the resulting data are not normally distributed, and as well, a Hochberg adjustment for multiple comparisons may be used instead of the overly stringent Bonferroni correction.

Study 3:

Power Computations

Power computation for Study 3 is provided based on objective 1. It is expected that 628 patients (85% of the target 739 patients) will have specimens available. For simplicity in the power computation, the gene expression levels will be dichotomized at the median

value and patients will be classified as having either low (below or equal to the median value) or high (above the median) levels. The Bonferroni correction will be used (0.05/12 test) with significance level of 0.004 due to the multiplicity of biomarkers being tested. With 272 OS events, the log-rank test has 80% and 85% to detect hazard ratios of 1.61 and 1.57 assuming a death rate of 43% (which is similar to the treatment trial), a two-sided type I error rate of 0.004 and that the OS endpoint follows an exponential distribution. It should be noted that if the continuous measures of each marker are used, the power to detect associations will be greater.

Data Analysis

The log-rank [51] statistic will be used to compare the DFS and OS by high- and low-expression groups for each gene signature, and by tumor molecular subtypes, respectively. Secondary analysis will be performed utilizing the proportional hazards model within each tumor molecular subtype. Furthermore, the proportional hazards model will be used to test the prognostic significance of individual gene expression signatures adjusting for the other clinical and genomic variables. The Spearman's rank correlation (Spearman's rho) will be used to evaluate the correlation between immune genes.

Study 4:

Power Computations

Power computation for Study 4 is provided based on objective 1. It is expected that 628 patients (85% of the target 739 patients) to have specimen available.

Shannon entropy [53] will be used as the index of diversity of TCR clonotypes. The proportional hazards model will be applied with this index as non-negative continuous variable. We do not know the variance of this index. Under a range of the variance of this index, assuming a two-sided type I error rate of 0.05, power of 0.80, Table 2 provides the minimum detectable HR for per one unit increase in Shannon entropy diversity index based on 326 DFS events.

Table 2. Minimum detectable HR for per one unit increase in Shannon entropy diversity index under a range of the variance of this index assuming two-sided type I error rate of 0.05 and power of 0.80

Variance of Shannon entropy diversity index	HR for DFS
1	1.17
2	1.08
3	1.06
5	1.03
10	1.02

Data Analysis

The Kaplan-Meier product [50] limit method will be used to estimate the probability of DFS and OS distributions. The log-rank [51] statistic will be used to compare the DFS and OS between patients with and without persistence in TCR clonotypes. The proportional hazards model [52] will be applied to evaluate the prognostic importance of persistence in TCR clonotypes adjusting for the treatment effect.

Furthermore, the proportional hazards model will be used to assess the prognostic importance of Shannon entropy diversity index in predicting DFS and OS.

Study 5:

Power Computations

Power computation for study 5 is provided based on objective 1. It is expected that 628 patients (85% of the target 739 patients) to have specimen available.

For the power computation, the two measures (i.e. tumor burden and neo-antigens burden) will be dichotomized at the median value although these measures will be analyzed as continuous covariates. It is expected that deaths will be observed in the training set. With 272 OS events, the log-rank test has 80% and 85% to detect hazard ratios of 1.45 and 1.49 assuming death rate of 43%, a two-sided type I error rate of 0.025 (adjusting for the two measures) and that the OS endpoint follows an exponential distribution.

Data Analysis

The Kaplan-Meier product-limit [50] method will be used to estimate the probability of DFS and OS distributions. The log-rank [51] statistic will be used to compare the DFS and OS by high- and low- tumor burden and neo-antigen burden, respectively. DFS and OS of patients with HLA-subtype-positive and HLA-subtype-negative will also be compared based on log-rank statistics. The proportional hazards model [52] with treatment, marker and marker-treatment interaction term will used to test for the predictive ability of the marker in predicting OS and DFS. Furthermore, the proportional hazards model will be utilized to assess the prognostic importance of these measures in predicting DFS and OS.

Study 6:

Power Computations

Power computation for study 6 is provided based on objective 1. It is expected that 628 patients (85% of the target 739 patients) to have specimen available. The sample size will be randomly divided into 2:1 allocation ratio with 420 and 208 patients in the training and testing sets, respectively.

No preliminary data are available regarding modeling the association between mutational load that distinguishes patients by DFS. Assuming a one-sided type I error rate of 0.025, power of 0.80, Table 3 provides the minimum detectable HR with 218 DFS events from the training set for different choice of the cut point. The power computations are based on the following assumptions: DFS endpoint follows an exponential distribution, a one-sided significance level of 0.025 and 218 DFS events (rate of 52% which is similar to the treatment trial).

Table 3. Minimum detectable HR under a range of conditions and assuming a one-sided type I error rate of 0.025, and 0.80 power.

% of patients with high mutational load	HR
10 or 90	1.88
20 or 80	1.61
30 or 70	1.51
40 or 60	1.47
50 or 50	1.46

Data Analysis

The Kaplan-Meier product-limit [50] method will be used to estimate the probability of DFS and OS distributions. The log-rank [51] statistics will be used to compare the DFS by mutational load. Moreover, the proportional hazards model [52] will be used to assess the prognostic importance of mutational load in predicting PFS adjusting for treatment effect and the interaction between mutational load and treatment. The training set will be used to

explore optimal cutpoint for the mutational load. The testing set will be utilized to validate the choice of the mutational load cut-point. In addition, by classifying patients into 4 groups based on a pre-defined clinical benefit (yes or no) and treatment arm (pembrolizumab and observation), two-way ANOVA will be used to compare mutational load among these four groups. Secondary analysis, such as one-way ANOVA or Mann-Whitney U test, will be performed in patients with clinical benefit in order to compare mutational load between patients with pembrolizumab and those randomized to observation.

14.2 Pharmacogenomic studies in Alliance A031501 - Alliance A031501-PP1

14.2.1 Background

There is now a preponderance of evidence that antibody therapies targeting the programmed death-1 (PD1)/programmed death ligand-1 (PDL1) interactions of tumor-infiltrating lymphocytes and tumor cells have profound clinical effects on outcomes in metastatic refractory urothelial cancer [31, 33, 54, 55, 70-72]. Pembrolizumab, an anti-PD1 antibody, is one such therapy under intense investigation for this disease. Pembrolizumab resulted in objective responses in significant percentages of platinum-refractory metastatic urothelial cancer patients in a prior phase I trial [30, 73], with responses preferentially occurring in patients whose tumor-infiltrating cells expressed PDL1. The primary study to which this companion correlative study is attached now proposes to examine whether pembrolizumab given in the adjuvant setting after urothelial cancer surgery improves disease-related outcomes for this disease.

While there is significant enthusiasm for eventual approval of drugs targeting PD1/PDL1 in urothelial cancer, development of these drugs has not been without recognition of important clinical toxicities. Most importantly, severe (grade 3 and higher) immune-related organ-specific toxicities including pneumonitis, colitis, hepatitis, and skin reactions have been observed. These and other similar grade 3/4 toxicities occur in approximately 15% of treated patients [30, 33, 56, 72-73]. While immune-related toxicities typically respond to drug withdrawal and steroid therapy, affected patients often require prolonged courses of immune suppression, and furthermore, these patients are typically ineligible for further use of immune-stimulatory anti-cancer therapy.

Relatively little is known about host factors that predispose patients to development of severe immune-related toxicities from anti-PD1 and anti-PDL1 treatments. HLA genotypes are likely to be important [57, 74]. HLA typing will be conducted as part of the proposed secondary endpoints of this trial.

Also of interest are germline polymorphisms in PD1 (also known as PDCD1) and PDL1. Intriguingly, a prominent investigation conducted more than 10 years ago showed that A allele carriers for rs11568821 (PD1.3 G>A) were more likely to develop the autoimmune disease lupus (Relative Risk 2.6, P=0.00001) than G allele homozygotes [58, 75]. This locus is located in an enhancer-like element within intron 4 of PDCD1, which is believed to be a binding site for transcription factors involved in inflammation, especially RUNX1. Elegant work in the field of multiple sclerosis similarly implicated this same PD1 polymorphism, with A allele carriers having significantly higher rates of primary recurrence, and with AA homozygotes occurring at unusually high frequencies (>10 X those normally expected) among cohorts of primary recurrence patients [59, 76] Functional assays showed that A-allele carriers were significantly less likely to inhibit interferon-gamma secretion. Multiple other reports have consistently found polymorphisms of PD1 associated with conditions related to immune activation and autoimmunity, including type I diabetes, rheumatoid arthritis, and subacute sclerosing panencephalitis [60-63, 77-80]. These findings provide intriguing initial justification for examining the potential role of

germline PD1 polymorphisms in governing immune-related toxicities in response to anti-PD1 anti-cancer therapy.

Because of the relatively recent development of immune oncology therapies, there is also a need to think broadly about potential germline targets, both within the PD1 and PDL1 genes, and across the entire genome. Therefore, hypothesis-generating genome-wide approaches (GWAS) are likely to be highly valuable in the setting of this trial because of the large trial size and the prospectively collected and well-phenotyped data that will be available. Prior studies have shown the powerful role of GWAS in identifying important genes in serious adverse drug reactions [64, 81]. In contrast to candidate gene studies, GWAS collect SNP data across the entire human genome and have significant power to detect common variants that confer a modest risk for a complex phenotype. Whole-genome sequencing takes genome-wide approaches even further and has the ability to interrogate the entire genome, rather than only common SNPs. Technological advances have made GWAS relatively common and technically easy to perform. Advances in whole-genome sequencing proficiency are similarly making this technology more readily available and affordable quite rapidly.

The identification of SNPs that contribute to toxicity with pembrolizumab will lead to additional studies to understand the mechanism for these associations and to investigate the application of genetic information for the optimization of this drug and likely other immune cancer therapies.

14.2.2 Objectives

1. To investigate the effect of PDCD1 SNP rs11568821 on severe (grade 3 or higher) immune-related toxicity in the pembrolizumab-treated cohort.
2. To investigate whether other SNPs commonly polymorphic within or near PDCD1 associate with development of pembrolizumab toxicity in the treated cohort.
3. To identify novel germline genetic markers of treatment-related toxicity through genome-wide association analysis of pembrolizumab-treated patients.
4. To identify novel germline genetic markers that are associated with disease-free survival through genome-wide association analysis.

14.2.3 Methods

All patients enrolled in the accompanying study will be given the option to consent for germline DNA analysis. A single blood sample (10 ml in a lavender top) will be collected at baseline (or any follow-up visit) for pharmacogenomic analysis. Germline DNA will be extracted using standard techniques and genetic testing appropriate to specific study questions will be performed. The concentration and quality of DNA will be quantified by ultraviolet spectroscopy. All DNA samples and unprocessed blood will be frozen and will be stored at the Alliance Biorepository at Mayo Clinic (AB Mayo) until they are distributed to the appropriate laboratory for analysis. The typical yield of DNA from a 10ml blood sample is 100 ug (range of 80-150 ug). For the studies described below we will need up to 5 ug for the proposed analyses, leaving the majority of the sample stored at the Alliance Biorepository at Mayo and available for additional future genotyping projects.

Genotyping for SNP rs11568821 will be performed using previously published methods and assay conditions [65, 82]. Genome-wide genotyping will be performed using a contemporary genome-wide platform to be determined. This decision will be based on available genome-wide platforms at the time of analysis, the number of samples collected, the clinical results, and the availability of funding. If genome-wide typing is performed, the results will be deposited into dbGAP, in accordance with NIH policy.

Consideration will also be given to exome or whole genome sequencing on some or all of the patients. This decision will be based on the clinical results and the availability of funding. Data deposition will be in accordance with any applicable NIH policy.

14.2.4 Statistical Considerations

The primary objective for the proposed pharmacogenomic companion is to validate rs11568821 as a SNP associated with severe immune-related toxicity. Specifically, an additive genetic risk model, with A as the risk allele, is hypothesized.

The immune toxicity hypotheses will be restricted to the self-reported white, non-Hispanic population treated with pembrolizumab. Evidence from a series of GWAS completed by the CALGB suggests that using a combination of self-reported race (white) and ethnicity (non-Hispanic) serves as a reasonable surrogate filter to identify a genetic European population. The patient population selection can of course be refined using genome-wide SNP data. The hypotheses related to clinical outcome will be tested in the entire patient population.

This companion will be designed under the assumption that 369 patients will be randomized to the treatment arm. It is expected that 85% of the patients will provide usable DNA along with consent to the pharmacogenomic analyses and that 85% will self-report as non-Hispanic whites. Thus, the expected sample size for the pharmacogenomic analyses will be n=266.

The SNP by toxicity association will be tested using a logistic model for additive risk effects at the one-sided α=0.01 level. The assumed relative frequency for the minor allele (A) is 0.12 (based on the putative relative allelic distribution for the EUR population reported in the dbSNP 1000 Genomes data, www.ncbi.nlm.nih.gov). The hypothesized severe (grade 3 or higher) immune-related toxicity probability for the treatment arm is 0.15. We will assume that the event probability follows a mixture of the form 0.15= (1-p0)^2*pi0 + 2*p0*(1-p0)*pi0*GRR + p0^2*pi0*GRR^2, where p0=0.12, pi0 denotes the probability of immune-related toxicity given no copies of the risk allele, and GRR denotes the genotype relative risk. The minimum effect size (GRR) detectable with a power of 0.8, at the one-sided α=0.01 level, is 2.25.

As a secondary objective, we will conduct a GWAS to validate or identify novel candidates, or, as next generation sequencing platforms become more cost effective, consider exome or whole-genome sequencing. Correction for multiple testing will be performed by accounting for the false-discovery rate (FDR).

All SNPs will be evaluated for deviation from Hardy-Weinberg. In the absence of a hypothesized effect, the association analyses will be powered for allele dosing (i.e., additive) effects. To this end, the Cochran-Armitage test (for binary endpoints), Jonkheere-Terpstra test (for quantitative traits including biomarker or gene expressions in serum or tumor RNA) and the Cox score test (for censored time-to-event outcomes including disease-free survival) will be used to quantify marginal associations. Multivariable models, with molecular, clinical and demographic variables, will be constructed using conditional inference trees and random forests.

14.3 Quality of Life studies in A031501 - Alliance A031501-HO1

14.3.1 Background

Patient-reported quality of life (QOL) after treatment for muscle-invasive bladder cancer has not been well-studied in cooperative group clinical trials, and this trial provides an opportunity to assess QOL changes after radical cystectomy plus/minus pembrolizumab,

at no additional cost to the NCI. Because this is an international trial, we will use the EORTC QLQ-C30 plus the muscle invasive bladder cancer module QLQ-BLM30 [66].

The European Organization for Research and Treatment of Cancer (EORTC) quality of life instruments consist of a common component (QLQ-C30) which includes questions broadly applicable to all cancer patients, which is meant to be combined with a disease-specific component, in this case QLQ-BLM30. QLQ-C30 assesses 5 functional domains (physical, role, cognitive, emotional, social) and 8 symptoms (fatigue, pain, nausea/vomiting, dyspnea, loss of appetite, insomnia, constipation, diarrhea), together with financial problems and global quality of life. The BLM-30 bladder-specific module further assesses symptoms in several domains, including urinary function, bowel symptoms, and sexual function (for men and women). Together, the EORTC instrument consists of a total of 60 questions, which takes approximately 10 minutes to complete.

Several questions of the EORTC instrument specifically measure described potential side effects of pembrolizumab, including fatigue, diarrhea, and pain. In addition, QOL data will allow an exploratory analysis to examine whether immune-therapy with pembrolizumab exacerbates urinary and other QOL effects after radical cystectomy/nephrectomy/ureterectomy – which has not been previously studied.

EQ5D-5L is a short, 6-question, instrument which assesses a patient's overall quality of life or health state[67]. The addition of the EQ5D-5L will allow for computation and comparison between arms of health utilities and quality adjusted life years (QALYs).

14.3.2 Objectives

Primary Objectives

1. Compare health-related quality of life (HRQL) as assessed by the EORTC QLQ-C30 between patients randomized to pembrolizumab vs. observation. Hypothesis: HRQL (based on the EORTC QLQ-C30 HRQL summary score at months 6, 12 and 24 months post-randomization) will be worse in patients randomized to pembrolizumab compared to observation over the first 24 months of the study.
2. Compare urinary symptoms as assessed by EORTC QLQ-BLM30 between patients randomized to pembrolizumab vs. observation. Hypothesis: Urinary symptoms (based on the EORTC QLQ-BLM30 urinary symptoms score at months 6, 12 and 24 months post-randomization) will be worse in patients randomized to pembrolizumab compared to observation over the first 24 months of the study.
3. Compare patient-reported fatigue, diarrhea, and pain between patients randomized to pembrolizumab vs. observation. Hypothesis: Fatigue, diarrhea, and pain (based on the EORTC QLQ-C30 fatigue, diarrhea, and pain scores at months 6, 12, and 24 months post-randomization) will be worse in patients randomized to pembrolizumab compared to observation over the first 24 months of the study.

Secondary Objectives

1. Compare health utilities and QALYs between patients randomized to pembrolizumab vs. observation. Hypothesis: Health utilities (based on the EQ5D-5L index score at months 6, 12, and 24 months post-randomization) will be worse in patients randomized to pembrolizumab compared to observation over the first 24 months of the study; however, we also hypothesize that QALYs will continue to favor pembrolizumab over observation.

Exploratory Objective

1. Compare other scale scores of the EORTC QLQ-C30, EORTC QLQ-BLM30, and EQ5D-5L between patients randomized to pembrolizumab vs. observation. Hypothesis: Other HRQL domains and symptoms (based on all other EORTC QLQ-C30, QLQ-BLM30, and EQ5D-5L scale/item scores at months 6, 12, and 24 months post-randomization) may be worse in patients randomized to pembrolizumab compared to observation over the first 24 months of the study.
2. Within subgroups defined by each of the stratification factors, to compare global quality of life, symptoms, health utilities, QALYs, and other scale scores of the three questionnaires between patients randomized to pembrolizumab vs. observation.

14.3.3 Methods

For Schedule of Assessments for this quality of life study, see Section 6.3. All participating institutions must ask patients for their consent to participate in this quality of life study (A031501-HO1), although patient participation is optional. Paper booklets will be used for this study. For information regarding ordering the booklets, see Section 4.5. For all patients who consent to participate in this quality of life study (A031501-HO1), a booklet will be given to the patient to complete at the specified planned clinic visits before any procedures/tests are initiated at the site visit and prior to any discussion of their status with healthcare personnel at the site. Booklets will be collected at the following time points: during screening and approximately 6, 12, and 24 months post-randomization. The booklet contains 66 questions and it is anticipated that the booklet will take approximately 10-15 minutes for the patient to complete at each administration time point. We anticipate having booklets available in a range of languages. Patients who consent to participate in this quality of life study (A031501-HO1) may decline to complete a booklet at any time. The primary reason for each missed booklet will be collected on a case report form.

14.3.4 Statistical Considerations

All questionnaires will be scored according to published scoring algorithms. The primary analysis will involve a single mixed model for each of the five primary patient-reported endpoints: EORTC QLQ-C30 HRQL summary score, QLQ-BLM30 urinary symptoms score, QLQ-C30 fatigue score, QLQ-C30 diarrhea score, and QLQ-C30 pain score. In order to give patients and physicians the opportunity to weigh the outcomes according to their own personal preferences, we have elected to analyze and report each outcome separately instead of constructing an ad hoc summary measure across domains. Construction of such a summary measure is generally discouraged because its measurement properties would be unknown, it would force a single weighting scheme across outcomes, and could potentially obfuscate important differences in individual domains. We have employed a Bonferroni approach to strictly control the type I error across these 5 primary endpoints.

For each primary patient-reported endpoint, a mixed model will compare the post-randomization endpoint (6, 12, and 24 months post-randomization) between randomized arms. In addition to a randomized arm covariate, each model will include a covariate for the baseline value of the endpoint and will use the planned month of assessment as the categorical time value. Unstructured covariance will initially be used, though alternative covariance structures will be investigated with the final covariance structure selected based on minimization of the Akaike information criterion. The statistical significance of the randomized arm covariate will be employed for hypothesis testing (one hypothesis test per primary endpoint) across the five primary endpoints. Within each model, this covariate represents the estimated average difference between randomized arms across the three post-randomization time points. We have chosen this approach to reduce the number of

hypothesis tests to one per endpoint. We feel that this is a reasonable approach because we anticipate a consistent effect across time points. We will modify this approach to include a time-by-arm interaction effect and report time-specific comparisons between arms (primary time point would be 6 months with 12 and 24 months considered as secondary) based on mixed model contrasts in the event that preliminary descriptive point estimates suggest that the direction of effect differs across time points.

Primary patient-reported outcome analysis will be conducted at the time that all consented patients have completed the 24-month assessment visit (or are no longer being followed for QOL). Release of the data for presentation will be at the discretion of the DSMB. Patients will be analyzed according to the randomized treatment arm assignment. All patients who consent for participation in the PRO component with a baseline endpoint value and at least one endpoint value post-randomization will be included in the analysis for a given endpoint. In the primary analysis, all observations available will be used. See below for information regarding analyses to account for missing data.

In secondary analysis, patient QALYs will be computed using the area-under-the-curve approach (with and without discounting) and will include all data through the follow-up of the last consented patient (i.e., the earliest censored patient). A population-based approach will also be used such that the area-under-the-curve of a quality-adjusted survival curve (mean health utility multiplied by the proportion of patients surviving based on Kaplan-Meier estimates) is the mean quality-adjusted survival for the population. Mean quality-adjusted survival will be compared between arms using a bootstrap approach. Exploratory analysis will include similar mixed model comparisons of other EORTC QLQ-C30, EORTC QLQ-BLM30, and EQ5D-5L scale/item scores. Supplemental comparisons of primary and other patient-reported endpoints at fixed time points may also be undertaken using analysis of covariance (ANCOVA) accounting for the baseline endpoint value. Exploratory analysis will also include similar mixed model comparisons of the primary and other patient-reported endpoints and QALY comparisons within subgroups by each stratification factor. Separate analyses will be carried out within each subgroup and interaction effects will be used to formally test for heterogeneity of effect.

Graphical procedures will include plots of average values over time by arm for each primary and other patient-reported endpoint. Exploratory analysis will include computation of Pearson correlations among patient-reported endpoints at fixed time points and univariate and multivariate linear mixed models of EORTC QLQ-C30 HRQL summary score and other EORTC QLQ-C30 functioning scale scores to investigate the impact of symptoms and randomized treatment assignment on QOL/functioning. Lastly, mediation models [68] will be developed to explore the mediating effects of patient-reported symptoms between randomized treatment assignment and patient-reported QOL/functioning as measured by the EORTC QLQ-C30 HRQL summary score and other EORTC QLQ-C30 functioning scale scores.

For all statistical analyses other than the primary patient-reported analyses, p-values <0.05 will be considered statistically significant (though interpretation will take into consideration that the Type 1 error is strictly controlled across the 5 primary endpoints only). For interpreting the clinical significance of effects, 0.2, 0.5, and 0.8 standard deviation (SD) effects will be considered as small, moderate, and large based on Cohen [69] throughout.

Missing data will be handled in a number of ways. Missing items within a summary or scale score will be handled according to each questionnaire's published scoring algorithms. Missing data at the summary or scale score level will be handled as follows. Baseline patient/disease characteristics will be compared between patients who do and do not

provide data for the primary analysis. We will also graphically explore patterns of missing data. All analyses will be completed using all available data, followed by analyses completed using a range of imputation methods. Lastly, we will employ pattern mixture models for longitudinal analyses. Output from all analyses will be tabulated and descriptively compared to assess the degree to which missing data impacts study results.

Power: Assuming that 490 patients are evaluable for the primary analysis (i.e., allowing 30-35% of patients to decline consent or be nonevaluable for the primary analysis), this QOL study has 90% power for each individual test to detect a 0.35 standard deviation difference and 97% power for each individual test to detect a 0.40 standard deviation difference between arms using a two-sided alpha=0.05/5 t-test. See below for a table showing power for each individual test as well as power overall across 5 tests assuming different levels of correlation among the primary endpoints. Assuming that all 5 primary hypothesis tests are independent, the likelihood of detecting a 0.40 standard deviation effect in 5 of 5 tests is 85%, which we feel is reasonable.

For secondary comparison of QALYs, 490 patients provides 76% power for this individual test assuming a 0.40 standard deviation difference in health utilities between arms and a 5-year OS of 43.5% in the observation arm and an OS hazard ratio of 0.65 between arms using a two-sided alpha of 0.05.

Effect size	**Power for each individual test**	**Pairwise correlation between endpoints**	**Power across all 5 tests**
0.35	90%	0	59%
		0.1	62%
		0.3	66%
		0.5	71%
0.40	97%	0	85%
		0.1	85%
		0.3	86%
		0.5	88%

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APPENDIX I REGISTRATION FATIGUE/UNISCALE ASSESSMENTS

Registration Fatigue/Uniscale Assessments

At patient registration, this form is to be administered by a nurse/CRA, completed by the patient, and entered into Medidata Rave at the time of registration.

If needed, this appendix can be adapted to use as a source document. A booklet containing this assessment does not exist – please do not order this booklet.

How would you describe:

your level of fatigue, on the average in the past week including today?

0	1	2	3	4	5	6	7	8	9	10
No Fatigue										Fatigue as bad as it can be

your overall quality of life in the past week including today?

0	1	2	3	4	5	6	7	8	9	10
As bad as it can be										As good as it can be

APPENDIX II QOL MODULES FOR A031501-HO1

EORTC QLQ - BLM30

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

PLEASE ANSWER QUESTIONS 31 - 37 ONLY IF YOU DO NOT HAVE A UROSTOMY

During the past week:	Not at all	A little	Quite a bit	Very much
31. Have you had to urinate frequently **during the day**?	1	2	3	4
32. Have you had to urinate frequently **at night**?	1	2	3	4
33. When you felt the urge to pass urine, did you have to hurry to get to the toilet?	1	2	3	4
34. Was it difficult for you to get enough sleep, because you needed to get up frequently at night to urinate?	1	2	3	4
35. Have you had difficulty going out of the house, because you needed to be close to a toilet?	1	2	3	4
36. Have you had any unintentional release (leakage) of urine?	1	2	3	4
37. Have you had pain or a burning feeling when urinating?	1	2	3	4

PLEASE ANSWER QUESTIONS 38 - 43 ONLY IF YOU HAVE A UROSTOMY

During the past week:	Not at all	A little	Quite a bit	Very much
38. Has urine leaked from your urostomy bag?	1	2	3	4
39. Did you have problems with caring for your urostomy?	1	2	3	4
40. Was your skin around the urostomy irritated?	1	2	3	4
41. Have you felt embarrassed because of your urostomy?	1	2	3	4
42. Have you been dependent on others for caring for your urostomy?	1	2	3	4
43. Did you frequently have to change the urostomy bag?	1	2	3	4

PLEASE ANSWER QUESTION 44 ONLY IF YOU HAVE USED A CATHETER DURING THE PAST WEEK

44. Have you had problems with self-catheterization? (inserting a tube in the bladder to pass urine)	1	2	3	4

Please go on to the next page

During the past week:	Not at all	A little	Quite a bit	Very much
45. Were you worried about your health in the future?	1	2	3	4
46. Did you worry about the results of examinations and tests?	1	2	3	4
47. Did you worry about possible future treatments?	1	2	3	4
48. Have you had a bloated feeling in your abdomen?	1	2	3	4
49. Have you had flatulence or gas?	1	2	3	4
50. Have you felt physically less attractive as a result of your illness or treatment?	1	2	3	4
51. Have you been dissatisfied with your body?	1	2	3	4
52. Have you felt less feminine/masculine as a result of your illness or treatment?	1	2	3	4

During the past 4 weeks:	Not at all	A little	Quite a bit	Very much
53. To what extent were you interested in sex?	1	2	3	4
54. To what extent were you sexually active (with or without sexual intercourse)?	1	2	3	4
55. **For men only:** Did you have difficulty gaining or maintaining an erection?	1	2	3	4
56. **For men only:** Did you have ejaculation problems (e.g. dry ejaculation)?	1	2	3	4

Please answer the following 4 questions only if you have been sexually active during the past 4 weeks:	Not at all	A little	Quite a bit	Very much
57. Have you felt uncomfortable about being sexually intimate?	1	2	3	4
58. Have you worried that you may contaminate your partner during sexual contact with the bladder treatment you have been receiving?	1	2	3	4
59. To what extent was sex enjoyable for you?	1	2	3	4
60. **For Women only:** did you have a dry vagina or other problems during intercourse?	1	2	3	4

EORTC QLQ - BLM30

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

PLEASE ANSWER QUESTIONS 31 - 37 ONLY IF YOU DO NOT HAVE A UROSTOMY

During the past week:	Not at all	A little	Quite a bit	Very much
31. Have you had to urinate frequently during the day?	1	2	3	4
32. Have you had to urinate frequently at night?	1	2	3	4
33. When you felt the urge to pass urine, did you have to hurry to get to the toilet?	1	2	3	4
34. Was it difficult for you to get enough sleep, because you needed to get up frequently at night to urinate?	1	2	3	4
35. Have you had difficulty going out of the house, because you needed to be close to a toilet?	1	2	3	4
36. Have you had any unintentional release (leakage) of urine?	1	2	3	4
37. Have you had pain or a burning feeling when urinating?	1	2	3	4

PLEASE ANSWER QUESTIONS 38 - 43 ONLY IF YOU HAVE A UROSTOMY

During the past week:	Not at all	A little	Quite a bit	Very much
38. Has urine leaked from your urostomy bag?	1	2	3	4
39. Did you have problems with caring for your urostomy?	1	2	3	4
40. Was your skin around the urostomy irritated?	1	2	3	4
41. Have you felt embarrassed because of your urostomy?	1	2	3	4
42. Have you been dependent on others for caring for your urostomy?	1	2	3	4
43. Did you frequently have to change the urostomy bag?	1	2	3	4

PLEASE ANSWER QUESTION 44 ONLY IF YOU HAVE USED A CATHETER DURING THE PAST WEEK

44. Have you had problems with self-catheterization? (inserting a tube in the bladder to pass urine)	1	2	3	4

Please go on to the next page

Health Questionnaire

English version for the USA

Under each heading, please check the ONE box that best describes your health TODAY

MOBILITY

- I have no problems walking ❑
- I have slight problems walking ❑
- I have moderate problems walking ❑
- I have severe problems walking ❑
- I am unable to walk ❑

SELF-CARE

- I have no problems washing or dressing myself ❑
- I have slight problems washing or dressing myself ❑
- I have moderate problems washing or dressing myself ❑
- I have severe problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

- I have no problems doing my usual activities ❑
- I have slight problems doing my usual activities ❑
- I have moderate problems doing my usual activities ❑
- I have severe problems doing my usual activities ❑
- I am unable to do my usual activities ❑

PAIN / DISCOMFORT

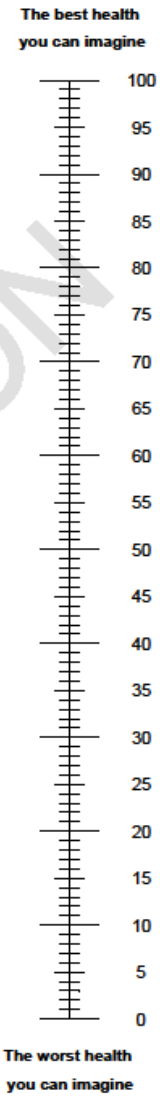
- I have no pain or discomfort ❑
- I have slight pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have severe pain or discomfort ❑
- I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

- I am not anxious or depressed ❑
- I am slightly anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am severely anxious or depressed ❑
- I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Health Questionnaire

English version for the USA

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ____

Your birthdate (Day, Month, Year): ____

Today's date (Day, Month, Year): 31 ____

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ____

Your birthdate (Day, Month, Year): ____

Today's date (Day, Month, Year): 31 ____

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

APPENDIX III: NCI/DCTD COLLABORATIVE AGREEMENTS LANGUAGE

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm).-Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

APPENDIX IV: CTDNA CORRELATIVE SCIENCE

1.0 Correlative Endpoints

To determine if ctDNA status (positive vs negative) and clearance (from positive to negative) are associated with OS and DFS in all patients with muscle-invasive bladder and upper-tract urothelial carcinoma treated with adjuvant pembrolizumab or observation.

1. The OS and PFS outcomes in the patients with evaluable ctDNA are similar to the ITT population

   Analysis: The first analysis will determine whether the subset of patients with evaluable ctDNA has similar OS and DFS outcomes compared to the entire ITT population. Kaplan-Meier estimates of OS (DFS) will be computed for the ctDNA evaluable and the intent to treat cohorts. The area under the OS (DFS) Kaplan-Meier curve will be calculated for each cohort along with a bootstrap confidence interval for the difference in the area under the curves, using the individual as the resampling unit. The computed confidence interval will be used to evaluate similarity between the evaluable and the intent to treat cohorts. The second analysis will determine whether there are differences in OS and DFS between patients with evaluable ctDNA (included in this correlative analysis) and those without evaluable ctDNA (patients not included in this correlative analysis). A log rank test will be used to test whether the OS (DFS) differs between patients with evaluable ctDNA and those without evaluable ctDNA. If there is a statistically significant difference, a Cox model will be used to determine if the adjusted hazard ratio (HR) differs significantly from 1 as a confirmation to determine whether the difference in survival curves could be due to other variables. The Cox model will contain the randomization stratification variables, the treatment arm (pembrolizumab versus observation), and ctDNA status (evaluable versus not evaluable) at a minimum. It may also contain prognostic baseline variables that differ between the group of patietns with evaluable ctDNA and those without evaluable ctDNA.

2. ctDNA status is associated with OS and DFS

   Analysis: An association of ctDNA status at baseline (positive versus negative) with outcome (DFS or OS) could mean ctDNA status at baseline is prognostic and/or predictive of benefit from treatment with pembrolizumab. The initial analysis will determine whether the association with ctDNA status at baseline differs by treatment arm. If it does, it would be evidence that ctDNA status is predictive of OS (DFS) benefit from pembrolizumab treatment. This will be assessed with a Cox model that contains the randomization stratification variables, treatment arm (pembrolizumab versus observation), ctDNA status at baseline, and the treatment arm by ctDNA status interaction term; separate analyses will be done with OS as the outcome and DFS as the outcome. If the interaction term is statistically significant at a 0.15 significance level, then an estimate of the strength of the association of ctDNA status with outcome (DFS or OS) will be determined with separate analyses for each treatment arm. A 0.15 level of significance is used to increase power (study is not powered adequately to detect an interaction). If the interaction term is not statistically significant, then ctDNA status will be assumed to be prognostic. An estimate of the association of ctDNA status with outcome (DFS or OS) will be made with a Cox model that includes the randomization stratification variables and treatment assignment as adjustment variables and baseline ctDNA status as the variable of interest. Finally, we will also generate separate estimates

of the association of baseline ctDNA status with outcome (DFS or OS) with Cox models that include the randomization stratification variables.

3. ctDNA clearance (+ → -) at 3 months (will also assess 6, 9, and 12 months) is associated with OS and DFS benefit vs no clearance (+ → +) in patients treated with adjuvant pembrolizumab

   Analysis: This analysis only includes patients treated with adjuvant pembrolizumab and who where ctDNA positive at baseline. The score test from a time-dependent variable (ctDNA status evaluated at 3, 6, 9, and 12 months) in a Cox model with the randomization stratification variables as adjustment variable and OS (DFS) as the outcome variable will be used to determine if there is an association between ctDNA clearance status (cleared meaning + → - versus not cleared meaning + → +) and outcome.

4. Estimate the time from ctDNA appearance (- → +) to disease recurrence by standard imaging

   Analysis: This analysis is restricted to patients with a negative ctDNA result at baseline and then had a subsequent ctDNA positive result. The cumulative incidence function using Kaplan-Meier estimates will be computed to estimate the probability of disease recurrence starting from the time of the first ctDNA positive result based. Patients who are alive without a disease recurrence (via standard imaging) will be censored at the time of their last disease evaluation using standard imaging. Patients who died prior to a disease recurrence will be censored at time of death. The median time (if reached) from first positive ctDNA result to disease recurrence detected with standard imaging and corresponding 95% confidence interval will be computed. In addition, the positive predictive and negative predictive value over the time of ctDNA landmarked at baseline, 3, 6, and 12 months will be determined using disease recurrence by standard imaging as ground truth. The analysis will be repeated with the addition of patients who are ctDNA positive at baseline. Their first positive ctDNA result will be the date of randomization.

5. ctDNA clearance (+ → -) rate after the completion of treatment is higher in patients treated with adjuvant pembrolizumab vs observation

   Analysis: This analysis is restricted to patients with positive ctDNA at baseline. The proportion of patients with ctDNA clearance (had a negative ctDNA result during treatment, essentially by one year after enrollment) will be determined for each treatment arm (pembrolizumab and observation). The proportions will be compared with a chi-square test or a Fisher's exact test if more appropriate.

6. Patients with combined ctDNA+ at baseline and PD-L1+ have an improved OS and DFS compared to patients with ctDNA+ at baseline and PD-L1-

   Analysis: This analysis is restricted to patients who had a ctDNA+ result at baseline. Association with PD-L1 status (positive versus negative) with outcome (DFS or OS) could mean PD-L1 status is prognostic and/or predictive of benefit from treatment with pembrolizumab in this patient subgroup. The initial analysis will determine whether the association of PD-L1 status and outcome (OS or DFS) differs by treatment arm. If it does, this would be evidence that PD-L1 status is predictive for OS (DFS) benefit from pembrolizumab treatment. This will be assessed with a Cox model that contains the

randomization stratification variables, treatment arm (pembrolizumab versus observation), PD-L1 status (positive versus negative), and the treatment arm by PD-L1 status interaction term; separate analyses will be done with OS as the outcome and DFS as the outcome. If the interaction term is statistically significant at a 0.15 significance level, then an estimate of the strength of the association of PD-L1 status with outcome (DFS or OS) will be determined with separate analyses for each treatment arm. A 0.15 level of significance is used to increase power (study is not powered adequately to detect an interaction). If the interaction term is not statistically significant, then PD-L1 status will be assumed to be prognostic. An estimate of the association of PD-L1 status with outcome (DFS or OS) will be made with a Cox model that includes the randomization stratification variables and treatment assignment as adjustment variables and PD-L1 status as the variable of interest. Finally, we will also generate separate estimates of the association of PD-L1 status with outcome (DFS or OS) with Cox models that include the randomization stratification variables.

7. Patients with combined ctDNA+ and TMB+ (≥ 10 mut/mb) have an improved OS and DFS compared to patients with ctDNA+/TMB-

   Analysis: This analysis is restricted to patients who had a ctDNA+ result at baseline. Association with TMB status (positive versus negative) with outcome (DFS or OS) could mean TMB status is prognostic and/or predictive of benefit from treatment with pembrolizumab in this patient subgroup. The initial analysis will determine whether the association of TMB status and outcome (OS or DFS) differs by treatment arm. If it does, this would be evidence that TMB status is predictive for OS (DFS) benefit from pembrolizumab treatment. This will be assessed with a Cox model that contains the randomization stratification variables, treatment arm (pembrolizumab versus observation), TMB status (positive versus negative), and the treatment arm by TMB status interaction term; separate analyses will be done with OS as the outcome and DFS as the outcome. If the interaction term is statistically significant at a 0.15 significance level, then an estimate of the strength of the association of TMB status with outcome (DFS or OS) will be determined with separate analyses for each treatment arm. A 0.15 level of significance is used to increase power (study is not powered adequately to detect an interaction). If the interaction term is not statistically significant, then TMB status will be assumed to be prognostic. An estimate of the association of TMB status with outcome (DFS or OS) will be made with a Cox model that includes the randomization stratification variables and treatment assignment as adjustment variables and TMB status as the variable of interest. Finally, we will also generate separate estimates of the association of TMB status with outcome (DFS or OS) with Cox models that include the randomization stratification variables.

8. Patients with TMB+ (≥ 10 mut/mb) have an improved OS and DFS compared to patients with TMB- when treated with adjuvant pembrolizumab or observation

   Analysis: An association of TMB status at baseline (positive versus negative) with outcome (DFS or OS) could mean TMB status at baseline is prognostic and/or predictive of benefit from treatment with pembrolizumab. The initial analysis will determine whether the association with TMB status at baseline differs by treatment arm. If it does, it would be evidence that TMB status is predictive of OS (DFS) benefit from pembrolizumab treatment. This will be assessed with a Cox model that contains the randomization stratification variables, treatment arm (pembrolizumab versus observation), TMB status at baseline, and the treatment arm by TMB status interaction

term; separate analyses will be done with OS as the outcome and DFS as the outcome. If the interaction term is statistically significant at a 0.15 significance level, then an estimate of the strength of the association of TMB status with outcome (DFS or OS) will be determined with separate analyses for each treatment arm. A 0.15 level of significance is used to increase power (study is not powered adequately to detect an interaction). If the interaction term is not statistically significant, then TMB status will be assumed to be prognostic. An estimate of the association of TMB status with outcome (DFS or OS) will be made with a Cox model that includes the randomization stratification variables and treatment assignment as adjustment variables and TMB status as the variable of interest. Finally, we will also generate separate estimates of the association of TMB status with outcome (DFS or OS) with Cox models that include the randomization stratification variables.

2.0 Background

Circulating tumor DNA (ctDNA) is a molecular invasive "liquid biopsy" technology currently under investigation that may potentially improve the detection of measurable residual disease and enhanced risk stratification that could enhance patient selection for therapy and consequently prolong survival in the highest risk population. ctDNA may also be prognostic and has been shown to predict recurrence after a cystectomy with a lead time of 3 months sooner than conventional imaging (1).

Better biomarkers for risk-stratification are necessary to select those patients who would benefit most from adjuvant immunotherapy after cystectomy. Detection of ctDNA post-cystectomy is considered detection of "measurable residual disease" (MRD) and may potentially serve as a biomarker to guide treatment selection (2).

3.0 Sample Processing

Tumor DNA

- Tumor DNA will be extracted from formalin-fixed paraffin-embedded (FFPE) sections of tumor by the Alliance repository. Requirements will include at least 5 FFPE tumor sections of 10μM in thickness.
- Approximately ½ of the extracted DNA from the primary tumor will be shipped to Natera for whole exome sequencing and the other ½ to Foundation Medicine for FoundationOne analysis.

Peripheral Blood Plasma

- Plasma will be collected from a 1 x 10 ml lavender top (EDTA) tube. See Section 6 for more detail regarding collection.

- **For Natera**
  - For each patient, 3- 4 mL of frozen never thawed plasma will be shipped for each time point for eligible cases. Samples not meeting these criteria may still be accepted but may affect Signatera calling performance. As such, samples not passing QC will be flagged at time of results reporting.

4.0 Techniques

1. Tumor tissue will be sequenced using two methods [1] Foundation Medicine's tissue-based CGP assay that detects genomic alterations in DNA extracted from formalin-fixed paraffin-

embedded tissue.[2] Signatera's WES assay will sequence tumor tissue and matched normal germline material

2. Up to 16 mutations for personalized mPCR ctDNA assay will be identified for each patient
3. Plasma samples will be sequenced using FoundationOne®Tracker ctDNA assay. FoundationOne®Tracker is intended to provide ctDNA burden measurements for longitudinal tracking of cfDNA extracted from plasma as a marker for tumor burden in a cancer patient previously tested for specific somatic variants identified through baseline testing of FFPE (formalin-fixed paraffin-embedded) tumor tissue.. Signatera assay will be starting from F1Tracker libraries
4. If ≥2 mutations are detected, sample was defined as ctDNA(+)
5. MRD sample timepoint before adjuvant treatment (C1D1) will be collected
6. On-treatment sample every 12 weeks x 5 (total) timepoints will also collected

5.0 References

1) Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma Emil Christensen, Karin Birkenkamp-Demtröder, et al. Journal of Clinical Oncology 2019 37:18, 1547-1557
2) Powles et al. IMvigor010 ctDNA